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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Alteration of Wheat Source-Sink Relation by Nitrogen and Spikelet Removal

Hassan HEIDARI[1*]

Abstract

The source and sink relationships determine the amount and distribution of biomass in plants. Field and laboratory experiments were conducted to study the effect of nitrogen rate and spikelet removal on seed yield and germination traits of wheat. The field experiment was conducted employing sink manipulation (no spikelet removal and ½ spikelet removal) and source manipulation (nitrogen rate of 0, 75, and 150 kg ha^{-1}). This study was performed as a factorial experiment in a randomized complete block design with three replications. Seeds obtained from the field experiment were subjected to determine the effect of the sink and source manipulation on seed germination traits in the laboratory experiment. Results showed that most traits under study were not affected by source and sink manipulation. Seed yield and seed weight were not affected by spikelet removal and varying nitrogen applications. Although some of the wheat spikelets have been removed, those plants have been able to maintain the number of seeds per spike and the weight of a single seed. The use of nitrogen at the spike emergence stage did not affect the seed yield of the Pishtaz cultivar. Nitrogen needed for the seeds could be compensated by the re-mobilization of nitrogen from various plant organs such as the stem. Nitrogen application of 150 kg ha^{-1} with the removal of ½ spikelets improved seed germination (%) and vigor comparing control (no spikelet removal with no nitrogen application). Considering that seed yield and seed weight did not change under the influence of source and sink manipulation, it can be concluded that Pishtaz wheat is more sink-limited than source-limited.

Keywords: Biomass, Germination, Harvest index**,** Plant nutrition**,** Seed vigor, *Triticum aestivum* L.

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1. Introduction

In crops, the organ in which food is made is called the source and the place where food is stored is called the sink. If the sink is small, the plant yield will not be high. Even if the sink is large but the source is limited, the yield will be low (Smith et al., 2018). More dry matter accumulation in some new wheat cultivars is associated with a higher allocation of photosynthate to the reproductive parts of the plant, which increases by more nitrogen uptake from the soil (Zhang et al., 2020). Nitrogen fertilization significantly increased grain yield in wheat. Nitrogen applied in spring and split-application of nitrogen led to higher seed yield than in autumn application (Ghimire et al., 2021). Elimination of old fruits decreased the respiration rate and increased the sugar content of new fruits (Zhou et al., 2000). In maize, the removal of 50% of the cob due to sink limitations had minimal remobilization (Falihzade et al., 2013). Uhart and Andrade (1991) reported that maize grown in the temperate zone had both sink and source constraints. While Seebauer et al. (2010) reported that in maize, grain compounds have source limitations. Mohammadi et al. (2014) reported that there is more sink limitation in new wheat and work needs to be done to increase the sink capacity.

In two tomato cultivars, photosynthesis was not affected by the source-sink change, but the net assimilation rate of tomatoes increased with an increasing source-sink ratio (Matsuda et al., 2011). In a study of the relationship between the source and the sink of three wheat varieties, it was observed that the single-grain weight decreased with the removal of the flag leaf and penultimate leaf and increased with the removal of the spikelet. This shows that wheat grain yield is more source-limited than sink-limited and more attention should be paid to increasing the source (Xiao-li et al., 2022). Manipulation of wheat source and sink showed that removal of half spikelets caused an increase, but defoliation caused a decrease in single grain growth. These changes show that the studied wheat cultivar has both sink and source limitations (Lv et al., 2020). Manipulating the relationship between source and sink with methods such as nitrogen fertilizer and trimming in wheat genotypes did not affect grain weight, but it affected grain quality (Arata et al., 2023). Ma et al. (1996) observed that the removal of wheat spikelets increased the grain weight of the responsive cultivars, but there was no difference in final grain weight between 50 and 75% removal of spikelets. In the study of the relationship between the source and the sink, it was observed that the removal of half of the spikelets caused a decrease in the number of grains in the spike, the weight of a single spike, and the weight of the whole grain of wheat (Wang et al., 2021). In the conditions of drought stress, the removal of wheat spikelets increased grain yield and 1000-grain weight by 35 and 21%, respectively (Abdoli et al., 2013). In maize, leaf removal resulted in significant remobilization of stem reserves, which did not completely alleviate seed weight loss. Seed weight was more sensitive to decreasing source-sink ratio than green leaf area duration (Abeledo et al., 2020). In canola, partial removal of pods and complete leaf removal at full flowering reduced pods per plant and yield. Supplementary irrigation increased yield without affecting the average grain weight. Canola yield is limited by the size of the sink at the flowering stage and by the source at the grain-filling stage (Zhang and Flottmann, 2018). Nitrogen is one of the most consumed nutrients that can be involved in the power of the source. Nitrogen consumption increased yields in crops such as wheat (Akdag and Zengin, 2020) and soybean (*Glycine max* (L.) Merrill) (Basal and Szabo, 2020).

The maternal plant environment can affect the germination characteristics of produced seeds (Kathleen, 2009; Sales et al., 2013; Sánchez et al., 2022). In *Sinapis arvensis*, it was observed that nitrogen enrichment of the maternal plant environment reduced the germination percentage of produced seeds, which is probably due to the induction of dormancy in the seeds (Luzuriaga et al., 2006). The addition of nitrogen to the maternal plant environment in *Potentilla tanacetifolia* significantly affected seed production, seed weight, and seed germination (Li et al., 2011). Environmental conditions during sunflower seed growth on the mother plant regulated seed dormancy rate. Seed dormancy at harvest time was a function of growth cycle length and seed drying rate on the mother plant (Lachabrouilli et al., 2021). Adding nitrogen to the medium of the mother plant increased the germination percentage and decreased the germination time of the Perennial Grass *Leymus chinensis* (Trin.) Tzvel seeds (Zhao et al., 2021).

There are limited studies on the effect of source and sink constraints on the germination characteristics of crop seeds. Therefore, this research was designed to determine the seed yield and germination characteristics of wheat seeds under varying source and sink relationships.

2. Materials and Methods

2.1. Field experiment

The field experiment was conducted on Chamchamal Plain located 40 km from Kermanshah (latitude 34 degrees north, longitude 47 degrees east, and altitude 1300 meters above sea level). The mean annual precipitation in the region is 442 mm (IMO, 2012). Chamchamal Plain has a high groundwater level and the groundwater level in April reaches about 1.5 meters from the soil surface (Razzaghmanesh et al., 2004).

2.1.1. Agronomical practices

The land used last year was under maize cultivation. In the fall of 2012, the land was plowed by a moldboard plow. Then phosphorus fertilizer at the rate of 266 kg ha^{-1} was used. Wheat (*Triticum aestivum*) seeds of the Pishtaz cultivar with 98% germination at the rate of 333 kg ha^{-1} were sown manually on November 10th. This cultivar was introduced by the Cereal Research Department, Seed and Plant Improvement Institute, Karaj, for cultivation in the temperate climate of Iran. Pishtaz wheat is relatively late and somewhat sensitive to plant loading and high yield. In spring, at the beginning of stem elongation, 300 kg ha^{-1} of urea fertilizer was applied as a top dressing. The most important weeds were wild oats (*Avena sp*), wild mustard (*Sinapis arvensis*), lady's bedstraw (*Galium verum*), and reed (*Phragmites sp*.). 2, 4-D + MCPA herbicide was used to control broad-leaved weeds. Wheat sunn pest was one of the pests observed in the field which was controlled with a suitable pesticide. During the growing season, the plants were irrigated three times (spike emergence stage, grain milk stage, and grain dough stage) by the surface method.

2.1.2. Experimental design and sampling

This study was performed as a factorial experiment in a randomized complete block design with three replications. The size of the plots was one square meter and the distance between the plots was considered to be one meter and the distance between the repetitions was two meters. The time of the wheat spike's emergence was May 10, 2013. The time of applying the treatments was May 15th. Experimental treatments included sink manipulation (no spikelet removal and removal of half spikelets) and source manipulation (consumption of 0, 75, and 150 kg N ha^{-1}) at the spike emergence stage. The spikelets were removed with a cutter. Nitrogen was used as a top dressing. The amounts of nitrogen used at the spike emergence stage are in addition to the amounts used at the start of stem elongation.

At the time of harvest (June 27), three plants per plot were randomly selected. These plants represented that plot. Spike length, spike weight, stem and leaf weight, number of seeds per spike, single seed weight, seed yield, and biological yield were scored. The biological yield was obtained from the total weight of the leaf, stem, and seed. Plant samples were weighed on a digital scale. A ruler with millimeter precision was used to measure the spike length. To measure the weight of a single seed, firstly 100 seeds were selected and weighed, then divided by 100. The harvest index was computed by dividing seed yield by biological yield.

2.2. Laboratory experiment

This study was designed to investigate the effect of the maternal plant environment (source and sink manipulation) on the germination characteristics of seeds obtained from the field experiment. After separating the seeds from the mother plant, the seeds were kept at room temperature for one week. This study was conducted at Razi University as a factorial experiment in a completely randomized design with three replications in July 2013. Wheat seeds were sterilized with 1% sodium hypochlorite solution for 10 minutes and washed immediately with water. Twenty wheat seeds were placed on a filter paper in each disinfected Petri dish. 8 mm of sterile distilled water was poured into each Petri dish and the Petri dishes were kept at 30°C for one week. Three seedlings per Petri dish were used to measure caulicle length, radicle length, seedling weight, and seed vigor. To measure the seed germination percentage, seeds that had two millimeters of caulicle growth were counted as germinated. The germination percentage was obtained by dividing the number of germinated seeds by all the seeds inside each Petri dish. Seed vigor was calculated using Heidari et al. (2013) equations (Eq. 1, 2).

$$\text{Seed vigor based on length (\% cm)} = \text{Seed germination percentage} \times \text{Seedling length (cm)} \quad \text{(Eq. 1)}$$

$$\text{Seed vigor based on weight (\% g)} = \text{Seed germination percentage} \times \text{Seedling weight (g)} \quad \text{(Eq. 2)}$$

In the above equations, the seedling length is the sum of the radicle length and the caulicle length. The seedling weight is the sum of the radicle weight and the caulicle weight.

2.3. Statistical analysis

MINITAB statistical software was used to find outlier data and test normality. The data were first analyzed by SAS statistical software and PROC GLM procedure. Then Duncan's test was used at the 5% probability level to compare the mean of the data. SPSS statistical software was used to calculate correlation coefficients between traits using Pearson's method.

3. Results and Discussion

3.1. Field experiment

Variance analysis of data showed that nitrogen and removal of spikelets had no significant effect on stem and leaf weight, spike weight, seed yield, seed weight, biological yield, and harvest index in wheat (*Table 1*). The removal of half spikelets had a significant effect on the number of seeds per spike and the length of the spike in wheat (*Table 1*).

3.1. 1. Stem and leaf weight and spike length

A comparison of the mean data showed that there was no difference between the studied treatments in terms of stem and leaf weight (*Table 2*). Probably the weight of the stem and leaves reached its maximum weight at the stage of spike emergence, and from this stage onwards, the reproductive part should have gained weight. The length of the spike was reduced by removing half of the spikelets (*Table 2*). This is perfectly normal because half of the spike is removed at the time of flowering. Nitrogen consumption of 75 kg ha^{-1} improved the spike length compared to nitrogen consumption of 150 kg ha^{-1}. Excess nitrogen may stimulate the growth of vegetative parts of the plant, although in this study the difference in stem and leaf weight was not significant. She et al. (2023) reported that nitrogen consumption of more than 150 to 240 kg per hectare did not affect wheat grain yield and growth. Spike length had a significantly positive correlation with most of the studied traits (*Table 3*). Spike length had a significantly negative correlation with seed weight (*Table 3*). This indicates that the seed weight increases with a decreasing number of seeds per spike.

3.1.2. Number of seeds per spike and weight of spike

Removing half of the florets only when nitrogen was not used or a small amount of nitrogen (75 kg ha^{-1}) was used, reduced the number of seeds per spike compared to treatment of 75 kg N ha^{-1} with no spikelet removal (*Table 2*). Spike's weight was not affected by any of the treatments. It seems that if, at the early stages of spike emergence, stress occurs, the plant loses some of its seeds, and the Pishtaz cultivar can compensate. Source constraints at the time of wheat seed filling reduced the number of seeds per unit area (Uhart and Andrade, 1991). The number of seeds per spike and the weight of the spike had a significantly positive correlation with most of the studied traits (*Table 3*). The number of seeds per spike had a significantly negative correlation with seed weight (*Table 3*). This indicates that the seed weight increases with a decreasing number of seeds per spike.

3.1.3. Seed yield and seed weight

Seed yield and seed weight were not affected by experimental treatments (*Table 2*). It seems that although some of the wheat florets have been removed, this plant has been able to maintain the number of seeds per spike and the weight of a single seed, which, ultimately, seed yield was not affected by treatments. The use of more nitrogen at the spike emergence stage did not affect the seed yield of the Pishtaz cultivar. Perhaps the plant provided the nitrogen needed for the seeds by re-mobilization nitrogen from organs such as the stem or soil nitrogen was enough for the seeds to grow. It is not possible to say here which factor (source or sink) limits the yield of the Pishtaz cultivar because seed yield has not changed under the influence of these two factors. Uhart and Andrade (1991) reported that source limitation during wheat grain filling reduced grain weight. In the study of the relationship between the source and sink in rice, it was seen that increasing the ratio of spikelet to leaf led to more allocation of nitrogen and biomass to the spike instead of the leaf and caused an imbalance between the source and the source (Li et al., 2023). Seed yield had a significantly positive correlation with most traits except seed weight and stem and leaf weight (*Table 3*).

3.1.4. Biological yield and harvest index

Biological yield and harvest index were not affected by source and sink manipulation (*Table 2*). The formed biomass of wheat may have been relatively completed at the spike emergence stage and at this stage, there has been a re-mobilization of material from the vegetative parts to the reproductive parts. Respiration of this cultivar may have lost part of the current photosynthetic reserves of the leaves, or it can be said that the current photosynthesis of the plant was not to the extent that it caused a significant increase in plant dry matter. However, some carbohydrates are lost by re-remobilizing material from vegetative parts such as the stem, which act as temporary food storage. Removal of wheat spikelets did not increase sucrose flow and starch accumulation in the remaining grains (Jenner, 1980), which is consistent with the results of our study. The harvest index had a significantly positive correlation only with seed yield and was not correlated with other traits (*Table 3*). This indicates that the harvest index is more important than other traits in increasing plant yield, and plant breeding work to manipulate the source and sink of wheat can focus on this trait.

Table 1. Analysis of variance (mean square) of the effect of spikelet removal and nitrogen on wheat traits

Source of variation	df	Stem and leaf weight	Seed number per spike	Spike length	Spike weight	Seed yield	Seed weight	Biological yield	Harvest index
Block	2	0.052^{*}	57.4^{ns}	2.28^{ns}	0.085^{ns}	0.089^{ns}	0.00003^{ns}	0.211^{ns}	164.7^{ns}
Spikelet removal (S)	1	0.042^{ns}	317.5^{*}	30.42^{**}	0.411^{ns}	0.228^{ns}	0.00007^{ns}	0.186^{ns}	239.8^{ns}
Nitrogen (N)	2	0.012^{ns}	25.7^{ns}	2.40^{ns}	0.019^{ns}	0.004^{ns}	0.00005^{ns}	0.030^{ns}	9.7^{ns}
S×N	2	0.001^{ns}	59.2^{ns}	2.22^{ns}	0.077^{ns}	0.049^{ns}	0.00003^{ns}	0.090^{ns}	3.4^{ns}
Error	10	0.011	36.6	1.44	0.132	0.072	0.00004	0.205	64.0

**, *=significant at the probability level of 1 and 5%, respectively. ^{ns}=non-significant

Table 2. Mean comparisons of wheat seed yield and yield-attributed traits under spikelet removal and nitrogen fertilizer

Treatments	Stem and leaf weight (g/plant)	Seed number per spike	Spike length (cm)	Spike weight (g/plant)	Seed yield (g/plant)	Seed weight (g)	Biological yield (g/plant)	Harvest index (%)
S1N1 [a]	0.632 [a]	29.3 [ab]	8.0 [ab]	1.53 [a]	1.08 [a]	0.036 [a]	2.16 [a]	49.8 [a]
S1N2	0.691 [a]	32.9 [a]	8.9 [a]	1.65 [a]	1.14 [a]	0.034 [a]	2.35 [a]	47.9 [a]
S1N3	0.708 [a]	22.9 [ab]	6.6 [bc]	1.33 [a]	0.96 [a]	0.044 [a]	2.04 [a]	50.0 [a]
S2N1	0.719 [a]	18.3 [b]	4.8 [c]	1.14 [a]	0.76 [a]	0.041 [a]	1.86 [a]	40.8 [a]
S2N2	0.781 [a]	19.9 [b]	5.5 [c]	1.18 [a]	0.81 [a]	0.043 [a]	1.96 [a]	41.1 [a]
S2N3	0.823 [a]	21. 7 [ab]	5.4 [c]	1.29 [a]	0.94 [a]	0.043 [a]	2.11 [a]	44.0 [a]

[a] S1 and S2 represent no spikelet removal and ½ spikelet removal, respectively. N1, N2, and N3 represent nitrogen applications of 0, 75, and 150 kg ha $^{-1}$, respectively.

[b] Means with the same letter in each trait have no significant difference according to Duncan's Test ($P < 0.05$).

3.2. Laboratory experiment

Analysis of the variance of the data showed that nitrogen and removal of spikelets had no significant effect on germination percentage, caulicle length, radicle length, and seedling weight of wheat (*Table 4*). The interaction effect of nitrogen and the removal of spikelets had a significant effect on the seed vigor based on weight of wheat (*Table 4*). Nitrogen had a significant effect on the seed vigor based on length of wheat (*Table 4*).

3.2.1. Seed germination percentage

A comparison of mean data showed that removing half of the florets with 150 kg N ha^{-1} had the highest germination percentage (*Table 5*). The removal of several florets may have increased the share of the remaining florets in obtaining the nutrients needed for seed growth, although this became significant when high nitrogen was available to the plant. Seed germination percentage is significantly positively correlated only with seed vigor (*Table 6*). Seed germination percentage is one of the components of seed vigor, so the correlation between these

two traits has become significant. In the study of the effect of fertilization of wheat mother plant with four amounts of nitrogen (0, 50, 100, and 150 kg ha^{-1}), it was observed that 150 and 100 kg N ha^{-1} had the highest seed germination percentage and seed vigor, respectively (Paneru et al., 2017).

Table 3. Correlation coefficients between wheat seed yield and yield-attributed traits under spikelet removal and nitrogen

	Stem and leaf weight	Seed number per spike	Spike length	Spike weight	Seed yield	Seed weight	Biological yield	Harvest index
Stem and leaf weight	1	-.648	-.688	-.604	-.529	.684	-.320	-.651
Seed number per spike	-.648	1	.990**	.992**	.952**	-.881*	.922**	.730
Spike length	-.688	.990**	1	.977**	.943**	-.831*	.890*	.785
Spike weight	-.604	.992**	.977**	1	.981**	-.842*	.948**	.770
Seed yield	-.529	.952**	.943**	.981**	1	-.734	.953**	.834*
Seed weight	.684	-.881*	-.831*	-.842*	-.734	1	-.731	-.413
Biological yield	-.320	.922**	.890*	.948**	.953**	-.731	1	.652
Harvest index	-.651	.730	.785	.770	.834*	-.413	.652	1

*, **. Significance at the level of 0.05 and 0.01

3.2.2. Caulicle and radicle length and seedling weight

A comparison of mean data showed that source and sink manipulation did not affect caulicle and radicle length and seedling weight (*Table 5*). Although the removal of some florets was expected to increase the chances of other florets absorbing nutrients and nitrogen consumption could increase caulicle and radicle length and seedling weight, these traits were not affected. There may have been enough nitrogen in the soil, or the plant may have had enough nitrogen storage in the vegetative organs during the spike stage, or nitrogen may not be absorbed from the soil at this stage. Caulicle length had a significantly positive correlation with radicle length and seedling weight (*Table 6*). The addition of nitrogen to the environment of the mother plant in *Potentilla tanacetifolia* significantly reduced the seedling weight produced by adding nitrogen to the seedling environment (Li et al., 2011).

Table 4. Analysis of variance (mean square) of the effect of spikelet removal and nitrogen on seed germination traits in wheat

Source of variation	df	Germination	Caulicle length	Radicle length	Seedling weight	Seed vigor (based on weight)	Seed vigor (based on length)
Spikelet removal (S)	1	830.0ns	0.022ns	0.005ns	0.000003ns	0.000019ns	28.4ns
Nitrogen (N)	2	885.6ns	10.34ns	1.770ns	0.000008ns	0.000026ns	75.9*
S×N	2	1085.9ns	3.75ns	2.150ns	0.000003ns	0.000034*	22.1ns
Error	12	277.7	3.218	2.685	0.000005	0.000008	9.5

*=significant at the probability level of 5%. ns=non-significant

3.2.3. Seed vigor

Under conditions where high nitrogen (150 kg ha^{-1}) was used and half of the florets were removed, seed vigor increased compared to the treatment in that the source and sink were not manipulated (*Table 5*). An increase in seed vigor based on length was also observed when the highest amount of nitrogen was used and spikelets were not removed. Seed vigor is a good indicator of seed germination, especially in adverse conditions such as dryland soils. Therefore, it can be said that a cultivar such as Pishtaz in terms of seed vigor has both source and sink limitations. The recommendation that can be made based on the results of this section is that it is necessary for the mother plants used to produce seeds to be supplied with nitrogen, even at the stage of spike emergence, to have seeds with high seed vigor. These results also confirm nitrogen restriction as the most important nutritional factor

in plants. Therefore, it is necessary to use nitrogen as a split application even at the beginning of the reproductive stage. Heidari et al. (2013) reported that high nitrogen consumption with complete defoliation reduced seed vigor compared to control. The difference in results is probably due to the difference between defoliation and spikelet removal because, under defoliation conditions, the source is limited, but by removing part of the spikelets, sufficient photosynthetic material is provided for other spikelets.

Table 5. Mean comparisons of wheat seed germination traits under spikelet removal and nitrogen fertilizer

[a] Treatments	Germination (%)	Caulicle length (cm)	Radicle length (cm)	Seedling weight (g)	Seed vigor (%g)	Seed vigor (% cm)
S1N1 [a]	35.0 [b]	6. 6 [a]	9.2 [a]	0.013 [a]	0.0046 [b]	5.65 [b]
S1N2	65.0 [ab]	10.1 [a]	10.0 [a]	0.017 [a]	0.0111 [ab]	8.77 [ab]
S1N3	66. 7 [ab]	10.3 [a]	11.2 [a]	0.016 [a]	0.0106 [ab]	14.32 [a]
S2N1	68.3 [ab]	8.2 [a]	9.9 [a]	0.016 [a]	0.0106 [ab]	12.18 [ab]
S2N2	30.0 [b]	9.7 [a]	10.9 [a]	0.017 [a]	0.0048 [b]	5.23 [b]
S2N3	83.3 [a]	8.8 [a]	9.9 [a]	0.016 [a]	0.0130 [a]	15.62 [a]

[a] S1 and S2 represent no spikelet removal and ½ spikelet removal, respectively. N1, N2, and N3 represent nitrogen applications of 0, 75, and 150 kg ha $^{-1}$, respectively.

[b] Means with the same letter in each trait have no significant difference according to Duncan's Test ($P < 0.05$).

Table 6. Correlation coefficients between wheat seed germination traits under spikelet removal and nitrogen

	Germination percent	Caulicle length	Radicle length	Seedling weight	Vigor (based on weight)	Vigor (based on length)
Germination percent	1	.323	-.009	.312	.988**	.921**
Caulicle length	.323	1	.820*	.900*	.446	.320
Radicle length	-.009	.820*	1	.643	.075	.201
Seedling weight	.312	.900*	.643	1	.431	.226
Seed vigor (based on weight)	.988**	.446	.075	.431	1	.887*
Seed vigor (based on length)	.921**	.320	.201	.226	.887*	1

*, ** Significance at the level of 0.05 and 0.01

4. Conclusions

Manipulation of source and sink did not affect seed yield and seed weight, it can be concluded that the Pishtaz wheat is more sink-limited than source-limited. It is recommended that breeders focus on increasing the sink strength of Pishtaz wheat. It is also recommended to evaluate the effect of manipulation of source and sink by nitrogen and removal of spikelets on other wheat cultivars.

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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Makarnalık Yerel Buğday Genotiplerinin (*Triticum durum* L.) Bazı Tarımsal Özellikler Bakımından Karakterizasyonu

Characterization of Durum Wheat (*Triticum durum* L.*)* Landraces Regarding to Some Agronomic Traits

Abdurrahman DURMAZ[1], Hüsnü AKTAŞ[2*]

Öz

Bu çalışma, 2019-2020 buğday yetiştirme sezonunda Mardin ili Artuklu İlçesi yağışa dayalı şartlarında Augmented Deneme Desenine göre planlanarak Güneydoğu Anadolu Bölgesine özgü 80 adet yerel makarnalık ve 10 adet standart çeşit kullanılarak yürütülmüştür. Deneme, her blokta 20 adet yerel genotip ve 10 adet kontrol çeşit olacak şekilde düzenlenmiştir. Güneydoğu Anadolu Bölgesine özgü bu yerel makarnalık çeşitlerin karakterizasyonunun amaçlandığı çalışmada; yerel buğday çeşitlerinin ve kontrol çeşitlerinin alınan gözlemleri sırasıyla tane verimi 229 kg/da- 371 kg/da; biyolojik verimleri 1313 kg/da –1218 kg/da; bin tane ağırlıkları 42.9 g – 40.15 g, başaklanma gün sayısı 117.8 gün-111 gün; protein oranı % 18.02 - % 14.94 arasında değişmiştir. Elde edilen verilere göre, yerel buğday çeşitlerinin bitki boyu, biyolojik verim, tanede protein oranı bakımından kontrol çeşitlerinden daha yüksek değerlere sahip olduğu tespit edilirken, tane verimi bakımından ise kontrol çeşitlerin daha yüksek değerlere sahip olduğu tespit edilmiştir. Yerel çeşitlerin, modern ıslah çeşitlerine göre daha geç başaklandığı ve yeşil kalma süresinin daha uzun olduğu gözlemlendiği çalışmada, yerel çeşitlerin daha çok kışlık gelişme tabiatına sahip özellikler taşıdığı, özellikle tanede protein oranın ve biyolojik verimin artırılması çalışmalarında gen kaynağı olarak kullanılabilecek çok sayıda potansiyel yerel çeşit olduğu tespit edilmiştir. Elde edilen verileri GGE biblot analiz metodolojisine göre değerlendirilmiş, yapılan değerlendirmede, tane verimi (TV), başakta tane sayısı (BSTS), başakta tane ağırlığı bakımından ST8 (kontrol) G80 (yerel) genotipleri en yüksek değerlere sahip olmuşlardır. Yerel genotipler bin tane ağırlığı (BTA), peduncle uzunluğu (PU), bitki boyu, tanede protein oranı (TPO), biyolojik verim (BV) ve başaklanma gün sayısı (BGS) bakımından daha yüksek değerlere sahip olurken, bu özellikler açısından G8 (yerel) en yüksek değerlere sahip olmuştur. İncelenen özellikler bakımından geniş bir varyasyona sahip olduğu tespit edilen bu yerel çeşitlerinin korunması ve gelecek kuşaklara aktarılması konusunun önemli olduğu ve aynı zamanda ulusal ve uluslararası buğday ıslah programlarında kullanılabilecek yararlı özelliklere sahip olduğu tespit edilmiştir.

Anahtar Kelimeler: Yerel çeşit, Makarnalık buğday, Tane verimi, Kalite

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Abstract

This research was conducted in 2019-20 wheat growing season under rainfall condition of Mardin - Artuklu province. 80 durum wheat landraces originated from Southeast Anatolia and 10 registered durum wheat cultivars were evaluated according to Augmented Trail Design. 20 landraces and 10 cultivars were used for each bloc. We determined large variations in durum wheat landraces for observed traits. Results indicated that mean of observed traits of landraces and varieties were ranged between 229 kg/da - 371 kg/da for grain yield; 1313 kg/kg – 1218 kg/kg for biomass, 18.02 % - 14.94 % for grain protein content. Mean thousand kernel weight of landraces and standard varieties changed between 42.9 g and 40.15 g; heading days ranged from 117.8 to 111 days respectively. According to observed data, landraces had longer heading days and grain stage and also higher grain protein content and biomass compare to standard varieties. Results of this study showed that landraces has high potential to increase biomass and grain protein content and they can be used as a genitor in wheat breeding programs to improving desirable durum wheat genotypes. Observation according to GGE biplot methodology (which-won-where) indicated that ST8 (Check) and G80 (landrace) have high values for grain yield, number of seed pers spike, seed weight per spike while G8 (landrace) showed high values for thousand grain weight, length of peduncle, plant height, grain protein content, biomass and heading days. Obtained results from this study indicated that landraces should be preserved for sustainable agriculture activities specially for marginal areas, also they have high diversity and useful traits for national and international wheat breeding programs.

Keywords: Landraces, Durum wheat, Yield, Quality

1. Giriş

Dünyada genel olarak üretimi yapılan buğdaylar ekmeklik (*Tr. aestivum* L., 2n =42 AABBDD) ve makarnalık (*Tr. durum* L.; 2n= 28 AABB) buğdaylardır (Özkan ve ark., 2002). Durum buğdayı dünya genelinde daha az oranda üretim imkanı olan özel bir üründür. Dünya genelinde tüketilen Makarnalık buğday Latince dilinde sert anlamına gelen ‘’durum’’ kelimesinden türetilmiştir. Yüksek protein oranı, sert tane yapısı ve yoğun sarı renk pigmenti nedeniyle kaliteli makarna, bulgur ve irmik yapımına uygun olan durum buğdayı (*Tr. durum* L.) dünya genelinde 16.7 milyon hektar ekim alanına ve yaklaşık 38 milyon ton üretime sahiptir (International Grains Council, 2020). Dünyada en fazla durum buğdayı üreten ülkeler Kanada, İtalya, Avrupa ülkeleri ve Türkiye ilk sıralarda yer almaktadır (International Grains Council, 2020). Türkiye’de durum buğdayı ekim alanı 1.3 milyon hektar olup, 4.5 milyon ton üretime sahiptir (TÜİK., 2018). Tüm dünya genelinde olduğu gibi Türkiye’de de durum buğdayı üretimi modern ıslah çeşitlerinin tohumluğu kullanılarak yapılmaktadır. Bu ıslah çeşitlerinin eski çeşitler ve yerel çeşitlere kıyasla kısa bitki boyuna sahip olması, sulu tarıma uygun olması, azotlu gübrelere tepkisinin yüksek olması ve tane veriminin çok yüksek olması nedeniyle geçmişte üretimde kullanılan yerel durum buğday çeşitlerinin marjinal alanlarda düşük oranda üretimde kullanılmasına sebep olmuştur. Günümüzde, gelecekte yaşanacak muhtemel küresel iklim değişikliği gibi olumsuz koşullara adapte olabilecek geniş bir varyasyona sahip bu yerel çeşitlerin korunması ayrı bir öneme sahiptir. Islah çalışmalarında, eski çeşitler, yerel çeşitler, yabani türler stres koşullarında adaptasyonu sağlayan gen varyasyolarına sahip olup bunların kaynak olarak kullanılması veya gelecekte oluşması muhtemel olumsuz şartlara adaptasyon için genetik kaynakların korunması ve kayıt altına alınması ayrıca büyük önem arz etmektedir.

Yerel buğday çeşitleri geniş bir varyasyona, genetik olarak dinamik ve mutasyona uğradıkları çevrelerdeki biyotik ve abiyotik stres faktörlerine karşı rekabet edebilme kabiliyetine sahip oldukları için, yeni çeşitler geliştirirken, özellikle stres koşulları altında ve gelecekteki iklim değişikliği koşulları altında verim artışı, verim bakımından stabil çeşit geliştirme çalışmalarında yerel buğday çeşitlerinin kullanılması pratik bir strateji olarak öne çıkmaktadır (Akçura, 2011; Lopes ve ark., 2015). Son yıllarda küresel iklim değişikliği ve buğdayda genetik çeşitlilik kaybının artması, buğday gen kaynaklarının önemini daha iyi anlamamıza vesile olmuştur. Özellikle mevcut buğday gen kaynaklarının biyotik ve abiyotik stres koşullarına karşı yetersiz kalmaya başlaması, buğday ıslahçılarını, yerel buğday çeşitlerine ve yabani buğdaylar kullanılarak elde edilen sentetik ekmeklik ve makarnalık buğday genotiplerine yöneltmiştir (Aktaş, 2016). Buğdayda çok sayıda modern çeşit ve aynı zamanda diğer tahılların modern çeşitleri genetik tabanları dar ve genetik olarak benzer olmaları nedeniyle yeni genetik kaynakların kullanımını gerektirmektedir. Dünyada kültür buğdaylarının genetik tabanının zenginleştirilmesi için de yerel buğday çeşitleri üzerinde CIMMYT (Uluslararası Buğday ve Mısır Geliştirme Merkezi), ICARDA (Kurak ve Yarı Kurak Alanlar için Uluslararası Tarımsal Araştırma Merkezi) ve uluslararası buğday tohumculuk şirketleri tarafından yoğun çalışmalar yapılmaktadır (Aktaş ve ark., 2017). Ülkemiz buğday gen kaynakları bakımından oldukça zengin olmasına rağmen yerel buğday genotipleri üzerinde çalışmalar ve bu gen kaynaklarının buğday ıslahında kullanımı sınırlı olmuştur (Aktaş ve ark., 2017).

Yerel buğdaylar Türkiye’de daha çok makineleşmenin az, ekonomik olarak düşük seviyedeki çiftçi ve bölgelerde, sulama imkanı olmayan, çeşitli stres faktörlerinin hakim olduğu alanlarda, aynı zamanda renk, tat koku, kalite gibi istenilen özelliklere sahip olmalarından dolayı, daha çok ev içi tüketimi sağlamak amacıyla üretimi yapılmaktadır. Buğday ıslahçıları da biyotik ve abiyotik stres koşulları için yeni çeşit geliştirme çalışmalarında, verimliliği ve kaliteyi artırmak için, bu uygun genetik kaynaklara ilgi duymaktadır. Bu çalışmalar yapılırken, bu yerel buğdaylardaki genetik varyasyonun anlaşılması, tespit edilmesi gerekmektedir. Bu süreç, istenilen özellikler bakımından genetik varyasyonun varlığı, bu özelliklerin kolay ve pratik bir şekilde tespit edilmesi, seleksiyon ve istenilen genlerin aktarılmasından ibarettir.

Verimli Hilal (Mezopotamya) içerisinde yer alan Güneydoğu Anadolu bölgesi buğdayın kültüre alındığı alanlardan birisi olup, yerel buğday genotiplerinin halen yetiştirildiği ve bu anlamda genetik çeşitliliğin var olduğu alanlardan birisidir (Heun ve ark., 1997). Yerel buğday gen kaynakları bakımından ülkemiz ve özellikle Güneydoğu Anadolu Bölgesi oldukça zengin olmasına rağmen bu gen kaynaklarının karakterizasyonu sınırlı olarak yapılmış ve ülkemiz buğday ıslah programlarında yeterince kullanılamamıştır. Fakat ülkemizin genelinde olduğu gibi, Güneydoğu Anadolu Bölgesinde de tohumculuk faaliyetleri, makineleşme ve sertifikalı tohum desteklemeleri gibi faktörler nedeniyle yerel çeşitlerin kullanımı gittikçe azalmakta ve var olan genetik çeşitlilik

yok olmaya başlamaktadır. Özellikle, yerel buğday çeşitlerinin yetiştirildiği bölgelerde geniş bir adaptasyon kabiliyetine sahip olması ve yüzyıllar boyunca değişik stres faktörlere rağmen varlığını sürdürebilme yetenekleri nedeniyle, son yıllarda dünyada yaşanan küresel iklim değişikliği ve bu değişikliğin gen kaynaklarında oluşturacağı düşünülen olumsuz etkisinden dolayı, ülkemiz ve dünya gıda güvenliği bakımından yerel buğdaylar çok önemlidir (Lopes ve ark., 2015). Bu nedenle bu bölgedeki yerel buğday genotiplerinin kayıt altına alınması, bunlardan yararlanarak buğday hatlarının geliştirilmesi ve bunların ileride amaca uygun şekilde karakterize edilmesi ve kullanılması aynı zamanda bu gen kaynaklarının gelecek kuşaklara problemsiz bir şekilde aktarılması hayati öneme sahiptir.

2. Materyal ve Metot

2.1. Bitki Materyali

Çalışmada, Güneydoğu Anadolu Bölgesinden toplanmış olan 80 adet yerel makarnalık genotip ve 10 adet modern makarnalık buğday çeşidi materyal olarak kullanılmıştır (*Tablo 1*).

Tablo 1. Çalışmada kullanılan bitki materyali

Table 1.Plant material used in study

Genotip	Orjin	Genotip	Orjin	Genotip	
G1	Adıyaman-Gerger	G31	Mardin-Midyat-Alıçlı Köyü	G61	Çermik
G2	Adıyaman-Gerger	G32	Mardin-Ömerli	G62	Çermik
G3	Adıyaman-Gerger	G33	Mardin-Ömerli	G63	Çermik
G4	Kahta-Gerger Yol- Yüksek K	G34	Mardin-Midyat-Söğütlü Köy.	G64	Çermik
G5	Kahta-Gerger Yol- Yüksek K	G35	Mardin-Midyat-Söğütlü Köy.	G65	Siverek
G6	Kahta-Gerger Yol- Yüksek K	G36	Mardin-Midyat-Söğütlü Köy.	G66	Siverek
G7	Kahta-Gerger Yol- Yüksek K	G37	Mardin-Midyat	G67	Siirt-Kozluk Y
G8	Gerger-Kesentaş Köyü	G38	Mardin-Midyat	G68	Siirt-Kozluk Y
G9	Gerger-Kesentaş Köyü	G39	Mardin-Midyat	G69	Siirt-Kozluk Y
G10	Gerger-Kesentaş Köyü	G40	Mardin- Savur	G70	Şırnak-Uludere
G11	Gerger-Kesentaş Köyü	G41	Mardin- Savur	G71	Şırnak-Uludere
G12	Gerger-Kesentaş Köyü	G42	Mardin- Savur	G72	Şırnak-Uludere
G13	Gerger-Kesentaş Köyü	G43	Mardin- Savur	G73	Şırnak-Uludere
G14	Kahta-Gerger Yol- Yüksek K	G44	Midyat-Ömerli Yolu	G74	Şırnak-Uludere
G15	Kahta-Gerger Yol- Yüksek K	G45	Midyat-Ömerli Yolu	G75	Şırnak-Uludere
G16	Kahta-Gerger Yol- Yüksek K	G46	Midyat-Ömerli Yolu	G76	Şırnak-Uludere
G17	Adıyaman-Kahta-Gümüş K.	G47	Midyat-Ömerli Yolu	G77	Siirt-Eruh Yolu
G18	Adıyaman-Kahta-Gümüş K.	G48	Mardin-Midyat-Ovabahçe K	G78	Siirt-Eruh Yolu
G19	Adıyaman-Kahta-Gümüş K.	G49	Mardin-Midyat-Ovabahçe K	G79	Siirt-Eruh Yolu
G20	Adıyaman-Kahta-Gümüş K.	G50	Mardin-Midyat	G80	Siirt-Eruh Yolu
G21	Gerger-Kahta Yolu	G51	Mardin-Midyat	G81	ST1 (Sümerli)
G22	Gerger-Kahta Yolu	G52	Mardin-Midyat	G82	ST2 (Şahinbey)
G23	Gerger-Kahta Yolu	G53	Mardin-Ömerli	G83	ST3 (Hasan Bey)
G24	Gerger-Çifthisar Köyü	G54	Mardin-Ömerli	G84	ST4 (Sarıçanak)
G25	Gerger-Çifthisar Köyü	G55	Mardin-Ömerli	G85	ST5(Güney Yıldız)
G26	Adıyaman-Kahta-Kırkpınar K	G56	Diyarbakır- Çüngüş	G86	ST6 (Artuklu)
G27	Adıyaman-Kahta-Kırkpınar K	G57	Diyarbakır- Çüngüş	G87	ST7 (Eyyubi)
G28	Mardin-Midyat-Alıçlı Köyü	G58	Diyarbakır- Çüngüş	G88	ST8 (Aydın-93)
G29	Mardin-Midyat-Alıçlı Köyü	G59	Diyarbakır- Çüngüş	G89	ST9 (Fırat-93
G30	Mardin-Midyat-Alıçlı Köyü	G60	Diyarbakır-Çermik	G90	ST10 (Zühre)

*G1-G80 = Yerel Makarnalık Genotipleri ; G81- G90= Islah Edilmiş Kontrol Çeşitler

2.2. Tarla Koşullarında Deneme Düzeni, Gübreleme ve Alınan Gözlemler

Deneme Augmented desenine göre kurulmuştur. Her genotip 2 metre uzunlukta ve sıra arası 50 cm olacak şekilde ekimler 15 Kasım 2019 tarihinde yapılmıştır. Her blokta 20 adet yerel genotip ve 10 adet kontrol çeşit olacak şekilde düzenlenmiştir. Ekimle beraber 7 kg/da azot ve 7 kg/da fosfor (35 kg/da 20.20.0 Kompoze gübre), üst gübrelemesinde ise 7 kg/da azot (15.3 kg/da Üre, % 46 azot) tatbik edilmiştir. Yabancı ot mücadelesi elle

yapılmıştır. Tane verimi (TV, kg/da) Başaklanma gün sayısı (gün, BGS), biyolojik verim (BV, kg/da), bitki boyu (BB, cm), bin tane ağırlığı (BTA, gr), başakta tane sayısı (BŞTS, adet/başak) ve peduncle uzunluğu (PU, cm) gözlemleri Geçit (1982)'ın kullandığı yöntemler esas alınarak elde edilmiştir. Tanede protein oranı ise NIT cihazı kullanılarak tanede (AACC 39-10 metoduna göre % olarak) tespit edilmiştir (Anonim, 1990).

2.3. Araştırma Yerinin Genel Özellikleri

Bu çalışma, Mardin İli, Artuklu İlçesi, Küçükköy Mahallesinde yürütülmüştür. Toprak Analiz Laboratuvarında yapılan incelemede deneme alanının bünyesinin killi-tınlı, pH'sının 7.71, tuz oranının % 0.03 olduğu, organik madde içeriğinin % 2.1, kireç oranının % 19.14, fosfor miktarının 13.11 kg/da ve potasyum miktarının 160.8 kg/da olduğu tespit edilmiştir.

2.4. İklim özellikleri

Denemenin yürütüldüğü bölge olan Mardin İlinin 2019-2020 yetiştirme sezonu meteorolojik verileri aşağıda bulunan *Tablo 2*'de verilmiştir.

Tablo 2. Mardin ili 2019-2020 iklim verileri

Table 2. Climate data of 2019-2020 season of Mardin

	Yıllar	Ocak	Şubat	Mart	Nisan	Mayıs	Haziran	Temmuz	Ağustos	Eylül	Ekim	Kasım	Aralık
Sıcaklık (°C)	**2019**	6.6	8.8	10.7	13.9	22.7	29.5	30.8	31.7	26.3	22.3	13.5	9.9
	2020	3.6	3.8	10.7	14.1	19.9	26.2	31.5	29.9	29.3	22.8	12.0	
	U.Y. Ort.	6.9	9.0	12.2	16.0	21.7	28.5	32.1	30.9	26.2	20.5	13.3	8.1
Yağış (mm)	**2019**	44.1	27.4	95.8	79.7	49.2	16.3	1.7	0.1	0.3	32.7	11.8	54.5
	2020	75.9	102.8	157.3	51.6	30.5	31.5	4	0	0	0	35.7	
	U.Y. Ort.	36.03	33.15	59.18	37.62	38.77	3.53	0.73	0.20	1.47	24.51	33.29	33.53
Nem (%)	**2019**	86.5	87.5	86.7	94.3	9.5	3.0	3.0	3.0	3.0	3.0	3.0	3.0
	2020	71.9	71.4	65	59.7	43.4	26	20.6	22.1	20.6	22.5	55.8	
	U.Y. Ort.	71.6	66.1	69.0	63.0	47.0	25.1	21.0	27.6	30.5	38.3	50.7	65.5

* 2019/2020 yıllarına ait veriler Mardin Meteoroloji İl Müdürlüğü kayıtlarından temin edilmiştir.

2.5. İstatistiki Analizler

İstatistiki analizle Peterson (1985)'e (Augmented for Peremilary Yield Trial) göre Microsoft Excel programı kullanılarak yapılmıştır.

3. Araştırma Bulguları ve Tartışma

İncelenen tüm özelliklere ilişkin Varyans Analiz Tablosu *Tablo 3*'te verilmiştir. İstatistiki analiz sonuçlarına göre çalışmada kullanılan kontrol çeşitlerin incelenen tüm özelliklere ait ortama değerler arasındaki fark istatistiki olarak ($P<0.05$) önemli bulunmuştur.

Tablo 3. Çalışmada İncelenen Özelliklere Ait Varyans Aanaliz Tablosu

Table 3. Table of variance analyse for examinated traits

		TV	BTS	BTA	BŞTA	BBS	BV	BU	BB	PU	BGS	TPO
VK	SD	KO	KO									
ST	9	4008**	425**	153**	1.2**	9.27**	76836**	5.1**	316**	91.4**	27.6	2.455
BLOK	3	144	2.6	0.97	0.009	3.39	2566	0.23	17	0.89	0.8	0.002
HATA	27	697.10	4.93	0.87	0.025	5.41	896	0.143	12.2	0.36	0.73	0.132
GENEL	39											
CV (%)		7.1	4.5	2.32	6.02	11.1	2.5	5.1	3.6	2.7	7.7	2.4

*Gen : Genotip; TV: tane verimi; BTS: Başakta tane sayısı; BTA: Bin tane ağırlığı; BŞTA: Başakta tane ağırlığı; BV: Biyolojik verim; BB: Bitki boyu; PU: Peduncle uzunluğu; TPO: Tanede protein oranı ; *, ** %5 ve %1 düzeyinde önemlidir. Öd: önemli değil,

3.1. *Tane Verimi (kg/da, TV)*

Kontrol çeşitlerin bloklardaki tane verimi değerleri *Tablo 4*'te verilmiştir. Elde edilen sonuçlara göre, bloklarda standart çeşitlere ait ortalama değerler 350 kg/da ile 456 kg/da arasında değişmiş, standart çeşitlerin ortalaması 371 kg/da olarak belirlenmiştir. Tane verimine etki yapan çok sayıda morfolojik ve fizyolojik karakter mevcut olup, çoğu karakter aynı zamanda çevre şartlarından etkilenmektedir (Aktaş, 2016).

Denemede kullanılan yerel buğday çeşitlerinin tane verimi değerleri *Tablo 5*'te verilmiştir. Yerel çeşitlerin birim alandaki tane verimi 92.98 kg/da ile 378.1 kg/da arasında değişmiştir. Yerel çeşitlerin ortalama tane verimi 229 kg/da, kontrol çeşitlerin ise 371 kg/da olarak tespit edilmiştir (*Tablo 5*). Yerel çeşitler ile kontrol çeşitler karşılaştırıldığında, yerel çeşitler en yüksek tane verimine sahip standart çeşit S7 (456 kg/da) çeşidinden daha yüksek tane verimine ulaşamazken, 3 adet yerel çeşit G46 (378.13 kg/da), G79 (363.97 kg/da) ve G80 (362.03 kg/da) en düşük verime sahip ST10 (350 kg/da) kontrol çeşidinden değerinden daha yüksek tane verimine sahip olmuş, sadece G46 genotipi (378.13 kg/da) kontrol çeşitlerin ortalamasından (371 kg/da) daha yüksek tane verimine sahip olmuştur.

Yerel buğday çeşitlerini konu alan birçok çalışmada bu genetik kaynakların daha çok taşlık, eğimli, toprak verimliliği düşük, kuraklık, yüksek sıcaklık gibi tane verimini olumsuz olarak etkileyen stres koşullarının yaşandığı marjinal alanlarda özel adaptasyon geliştirdikleri, bu söz konusu alanlarda çok yüksek olmayan fakat modern ıslah çeşitlerine kıyasla, kayda değer tane verimine ulaşılabildikleri rapor edilmiştir (Kan ve ark., 2017; Aktaş ve ark., 2018). Çalışmamızın yürütüldüğü alanın toprak yapısı, bu alanın taban arazi olma özelliği ve en önemlisi deneme süresince düşen yağış miktarının uzun yıllara göre çok yüksek olması standart çeşitlerin yerel buğday genotiplerinden daha yüksek tane verimine sahip olmasına katkı yaptığını söyleyebiliriz. Philipp ve ark. (2018) 180 adet eski ve yerel çeşit ile 210 adet elit ıslah çeşidinin verim ve verim öğeleri bakımından değerlendirdiği çalışmasında; elit ıslah çeşitlerinin eski çeşit ve yerel çeşitlere kıyasla % 38 daha verimli olduğunu rapor etmiştir. Yerel çeşitlerin marjinal alanlara adaptasyonunu sağlayan, morfolojik, fizyolojik özelliklerinin belirlenip, buğday ıslah programları kapsamında bu özelliklerin ıslah çeşitlerine aktarılması noktasında yararlanılması gereken bu genetik kaynakların korunması ve bu anlamda değerlendirilmesinin çok önemli olduğu bildirilmiştir (Akçura, 2011; Aktaş, 2016).

Tablo 4. Çalışmada kullanılan standart çeşitlerde incelen özelliklerin ortalaması

Table 4. Mean of examined traits for standart varieaties

		TV	BTS	BTA	BŞTA	BV	BB	BGS	PU	TPO
ST1 (Sümerli)		378	52.8	46.6	3.15	1067	91.3	110	20.5	15.15
ST2 (Şahinbey)		366	36.6	41.7	2.16	1163	90.0	110	19.3	14.97
ST3 (Hasan Bey)		354	58.0 b	39.6	2.60	1026	90.0	112	21.5	14.37
ST4 (Sarıçanak)		361	45.7	44.4	2.97	1159	90.0	113	24.3	15.15
ST5 (G. Yıldızı)		373	46.9	39.3	2.04	1070	103.8	113	25.0	13.98
ST6 (Artuklu)		352	47.8	45.7	3.18	1313	107.5	106	26.5	13.69
ST7 (Eyyubi)		456	54.4	35.2	2.58	1299	108.8	114	26.5	14.94
ST8 (Aydın-93)		364	71.5	33.8	3.41	1295	105.0	108	20.5	15.04
ST9 (Fırat-93		351	38.6	34.2	1.78	1371	87.5	112	10.8	15.74
ST10 (Zühre)		350	41.9	41.0	2.47	1418	87.5	113	25.5	16.35
Ort		371	49.4	40.2	2.63	1218	96.1	111	22.0	14.94
AÖF (0.05)		12.1*	1.02*	0.43*	0.07*	13.8*	1.6*	0.39*	0.27*	0.17*
	Blok 1:	-2.1	-0.3	0.40	-0.04	20	0.9	-0.4	0.4	0.0
	Blok 2:	5.4	-0.3	-0.33	0.01	-17	-1.1	0.2	0.1	0.0
Düzeltme	**Blok 3:**	-3.1	0.8	-0.32	0.02	-8.0	1.4	0.3	-0.2	-0.02
Terimi	**Blok 4:**	-0.2	-0.2	0.25	0.01	5.0	-1.2	-0.1	-0.3	0.02

*Gen : Genotip; TV: tane verimi; BTS: Başakta tane sayısı; BTA: Bin tane ağırlığı; BŞTA: Başakta tane ağırlığı; BV: Biylojik verim; BB: Bitki boyu; PU: Peduncle uzunluğu; TPO: Tanede protein oranı; *, ** %5 ve %1 düzeyinde önemlidir.

3.2. *Başakta Tane Sayısı (adet/başak, BTS)*

Standart çeşitlerin tane verimi değerleri ortalaması *Tablo 4*'te verilmiş olup, en düşük değer 36.6 adet/başak ile ST2 çeşidinden, en yüksek değer ise 71.5 ile ST8 kontrol çeşidinden elde edilmiş, kontrol çeşitlere ait ortalama değer ise 49.4 adet/başak olarak kaydedilmiştir. Başakta tane sayısı, en önemli ana verim komponentlerinden olup, tane verimini doğrudan etkileyen bir parametredir (Karagöz ve Zencirci, 2005). Yoğun ıslah çalışmaları ile modern buğday çeşitlerinde geçmişten günümüze tarihsel buğday ıslahı (Historical improvement of breeding varieties)

boyunca başakta tane sayısı artış gösteren çeşitler geliştirildiği rapor edilmiştir (Tomás ve ark., 2020). Başakta tane sayısının artması verime olumlu yansırken, tane iriliğinde ise düşük olan genotipler ile sonuçlandığı rapor edilmiştir (Alipour ve ark., 2017).

Yerel çeşitlere ait başakta tane sayısı değerleri Tablo 5'te verilmiştir. Yerel çeşitlerin başakta tane sayısı 27.81 tane/başak ile 61.95 tane/başak arasında değişmiştir. Yerel çeşitlerin başakta tane sayısı ortalaması 42.4 adet/başak, kontrol çeşitlerin ortalaması ise 49.4adet/başak olarak tespit edilmiştir. Yerel çeşitler ile kontrol çeşitler karşılaştırıldığında, yerel çeşitler en yüksek başakta tane sayısına sahip standart çeşit S8 (71.5 tane/başak) çeşidinden daha yüksek değere ulaşamazken, 9 adet yerel çeşit, G8 (53.29 tane/başak), G10 (54.72 tane/başak), G35 (58.8 tane/başak), G36 (53.10 tane/başak) G71 (61.95 tane/başak) G74 (52.38 tane/başak), G78 (54.66 tane/başak), G79 (51.24 tane/başak) ve G80 (57.52 tane/başak) kontrol ortalamasından (49.4 tane/başak) daha yüksek değerlere sahip oldukları belirlenmiştir. Kontrol çeşitlerin ortalama değerinden daha yüksek başakta tane sayısına sahip bu yerel çeşitler içerisinden tane irilikleri de göz önüne alınarak planlanacak buğday ıslah programlarında, hem başakta tane sayısı hem de tane irilikleri yüksek genotipler elde edilmesi için melezleme çalışmalarında genitör olarak kullanılması için değerlendirilebileceğini düşünmekteyiz. Philipp ve ark. (2018) 180 adet yerel çeşit ve 210 adet elit ıslah çeşidini tarla koşullarında tane verimi ve verim komponentleri bakımından karşılaştırdığı çalışmasında; ıslah çalışmaları sonucu buğday tane veriminde artışa % 23 oranında başakta tane sayısının katkıda bulunduğunu, model başak yapısı için de çalışmaların devam ettiğini belirtmiştir. Tomás ve ark. (2020) Portekize ait yerel ekmeklik buğday çeşitlerini yüksek sıcaklık stesine karşı test yaptığı çalışmasında "Ardito'' yerel buğday çeşidinin stres koşullarında başakta tane sayısı bakımından tüm genotiplerden daha yüksek değere ulaşırken, stres koşullarının olmadığı uygulamalarda ise modern ıslah çeşitlerinin daha yüksek değerlere sahip olduğunu belirtmiştir.

3.3. *Bin Tane Ağırlığı (gr, BTA)*

Kontrol çeşitlerin bin tane ağırlığı ortalama değerleri *Tablo 4*'te verilmiştir. Kontrol çeşitlerin bin tane ağırlığı değerleri 33.80 g (ST8) ile 46.63 g (ST1) arasında değişmiş, kontrol çeşitlerin bin tane ağırlığı genel ortalaması 40.15 g olarak tespit edilmiştir. Bin tane ağırlığı, ana verim öğelerinden birisi olup, tane verimine etkisi yanında un ve irmik randımanının yüksek olmasına katkı sağlayan bir fiziksel özelliktir (Kara ve Akman, 2008). Aynı zamanda, yüksek bin tane ağırlığına sahip çeşitler genel olarak hem üreticiler tarafından iyi özelliklere sahip tohumluğun belirteci sayılırken, sanayiciler için ise son ürüne kolay işlenebilen ve daha yüksek ham madde sağlayan bir parametre olarak kabul görür (Alp ve Kün, 1999). Kalıtsal özellik olan BTA, yıl içerisinde düşen yağış miktarı ve dağılışı, vejetasyon süresince özellikle tane doldurma dönemindeki sıcaklık, gübreleme ve agronomik uygulamalardan etkilenen bir karakterdir (Akıncı ve ark., 1999). Bu nedenle bin tane ağırlığı özellikle yetiştiriciliği yapılan ve ticarete konu olan modern ıslah çeşitleri için de çok önemli bir karakter olarak değerlendirilmektedir.

Denemede kullanılan yerel çeşitlerin bin tane ağırlığı değerleri *Tablo 5*'te verilmiştir. Elde edilen verilere göre yerel çeşitlerin bin tane ağırlığı değerleri 27.51 g (G12) ile 61.68 g (G24) arasında değişmiş ve mevcut materyalin bu anlamda geniş bir varyasyona sahip olduğu tespit edilmiştir. Yerel çeşitlerin bin tane ağırlığı ortalaması 42.9 g, kontrol çeşitlerinin ise 40.15 g olarak tespit edilmiştir. Deneme sonuçlarına göre 18 adet yerel çeşit, en yüksek bin tane ağırlığına sahip olan genotipten (ST1; 46.63 g) daha yüksek değere ulaşırken, 55 adet hatta ise kontrol çeşit ortalamasından (40.15 g) daha yüksek değere sahip olmuştur. Lopes ve ark. (2015) yerel buğday çeşitlerinin karakterizasyonu için yaptığı çalışmada, çok sayıda yerel çeşidin standart olarak kullanıldığı çeşitlerin ortalamasından daha yüksek bin tane ağırlığına sahip olduğunu ve yerel makarnalık çeşitlerin muhtemelen tane iriliği ile ilişkili farklı alleller içerebileceğini rapor etmiştir. Bu çalışma sonucunda elde ettiğimiz veriler, yerel çeşitlerin bin tane ağırlığı bakımından geniş bir varyasyona sahip olduğunu, bunun da çalışmada kullanılan materyalin bin tane ağırlığına ilişkin farklı allellere sahip olabileceğini göstermektedir. Elde edilen bu veriler çalışma materyali için aynı zamanda BTA ile ilişkili allellerin tespitinde Genom Çaplı Çalışmalar (Wide-assocation Mapping) için kullanılabileceğini söyleyebiliriz.

Tablo 5. Yerel durum buğday çeşitlerinde incelenen bazı özelliklere ait düzeltilmiş değerler

Table 5. Mean of examinated traits for durum wheat landraces

Blok	Gen	TV	BTS	BTA	BŞTA	Blok	Gen	TV	BTS	BTA	BŞTA
1	G1	205.7	37.3	37.9	1.6	3	G41	312.5	44.8	44.3	2.2
1	G2	203.8	35.2	43.8	1.7	3	G42	181.9	47.8	37.2	1.9
1	G3	239.7	55.4	40.0	2.4	3	G43	210.4	42.1	43.5	2.0
1	G4	220.9	49.3	45.7	2.5	3	G44	358.4	44.4	46.5	2.2
1	G5	171.8	41.2	47.6	2.2	3	G45	281.8	47.2	40.5	2.0
1	G6	180.7	37.4	46.0	1.9	3	G46	378.1	47.0	40.0	2.0
1	G7	191.5	38.9	38.4	1.7	3	G47	253.9	43.0	47.9	2.2
1	G8	93.0	53.3	35.8	2.1	3	G48	198.1	48.0	49.6	2.6
1	G9	201.1	41.3	46.2	2.1	3	G49	245.5	47.4	50.7	2.6
1	G10	255.6	54.7	35.6	2.1	3	G50	162.3	40.7	40.2	1.7
1	G11	266.4	33.9	39.1	1.5	3	G51	194.1	29.4	44.6	1.4
1	G12	254.1	39.6	27.5	1.2	3	G52	258.1	41.2	44.6	2.0
1	G13	160.5	30.7	38.8	1.3	3	G53	245.5	34.7	40.8	1.5
1	G14	103.8	36.0	34.1	1.4	3	G54	326.6	47.5	37.4	1.9
1	G15	241.3	33.0	39.6	1.5	3	G55	203.2	36.8	40.9	1.6
1	G16	228.0	41.0	36.7	1.7	3	G56	185.4	37.7	46.0	1.9
1	G17	214.7	44.9	45.4	2.2	3	G57	146.6	47.2	48.2	2.4
1	G18	170.5	39.7	42.5	1.9	3	G58	228.6	49.1	44.7	2.3
1	G19	268.0	44.9	44.2	2.2	3	G59	227.7	45.2	42.7	2.1
1	G20	180.0	38.6	42.8	1.8	3	G60	179.1	37.1	53.2	2.1
2	G21	305.2	42.4	45.8	2.1	4	G61	283.5	51.8	39.6	2.2
2	G22	250.9	36.8	43.1	1.7	4	G62	300.6	41.7	47.7	2.1
2	G23	230.5	34.7	57.4	2.1	4	G63	213.1	37.4	41.9	1.7
2	G24	349.1	45.4	61.7	3.0	4	G64	180.2	39.2	40.6	1.7
2	G25	161.6	37.8	47.5	1.9	4	G65	226.4	44.0	40.6	1.9
2	G26	260.9	42.0	40.9	1.8	4	G66	195.2	43.7	42.9	2.0
2	G27	312.4	39.7	39.1	1.7	4	G67	209.0	42.8	36.3	1.7
2	G28	296.0	45.1	42.5	2.1	4	G68	136.5	43.7	30.7	1.4
2	G29	214.6	30.2	48.2	1.4	4	G69	221.6	46.0	39.0	1.9
2	G30	234.6	35.0	41.0	1.5	4	G70	239.3	37.5	41.4	1.7
2	G31	223.4	42.7	48.0	2.2	4	G71	320.8	62.0	38.5	2.6
2	G32	116.0	37.1	48.0	1.9	4	G72	211.4	43.7	40.3	1.9
2	G33	251.8	31.7	48.5	1.6	4	G73	225.5	37.4	43.5	1.7
2	G34	234.8	38.8	37.5	1.6	4	G74	163.7	52.4	42.1	2.4
2	G35	278.2	58.8	46.5	2.9	4	G75	213.5	36.0	42.6	1.6
2	G36	218.7	53.1	38.8	2.2	4	G76	219.2	41.1	42.3	1.9
2	G37	286.3	43.5	46.6	2.2	4	G77	196.5	46.2	35.1	1.7
2	G38	234.7	27.8	52.2	1.5	4	G78	187.4	54.7	30.4	1.8
2	G39	193.9	30.2	34.6	1.1	4	G79	364.0	51.2	52.7	2.9
2	G40	195.4	41.1	51.6	2.3	4	G80	362.0	57.5	49.5	3.1
Dü	Blok1	-2.15	-0.29	0.27	-0.04		Blok3	-2.12	0.77	-0.45	0.03
z.			-0.24	0.06	0.01				-0.24	0.11	0.01
Ter.	Blok2	5.38					Blok4	-0.12			
		TV	BTS	BTA	BŞTA						
StdOrt		371	49.4	40.2	2.63						
Yerel Ç.Ort		229	42.4	42.9	1.95						
AÖF (0.05) Kontrol ve Hatlar için		20,1*	2.45*	0.71*	0.12*						
AÖF (0.05) Hatlar için		24.8*	2.09*	0.88*	0.15*						

*Gen :Genotip; TV: tane verimi; BTS: Başakta tane sayısı; BTA: Bin tane ağırlığı; BŞTA: Başakta tane ağırlığı

*, ** %5 ve %1 düzeyinde önemlidir.

3.4. *Başakta Tane Ağırlığı (gr, BŞTA)*

Kontrol çeşitlerin başakta tane ağırlığı ortalaması değerleri *Tablo 4*'te verilmiştir. Kontrol çeşitlerin başakta tane ağırlığı ortalama değerleri 1.78 g (ST9) ile 3.41 g (ST8) arasında değişmiş, kontrol çeşitlerin başakta tane ağırlığı genel ortalaması 2.63 g olarak tespit edilmiştir. Başakta tane ağırlığı tane verimini doğrudan etkileyen bir karakter olup, Yeşil Devrim ile beraber buğdaya kısa boy genleri aktarılırken, yatmaya ve azota responsu yüksek buğday çeşitleri geliştirilmiş, geçmişten günümüze kadar devam eden ıslah çalışmaları ile birim alanda başak sayısı, başakta tane sayısı ve başakta tane ağırlığı yüksek olan başak tipine sahip buğday çeşitleri geliştirilmiştir (Botwright ve ark., 2005). Bu modern ıslah çeşitlerinin yerel buğday çeşitlerinden bazı özellikler bakımından üstün veya farklı özelliklere sahip olması buğday ıslah programlarında yoğun seleksiyon baskısından ileri geldiği birçok araştırmacı tarafından belirtilmiştir (Liatukas ve Ruzgas, 2011).

Denemede kullanılan yerel çeşitlerin başakta tane ağırlığı değerleri ve düzeltilmiş değerleri *Tablo 5*'te verilmiştir. Elde edilen verilere göre yerel çeşitlerin başakta tane ağırlığı değerleri 1.11 g (G39) ile 3.06 g (G80) arasında değişmiş, yerel çeşitlerin ortalaması 1.95 g, kontrol çeşitlerinin ortalaması ise 2.63 g olarak tespit edilmiştir. Bu sonuç, modern ıslah çeşitlerinin yerel çeşitlerden daha yüksek tane verimine sahip olmalarında, başakta tane ağırlığının çok önemli bir etkiye sahip olduğunu göstermektedir. Deneme sonuçlarına göre sadece 4 adet yerel çeşit (G24: 3g; G35: 2.93 g; G79: 2.83 g ve G80: 3.06 g) kontrol çeşit ortalamasından (2.63 g) daha yüksek değere sahip olmuştur. Yerel makarnalık buğday çeşitleri konusunda çalışma yapmış birçok araştırmacı, yerel çeşitlerin zengin gen kaynakları sağladığını fakat aynı zamanda ıslahçıların yüksek verimli çeşitler geliştirmek amacıyla elit ıslah hatları arasında melezleme yaparak, birim alanda daha fazla başak sayısına, başak verimi ve hasat indeksi yüksek daha verimli çeşitler geliştirdiklerini, geliştirilen bu modern ıslah çeşitlerinin yerel buğday çeşitlerinden daha verimli olduğunu rapor etmişlerdir (Royo ve ark., 2008; Baenziger ve DePauw, 2009; Fayaz ve ark., 2013).

3.5. *Biyolojik Verim (kg/da, BV)*

Kontrol çeşitlerin biyolojik verim ortalaması değerleri *Tablo 4*'te verilmiştir. Kontrol çeşitlerin biyolojik verim ortalama değerleri 1026 kg/da (ST3) ile 1418 kg/da (ST10) arasında değişmiş, kontrol çeşitlerin biyolojik verim ağırlığı genel ortalaması 1218 kg/da olarak tespit edilmiştir. 20. Yüzyılda Yeşil Devrim ile kısa boyluluk genlerine sahip buğday çeşitlerinin geliştirilmesi ile bitki boyunun kısalması biyomasın stabil ve hasat indeksinin artmasına sebep olmuştur (Rebetzke ve Richards, 2000**)**. Günümüzde biyolojik verim ile beraber tane verimini de artırılmasını amaçlansa da, bunun için bitki boyunun artması gerekmekte, bitki boyunun artması yatmaya hassasiyeti artırdığı için, buğday ıslahçıları bunun bir darboğaz olarak nitelendirmekte ve bu konuda yoğun çalışmalar yapılmaktadır. Bu konuda farklı bakış açıları mevcut olup, yerel buğdayların eksik yönlerinin (bitki boyu gibi) giderilmesi için modern ıslah çeşitlerinin genitör olarak kullanılması veya yerel çeşitlerde arzu edilen özelliklerin ıslah çeşitlerine aktarılmasında yerel çeşitlerin genitör olarak kullanılması yolları izlenebilir fikirleri öne sürülmektedir (Kan ve ark., 2017).

Denemede kullanılan yerel çeşitlerin biyolojik verim değerleri *Tablo 6*'da verilmiştir. Elde edilen verilere göre yerel çeşitlerin biyolojik verim değerleri 755 kg/da (G20) ile 1820 kg/da (G7) arasında değişmiş, yerel çeşitlerin ortalama biyolojik verimi 1313 kg/da, kontrol çeşitlerin ise 1218 kg/da olarak kaydedilmiştir. Deneme sonuçlarına göre 60 adet yerel çeşit, kontrol çeşitlerin ortalamasından daha yüksek biyolojik verime sahip olmuştur. Çalışmada, yerel çeşitler içerisinden (G20, G25, G32, G47, G50) ekstrem düşük biyolojik verim değerlerine sahip olması bu çeşitlerde meydana gelen yatmadan kaynaklandığını öngörmekteyiz. Yerel buğday çeşitleri konusunda çalışma yapmış birçok araştırmacı, yerel çeşitlerin modern ıslah çeşitlerine kıyasla, uzun bitki boyuna sahip olduğunu, vejetatif aksam gelişimi ve oranının ve yeşil kalma süresinin daha yüksek olduğunu ve bunun da daha yüksek biyolojik verim değerleri ile sonuçlandığını rapor etmişlerdir (Moghaddam ve ark., 1997; Akçura, 2011). Çalışmamızda yerel buğday çeşitlerinin genel olarak modern ıslah çeşitlerinden daha yüksek biyolojik verim değerlerine sahip olduğu tespit edilmiş olup, bu yerel çeşitler içerisinden genotipler kullanılarak yüksek biyolojik verim ile ilişkili markörlerin tespit edilmesi için Genom-Çaplı çalışmaları (Genom- Wide Association) için hazır veriler elde edilmiştir. Yerel çeşitlerin yüksek biyolojik verim değerine sahip olması, özellikle hayvancılık yapan küçük tarım işletmelerinde tercih edilmesini sağlayan bir özelliktir, çünkü bu yerel çeşitler yüksek oranda saman sağladığı gibi saman kalitesi olarak hayvanların sevdiği, tercih ettiği özelliklere sahip olduğu rapor edilmiştir (Kan ve ark., 2017).

Tablo 6. Yerel durum buğday çeşitlerinde incelenen bazı özelliklere ait düzeltilmiş değerler

Table 6. Mean of examinated traits for durum wheat landraces

Blok	Gen	BV	BB	BGS	PU	TPO	Blok	Gen	BV	BB	BGS	PU	TPO
1	G1	1270	124	120	25.6	17.4	3	G41	1373	129	116	30.1	18,7
1	G2	1475	144	118	29.6	16.7	3	G42	993	119	101	38.1	17,7
1	G3	1255	129	116	28.6	19.0	3	G43	1093	129	117	18.1	17,0
1	G4	1415	144	117	25.6	18.1	3	G44	1173	119	113	32.1	16,9
1	G5	1750	144	120	29.6	18.0	3	G45	1268	114	100	40.1	17,0
1	G6	1765	129	118	21.6	17.7	3	G46	1323	119	114	27.1	17,8
1	G7	1820	149	120	28.6	18.5	3	G47	883	119	113	32.1	17,2
1	G8	1395	149	122	47.6	18.9	3	G48	1198	129	120	33.1	17,8
1	G9	1365	154	120	27.6	18.1	3	G49	1248	129	125	22.1	16,6
1	G10	1335	149	120	27.6	18.7	3	G50	928	134	122	24.1	17,3
1	G11	1495	149	120	26.6	18.9	3	G51	1248	119	118	29.1	16,8
1	G12	1370	134	119	22.6	19.0	3	G52	1293	129	120	25.1	17,2
1	G13	1505	154	120	24.6	18.2	3	G53	1073	129	120	22.1	17,7
1	G14	1160	154	125	17.6	18.0	3	G54	1728	129	116	28.1	17,2
1	G15	1420	144	120	26.6	18.9	3	G55	1073	129	117	27.1	18,5
1	G16	1220	154	120	23.6	19.3	3	G56	1033	144	118	28.1	17,5
1	G17	1325	139	120	31.6	20.4	3	G57	1073	149	125	33.1	17,4
1	G18	1305	144	112	44.6	16.5	3	G58	1218	144	125	26.1	17,9
1	G19	1495	134	120	27.6	19.1	3	G59	1068	139	116	28.1	18,7
1	G20	755	134	120	31.6	18.5	3	G60	1673	139	116	38.1	17,3
2	G21	1262	116	104	27.9	18.5	4	G61	1275	136	125	20.3	18,5
2	G22	1412	136	118	33.9	15.7	4	G62	1395	131	113	41.3	18,3
2	G23	1662	141	120	24.9	17.6	4	G63	1355	141	113	25.3	20,9
2	G24	1202	156	120	22.9	17.5	4	G64	1220	136	125	15.3	20,1
2	G25	897	131	112	36.9	14.3	4	G65	1470	136	122	19.3	19,4
2	G26	1412	141	106	26.9	16.0	4	G66	1565	141	125	26.3	18,6
2	G27	1212	136	113	25.9	18.2	4	G67	1640	136	119	19.3	19,2
2	G28	1262	131	112	34.9	18.3	4	G68	1370	131	119	18.3	18,3
2	G29	1262	136	112	33.9	15.7	4	G69	1150	131	125	30.3	17,5
2	G30	1367	126	115	38.9	17.6	4	G70	1510	136	120	29.3	18,0
2	G31	1057	136	120	29.9	16.8	4	G71	1210	141	125	28.3	19,5
2	G32	917	146	120	27.9	17.6	4	G72	1245	136	119	19.3	18,7
2	G33	1382	131	125	20.9	17.5	4	G73	1570	116	113	22.3	17,8
2	G34	1182	131	112	27.9	18.3	4	G74	1545	126	120	20.3	18,3
2	G35	1277	121	112	24.9	17.3	4	G75	1430	121	120	24.3	20,2
2	G36	1052	111	118	22.9	16.6	4	G76	1630	136	120	26.3	18,9
2	G37	1667	121	113	28.9	17.0	4	G77	975	151	122	27.3	19,6
2	G38	1642	116	115	18.9	16.7	4	G78	1385	131	118	23.3	18,4
2	G39	1137	111	116	20.9	18.4	4	G79	1465	121	118	28.3	20,7
2	G40	1002	126	116	21.9	17.2	4	G80	1475	131	125	37.3	20,4
			0.88		0.38	0.0		Blok 3	-8.0	1.38	0.22	-0.13	0,02
Düz. Ter.	Blok1	20	-1.13		0.07	0.0		Blok 4	-5.0	-1.13	-0.08	-0.32	0,01
	Blok2	-17											
		BV	BB	BGS	PU	TPO							
Std Ort		1218	96.1	111	22.0	14.9							
Yerel Ç.Ort		1313	133.9	118	27.9	18.0							
AÖF (0.05) Kontrol ve Hatlar için		22,8*	2.7*	0.65	0.45*	0.28*							
AÖF (0.05) Hatlar için		28,2	3.3*	0.81	0.56*	0.46*							

*Gen :Genotip; BV: Biylojik verim; BB: Bitki boyu; PU: Peduncle uzunluğu; TPO: Tanede protein oranı; *, ** %5 ve %1 düzeyinde önemlidir.

3.6. *Bitki Boyu (cm, BB)*

Kontrol çeşitlerin bitki boyu ortalama değerleri*Tablo 4*'te verilmiştir. Kontrol çeşitlerin bitki boyu ortalama değerleri 87.5 cm (ST9-10) 108.8 cm (ST7) arasında değişmiş, kontrol çeşitlerin bitki boyu değerleri genel ortalaması 96.1 cm olarak tespit edilmiştir. Buğdaya yarı-cücelik genlerinin aktarılması ile geliştirilen çeşitler, yerel ve eski çeşitlere kıyasla kısa boylu, yatmaya dayanıklı ve azotlu gübre responsu yüksek olmaları nedeniyle dünya genelinde yoğun olarak üretilmeye başlanmıştır. Söz konusu cücelik genlerinin Gibberilik Asite duyarlılığının düşük olması bu çeşitlerde bitki boyunu kısaltmasında etki yapmıştır (Keyes ve ark., 1989; Rebetzke ve ark., 2001). CIMMYT, ICARDA gibi uluslararası ıslah kuruluşları, melez bahçelerinde bitki boyunu kısaltan ebeveynleri daha geniş adaptasyon yeteneğine sahip genotipler geliştirmek amacıyla kullanırlar. Bu cücelik genlerinin bitki boyunu kısaltma etkisine sahipken, aynı zamanda tane verimi ve yatmaya karşı dayanıklılık, hasat indeksinin artması ile ilişkili olduğu bilinmektedir (Kaya ve ark., 2015).

Denemede kullanılan yerel çeşitlerin bitki boyu değerleri *Tablo 6*'da verilmiştir. Elde edilen verilere göre yerel çeşitlerin bitki boyu uzunluğu değerleri 111.3 cm (G36, G39) ile 156.13 cm (G24) arasında değişmiş, yerel çeşitlerin ortalama bitki boyu 133.9 cm, kontrol çeşitlerin ise 96.1 cm olarak tespit edilmiştir. Deneme sonuçlarına göre çalışmada kullanılan tüm yerel çeşitler kontrol çeşitlerinin ortalamasından daha yüksek bitki boyu değerine sahip olurken, söz konusu karakterler ile geniş bir varyasyon tespit edilmiştir. Yerel ve cücelik genleri taşımayan eski çeşitlerde, bitki boyu ve vejetatif gelişimi teşvik eden Giberilik Asit responsunun daha yüksek olduğu (Rebetzke ve ark., 1999; Ellis ve ark., 2004), özellikle yağışın çok yüksek olduğu alanlar ile sulama yapılan alanlarda yerel çeşitlerin uzun boylu olmaları yatma nedeniyle büyük verim kayıpları olduğu, bu nedenle bu çeşitlerin yerine genel olarak CIMMYT orjinli kısa bitki boyuna sahip çeşitlerin dünya genelinde yayılım gösterdiği birçok araştırmacı tarafından rapor edilmiştir (Keyes ve ark., 1989; Rebetzke ve ark., 1999; Rebetzke ve ark., 2001; Ellis ve ark., 2004).

3.7. *Peduncle Uzunluğu (cm, PU)*

Kontrol çeşitlerin peduncle uzunluğu ortalama değerleri 10.75 cm (ST9) ile 26.5 cm (ST6-7) arasında değişmiş, kontrol çeşitlerin peduncle uzunluğu değerleri genel ortalaması 22.03 cm olarak tespit edilmiştir (*Tablo 4*). Khaliq ve ark. (2004), CIMMYT orjinli modern ıslah genotiplerini yarı-kurak koşullarda değerlendirdiği çalışmada, peduncle uzunluğu ile başak uzunluğu ve tane verimi arasında istatistiki olarak önemli korelasyon tespit ettiklerini rapor etmiştir.

Denemede kullanılan yerel çeşitlerin peduncle uzunluğu değerleri ve düzeltilmiş değerleri *Tablo 6*'da verilmiştir. Elde edilen verilere göre yerel çeşitlerin peduncle uzunluğu değerleri 15.33 cm (G64) ile 47.63 cm (G8) arasında değişmiş, yerel çeşitlerin ortalama peduncle uzunluğu 27.60 cm, kontrol çeşitlerin ortalaması ise 22.03 cm olarak tespit edilmiştir. Deneme sonuçlarına göre çalışmada kullanılan tüm yerel çeşitler kontrol çeşitlerin ortalamasından daha yüksek peduncle uzunluğu değerine sahip olurken, söz konusu karakter bakımından geniş bir varyasyon tespit edilmiştir. Yeşil Devrim ile cücelik genleri tane verimine katkı yaparken, kısa boylu ıslah çeşitlerinin bitki boyu ve peduncle uzunluğunun eski çeşitlere göre daha kısa olduğu, peduncle uzunluğunun kuraklığa dayanıklılık ile ilişkili olabileceği, peduncle uzunluğunun fizyolojik olum süresinin daha uzun olmasına ve dolayısıyla toplam fotosentez süresi ve oranının daha yüksek olduğu bazı araştırmalarda belirtilmiştir (Khaliq ve ark., 2004; Botwright ve ark., 2005).

3.8. *Başaklanma Gün Sayısı (Gün, BŞGS)*

Kontrol çeşitlerin başaklanma gün sayısı değerleri *Tablo 4*'te verilmiştir. Kontrol çeşitlerin başaklanma gün sayısı ortalama değerleri 106 gün (ST6) ile 114 gün (ST7) arasında değişmiş, kontrol çeşitlerin başaklanma gün sayısı genel ortalaması 111 gün olarak tespit edilmiştir. Denemede kullanılan yerel çeşitlerin başaklanma gün değerleri ve düzeltilmiş değerleri *Tablo6*'da verilmiştir. Elde edilen verilere göre yerel çeşitlerin başaklanma gün değerleri 99.77 gün (G45) ile 125.3 gün (G14) arasında değişmiştir (*Tablo 6*). Yerel çeşitlerin ortalama başaklanma gün sayısı 117.8 gün, kontrol çeşitlerin ortalaması ise 111 gün olarak tespit edilmiştir. Başaklanma gün sayısı bakımından yerel buğday çeşitlerinde geniş bir varyasyon tespit edilmiştir. Deneme sonuçlarına göre 3 adet yerel çeşit (G21: 103.8 gün; G26: 105.8 gün; G45: 99.8 gün) dışında tüm yerel çeşitler kontrol çeşitlerinin ortalamasından (111 gün) daha yüksek başaklanma gün sayısı değerlerine sahip olmuşlardır. Bu veriler yerel buğday çeşitlerinin genel olarak geççi-kışlık gelişme karakterli bir yapıya sahip olduğunu göstermekte olsada, bu

yerel çeşitlerdeki gün uzunluğuna hassasiyet genleri (Ppd genleri), vernelizasyon genleri (Vrn-1, Vrn-2 gibi) bakımından farklı veya yeni (novel) gen allelleri bakımından moleküler markörler ile karakterizasyonunun yapılması gerektiğini göstermektedir. Bu çalışmadan başaklanma gün sayısı ve diğer özellikler için hali hazırda Genom-Çaplı Genetik Karakterizasyon (Genom-Wide Association) ile validasyon (fenotipik ve genetik verilerin karşılaştırılması) işlemleri için de önemli veriler sağlamaktadır. Başaklanma ve çiçeklenme gibi karakterler vernelizasyon, gün uzunluğu-ışıklanma süresi (fotoperyodizm) ile yakından ilişkili olup, bir bitki türünün veya çeşidinin farklı ekolojilere adaptasyonunu sağlayan önemli parametrelerdir (Kaya ve ark., 2015). Birçok çalışmada modern makarnalık buğday çeşitlerinin, yerel çeşitlere göre çok düşük vernelizasyona gereksinim duyduğunu, fotoperyodizm bakımından çok daha düşük oranda hassas olduklarını, son ürüne işleme kalite kriterlerince daha standart özelliklere sahip olduklarını fakat doğa ve insan eliyle gerçekleşen seleksiyonlar neticesinde bu yerel çeşitlerin genetik olarak geniş bir varyasyona sahip olmalarını sağladığını; bu geniş varyasyonun kuraklığa, zararlı ve hastalıklara toleranslı, daha çok küçük ölçekli tarım sistemlerine adapte olmalarına katkı sağladığını rapor etmişlerdir (Kyzeridis ve ark., 1995; Motzo ve Giunta, 2007; Talas ve ark., 2011; Nazco ve ark., 2012). Özellikle çiçeklenme, döllenme ve tane doldurma süresine denk gelen evrelerdeki yüksek sıcaklıklar başaktaki tanelerin fiziksel özellikleri üzerine olumsuz etkilere neden olmakta, erkencilik bu anlamda söz konusu stres faktörlerinden kaçışı sağlayarak bu olumsuz etkilerin sonuçlarını minimize etmektedir (Beharav ve ark., 1998; Başer ve ark., 2005). Diğer yandan, yüksek sıcaklık ve kuraklığın olmadığı, vejetasyon süresinin uzun olduğu ekoloji ve yıllarda geç olgunlaşan veya generatif döneme geçişin uzun sürdüğü zamanlarda geççi çeşitlerin tane verimi bakımından avantajlar sağladığı bilinmektedir (Worland ve ark., 2001). Bu nedenle başaklanma gün sayısı bakımından seleksiyon ve karakterizasyon çok önemli bir parametre olarak kabul edilir (Kaya ve ark., 2015).

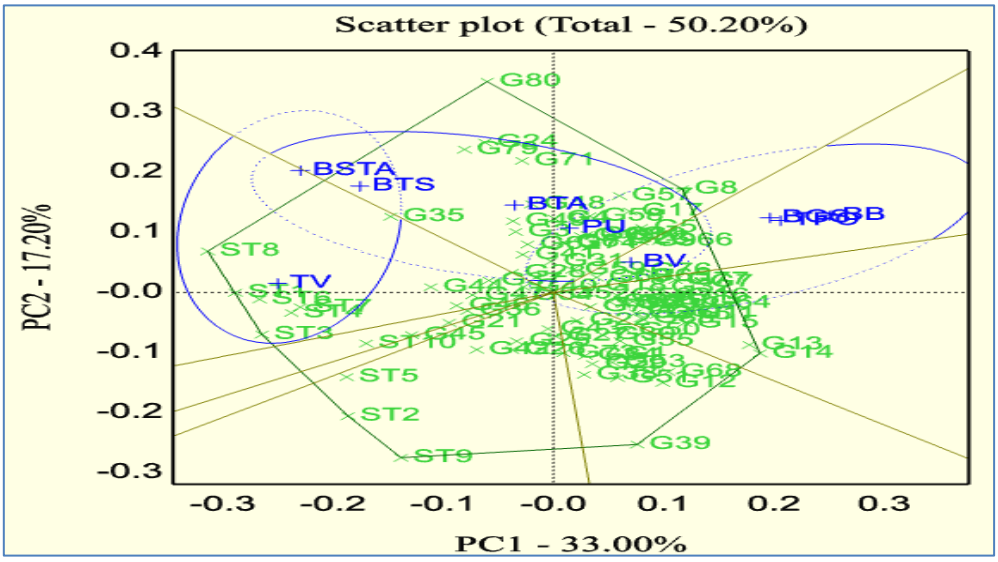
3.9. *Tanede Protein Oranı (%, TPO)*

Kontrol çeşitlerin tanede protein oranı ortalama değerleri *Tablo 4*'te verilmiştir. Kontrol çeşitlerin tanede protein ortalama değerleri % 13.69 (ST6) ile % 16.35 (ST10) arasında değişmiş, kontrol çeşitlerin tanede protein genel ortalaması % 14.94 olarak tespit edilmiştir. Tanede protein oranı makarna ve ekmek yapımında en önemli kalite kriteri aynı zamanda insan beslenmesi için de çok önemli olup, buğday yetiştiricileri için yüksek proteine sahip ürünün daha yüksek fiyatla alıcı bulmasını sağlamaktadır. Tanede protein oranının ekonomik önemine rağmen konvansiyonel ıslah çalışmaları ile tanede protein oranının artırılması, bu özelliği idare eden kompleks genetik sistem, çevrenin protein oranı üzerindeki etkisi, yüksek verim ile protein oranı arasındaki negatif korelasyon nedeniyle çok yavaş ilerlemektedir (Simmonds, 1995; Öner ve ark., 2016). Bu nedenle tanede protein oranını idare eden gen allellerinin moleküler tekniklerle tespiti, yüksek proteine sahip genotiplerin geliştirilmesine olanak sağlayacağı ve direk olarak bu özelliklere sahip genotiplerin seçiminde pozitif etki yapacaktır. Bu çalışmada yüksek protein oranına sahip yerel çeşitlerin içerisinden tanede yüksek proteini idare eden genlerin tanımlanması ve planlanacak ıslah programlarında yüksek proteine sahip genotiplerin geliştirilmesine katkı yapacak veriler elde edilmiştir.

Denemede kullanılan yerel çeşitlerin tanede protein oranı değerleri ve düzeltilmiş değerleri *Tablo6*'da verilmiştir. Elde edilen verilere göre yerel çeşitlerin tanede protein oranı değerleri % 14.26 (G25) ile % 20.91 (G63) arasında değişmiş, kontrol çeşitleri ile yerel çeşitler arasındaki asgari önemi fark (AÖF =0.28) olarak belirlenmiştir. Yerel çeşitlerin ortalama tanede protein oranı % 18.02, kontrol çeşitlerin ortalaması ise % 14.94 olarak tespit edilmiştir. Tanede protein oranı bakımından yerel buğday çeşitlerinde geniş bir varyasyon tespit edilmiştir. Deneme sonuçlarına göre 4 adet yerel çeşit (G22: %15.698; G25: % 14.26; G26: % 16.02 ve G29: %15.69) dışında tüm yerel çeşitler, en yüksek protein oranına sahip kontrol çeşidinden (ST-10: % 16.35) daha yüksek protein oranına sahip olduğu tespit edilmiştir. Elde edilen bu sonuçlar, çalışmada kullanılan yerel çeşitlerin kalite değerlendirilmesinde en önemli kriter olan protein oranı bakımından kontrol çeşitlerine bariz bir üstünlüğünün olduğunu, bu yerel çeşitlerin tane protein oranının artırılmasında kullanılabilecek çok değerli genotipler olduğunu göstermektedir. Türkiye orjinli yerel ve modern durum buğday çeşitlerinin kalite bakımından değerlendirdiği bir çalışmada protein oranını % 10.7 ile % 16.8 arasında değiştiği, kümülatif olarak yerel çeşitlerin modern ıslah çeşitlerinden daha yüksek protein oranına sahip olduğunu belirtilmiştir (Saylan ve ark., 2012). Güneydoğu Anadolu Bölgesine ait 145 yerel durum buğdayı çeşidi ve 10 adet modern ıslah çeşidi ile yürütülen çalışmada protein oranının %12.22 ile % 18.11 arasında değiştiği, yerel genotiplerin % 70'nin modern ıslah çeşitlerinden bariz bir şekilde daha yüksek protein oranına sahip olduğu ve bu genotiplerin ıslah çalışmalarında genitör olarak kullanılabileceği belirtilmiştir (Kendal ve ark., 2019).

3.10. *Biplot Grafiği ile Genotip Özellik İlişkisinin Yorumlanması*

Biplot metodolojisi çok sayıda genotip ve özelliğin incelendiği, çoklu lokasyonlarda yürütülen denemelerde, genotip – özellik, genotip – lokasyon ilişkisini kolaylıkla grafik üzerinde yorumlanmasını sağlamaktadır (Kendal ve ark.,2019). Biplot grafiğine göre, standart olarak kullanılan modern ıslah çeşitleri, tane verimi (TV), başakta tane sayısı (BSTS), başakta tane ağırlığı bakımından öne çıkmış bu özellikler bakımından ST8 (kontrol) G80 (yerel) genotipleri en yüksek değerlere sahip olmuşlardır. Yerel çeşitler ise bin tane ağırlığı (BTA), peduncle uzunluğu (PU), bitki boyu, tanede protein oranı (TPO), biyolojik verim (BV) ve başaklanma gün sayısı (BGS) bakımından daha yüksek değerlere sahip olurken, bu özellikler açısından G8 (yerel) en yüksek değerlere sahip olmuştur (*Şekil 1*). Biplot analizi sonuçları bu çalışmada elde edilen sonuçların kolaylıkla yorumlanmasını sağlamaktadır.



Şeki 1. Genotip – özellik ilişkisini gösteren biblot grafiği

Figure 1. Biblot graph for relation of Genotype-traits

4. Sonuç ve Öneriler

Elde edilen sonuçlara göre, Güneydoğu Anadolu Bölgesi yerel çeşitlerinde incelenen özellikler bakımından yüksek bir varyasyon tespit edilmiş, yerel çeşitlerin tanede protein oranı, biyolojik verim, bin tane ağırlığı bakımından modern ıslah çeşitlerine göre daha yüksek değerlere sahip olduğu ve bu özellikler bakımından yerel çeşitlerin ön-ıslah programlarına dahil edilerek bu özellikler bakımından üstün genotipler geliştirmek için gen kaynağı olarak kullanılabileceği sonucuna varılmıştır. Yerel buğday çeşitlerinin bitki boyunun modern ıslah çeşitlerine göre daha uzun olduğu, daha geç başaklanma süresine sahip olduğu ve daha çok kışlık gelişme tabiatı özellikleri taşıdığı belirlenmiş olup, tane verimi bakımından modern ıslah çeşitlerinin daha üstün performansa sahip olduğu tespit edilmiştir. Çalışmanın yürütüldüğü sezonun uzun yıllar ortalamasının çok üzerinde yağışlı geçmesi yerel çeşitlerin kuraklık veya kısıtlı yağış şartlarındaki performansına dair veriler elde edilmemesine rağmen, bitki yeşil kalma süresini uzun olduğu bu konuda daha kapsamlı fizyolojik çalışmaların yapılması gerektiği saptanmıştır. Özellikle tanede protein oranını, biyolojik verimi açısından elde edilen veriler, üzerinde çalıştığımız materyalde bu özellikleri idare eden genlerin tespitinin moleküler markörler ile yapılması için değerli veriler elde edilmiştir. İleride yapılacak çalışmalarda bu yerel buğday genotiplerinin protein ve biyolojik verim ile ilişkili genlerin tespitinde büyük kolaylıklar sağlayacağını öngörmekteyiz. Elde edilen sonuçlar yerel buğday çeşitlerinin morfolojik olarak modern ıslah çeşitlerinden bariz farklılıklara sahip olduğunu gösterirken, bu yerel buğday genotiplerinin ex-situ ve in-situ koruma metodlarıyla korunması gerektiği, günümüzde ve gelecekte buğday üretimini kısıtlayacak faktörlere karşı ilk olarak değerlendirilecek değerli genetik kaynaklar olduğu tespit edilmiş olup, bu genetik materyalin gelecek kuşaklara aktarılması noktasında ilgili tüm kesimlerin üstüne düşen görevi yapması gerektiği önerisi geçmişte bu konuda çalışmış araştırmacılar gibi bu çalışma sonucunda bizler de aynı önerilerde bulunmaktayız.

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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Determination of Oil Quality Factors and Fatty Acid Compositions of Some Peanut Varieties

Bazı Yerfıstığı Çeşitlerinin Yağ Kalitesi Özellikleri ve Yağ Asidi Bileşimlerinin Belirlenmesi

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Abstract

The aim of this study was to determine the oil properties and fatty acid compositions of peanut cultivars (*Arachis hypogaea* L.) grown as the main crop in the Eastern Mediterranean transition zone of Turkey. The field experiment was conducted at the Oil Seed Research Institute experiment area in the main crop seasons of 2018 and 2019. The experiment was designed according to the randomized complete block design (RCBD) with three replications. Oil ratio, saturated fatty acids (palmitic acid, stearic acid), unsaturated fatty acids (oleic acid, linoleic acid), iodine value, and oleic/linoleic acid ratios were investigated in the experiment. Runner (Georgia Green), Spanish (Florispan), and Virginia market types (Sultan, Brantley, BATEM-Cihangir, BATEM-5025, Arioglu-2003, Halisbey, NC-7, Flower-22, Wilson, NC-V-11, Com, Osmaniye-2005, Gazipasa) varieties were used as plant materials. As a result of this study, NC-V-11 (52.23%) cultivar with the highest oil content was determined, followed by Florispan (52.16%), Brantley (52.10%), and Gerogia Green (51.54%). The lowest oil content was obtained from BATEM-Cihangir (44.57%) variety. Brantley variety was found to have the least palmitic acid ratio with 8.04%, while Florispan variety was found the highest with 12.24%. In terms of stearic acid ratios, the lowest value (1.38%) was determined in Com variety, while the highest value (2.91%) was found in Brantley variety. Brantley variety was found to have the highest oleic acid content (71.83%), which is one of the unsaturated fatty acids. Florispan variety had the lowest oleic acid content (43.70%). While Brantley variety had the lowest linoleic acid ratio (9.78%), it was determined that Com variety had the highest linoleic acid ratio (35.77%). The iodine value varied between 78.71-100.71, the lowest value was in Brantley and the highest value was in Com. The oleic acid/linoleic acid ratio was determined to vary between 1.22 and 7.35, the lowest value was in Florispan and the highest value was in Brantley. As a result of the research, it was determined that the variety with the highest oleic acid ratio in the Eastern Mediterranean Transition Zone was Brantley. Peanuts with high oleic acid content are preferred by producers and consumers because they have good quality and extend the shelf life of products producing with them.

Keywords: *Arachis hypogaea* L., Fatty acid compositions, Iodine value, Oil content, Oleic/Linoleic acid ratio

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Öz

Bu çalışmanın amacı, Türkiye'nin Doğu Akdeniz geçit kuşağında ana ürün olarak yetiştirilen yerfıstığı çeşitlerinin (*Arachis hypogaea* L.) yağ özellikleri ve yağ asidi kompozisyonlarını belirlemek için yapılmıştır. Tarla denemeleri Yağlı Tohumlar Araştırma Enstitüsü deneme lokasyonunda 2018 ve 2019 yıllarının ana ürün sezonlarında yapılmıştır. Deneme tesadüf blokları deneme desenine göre (RCBD) üç tekerrürlü olarak tasarlanmıştır. Denemede yağ oranı, doymuş yağ asitleri (palmitik asit, stearik asit), doymamış yağ asitleri (oleik asit, linoleik asit), iyot değeri ve oleik/linoleik asit oranları incelenmiştir. Runner (Georgia Green), Spanish (Florispan) ve Virginia pazar tipleri (Sultan, Brantley, BATEM-Cihangir, BATEM-5025, Arıoğlu-2003, Halisbey, NC-7, Flower-22, Wilson, NC-V-11, Çom, Osmaniye-2005, Gazipaşa) olmak üzere 15 yerfıstığı çeşidi bitkisel materyal olarak kullanılmıştır. Bu çalışmanın sonucunda, en yüksek yağ içeriği NC-V-11 (%52,23) çeşidi tespit edilmiş olup, bunu sırasıyla Florispan (%52,16), Brantley (%52,10) ve Gerogia Green (%51.54) çeşitleri takip etmiştir. En düşük yağ içeriği ise (%44.57) BATEM-Cihangir çeşidinden elde edilmiştir. En düşük palmitik asit oranı %8.04 ile Brantley çeşidinde bulunurken, en yüksek ise %12.24 ile Florispan çeşidinde bulunmuştur. Stearik asit bakımından en düşük değer (%1.38) Çom çeşidinde bulunurken, en yüksek değer (%2.91) Brantley çeşidinde bulunmuştur. Doymamış yağ asitlerinden olan oleik asit içeriği en yüksek Brantley çeşidinde (%71.83) bulunmuştur. Florispan çeşidi ise en düşük oleik asit içeriğine (%43.70) sahip olmuştur. Linoleik asit oranı en az (%9.78) Brantley çeşidinde bulunurken, en fazla (%35.77) Çom çeşidinde olduğu tespit edilmiştir. İyot değeri 78.71 (Brantley) ile 100.71 (Çom) arasında değişirken; oleik asit/linoleik asit oranı %1.22 (Florispan) ile %7.35 (Brantley) arasında değiştiği belirlenmiştir. Yapılan araştırma sonucunda Doğu Akdeniz Geçit Kuşağında en yüksek oleik asit oranına sahip çeşidin Brantley olduğu tespit edilmiştir. Yüksek oleik asit oranına sahip yerfıstıkları kaliteli olmaları ve kullanıldığı ürünlerde raf ömrünü uzattığı için üretici ve tüketiciler tarafından tercih edilmektedir.

Anahtar Kelimeler: *Arachis hypogaea* L., Yağ asidi, İyot değeri, Yağ içeriği, Oleik/Linoleic asit oranı

1. Introduction

Groundnut (*Arachis hypogaea* L.), named peanut, is an unusual plant for the reason that it flowers above ground and pods containing one to five seeds are produced underground (Fabra et al., 2010). Peanut is a member of the family *Fabaceae*, and genus *Arachis* which has been categorized into the two subspecies *hypogaea* and *fastigiata*. Peanut is an important oil crop and used in both human and animal nutrition because of its protein, minerals (P, Ca, Mg, Mn, K), and carbohydrate contents (Onemli, 2005; Onemli, 2012; Awal and Aktar, 2015; Arioglu et al., 2016).

Peanut seeds contain approximately 45-55% oil and 25-30% protein depending on market types, years, and other conditions (Bakal and Arioglu, 2019). There are four market types of peanut like Virginia, Spanish, Valencia, and Runner. Every market type has its own nutritional composition, pod size, and flavor pod size (Zhao et al., 2017; Karabulut and Tuncturk, 2019). The fatty acid combination of peanut plays a prominent role in defining the shelf life, nutrition, and flavor of peanut. High oleic acid content provides an extended shelf life for peanut-derived products in food applications (Onemli, 2012; Yol and Uzun, 2018; Ozluoymak and Guzel, 2020). Even though eight grand fatty acids are present in peanuts four stearic, palmitic, oleic, and linoleic acids carve out approximately 90% of total peanut triacylglycerols (Hassan and Ahmed, 2012). Peanut oil is composed of 80% unsaturated fatty acids (oleic acid (|C18:1|) and linoleic acid (|C18:2|)). The rest of the fatty acids are saturated fatty acids (palmitic acid (|C16:0|), stearic acid (|C16:0| etc.) (Shin et al., 2010; Yasli et al., 2020; Yilmaz et al, 2022).

The property of high oleic to linoleic acid ratio (O/L) could provide the consumer with a significant health benefit and has the potential to greatly advance the marketability of peanuts. The nutritional quality of the seeds is violently impressed by the place of production, the variety, and the season, in particular, the soil moisture and temperature during plant growth and seed maturation (Hassan and Ahmed, 2012; Arioglu et al., 2016). A loud ratio of oleic acid to linoleic acid (O/L) in peanut (>10:1) results in an extended shelf-life (up to 10 times) and an advanced flavor compared to a normal O/L ratio (1.5:1). Moreover, the iodine value (IV) was used to specify the fatty acid content and stability of peanut oil. A high O/L ratio and a low iodine rate normally remark fine stability and long shelf life (Gali et al., 2021).

The World produced about 48.7 million tonnes of peanut in 29.5 million ha area in 2019. The top producers are China, India, Nigeria, Sudan, and the USA (Anonymous, 2022a). In 2019, the Republic of Turkey produced about 170 thousand tonnes of peanut in 42.2 thousand ha area. Adana and Osmaniye, located in the Mediterranean region, were the top producer provinces at about 90% (Anonymous, 2022b). In 2019, shelled peanut were one the most considerable market products in the Earth which has 3 billion USD in import and 2.8 billion USD in export values. The importers were the Netherlands, Indonesia, the UK, Mexico, and Canada while the top five exporters were India, China, the USA, Argentina, and Netherlands (Anonymous, 2022a).

Onemli (2012) and Bakal and Arioglu (2019) reported that the fatty acid composition and oil content of peanut were influenced by genotypic variation, market type, growing conditions, and maturity. Stearic, palmitic, oleic, and linoleic acids constitute virtually 90% of total peanut fatty acids. Higher temperatures during seed development concluded greater oleic contents while lower temperatures post-anthesis caused higher linoleic acid (Yol et al., 2018). Gali et al. (2021) indicated that higher temperatures over the last four weeks before harvest resulted in higher oil and oleic acid ingredients and according to higher O/L ratios.

The study aimed to state oil content, unsaturated and saturated fatty acids composition, and oil quality (O/L and IV) of some peanut varieties growing main crop season in the Mediterranean region in Turkey.

2. Materials and Methods

2.1. Materials

Georgia Green, Sultan, Brantley, BATEM-Cihangir, BATEM-5025, Arioglu-2003, Halisbey, NC-7, Florispan, Flower-22, Wilson, NC-V-11, Com, Osmaniye-2005, and Gazipasa were used as plant material in the present study (*Table 1*).

Table 1. Some characteristics of peanut varieties*

Varieties	Growing Type	Market Type	Origin
Florispan	Erect	Spanish	USA
BATEM-Cihangir	Semi-erect	Virginia	Turkey
Georgia Green	Spreading	Runner	USA
Sultan	Semi-spreading	Virginia	Turkey
Brantley	Semi-spreading	Virginia	USA
BATEM-5025	Semi-spreading	Virginia	Turkey
Arioglu-2003	Semi-spreading	Virginia	Turkey
Halisbey	Semi-spreading	Virginia	Turkey
NC-7	Semi-spreading	Virginia	USA
Flower-22	Semi-spreading	Virginia	China
Wilson	Semi-spreading	Virginia	USA
NC-V-11	Semi-spreading	Virginia	USA
Com	Semi-spreading	Virginia	Turkey
Osmaniye-2005	Semi-spreading	Virginia	Turkey
Gazipasa	Semi-spreading	Virginia	Turkey

*Asik et al. (2018)

The study was carried out over the experimental fields of Oil Seed Research Institute (37°03′41” N, 36°06′79” E; 50 m) in Turkey during the main growing seasons of 2018 and 2019 (*Figure 1*).

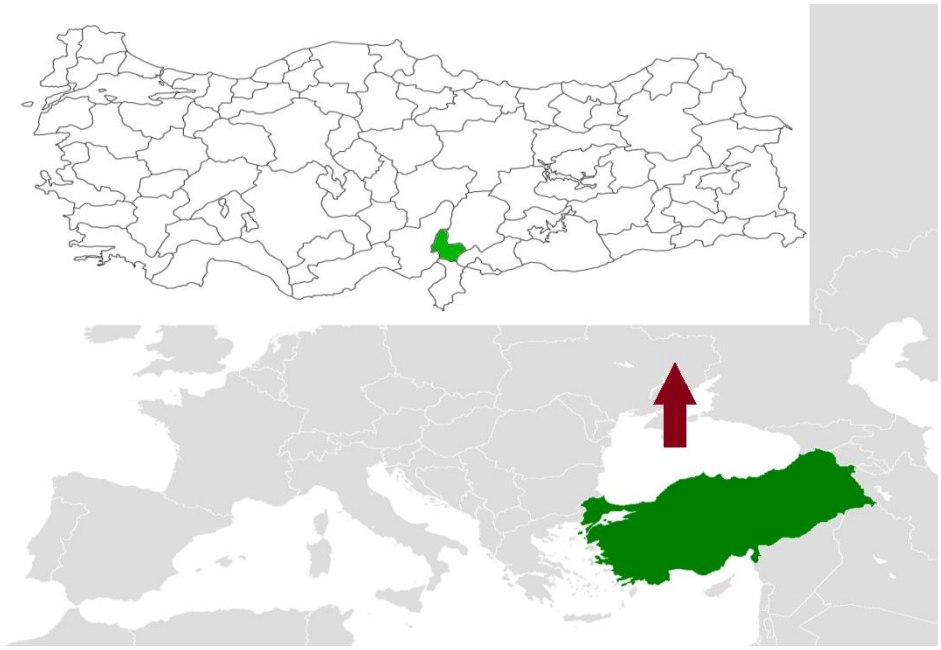

Figure 1. Map of the experimental area (Osmaniye, Turkey)

Table 2. Climate parameters of the research field (2018, 2019, and long-year average)

Months	Precipitation (mm)			Temperature (°C)			Relative Humidity (%)		
	LY	2018	2019	LY	2018	2019	LY	2018	2019
April	79.9	41.2	46.7	17.1	18.9	16.3	63.6	62.6	69.8
May	69.4	65.9	2.4	21.2	23.1	23.3	62.7	65.7	56.6
June	42.2	111.2	73.4	25.2	25.1	26.0	62.0	74.7	71.0
July	17.6	1.8	48.5	27.8	27.8	28.6	65.0	73.3	72.3
August	10.8	0	8.3	28.5	28.6	28.5	64.9	70.9	69.7
September	40.3	0	14.5	25.5	26.9	26.1	61.6	64.8	61.3
Total/Av.	260.2	220.1	193.8	24.2	25.1	24.8	63.3	68.7	66.8

Av.: Average; LY: Long Year.

The pH of the clay-loam soil used in the study was slightly alkaline (pH ~8). The lime substance of the soil was optimum (~10%) while the organic matter of the soil was low (~1.20%). Climate parameters -total precipitation,

relative humidity, and average temperature - during 2018 and 2019 growing period and long year (LY) were shown in *Table 2*. The total precipitation was 220.1 mm in 2018 and 193.8 mm in 2019. Although LY (260.2 mm) was similar to 2018 but a bit difference with 2019. The average temperature and relative humidity in the studied years and LY showed no significant differences. The average temperatures were 25.1°C and 24.8°C in 2018 and 2019, respectively. In addition, the relative humidity values were 68.7% in 2018 and 66.8% in 2019 while the LY was 63.3%.

2.2. Methods

The experiments were conducted in randomized complete block design (RCBD) with three replications. Each plot was composed of 5 m long and four rows with 70 cm row space and 15 cm plant spacing. Di-ammonium phosphate (DAP) fertilizer was used at the rate of 25 kg da^{-1} before sowing. Sowing was performed on April 6, 2018, in the first year and on April 30, 2019, in the second year. Weed control was achieved by hand weeding when it was needed. Irrigations were performed to prevent the plants against drought effects with a drip irrigation system. Harvests were performed on September 11, 2018, in the first year and on September 25, 2019, in the second year, manually. By taking into consideration side effects, two inner rows were harvested from every plot.

The typical conventional Soxhlet apparatus was used for extracting the seed oil. Diethyl ether was used as a solvent for extracting oil from peanut seeds. The fatty acid composition was analyzed as described by Sahin and Isler (2022) (*Figure 2*).

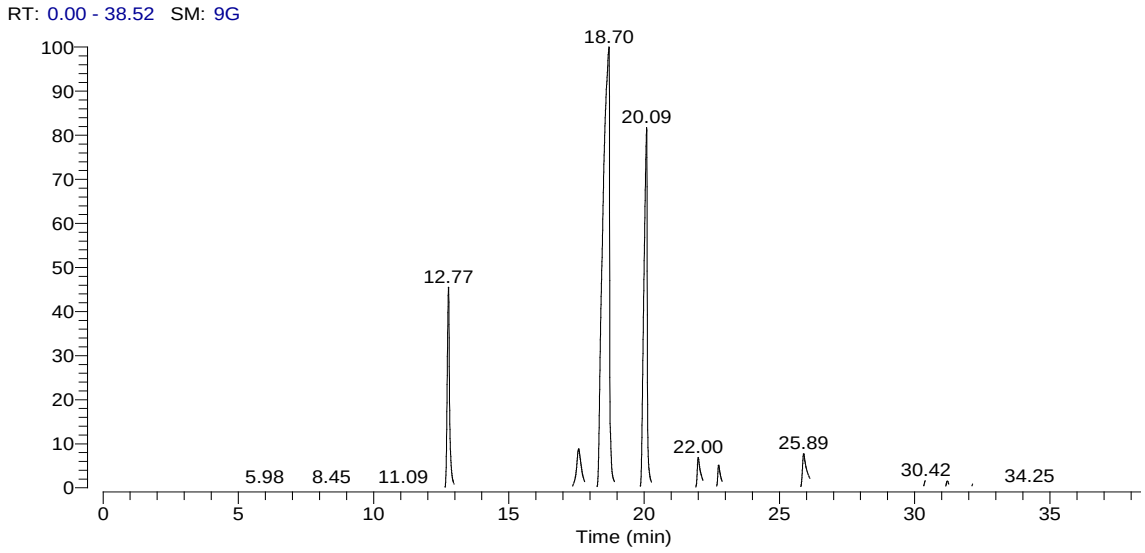

Figure 2. An example chromatogram generated by GC-MS

Oil quality factors (O/L and IV) were calculated using the equation given by Chowdhury et al. (2015) (Eq. 1 and 2).

$$\text{Iodine Values (IV)} = (\%\ \text{oleic acid} \times 0.8601) + (\%\ \text{linoleic acid} \times 1.7321) \quad \text{(Eq. 1)}$$

$$\text{Oleic/Linoleic Acid (O/L) Ratio} = (\%\ \text{oleic acid (18:1)})/(\%\ \text{linoleic acid (18:2)}) \quad \text{(Eq. 2)}$$

2.3. Statistical Analysis

Experimental data were subjected to analysis of variance in accordance with RCBD joined year with the aid of R v4 software. Means were compared to the aid of Duncan's multiple range test.

3. Results and Discussion

3.1. Oil Content

The result for oil content was significant ($p < 0.01$) for varieties but not for year and year x varieties interaction (*Table 3*).

Table 3. Results of the analysis of variance for characteristics studied in the experiment

SV	df	OC	PA	SA	OA	LA	O/L	IV
Block	4	ns	ns	ns	ns	ns	ns	ns
Year	1	ns	ns	ns	ns	ns	ns	ns
Varieties	14	**	**	**	**	**	**	**
Y x V	14	ns	ns	ns	ns	ns	ns	ns
CV (%)		1.79	4.10	7.12	3.68	4.87	5.72	1.85

SV: Source of variation, df: Degree of freedom, CV: Coefficient of variation, ** $p < 0.01$, OC: Oil content; PA: Palmitic acid; SA: Stearic acid; OA: Oleic acid; LA: Linoleic acid; O/L: Oleic/Linoleic Ratio; IV: Iodine Value

The oil content varied between 44.57-52.23% on a two-year average. The ultimate value of oil content was acquired from NC-V-11 at 52.23% and the lowest from BATEM-Cihangir at 44.57% in joint year analysis (*Table 4*). Gulluoglu et al. (2017) and Asik et al. (2018) stated that the oil percentage of peanut kernel varies between 35% and 56% depending on growing conditions and genotype and the oil content of peanut varieties impact by seed maturity, climatic conditions, genotype, geographical location, growing conditions and growing season. The oil content had a positive correlation with the number of pods per plant. Yol and Uzun (2018) detected that the oil percentage of peanut kernel varies between 47.9% and 52.4%. Also, Ozcinar (2022) stated that the oil percentage of peanut kernel varies by %50. The parallel consequences were found by Ozcinar (2022), Arioglu et al. (2016), Asik et al. (2018), and Yol and Uzun (2018). The oil content values of the present study were higher than Karabulut and Tuncturk (2019).

3.2. Saturated Fatty Acid Compositions

The grand saturated fatty acids in peanut oil are stearic (18:0) and palmitic (16:0) acids. The result for palmitic (16:0) acid was significant ($p < 0.01$) for varieties but not for year and year x varieties interaction (*Table 3*). Palmitic acid percentage values varied between 8.04-12.24% on two-year average. The grandest value of palmitic acid content was obtained from Florispan at 12.24% and the lowest from Brantley as 8.04% in joint year analysis (*Table 4*). Yu et al. (2020) palmitic acid ratio 2.92-5.5%; Shibli et al. (2019) found that the palmitic acid ratio varied between 9.32-12.03%; Kamdar et al. (2021) reported palmitic acid values of 8.1-14.2%. Gulluoglu et al. (2017), Yu et al. (2020) and Kamdar et al. (2021) rely on growing conditions and genotype, and the palmitic acid content of peanut varieties was influenced by climatic conditions, genotype, seed maturity, geographical location, growing conditions and growing season. The analog outcomes were found by Shibli et al. (2019) and Kamdar et al. (2021), Onemli (2012), Hassan and Ahmed (2012). The palmitic acid content values of the present study were less than Yol and Uzun (2018), and Yu et al. (2020).

The result for stearic (16:0) acid was significant ($p < 0.01$) for varieties but not for year and year x varieties interaction (*Table 3*). As a consequence, in a two-year average, the stearic acid percentage varied between 1.38-2.91% in two-year average. The highest value of stearic acid content was acquired from Brantley at 2.91% and the lowest from Com at 1.38% in joint year analysis (*Table 4*). Onemli (2012), Gulluoglu et al. (2016), Gulluoglu et al. (2017), Yol and Uzun (2018), and Salamatullah et al. (2021) determined that the stearic acid percentage of peanut kernel varies between 2.38% and 4.9% attaching to growing conditions and genotype, and the palmitic ingredient of peanut varieties efficacy by climatic conditions, genotype, seed maturity, growing season, geographical location, and growing conditions. The similar results were found by Hassan and Ahmed (2012) and Salamatullah et al. (2021). The palmitic ingredient values of the present study were less than Onemli (2012), and Yol and Uzun (2018).

3.3. Unsaturated Fatty Acid Compositions

The great unsaturated fatty acids in peanut oil are oleic (18:1) and linoleic (18:2) acids. The seeds have oleic and linoleic acids accounting for nearly 80% of total fatty acids at seed maturity. The result for oleic (18:1) acid content was significant ($p < 0.01$) for varieties but not for year and year x varieties interaction (*Table 3*). The oleic acid content varied between 43.70-71.83% on two-year average. The highest value of oleic content was obtained from Brantley at 71.83% and the lowest from Florispan at 43.70% in joint year analysis (*Table 5*). Onemli (2012), Hassan and Ahmed (2012), Wang et al. (2013), Yol ve Uzun (2018) and Gali et al. (2021) determined that the oleic acid percentage of peanut kernel varies between 38.85% and 62.04% relying on growing conditions and genotype, and the oleic acid content of peanut varieties impact by seed maturity, genotype, climatic conditions,

growing season and growing conditions. The oleic acid content had a positive correlation with the number of pods per plant. Similar results were found by Onemli (2012), Hassan and Ahmed (2012), Wang et al. (2013), Yol and Uzun (2018) and Gali et al. (2021).

Table 4. Results of the analysis of variance for characteristics studied in the experiment The-2-years average values of oil content and saturated fatty acid compositions (palmitic and stearic acids)

Varieties	Oil Content (%)	Palmitic Acid (%)	Stearic Acid (%)
Florispan	52.16±0.38 a	12.24±0.75 a	1.72±0.06 d
BATEM-Cihangir	44.57±0.34 e	10.65±0.15 de	2.01±0.01 c
Georgia Green	51.54±0.41 a	10.82±0.26 cd	1.57±0.11 de
Sultan	47.96±0.48 d	11.41±0.08 bc	2.05±0.06 c
Brantley	52.10±0.27 a	8.04±0.02 h	2.91±0.14 a
BATEM-5025	51.13±0.25 a	9.82±0.14 fg	2.50±0.14 b
Arioglu-2003	48.49±0.40 d	10.48±0.15 ef	1.72±0.05 d
Halisbey	48.55±0.50 cd	10.18±0.04 ef	1.68±0.11 d
NC-7	49.74±0.45 b	10.31±0.34 ef	2.13±0.08 c
Flower-22	48.50±0.37 d	11.55±0.03 ab	2.22±0.09 c
Wilson	49.65±0.17 bc	9.56±0.08 g	1.99±0.04 c
NC-V-11	52.23±0.34 a	9.75±0.26 fg	2.14±0.08 c
Com	49.01±0.20 cd	10.55±0.05 ef	1.38±0.03 e
Osmaniye-2005	51.21±0.52 a	11.06±0.14 bc	1.56±0.13 de
Gazipasa	49.05±0.17 cd	10.70±0.02 de	1.50±0.13 de
Average	49.73	10.47	1.94

Letters show different groups for varieties in each column. The values are Mean±Standard Error of the Mean.

Table 5. The-2-years average values of unsaturated fatty acid compositions (oleic and linoleic acids) and oil quality factors (O/L and IV)

Varieties	Oleic Acid (%)	Linoleic Acid (%)	Oleic/Linoleic Ratio (O/L)	Iodin Value (IV)
Florispan	43.70±2.51 e	35.68±2.77 a	1.22±0.17 g	99.38±2.64 ab
BATEM-Cihangir	48.63±0.17 d	30.95±0.07 c	1.57±0.01 e	95.43±0.14 cd
Georgia Green	46.97±0.37 de	32.79±0.31 bc	1.43±0.02 fg	97.18±0.47 bc
Sultan	53.45±0.14 c	27.04±0.52 d	1.98±0.04 d	92.80±0.85 d
Brantley	71.83±0.57 a	9.78±0.13 g	7.35±0.14 a	78.71±0.43 h
BATEM-5025	59.58±0.29 b	20.05±0.18 f	2.97±0.04 b	85.98±0.29 g
Arioglu-2003	48.80±0.06 d	30.44±0.08 c	1.60±0.01 e	94.69±0.13 cd
Halisbey	46.24±2.97 de	30.50±0.65 c	1.52±0.12 ef	92.60±2.22 de
NC-7	57.44±1.56 b	22.52±0.03 e	2.55±0.07 c	88.41±1.28 fg
Flower-22	48.72±0.06 d	31.61±0.30 c	1.54±0.01 ef	96.66±0.55 bc
Wilson	58.93±0.36 b	22.56±0.01 e	2.61±0.02 c	89.76±0.31 ef
NC-V-11	53.13±0.44 c	27.95±0.60 d	1.90±0.05 d	94.10±0.92 cd
Com	45.07±0.42 e	35.77±0.28 a	1.26±0.02 g	100.71±0.44 a
Osmaniye-2005	49.61±0.17 d	30.81±0.28 c	1.61±0.02 e	96.03±0.43 c
Gazipasa	46.10±0.26 de	34.48±0.21 ab	1.34±0.01 fg	99.37±0.32 ab
Average	51.88	28.19	2.16	93.45

Letters show different groups for varieties in each column. The values are Mean±Standard Error of the Mean.

The result for linoleic (18:2) acid content was significant ($p < 0.01$) for varieties but not for year and year x varieties interaction (*Table 3*). The linoleic acid content varied between 9.78-35.77% on two-year average. The ultimate value of linoleic content was gotten from Com as 35.77% and the lowest from Brantley as 9.78% in joint year analysis (*Table 5*). Hassan and Ahmed (2012), Bishi et al. (2015), Gulluoglu et al. (2017), Yol ve Uzun (2018), Gali et al. (2021) stated that the linoleic acid percentage of peanut kernel varies between 22.30% and 41.40% relying on growing conditions and genotype, and the oleic acid content of peanut varieties impact by seed maturity,

genotype, climatic conditions, growing season, growing conditions and geographical location. The linoleic acid content had a positive correlation with the number of pods per plant. The counterpart conclusions were found by Onemli (2012), Bishi et al. (2015) and Gali et al. (2021). The linoleic acid content values of the present study were lower than Hassan and Ahmed (2012) and Yol and Uzun (2018).

3.3. Oil Quality Values

The new ingredients in nutrition and exchanging inclinations in peanut marketing project a glossier future for the utilization of peanut cultivars with widely varied nutritional and chemical quality features. All of the constituents of peanut oil quality in this study were extremely impacted by genotype (*Table 5*). The maximum value of oleic/linoleic ratio (O/L) content was obtained from Brantley as 7.35 and the lowest from Florispan as 1.22 in joint year analysis (*Table 5*). Lopez et al. (2001), Yav et al. (2008) and Gali et al. (2021) stated that the ratio of oleic acid to linoleic acid (O/L ratio) and iodine value defines the storability, quality, and shelf life of groundnut oil and its products. The like outcomes results were found by Hashim et al. (1993), Lopez et al. (2001) and Gali et al. (2021).

The iodine value (IV), which ensures a measure of the degree of oil unsaturation, and the ratio of oleic to linoleic acid (O/L) has been mostly used as a means of predicting shelf-life and measuring stability of the oil. Higher (O/L) ratios and lower IV generally suggest better stability and longer shelf-life (Casini et al., 2003; Gulluoglu et al., 2017; Bakal and Arioglu, 2019). The iodine value (IV) content varied between 78.71-100.71 on two-year average. The highest value of iodine value (IV) content was obtained from Com as 100.71 and the lowest from Brantley as 78.71 in joint year analysis (*Table 5*). The similar results were found by Hashim et al. (1993). The iodine value (IV), content values of the present study were lower than Biermann et al. (2000).

4. Conclusions

The oil composition and oil content of peanut varieties may vary depending on environmental conditions and genetic factors. According to the results of this study, besides the significant effects of genotypic differences, years were also found to have a considerable influence on the following fatty acids: palmitic, stearic, linoleic, and behenic. Year effects were significant for the O/L ratios and iodine values as well as the saturated, unsaturated, and long-chain composites. This is in agreement with what has previously been reported regarding the yearly effect on peanut oil quality. Finally, it was recommended that Brantley could be the optimum variety because it had the highest oil content, oleic acid, and O/L ratio according to two-year experiment.

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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Formulation of SCP-Based Coconut Sugar Marketing Model Through Analysis of Marketing Patterns in Central Java Province, Indonesia

Pujiharto PUJIHARTO[1*], Sri WAHYUNI[2]

Abstract

This study aims to analyze the marketing pattern of coconut sugar products, describe the stakeholders involved in the marketing of coconut sugar, and develop a structure, conduct, performance (SCP) based marketing model for coconut sugar. This research method is a quantitative descriptive method. The research location was determined purposively in the coconut sugar production center of Central Java Province (Banyumas, Purbalingga, and Cilacap Regencies). The research respondents were 60 people consisting of coconut sugar makers who were members of the Joint Business Group (KUB) and 60 people, 10 collectors traders, 2 wholesalers, and 4 institutional exporters. Data were analyzed descriptively. Data analysis for the basic structure by measuring market share size and concentration (CR4). The basis of conduct is through analysis of sales and purchasing processes, payment systems, and institutional cooperation. The basis of performance is obtained from the analysis of the margins of the trading system and the level of prices received by farmers. The results showed that there are two marketing channels for coconut sugar. A tight oligopsony is formed based on the CR4 (Concentration Ratio for the Big Four) value, while the Herfindahl index value shows that the market formed is an oligopoly. Market behavior occurs when the payment system is made in cash at the level of collectors and non-cash at the level of wholesalers. In market performance, the largest marketing margin is in the marketing channel pattern 1 of Rp. 8415.4/kg, the lowest in the channel 2 pattern of Rp. 6500/kg. The highest Farmer share value is in the marketing channel pattern 2 as 74%, and the lowest is in the channel 1 pattern at 66.3%.

Keywords: Marketing model, Coconut sugar, SCP, Marketing sugar, Agricultural product marketing

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1. Introduction

The agroindustry is an industry that processes agricultural products into finished products oriented to consumer tastes (Djamali et al., 2018). It is known that 70.5% of students consume sugar as crystal sugar, and 83.2% prefer to consume sugar every day (Konyali, 2019). Empowerment of agroindustry through marketing aims to increase the product's added value so that farmers get a higher selling price (Amir et al., 2018). Marketing agro-industrial products, including coconut sugar, has an important role in improving the national economy. Coconut sugar is a processed coconut product that evaporates the juice until it reaches a saturated liquid and forms a coconut structure (Nurhadi et al., 2018). Indonesia is one of the world's leading producers of coconut sugar (Suliyanto, 2013). Most exported coconut sugar products come from Central Java Province, especially Banyumas, Purbalingga, and Cilacap Regencies. The coconut sugar agroindustry, one of the important subsystems in the agribusiness system, has great potential to encourage high economic growth due to its relatively large market share and added value.

It is believed that the coconut sugar agroindustry will continue to grow due to the availability of vast land and a sharp increase in international market demand. However, the welfare of coconut sugar farmers in Indonesia is still low because they cannot meet the export market's organic needs (Restianto et al., 2021), as well as low prices at the producer level (Suyono et al., 2020). The low price of coconut sugar products at the producer level is also inseparable from a less competitive market structure, where parties are more dominant in determining prices (price makers). Therefore, on the one hand, producers tend to be price takers. On the other hand, from the description above, it is very important to research a marketing model based on structure conduct performance (SCP) in the coconut sugar agroindustry.

Marketing of coconut sugar agroindustry products generally works in an imperfect market. High transaction costs cause these imperfections Imperfections in infrastructure (markets) and market structure and behavior can hinder optimal prices for farmers (Van Tilburg et al., 2008). Bagchi et al. (2022) research said that based on structure-behavior-performance theory and previous field experience research, smallholder collectivization helps explore a larger set of strategic options for better yields and the importance of addressing market imperfections through policy and institutional interventions. This study aims to analyze the marketing pattern of coconut sugar products, describe the stakeholders involved in marketing coconut sugar, and develop a marketing model for coconut sugar based on SCP. The research problem formulation is (1) how is the marketing pattern of coconut sugar agroindustry products? (2) who are the stakeholders involved in marketing coconut sugar agroindustry products? (3) how is the SCP-based marketing model for coconut sugar agroindustry products?

2. Materials and Methods

The basic method used in this research is the descriptive quantitative survey method, which is a research method that focuses on a current problem by collecting data, compiling, and analyzing it. The research location was determined purposively in the coconut sugar production center of Central Java Province, covering Banyumas, Purbalingga, and Cilacap Regencies, coconut sugar producer had become members of the Joint Business Group (KUB). The sample of coconut sugar producer farmers was determined by the Simple Random Sampling method from the population of coconut sugar producer who were members of the Joint Business Group (KUB) in the research area. In contrast, the Snowball Sampling method determined the sample of traders (collecting traders, wholesalers, retailers, and exporters). The data taken include primary data obtained from respondents by conducting direct interviews using a list of questions (questionnaires) that have been provided. The survey research variables include fixed production costs, variable costs, coconut sugar production volume, coconut sugar selling price, buying and selling volumes, revenues, and profits. This research will obtain the pattern of marketing of coconut sugar, stakeholders involved in marketing coconut sugar, market structure, market behavior, and market performance in the marketing of coconut sugar. Moreover, secondary data from relevant agencies is related to research, journals, and other literature.

Data analysis uses a descriptive and structured conduct performance (SCP) approach. Market structure is described by the size of market share (market share) and market concentration (CR4) (Martin, 2012; Tomek and Kaiser, 2014).

$$MS_i = \frac{S_i}{S_{tot}} \times 100\% \quad \text{(Eq. 1)}$$

Information:

MS_i = trading market share (%)
S_i = sales (Rp)
S_{total} = total of all sales researched (Rp)

The measurement of the Hirschman Index is based on the total number and size distribution of firms in the industry. Where the Hirschman – Herfindahl Index (HHI) equation can be solved by the formula:

$$HHi = (MS_1)^2 + (MS_2)^2 + \cdots + (MS_n)^2 \quad \text{(Eq. 2)}$$

Information:

HHI : Hirschman – Herfindahl Index
MS_i : commodity buyers from the i-th trader (i= 1,2,3...,n)
n : number of traders in a product market area

Criteria as follows:

HHI = 1 or 1,800-10,000 then it leads to monopoly or monopsony
HHI = 0 or 0-1000 then it leads to perfect competition
0 < HHI < 1 or 1000-1800 then oligopoly or oligopsony

The next step is to calculate the market concentration of the four largest buyers (CR4)

$$CR4 = \sum_1^4 Sij \quad \text{(Eq. 3)}$$

Information:

CR4 = concentration ratio of the 4 largest traders
Sij = market share of the 4 largest coconut sugar traders

If the value of CR4 < 33% (competitive market structure); 33-50% (weak oligopsonistic market structure); > 50% (strongly oligopsonistic market structure).

Conduct analyzed descriptively includes sales and purchase processes, equilibrium prices, payment systems (cash, credit), and cooperation with other trading institutions. Performance shows the level of marketing efficiency of coconut sugar. The analysis carried out is the margin of trade and the price level received by farmers (farmer share).

3. Results and Discussion

3.1. Coconut Sugar Marketing Channel Pattern

The pattern of marketing channels for coconut sugar in the research area starts from coconut sugar producers as producers and then to collectors and wholesalers (KUB) who eventually relate to exporters or consumers. From the study results, it was found that there are 2 patterns of coconut marketing channels. 58 people (96.67%) sugar makers use the channel 1 pattern, and 2 people (3.33%) use the channel 2 pattern. There are no coconut sugar producers who use more than one marketing pattern, and this is because each producer already has a fixed channel pattern. The selection of marketing channels is based on several things, including the selling price, distance (transportation), source of purchase, and purpose of sales. Some wholesalers determine the purchase price per kilogram, and then the collectors adjust the prices that the wholesalers have determined. The forms of the pattern of coconut sugar marketing channels in the research location are described below.

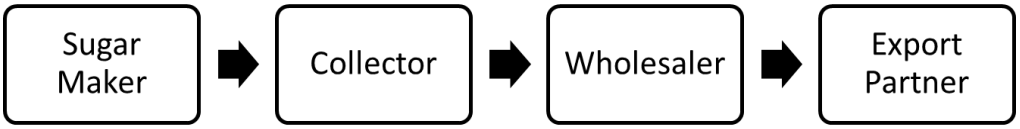

Figure 1. Marketing Channel Pattern 1

In this channel, coconut sugar producer sells their products to collectors, with the consideration of easy access to the collectors' place so that sugar producers do not have to go far to sell their products and a cash payment system that makes it easier for a producer to fulfill their daily needs and the purchase price of coconut sugar which the producer feel is already profitable. Collecting traders deposit to wholesalers, wholesalers then supply coconut sugar to exporter partners. In this pattern, sugar producers sell to collectors near their homes; the products sold by the producer are wet coconut sugar products that have not been dried. All products from sugar producers are sold to collectors who are deposited 2-3 times a week and paid in cash. Collecting traders collect and store, which will later be sold to wholesalers who pick up the place once a week and payment systems in cash and non-cash (credit). Wholesalers carry out the drying process by oven, sorting, and packaging, which will be sent to export partners. Coconut sugar products are marketed to several countries such as Singapore, Japan, United Arab Emirates, Qatar, the UK, Poland, and Russia. Exporting partners that work together are PT Haldin Pacific Semesta, PT Mega Innovation Organic (MIO), CV Realsa Natural Indonesia, and CV Itrade International.

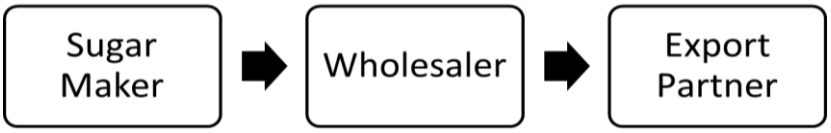

Figure 2. Marketing Channel Pattern 2

In the channel 2 pattern, coconut sugar producers sell wet coconut sugar products that have not been dried to wholesalers. All products from coconut sugar producers are sold to wholesalers who are deposited once a week. Wholesalers go directly to the coconut sugar producer and pay in cash. Then wholesalers carry out the drying process with ovens, sorting, and packaging, which will be sent to exporter partners.

3.2. Coconut Sugar Marketing Stakeholders

From the results of the study, it was found that the stakeholders involved in marketing coconut sugar are coconut sugar producer who are members of the Joint Business Group (KUB) as producers, collectors, wholesalers, and exporter partners, namely PT Haldin Pacific Semesta, PT Mega Innovation Organic (MIO), CV Realsa Natural Indonesia, and CV Itrade International.

3.3. SCP-Based Coconut Sugar Marketing Model

3.3.1. Market Structure

The market structure shows market characteristics, such as the number of buyers and sellers, the state of the product, the state of knowledge of buyers and sellers, and the state of market obstacles. These differences will determine the company's behavior and performance. To examine the market structure used, the concentration ratio of the four companies (CR4) and the Herfindahl-Hirschman index (HHI) (Sim and Hassan, 2020). Market share is the percentage of total sales in the target market obtained from a company. The size of the market share ranges from 0 to 100 percent of the total sales of the entire market. A large market share characterizes a large market power. On the other hand, a small market share means that the company cannot compete under competitive pressures. In the coconut sugar market structure that occurs in the Karanganyar and Kutasari sub-districts with a market share of mediators, there are 10 different collectors. The following is the market share size of trading collectors, which can be seen in *Table 2*.

In *Table 1*, the strength of each market share at the collectors is different because of the difference in the amount of production for each collector in one deposit. The greater the production power or the number of deposits, the higher the market share value. Factors that affect the size of the market share are the large number of producers who sell coconut sugar products, the amount of coconut sugar production produced by producers, and the number of coconut sugar deposits.

CR4 (Concentration Ratio for Biggest Four) measures the industry's share of total asset value. In general, the number of N traders whose market share proportion is calculated is 4 traders. In determining the CR4 value based on the output value or the amount of coconut production selected from the four largest traders.

Table 2 shows the CR4 value of the market structure for collectors at 56.8% or 0.56, meaning that the concentration ratio (CR4) is > 50% (strongly oligopsonistic market structure), meaning that the market has only a few buyers. In this case, the collectors, while the product has few buyers, have similar offerings with little price competition and relatively little product information.

Table 1. Market Share Percentage Value of Each Collecting Trader

Collecting Merchant	Total deposit (kg)	Percentage Market Share (%)
1	700	6.3
2	1000	9.0
3	840	7.5
4	1050	9.5
5	750	6.7
6	1800	16.2
7	2450	22.1
8	950	8.5
9	900	8.1
10	650	5.8
Total	11,090	100

Table 2. The Four Biggest Collecting Traders to Analyze CR4 Value

Collecting Merchant	Total deposit (kg)	Percentage Market Share (%)
1	1,000	9.0
2	1,050	9.5
3	1,800	16.2
4	2,450	22.1
Concentration Ratio Value	6,300	56.8

Measurements on the Hirschman – Herfindahl Index (HHI) are based on the total number and size distribution of coconut sugar collectors by adding up the square of the market share of all these collectors. The following results of calculations from HHI can be seen in *Table 3*.

Table 3. Hirschman – Herfindahl Index (HHI) Value for Each Collecting Trader

Collecting Merchant	Total deposit (kg)	Percentage Market Share (%)	Percentage² (% squared)
1	700	6.3	39.69
2	1,000	9.0	81.00
3	840	7.5	56.25
4	1,050	9.5	90.25
5	750	6.7	44.89
6	1,800	16.2	262.44
7	2,450	22.1	488.41
8	950	8.5	72.25
9	900	8.1	65.61
10	650	5.8	33.64
Total	11,090	100	1,297.79

Table 3 can be seen that the HHI value for collectors is 1297.79, indicating that competition in the market share of coconut sugar collectors is an oligopoly with a range between more than 1000 and less than 1800, which means the higher the Hirschman Herfindahl index value, the higher the size distribution. from merchants. This, of course, will result in a policy of reducing the price of goods by one trader to be followed by other traders.

3.3.2. Market Conduct

Market behavior is related to the behavior of traders. Constructive validity is found in the market behavior function, where indicators of consumer demand and product supply have a significant effect (Darma et al., 2022). Market behavior is related to the behavior of traders, the behavior of the coconut sugar producer, the collectors,

and wholesalers carried out several strategies to facilitate the distribution of coconut sugar. Collectors and wholesalers carry out purchasing activities for producers of coconut sugar producer. The cash and credit payment strategies between coconut sugar producers and collectors are carried out after depositing coconut sugar products and price agreements. Collectors and wholesalers determine the price of coconut sugar, and there is no price bargaining. According to coconut sugar producers, this is because the purchase price by traders is already profitable. It can cover the costs of daily needs and the costs incurred by a producer in producing coconut sugar. Sales activities occur to traders, from collectors to wholesalers, and from wholesalers to exporter partners. In sales activities from collectors to wholesalers, data on the name of the coconut sugar craftsman who deposited it must be included. In the middleman's trade, only storage activities occur, and later every week, wholesalers will take the goods.

Meanwhile, at wholesalers of coconut sugar, drying, sorting, and packaging are carried out before delivery to exporter partners. The payment system for coconut sugar at the collectors level to coconut sugar producers is in cash, which is done to make it easier for coconut sugar producer to cover their daily needs and sugar production costs. Meanwhile, payments from wholesalers to retailers are partly made in cash (Susilo, 2016) and partly on credit. Payments from exporter partners to wholesalers are non-cash with an upfront payment of 50%. The price determined by traders and wholesalers is based on the quality or color of the sugar to be sold; the darker the color of the coconut sugar, the lower the price offered. The dominant price determinants are traders, not farmers, even though there is bargaining between farmers and traders (Wa Ode Al, 2019). Market behavior in cooperative activities between coconut sugar marketing institutions, producers and collectors, wholesalers, and exporter partners is well established. Coconut sugar producer may sell their products to more than one collecting trader from the origin of these traders as members of the KUB. In addition, increasing farmers' knowledge about production and marketing is important and must be developed (Torun, 2014). Developing an integrated marketing channel system for agricultural products will advance industrial and agricultural production and increase farmers' income steadily (Wang, 2010).

3.3.3. Market Performance

Market performance is used to see the influence of market structure and behavior in the coconut sugar marketing process. Market performance is a combination of market structure and market behavior which shows the interaction between market structure, market behavior, and market performance which are not always linear but influence each other. Collaborating with suppliers (in this case, coconut sugar producers) companies will support sustainability values and maintain market performance (Ukko et al., 2022). Institutional relationships with government officials, local communities, and other companies affect market performance (Nwoba et al., 2021). A more effective food production strategy is critical to sustainable agro-industry development along with poverty reduction (Leite et al., 2017). *Table 4* shows the marketing margins of coconut sugar in the two marketing channels found in this study.

Table 4. Coconut Sugar Marketing Margin Summary

Marketing channel	Price (Rp/Kg)				
	Producer	**Consumer**	**Cost**	**Profit**	**Margin**
1	16,584.6	25,000	623.28	7,892.03	8,415.4
2	18,500	25,000	623.28	5,976.72	6,500

Table 4 shows the distribution of selling prices from sugar producers and the margins formed from the different marketing channel processes. In the marketing channel pattern 1, the margin formed is Rp. 8415.5/kg while in marketing channel 2, it is Rp. 6500/kg. The share received by coconut sugar producer is a percentage of the price at the producer level with the price paid by consumers (*Tabel 5*).

The amount of share received by coconut sugar producers differs in each marketing channel. In the marketing channel pattern 1, the farmer share received by the coconut sugar producer is 66.3%, while in channel 2 farmer share pattern received by the producer is 74%. From the description above, the share of the price received by the largest sugar producers is in the channel 2 pattern due to the short chain of marketing channels for Coconut sugar. While the share received by coconut sugar producers is lower in the channel 1 pattern, this is due to additional marketing agencies distributing coconut sugar products.

Table 5. Farmer Share Coconut Sugar Marketing

Marketing channel	Price at Producer Level (Rp/kg)	Price at Final Trader level (Rp/kg)	Farmer Share (%)
1	16,584.6	25,000	66.3
2	18,500	25,000	74

4. Conclusions

This study concludes that two marketing channels for coconut sugar can be chosen by producers, with the ultimate goal of marketing being exporter partners. Stakeholders involved in marketing coconut sugar are coconut sugar producers who are members of KUB, collectors, wholesalers, and exporter partners. The market formed from the marketing of coconut sugar in Central Java Province is a tight oligopoly market, as indicated by a CR4 value of 56.8%. But the price of coconut sugar is determined by the buyer by looking at the quality and color of the sugar. The performance of the coconut sugar market from both marketing channel pattern 1 and 2 is quite profitable for producers because it has a margin value of 8,415.4 IDR/kg in channeol pattern 1 and a high farmer share (above 50%). Of the two patterns, several factors influence marketing patterns, namely access (distance from producers to traders), payment systems (cash and non-cash), selling prices, number of products, and marketing agency cooperation. The results of this study only describe the SCP-based coconut sugar marketing channel in Central Java Province, without comparing the two marketing channels. It is necessary to carry out similar research in other regions to describe how the coconut sugar marketing channels are in Indonesia and to compare between marketing channels to see the advantages and disadvantages between channels.

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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Carbon Nanotubes (CNTS) and Frankincense Nanoparticles as Promising Insecticides to Control Onion Thrips

Abdulla N. Ali[1*], Akram A. Mohammed[2], Sahar M. Jawad[3]

Abstract

Thrips tabaci Lindeman (Thysanoptera: Thripidae) is one of the most common and devastating onion pests which is capable of causing substantial harm to onion crops. Synthetic pesticides are mainly used to control onion thrips. *T. tabaci* requires alternative, low-impact control measures since there are numerous difficulties with utilizing chemical pesticides, including pesticide resistance. This study aimed to evaluate the effectiveness of the nanomaterial compounds on adults and nymphs of the *T. tabaci* in vivo and study their physiological changes caused by pesticides. The findings demonstrate that using nanomaterials, such as carbon nanotubes (CNTs) and frankincense nanoparticles (FNPs), significantly impacts the number of onion thrips. It also has the potential to lower the risk of pesticide resistance. According to the preliminary results, using carbon nanotubes (CNTs) considerably increased the mortality rate of adults and nymphs of *T. tabaci* and decreased egg-hatching success. Carbon nanotube (CNTs) and frankincense nanoparticles showed a high death rate in adult and nymphal stages at a concentration of 0.05 percent. Carbon nanotubes (CNTs) demonstrated exceptional mortality rates in adult and nymphal stages, with 90 and 50 percent at 5 mg/mL concentrations. Frankincense nanoparticles (FNPs) treatment demonstrated a high adult mortality rate of around 60 percent compared to the control treatment. Eggs of onion thrips showed different hatching success rates after treatment with CNTs and FNPs. The egg hatch rate did not exceed 40 percent of hatched eggs in the CNTs treatment compared to 90 percent in the control treatment. On the other hand, number of laid eggs per female did not differ significantly, indicating that none of the treatments affected the fecundity of the females. The ability of thrips to develop resistance to CNTs and frankincense compounds requires additional investigation. These natural products could be a suitable alternative to control destructive pests like onion thrips.

Keywords: Nanomaterials, Eco-friendly insecticides, Onion thrips**,** Frankincense, Carbon nanotubes (CNTs).

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1. Introduction

Thrips tabaci (Thysanoptera: Thripidae) is a common pest of onion and other crops that can cause severe crop losses and economic impact owing to feeding on green leaf parts by the adults and nymphs (Ananthakrishnan, 1973; Lewis, 1997). This tiny insect destroys onion epidermal tissue (Chisholm and Lewis, 1984). They pierce surface tissues and absorb the contents of living cells (Koschier et al., 2002). When *T. tabaci* feeds on onions, it produces silvery leaf spots that evolve into white blotches, then silvery patches along with the leaves, which reduces the plant's ability to photosynthesize, lowering the weight of onion bulbs (Diaz-Montano et al., 2011). Onion thrips are most recognized for their ability to transmit numerous illnesses to onions (Gill et al., 2015). In warm and dry conditions, the population of onion thrips can multiply rapidly, leading to an outbreak (Rueda et al., 2007). *T. tabaci* has been observed to develop pesticide resistance, which might lead to control failures, agricultural losses, and environmental issues (Adesanya et al., 2020). Furthermore, recent research indicates that synthetic pesticides are being used more frequently, which has implications for public health, food safety, and the national economy (Lougraimzi et al., 2022). Consequently, discovering alternate materials to manage onion thrips is a crucial objective (Diaz-Montano et al., 2012). Alternative approaches to control pests are urgently needed because utilizing chemical pesticides can be extremely detrimental to the environment (Tayat and Özder, 2023). Especially in a warm location like Iraq, where the population of onion thrips might develop rapidly due to insufficient pest control and excessive chemical usage.

Plants and herbs contain potent compounds such as phenols, aldehydes, and alkaloids that have many therapeutic applications against various diseases caused by bacteria, molds, or viruses. There has been worldwide interest in their use (Al-Harrasi et al., 2018). The Boswellia tree is known for its fragrant resin, which is used to make frankincense. Frankincense resin (FR) is inexpensive, renewable, and non-toxic (Shi et al., 2012) and has many therapeutic uses. Various species of *Boswellia* that grow in tropical parts of Africa, Asia, and the Americas are used to make frankincense with varying properties (Al-Harrasi et al., 2018). *Boswellia sacra* is a good source of high-quality frankincense containing bioactive compounds with a variety of functions, including anti-cancer (Efferth and Oesch, 2020). Carbon nanotubes (CNTs) are nanomaterials made of rolled-up graphene sheets with a tube-like shape (Maruyama, 2021). In addition to their unique nanostructures, they have extraordinary characteristics; some are derived from graphite-like features, and others from their one-dimensional aspects. CNTs are used as nanofillers in polymer-based nanocomposites because they have great electrical, magnetic, and mechanical properties (Kaseem et al., 2017). Indeed, considerable research published in peer-reviewed journals demonstrates that adding CNTs to a polymer matrix reinforces mechanical qualities. Carbon-based nanoparticles have also been shown to have high antibacterial activity (Kang et al., 2008). Early research suggested that fullerenes, such as single-walled carbon nanotubes, have powerful microbicidal capabilities. These novel allotropic kinds of carbon were found in the previous two decades and have subsequently been utilized in various scientific applications (Cataldo and Da Ros, 2008). This paper's contribution and novelty are as follows: seeking unique and novel substances that can reduce populations of onion thrips, aiming to keep them below the economic threshold, thereby obviating the necessity for chemical pesticides. This study also aims to determine the mechanism of action of these chemicals on *T. tabaci*'s physiological function. The use of these novel insecticides may also inhibit or slow the development of pesticide resistance in onion thrips.

2. Materials and Methods

2.1.Onion Thrips population

Thrips samples were collected from green onions in the desert region of Najaf Provence, Iraq, during the winter months of 2021 and 2022 (November–March). Infested green onion plants were grabbed and brought from onion farms to the laboratory using a plastic bag. Onion plants were examined for the presence of onion thrips. It was ensured that no pesticides had been sprayed on the plants two weeks before collecting onion plants. The populations were maintained using green onion seedlings prepared previously for this purpose at 25±1°C, and 70-80% R.H. Experiments were carried out under the same conditions in an incubator. The host plant used in the experiments was also green onion. Following the collection of onion thrips, all onion thrips assays were conducted immediately. Three replicates with ten adults and nymphs of onion thrips were applied for each concentration, and a control treatment consisted of just distilled water.

2.2. Synthesis of carbon nanotubes (CNTs)

The carbon nanotubes were acquired from the Chemical Science Department, Faculty of Science, University of Kufa laboratory and prepared as mentioned in Ordoez-Casanova et al. (2013) and Lafta et al. (2016). Non-volatile carbonaceous compounds (derived from date palm seeds) must be formed as a gas phase, which needs the utilization of energy sources or devices from the substrate. MWNTs with a purity of roughly 85% and a diameter ranging from 166 to 200 nm were employed as support in this study, which were generated by modified CVD on ceramic boats without the use of a catalyst. The first phase of this segment was treated and placed in the center of the tube furnace (XIN YOO electronic components Co. Ltd.), which is the best site for heat recovery for precipitation. Ceramic boats were used as a support. The prepared seed samples were placed in the combustion chamber with a complete connection to the rest of the reactor The nitrogen gas was purged to complete the removal of air from all reaction chamber systems prior to operating the furnace. The reaction temperature was reached once the furnace was turned on. The synthesis was carried out at 750° C under atmospheric pressure, with a normal reaction time of 30 minutes in a nitrogen environment and a flow rate of 100cm^3/min. The nitrogen gas flow was gradually lowered to 50cm^3/min when the furnace achieved the necessary temperature. A waste date palm sample was then added to the reaction by turning on the combustion heater and running it in batches The furnace was turned off and allowed to cool to ambient temperature under a continuous nitrogen flow after precipitation. The product was then collected for purification before going through the characterization process. The purification of the manufactured CNTs was done in two steps: The first phase was to heat the product for 4 hours in an oven, and the second was to oxidize the residual product 30 percent with H_2O_2 at 50˚C for 4 days 24. Scanning electron microscopy (SEM) and Powder X-Ray Diffraction (PXRD), (EDX), Raman Spectroscopy, and FTIR spectra were used to characterize the produced CNTs. (Kumar and Ando, 2010). All The chemicals used in the experiment, including zinc chloride ($ZnCl_2$), ethanol, and all other substances, were of analytical purity and came from Merck (Merck and Co., Inc.). All glassware was washed with sterile distilled water and dried in an oven before use.

2.3.Frankincense nanoparticle synthesis (FNPs)

The aqueous extract of Frankincense resin (FR) obtained from the tree of *Boswellia serrata* was prepared. (5 g) of Frankincense resin (FR) was placed in a 250 ml beaker, adding 100 ml of distilled water. The beaker was placed on a hot plate stirrer for 30-45 minutes at a temperature not exceeding 50 °C. The mixture was then filtered through a filter funnel and set aside for the second day. Frankincense nanoparticle was prepared in the laboratory of the Chemical Sciences Department, Faculty of Science, University of Kufa as mentioned in (Jamdagni et al., 2018).

Preparation ZnO nanoparticles

Zinc oxide synthesis was carried out by dissolving 6.05g in 200 ml of distilled water according to the molarity equation (Mirzaei and Darroudi, 2017).

$$M = \frac{Wt}{MWt \times V/1000}$$

Where (M) represents molar concentration, (Wt) represents the weight of the materials utilized, (Mwt) represents the molecular weight of the materials (g/mole), and (V) represents the volume of distilled water (mL).

Frankincense nanoparticle preparation

The purpose of adding nanoparticles is intended to achieve a high level of adhesion. To prepare frankincense nanoparticles, a mixture of 100 µL hydrochloric acid (to improve solubility), 50 mL of aqueous frankincense extract and 200 mL of 0.2 M zinc chloride were put into a beaker. The beaker was placed onto a hot plate stirrer for one hour at a temperature as little as 80ºC. A magnetic stirrer was used to dissolve the solution at 80°C for 2 hours. The light-yellow tinted precipitate that had formed was then allowed to settle for another 18 hours. Centrifugation at 10000 rpm for 25 minutes was used to remove the precipitate, which was followed by numerous rinses with distilled water to remove impurities and overnight drying in a hot air oven at 90°C. Crystallinity and other organic impurities are removed during the calcination process. The powder was calcined for 2 hours (Jamdagni et al., 2018).

2.4.Treating onion thrips

Individuals of onion thrips were treated by using (Kondo and Takafuji, 1985) disc Leaflet method, with a slight modification. Three discs made from the edges of the uppermost leaves of a tomato plant, each 4 cm in diameter, were placed in a Petri dish 9 cm in diameter and 1.5 cm high. A layer of sponge soaked in water and covered with filter paper was place in the Petri dish under the leave. The petri dish has a sponge layer soaked with water and covered with filter paper, leaving no opening between the edges of the tomato leaflet and the perforations. For replicates in which the disc's outer cover is held in place by pins on all sides. Surround each replicate with cotton and wiping the plastic cover from the outside with a layer of Vaseline to prevent the thrips from escaping. Perforating the dish's outer cover with pins for the purpose of injecting water with a (1 mL) medical syringe from time to time to maintain the leaflet for a longer period and preserving its vitality. Finally, numbering the replicate with a sharpie pen.

Carbon nanotubes (CNTS) and frankincense nanoparticles (FNTs) were applied to adult and nymphal instars, since these instars are active feeding stages, using a manual sprayer in differing proportions (0.05, 0.03, 0.01% and 5,3,1 mg/L, respectively). Distilled water was used as a control treatment in compared to carbon nanotubes, and zinc oxide (as synthesized above) was used as a control treatment in comparison to frankincense. Thrips exhibiting ataxia (active, apparently untidy movement), as well as thrips resting on their backs, legs up, or not moving, were considered dead at 24, 48, and 72 hours (Heinz-Castro et al., 2021). In general, three replicates with ten individuals were used for each treatment within a trial.

2.5.Egg hatching rate (%)

Onion thrips females were given 24 hours to deposit eggs. Following that, the thrips were removed. Eggs were sprayed with (0.05%) frankincense nanoparticles (FNTs) and (5mg/L) Carbon nanotubes (CNTS). Zinc oxide (as mentioned above) was used as a control treatment in comparison to frankincense nanoparticles (FNTs). Distilled water was used as a control treatment in compared to carbon nanotubes (CNTS). The eggs were checked every day over the first ten days under a dissecting microscope to determine the egg hatching rate (%). Hatched eggs were those with a hatching hole or that transformed into nymph, whereas non-hatched eggs were those without a hatching hole or did not develop into nymph. Three replicates, each with ten adults were used for each treatment.

2.6.Female fecundity

Female onion thrips were exposed tocarbon and frankincense nanoparticles (0.05 percent and 5mg/L, respectively), and their fertility was measured after that. Ten females were selected and placed in a Petri dish. All females were placed in Petri dishes under typical circumstances. Each Petri dish with an onion leaf disc represents as one replicate. The number of eggs laid in their lifetime each day was counted until the females dead. Onion leaf discs were changed as needed.

2.7.Statistical analyses

Analysis of variance (ANOVA) was conducted using JMP16 Pro® (SAS Institute, Cary, NC). Two-way ANOVA was used to compare nanomaterials activities on *T. tabaci* population and means were separated by Tukey's HSD test. One-way ANOVA was used for the single trial.Cumulative mortality was corrected for natural death in control using Abbott's formula (Abbott, 1925). An unpaired *t*-test was used to assess which nanomaterial treatment differed significantly from the control in egg hatching rate and female fecundity. A (0.05) significance level was used for all the analyses.

3. Results

The nanomaterial compounds used in this study had varying impacts on the mortality rate, fertility, and hatching rate of onion thrips (both adults and nymphs), as shown below.

3.1.Effect of CNTs in adult and nymphal stages

The overall results from the CNTs trials revealed a signifiacnt effect for CNTs treatments and sexposure time in adult and nyphal instars time ($F(11, 22) = 50.14$, $P<0.0001$ and ($F(11, 22) = 38.6$, $P<0.0001$), respectively. Theadult mortality rate varied significantlyamong CNTs treatments and the control. There was a significant main effect for treatment, $F(3, 22) = 51.5$, $p < .01$, and a significant interaction, $F(6, 22) = 11.01$, $P<0.0001$). The

mortality rate of nymphal instar also differed significantly across treatments and time of exposure (F (3, 22) = 45.09, P<.0001and F (2, 22) = 94.7, P<0.0001., respectively). Compared to the control treatment, carbon nanotube (CNTs) showed a high death rate in adult and nymphal instars (76.7 and 83.2%), respectivly, (*Figure 1,3*) at concentrations of 0.05% after 72h of exposure.

3.2. *Effect of Frankincense nanoparticles (FNTs) in adult and nymphal stages.*

FNTs trials showed that there was a significant effect for FNTs treatments and exposure in adult and nymphal morilty rate of onoin thrips time (F (11, 22) = 9.8, P<0.0001and (F (11, 22) = 20.7, P<0.0001), respectively. Frankincense nanoparticles (FNPs) treatment at a concentration (5 mg/L) demonstrated a high nymphal mortality rate of around 43.3% (*Figure 2*). Additionally, the concentration (5 mg/L) showed the higher nymphal morility rate with about 565.8% (*Figure 4*). Frankincense nanoparticles (FNPs) also showed a significant impact on nymphal mortality, with around (60%) death (*Figure 4*).

3.3.Effect of CNTs and Frankincense nanoparticles on egg viability

The rate of egg hatching varied significantly between the CNTs and the controls (t= 9.192, df= 4, P = 0.0008). The control treatment had a 90 percent egg hatching rate, compared to around 40 percent of hatched eggs in the CNTs treatment (*Figure 5a*). In contrast, there was no significant difference in egg hatching rate between the frankincense nanoparticles and control treatments (t=1.789, df=4, p= 0.1481) (*Figure 5b*).

3.4.CNTs and frankincense nanoparticles effect on Female Fecundity

Fecundity of adult females in the CNTs treatment did not differ significantly from the control treatment (t=2.152, df=4, p=0.0978) (*Figure 6a*). Similarly, Fecundity of adult females in the FNPs treatment did not differ significantly from the control treatment (t=1.032, df=4, p= 0.3603) (*Figure 6b*).

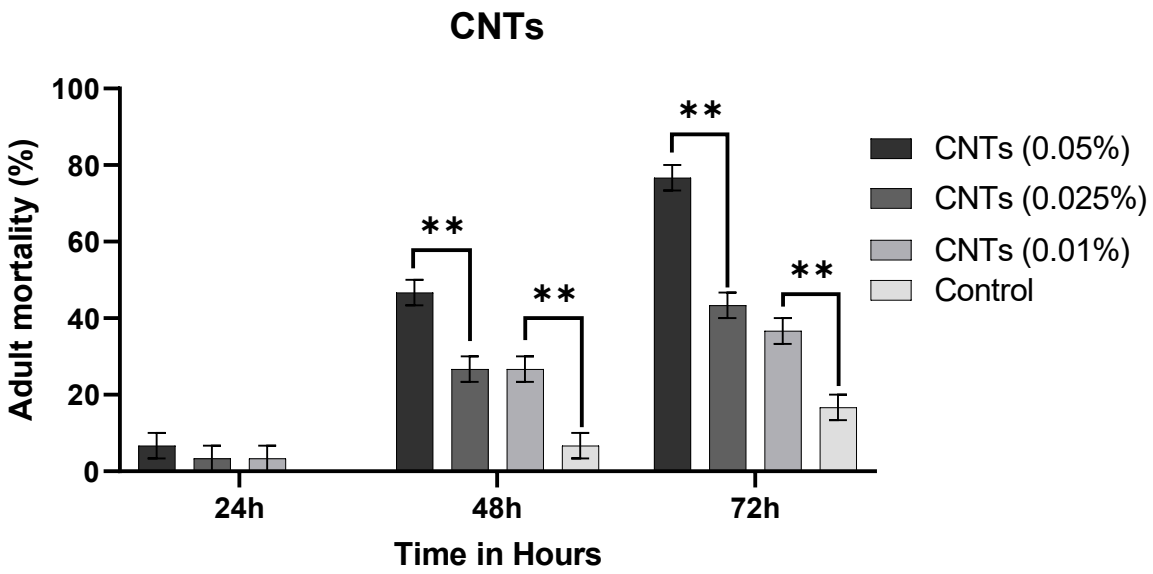

Figure (1). Onion thrips Thrips tabaci adult mortality rate (Mean ± SEM) after exposure to different concentrations of carbon nanotubes (CNTs) vs. the control (distilled water only) and exposure time in hours. P<0.0001, **= significant Bars without connectors were not significantly different.

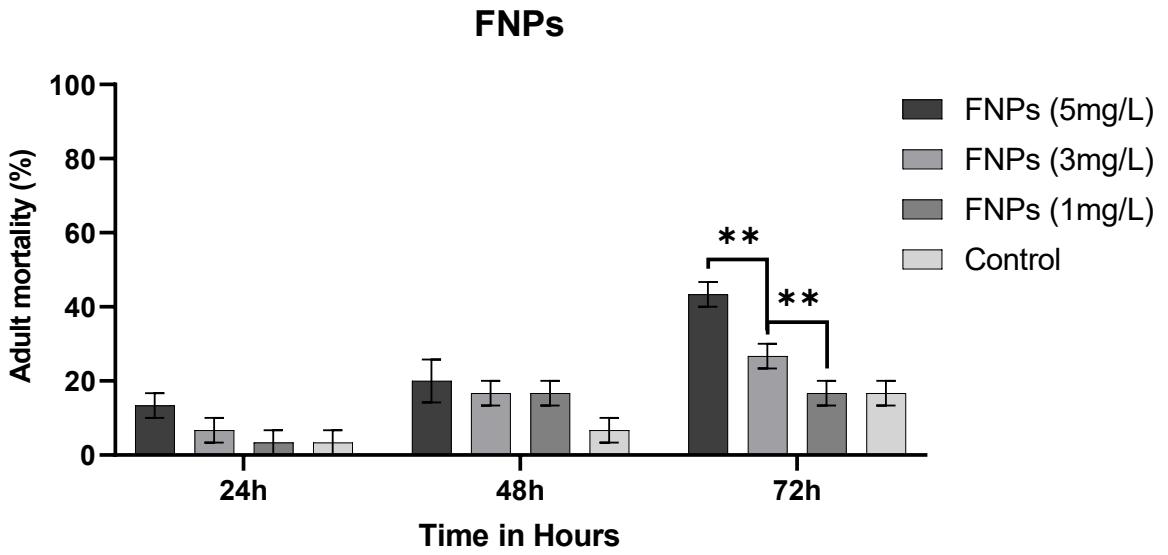

Figure (2). Onion thrips Thrips tabaci adult mortality rate (Mean ± SEM) after exposure to different concentrations of frankincense nanoparticles (FR) vs. the control (ZnO) and exposure time in hours. P<0.0001, **= significant. Bars without connectors were not significantly different.

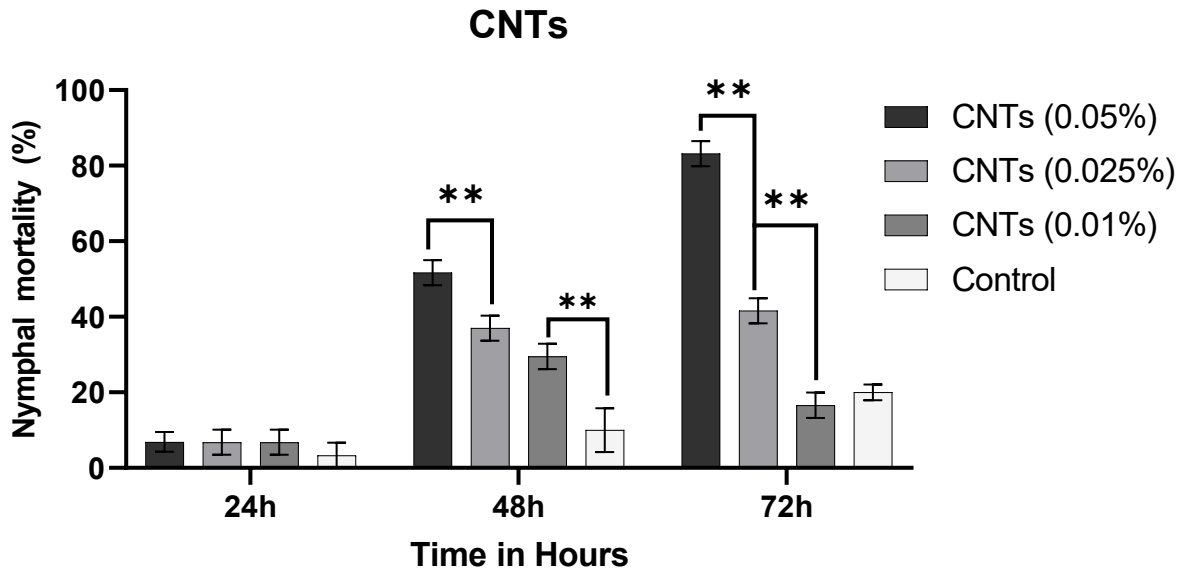

Figure (3). Onion thrips Thrips tabaci nymphal mortality rate (Mean ± SEM) after exposure to different concentrations of carbon nanotubes (CNTs) vs. the control (distilled water only) and exposure time in hours. P<0.0001, **= significant. Bars without connectors were not significantly different.

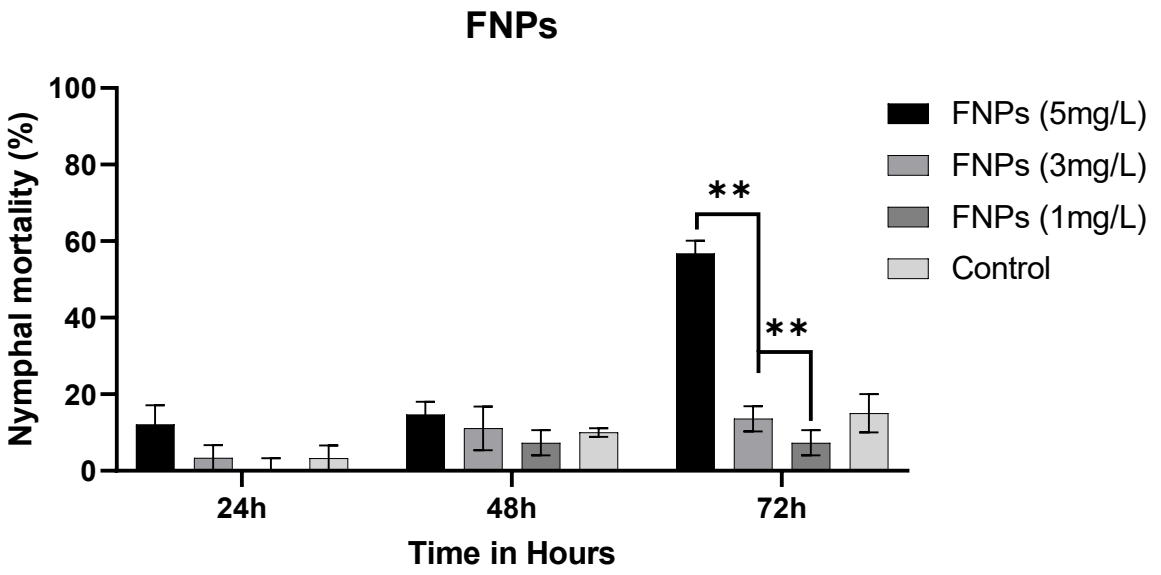

*Figure (4). Onion thrips Thrips tabaci nymphal mortality rate (Mean ± SEM) after exposure to different frankincense nanoparticles (FR) concentrations. P<0.0001, **= significant. Bars without connectors were not significantly different.*

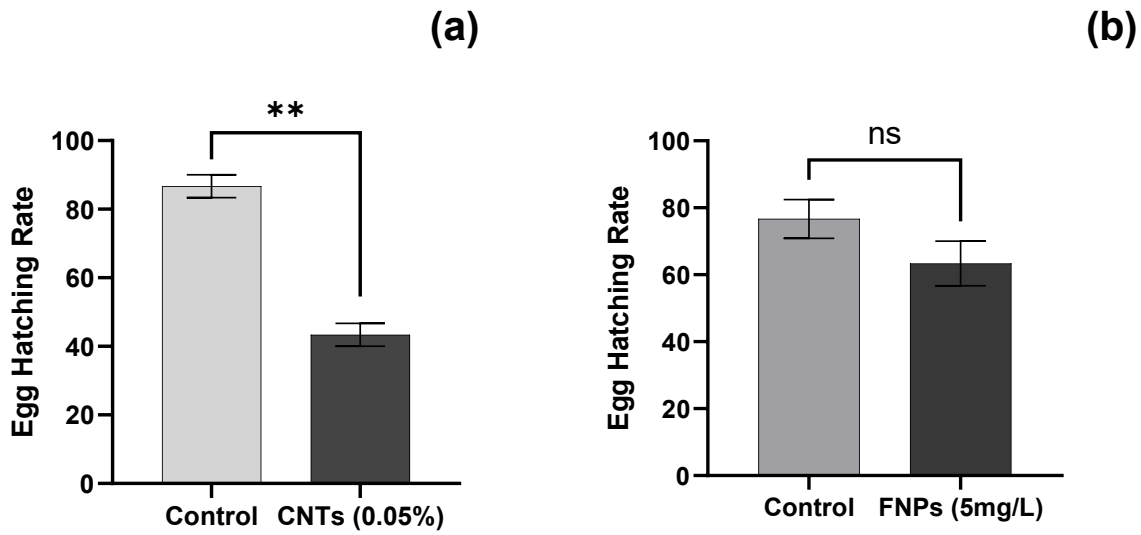

Figure (5). Onion thrips Thrips tabaci egg hatching rate (Mean ± SEM). (a) carbon nanotubes CNTs (0.05%) vs. the control (distilled water only). (b) frankincense nanoparticles FNPs (5mg/L)) vs the control. P<0.05.

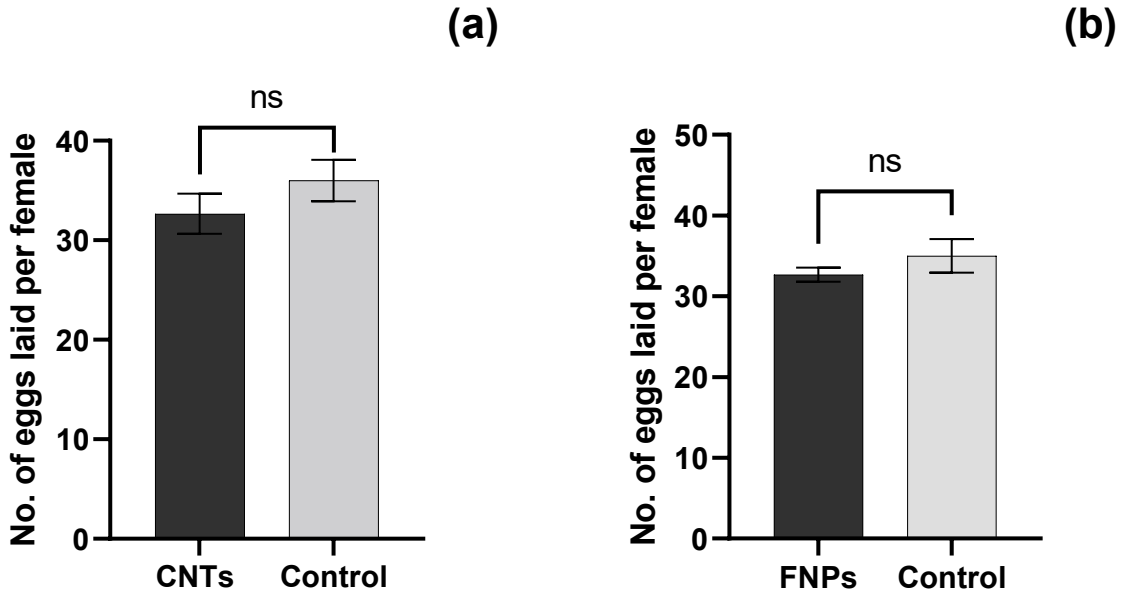

Figure (6). Mean(±SEM) number of eggs laid by Onion thrips Thrips tabaci female after surviving exposure to nanomaterials:(a) carbon nanotube (CNTs) and (b) frankincense nanoparticles (FNPs). P ≥ 0.05,ns= non-significant.

4. Discussion

Few studies have examined the effectiveness of various synthetic nanoparticle compounds against insect pests. This study tested the effectiveness of two distinct nanoparticle compounds against *T. tabaci* onion thrips. This research found that carbon nanotubes and frankincense are effective nanomaterials against onion thrips. The findings of this study indicate that carbon nanotubes and frankincense are efficient compounds against onion thrips. CNTs with different structural and chemical compositions may have different effects on onion thrips (Martins et al., 2019). Carbon nanotubes (CNTs) were shown to have antibacterial properties against numerous *Escherichia coli* cells (Kang et al., 2008). However, little study has been conducted on the influence of carbon nanotubes on insect biological parameters, and most of it has been done in the lab. One of the first was research on Drosophila (Drosophilidae: Diptera) by (Liu et al., 2009).

Salamn et al. (2021) evaluated the antibacterial activity of crude aqueous extracts of *Boswellia sacra* bark against clinical isolates of periodontitis-causing bacteria in vitro (*Streptococcus orails*, *Gemella morbillorum*, and *Rothia dentocariosa*). They discovered that the concentration (250 mg/L) is more effective than the others. According to a review by Efferth and Oesch (2020), the phytochemical compounds in frankincense have shown promise in treating a wide range of conditions, including osteoarthritis, asthma, psoriasis, erythema, plaque-induced gingivitis, and pain.

The research says nothing about the manufacturing process of zinc oxide, and nothing about safety of any of the compounds tested. We hypothesize that nanomaterials have essential nutrients to the onion thrips (e.g. de Vries et al., 2008). From the results provided here, CNTs were found to have an effect on egg viability. In addition, CNTs and FNPs, at certain concentrations, had an effect on adult and nymphal mortality rates. This could be because CNTs harm the digestive system of adults and nymph or their metabolic efficiency.

Carbon nanomaterials (CNMs) were shown to negatively impact the reproductive, digestive, and metabolic efficiency of *Spodoptera frugiperda*, according to recent research (Martins et al., 2019). In this investigation,

CNTs had no insecticidal action against female fecundity. CNMs were also not found to have an influence on female fertility in *Drosophila* larvae (Liu et al., 2009).

5. Conclusions

In conclusion, carbon nanotubes (CNTs) and frankincense nanoparticles (FNPs) exhibited potential eco-freindly insecticidal effects against onion thrips. The use of CNTs had the most significant effect on the mortality of adult and nymph of onion thrips, the fertility and hatching rate of eggs laid by females, and the overall population of onion thrips. More study is required to determine the biological mechanisms of these nanomaterials on onion thrips. This study might aid in the creation of efficient nanoparticle-based treatments.

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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Image Processing and Traditional Machine Learning Based Classification of Brown Marmorated Stink Bug (*Halyomorpha Halys*) Defected Hazelnut*

Görüntü İşleme ve Geleneksel Makina Öğrenmeye Dayalı Fındıkta Kahverengi Kokarca (*Halyomorpha Halys*) Zararının Sınıflandırılması*

Omsalma Alsadig Adam GADALLA[1*], Yeşim Benal ÖZTEKİN[2]

Abstract

Quality control of hazelnuts is a major concern in many regions across the world, but particularly in Turkey as the world's largest hazelnut producer. Using image processing and deep learning techniques, this study intended to detect and classify healthy hazelnuts and hazelnuts infected with the Brown Marmorated Stink Bug. Infected hazelnut samples were collected from the 2021 production period by experts. A Guppy Pro CCD camera-based image acquisition system was used to capture hazelnut images. A total of 400 RGB hazelnut images were captured to train machine learning models. Image segmentation process was carried out to subtract hazelnut images from the background using the Thresholding technique. Moment features were extracted from RGB and l*a*b* spaces to be used to train traditional machine learning models. Furthermore, the most relevant and discriminative feature set was selected using the Boruta feature selection method. Traditional machine learning models including Random Forest, Support Vector Machine, Logistic Regression, Naive Bayes, and Decision Tree were trained twice, once with all features and another with the selected feature set only. The overall accuracy, statistical characteristics of the confusion matrix, and model training time were all calculated to evaluate and compare models performances. As a result, threshold value of 50 was determined from the gray level histogram and was able to separate hazelnut image from the background perfectly. Only seven moment features were identified as the most discriminative features out of 24 features. The SVM model with all feature vectors had the greatest classification accuracy of 98.75 %. When only the selected features were employed, the performance of Random Forest and Logistic Regression models improved to 97.5 and 96.25 %, respectively.

Keywords: Support vector machine, Hazelnut, Feature selection, Feature extraction, Boruta

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*This study was summarized from the PhD thesis.

Öz

Fındığın kalite kontrolü, dünyanın birçok bölgesinde, özellikle de dünyanın en büyük fındık üreticisi olan Türkiye'de büyük bir problem kaynağıdır. Bu çalışma, görüntü işleme ve derin öğrenme tekniklerini kullanarak, Kahverengi Kokarca ile enfekte olmuş ve sağlıklı fındıkları birbirinden ayırarak belirlemek ve sınıflandırmak amaçlanmıştır. Kahverengi Kokarcalı fındık örnekleri, uzmanlar tarafından 2021 üretim döneminden elde edilmiştir. Fındık görüntülerini yakalamak için Guppy Pro CCD kamera tabanlı görüntü alma sistemi kullanılmıştır. Geleneksel makine öğrenme modellerini eğitmek için toplam olarak 400 RGB fındık görüntüsü alınmıştır. Fındık görüntülerinin arka plandan çıkarılması için görüntü bölüntüleme işlemi Eşikleme tekniği kullanılarak gerçekleştirilmiştir. Fındık moment özellikleri, geleneksel makine öğrenme modellerini eğitmek için kullanılmak üzere RGB ve l*a*b* renk çıkarılmıştır. Ayrıca, Boruta özellik seçim yöntemi kullanılarak en önemli ve en ayırt edici öznitelik seti seçilmiştir. Rastgele Orman, Destek Vektör Makinesi, Lojistik Regresyon, Naive Bayes ve Karar Ağacı dâhil olmak üzere geleneksel makine öğrenme modelleri, bir kez tüm özelliklerle ve bir kez daha yalnızca seçilmiş özelliklerle olmak üzere iki kez eğitilmiştir. Genel doğruluk, karışıklık matrisinin istatistiksel özellikleri ve model eğitim süresinin tümü, modelin sınıflandırma performansını değerlendirmek ve karşılaştırmak için hesaplanmıştır. Sonuç olarak, gri seviye histogramından 50 eşik değeri belirlenmiştir ve fındık görüntüsünü arka plandan mükemmel bir şekilde ayırabilmiştir. Çıkartılmış 24 özellik arasından en ayırt edici özellik olarak sadece yedi tane renk özelliği belirlenmiştir. Tüm çıkartılmış özellikler kullandıktan sonra Destek Vektör Makinesi modeli kullanılarak, %98.75 ile en yüksek sınıflandırma doğruluğu elde edilmiştir. Aynı zamanda tüm özelliklerden sadece seçilen özellikler kullanıldığında Rastgele Orman ve Lojistik Regresyon (sınılandırıcılarının) modellerinin performansı sırasıyla %97.5 ve %96.25'e kadar yükselmiştir.

Anahtar Kelimeler: Destek vektör makinesi, Fındık, Özellik seçme, Özellik çıkartma, Boruta

1. Introduction

Hazelnut (*Corylus avellana* L.) is a seasonal fruit related to the family of Betulaceae of Fagales ordo (Aydinoglu, 2010). Hazelnut grows in various places of the world such as USA, Italy, China, and Spain, while Turkey produces the majority of commercial hazelnuts (FAOSTAT, 2019). Hazelnuts are extensively utilized in the confectionery industry due to their flavor and taste. They have a great nutritional value because they contain a variety of constituents, primarily lipids, carbohydrates, proteins, sugar, and dietary fibers (Memoli et al., 2017). However, many factors influence hazelnut production and quality around the world. Temperature, humidity, mold, and pests all have a significant impact on hazelnut quality and yield.

One of the most serious defects facing the production of hazelnuts in Turkey is hazelnut defection generated by the Brown Marmorated Stink Bug (BMSB). During the development period, the BMSB penetrates the hazelnut and feeds on the nuts. The damage that occurs inside hazelnut rises as the pest grows, and even a minor attack might jeopardize hazelnut quality. When the insect feeds, saliva is sent down one maxillary stylet while food is sucked up via another (Mitchell, 2018). Through the feeding process, the fruit's skin is damaged, and enclosing tissue is removed, resulting in serious injuries (Short, 2010). Depending on where the insect feeds on the fruits, they can produce blanks, shrink or corking incidents, or injuries on the kernels (Saruhan, 2010). Hazelnut kernel infected with the Brown Marmorated Stink Bug varies in their infection severity level. Due to the pest's different behaviors, the external symptoms could appear as very clear dark skin injuries and discoloration or very small entry points resulting from the insertion of the mouthpart (Short, 2010). The insect has not been managed yet, and the consequent loss from this insect is sometimes hidden or not visually detectable on the fruit surface (Saruhan, 2010). However, post-harvest hazelnut evaluations revealed that BMSB feeding appears to have harmed a significant proportion of nuts. The damaged kernels seem shriveled and withered, with evident feeding points consisting of a noticeable depression encircled by necrotic tissue areas that can lead to mildew and rotting (Molnar, 2010). Christopher (2014), evaluated the destruction caused by H. halys adults feeding on hazelnut kernels. As a result, the author claims that when stink bugs feed on ripe nuts, a higher percentage of kernels develop corky, white, rotting kernel tissue, with no statistically significant differences variation in the proportion of damaged kernels exhibiting these symptoms in field or laboratory studies. Economically, in the last few years; the pest caused damages of 200 Million Dollars in 2017, 300 Million in 2018, and about 500 Million in 2019 for the total hazelnut production in Turkey which negatively affected the hazelnut export value (Anonymous, 2019). Applying machine vision with pattern recognition techniques, machine learning, and deep learning algorithms to identify hazelnuts has numerous advantages over traditional sorting methods.

During post-harvest processing operations the primary method for detecting defects in the fruits is visual inspection, which includes sample preparation, consecutive sampling methods, visual and sensory characteristics examination, and dismisses nut classification. This technique, which is focused on visual and organoleptic evaluation takes time and trained workers for accurate nuts classification. Other methods have been studied to develop smart inspection techniques for inspecting hazelnut quality characteristics. Machines supported by mechanical approaches such as calibrator devices are used to characterize the dimensions of the shell and empty nuts. These machines are advantageous for the pre-treatment of the products, but they are not quality management mechanisms. A unique approach for detecting blank hazelnuts has been proposed, depending on an examination of the acoustic signal generated by the nut's action on a steel surface (Onaran et al., 2005).

In field of hazelnut defect detection, few works have been done using artifiecial intellegence based technologies such as; Deep learning , Machine learning, and Image processing. Fungal contaminated hazelnut kernels were categorized using A two-dimensional local discriminant bases algorithm and multispectral imaging technique. For hazelnuts that were both contaminated and uncontaminated with aflatoxin, a classification accuracy of 92.3% was attained (Kalkan and Çetisli, 2011). Solak and Altinisik (2018) classified and detected hazelnuts using image processing and clustering techniques. The size and area features were extracted from hazelnut images and hazelnuts were divided into three classes. The use of mean-based classification and K-means clustering algorithms yielded 100 detection and classification accuracy. The whole defective hazelnuts were detected by Kivrak and Gürbüz (2019) using image processing and machine learning techniques. The goal of the work was to distinguish intact hazelnuts from damaged or defective ones. Images of hazelnut samples were captured using a cell phone and processed using image labelling techniques. Satisfactory results were obtained using the supervised learning

method. Furthermore, a computer vision system was used to classify the partly skin removed hazelnut kernel, skin removed and rotten hazelnuts kernels. The processed hazelnut kernels are classified with a classification accuracy rate of 93.57 % (Guvenc et al., 2015)

There is no satisfactory technique for detecting insect pests in hazelnuts rapidly after harvest. Under existing production procedures, the most common technique for detecting defected hazelnuts involves manual selection, which is time-consuming, subjective, labor-intensive, and does not recognize hazelnut with very low severity levels. As a result, there is a need to raise the level of subjectivity, stability and effectiveness in assessing hazelnut quality. The digital image processing Approaches and artificial intelligence methods can play a significant role in this endeavor as the method offers the potential for high speed, non-destructive classification of hazelnut (Yadhunath et al., 2022). Machine vision systems are suitable for inspecting rigid and predefined objects. However, visual characteristic of agricultural products such as color, shape, texture are difficult for machine vision system to discern. Artificial intelligence algorithms such as artificial neural network have presented to be powerful in dealing with the type of problems that require interpolation of huge amount of data. The main aim of this study was to apply image analysis and different machine learning algoriths to separate healthy hazelnut from BMSB-defected hazelnut.

2. Materials and Methods

2.1. Materials

2.1.1. Hazelnut Samples

For classification purpose, normal and Brown Marmorated Stink Bug-infected hazelnuts were used in this experiment. Brown Marmorated Stink Bug-infected hazelnut samples were taken from a commercial hazelnut processing factory, which were previously harvested in the Black Sea region of Samsun. Before using hazelnut samples in this experiment, specialist assessors from the Plant Protection Department identified the damaged hazelnut samples as Brown Marmorated Stink Bug-infested samples. Infected hazelnut samples contained a mixture of hazelnut varieties, however the most commonly observed type was the Tombul variety, which is the most widespread hazelnut variety in the Black Sea region. *Figure 1* shows hazelnut samples infested with brown marmorated stink bug at various levels of severity and healthy hazelnut samples.

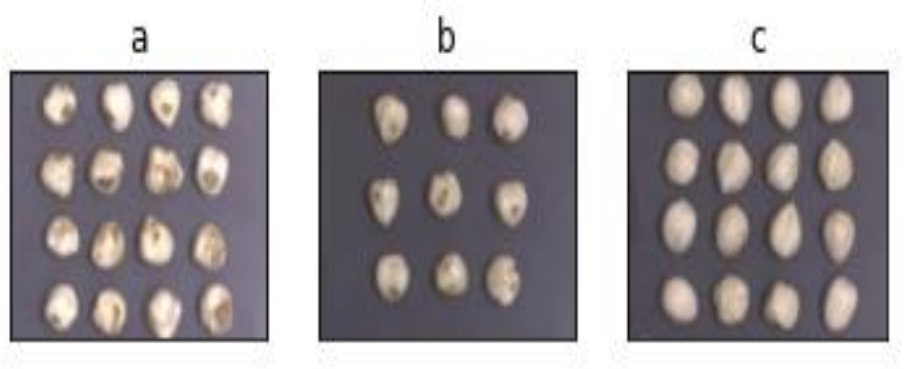

Figure 1. Normal and BMSB infected hazelnut samples, a; severely infected samples, b; slightly infected samples, c; normal samples

2.1.2. Computer Vision and Image Acquisition System

The image acquisition and machine vision system in this work was constructed and built in the biological material laboratory of Ondokuz Mayis University's Agricultural Machinery and Technologies Engineering Department (*Figure 2*). The components of the machine vision and image acquisition system were; darkened imaging chamber for adequate and well-distributed light, an image acquisition camera, a halogen lamp, a computer, and software.

Figure 2. Image acquisition system

2.1.2.1. Image Acquisition

For image acquisition, an Allied Vision Technology Guppy PRO F-032 economical FireWire camera with a Sony ICX424 CCD type sensor was employed. As demonstrated in *Figure 3* the sensor used for image acquisition was capable of capturing images in color and monochrome formats at wavelengths extending from 400 to 1000 nm at 82.0 frames p er second and 0.3 MP resolutions. The system showed high performance in RGB color space when used to classify cashew kernels using color features (Baitu et al., 2023).

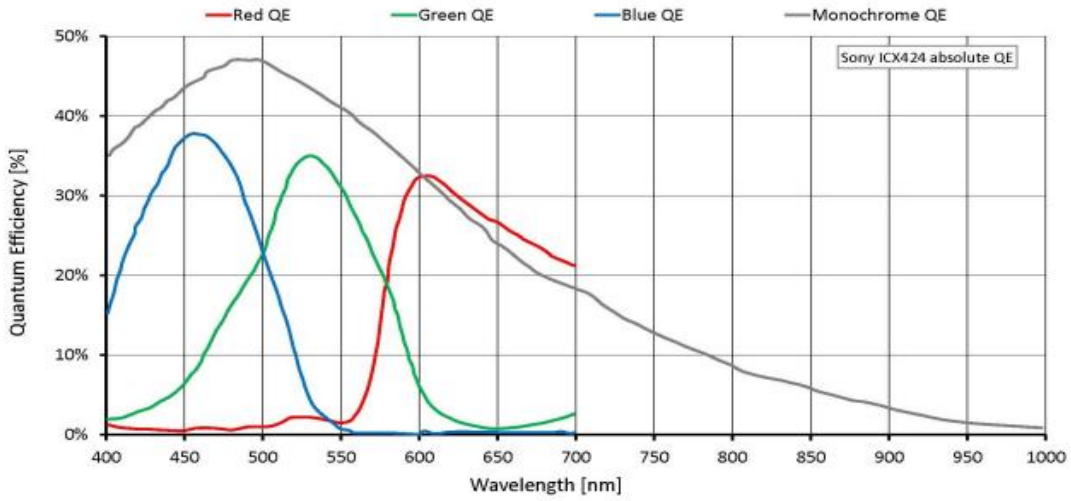

Figure 3. Absolute quantum efficiency of the CCD sensor in RGB and Monochrome color spaces

2.1.2.2. Image Processing and Image Analysis Software

In this study, Spyder (Python 3.8.8) was used as image analysis and image processing software (*Figure 4*), which is initially created and developed by Raybaut (2009) as an integrated development environment (IDE) for Python-based programming languages. Spyder software incorporates many well-known scientific Python packages. The packages that were used in this research included; NumPy for reading images in form of arrays, SciPy for solving mathematical, scientific, engineering, and technical problems besides manipulating and visualizing data, Matplotlib for plotting images and figures, Pandas for adjusting data in form of data frames, and other open-source software. On the other side, Open CV (Open Source Computer Vision Library) was used mainly as an open-source library for machine and deep learning models, and image processing purposes in this study.

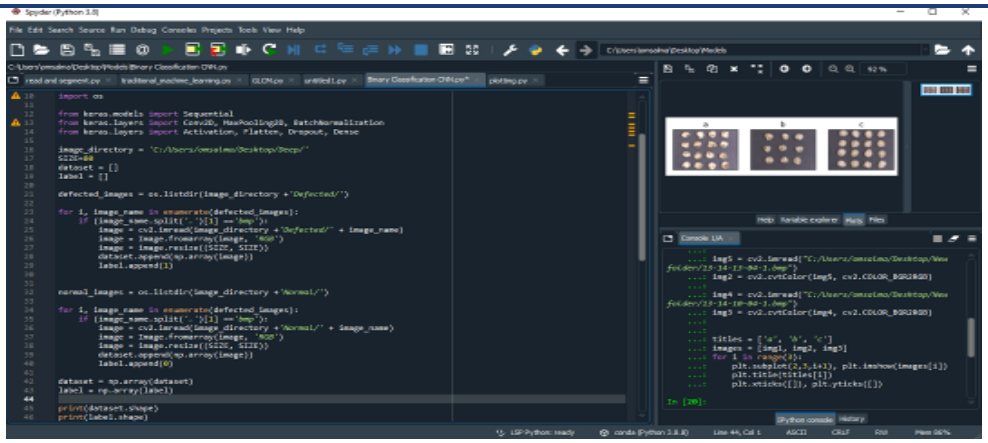

Figure 4. Spyder (Python 3.8.8) integrated development environment (IDE) Software

2. 2. Methods

2.2.1. Hazelnut Image Acquisition

Using the image acquisition system and the AVT Smart View image capture application, hazelnut samples were manually positioned on the sample holder and placed under the camera one by one. Two separate portions of the hazelnut surface were rotated and randomly captured for each sample. A total of 400 defective and normal hazelnut samples were captured in RGB (400-700) color space from a distance of 37 cm to be used in traing machine learning models as training and validation datasets. As the aim of the study was a classification target task, hazelnut images were captured at a resolution of 80x80 pixels.

2.2.2. Image Processing

2.2.2.1. Image Segmentation for Background Removal

Various image segmentation techniques are available, but the most applicable methods are; Otsu's thresholding, adaptive thresholding, and simple (global) thresholding techniques. Otsu thresholding technology is a powerful background extraction approach, but sometimes the algorithm uses large T values. Adaptive thresholding is a technique that calculates the threshold value for smaller regions, resulting in varying threshold values for different regions, making this technique unsuitable for our scenario. Global thresholding determines the threshold value based on the histogram of the overall pixel intensity distribution of the image as it performs better under controlled illumination conditions. Since in our case, it was capable to observe the image histogram clearly in the image acquisition software, global thresholding was the suitable method for separating hazelnut samples from the background. An image histogram is a graph that shows the distribution of image intensities in a given color space. In this study, a threshold value from the grayscale image histogram is used to transform hazelnut images from grayscale to binary images (black and white). The binary image was masked with the original image using the Bitwise-and operation to get the segmented RGB hazelnut images. The overall segmentation process is illustrated in *Figure 5*.

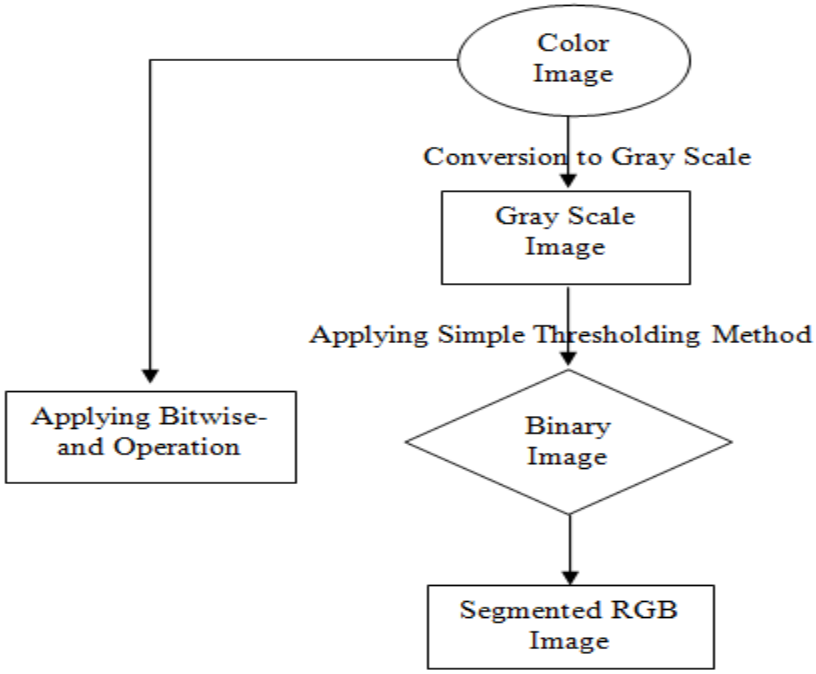

Figure 5. The overall process of hazelnut image segmentation algorithm

2.2.2.2. Feature Extraction

Proper feature extraction from the image is a key factor for a proper classification process. An appropriate feature set for a class should form a tight cluster around the centroid of that class. In this study, the insect performs shrivel or corking damage on hazelnut kernels and hazelnut kernel infected with the Brown Marmorated Stink Bug varies in their infection symptoms due to the pest's different behaviors. The external symptoms could appear as very clear dark skin injuries and discoloration or very small entry points resulting from the insertion of the mouthpart (Short, 2010). Also, changes in whole kernel color and changes in color where the mouth is inserted were observed (Ali, 2018). To improve the efficiency of hazelnut classification, color features from RGB and CIELAB (a color space specified by the International Commission on Illumination CIE) color spaces were extracted in this work. Therefore, hazelnut images in RGB and CIELAB (L*a*b*) color spaces were split into R (red), G (green), B (blue), L (l), A (a), and B (b). Moments features representing mean, variance, range, and skewness were captured from the split color spaces (Teimouri et al., 2016; Özlüoymak and Guzel, 2020).

2.2.2.3. Feature Selection

A feature selection algorithm was developed to select only the most relevant and discriminative features. Using BorutaPy from Boruta, a feature selector was created using the XGBoost classifier and utilized to fit in the train and test dataset. The contribution of all features was ranked and the features that ranked 1 were kept.

2.2.3. Machine learning Models

To determine the capability of RGB and lab color features in classifying BMSB-defected hazelnuts and healthy ones, the extracted features were used to train traditional machine learning classifiers. A total of 400 normal and defected hazelnut samples were used in this study for this purpose. 80% of the samples which count 320 hazelnut samples were applied for training purpose while the remaining 20% (80 samples) was used to test the classifiers. Here, the traditional machine learning classifiers were; Support Vector Machine, Logistic Regression, Naïve Bayes, Decision Tree, and Random Forest classifier with 100 estimators. To evaluate the efficiency of the selected features in hazelnut classification, the classifiers were trained two times, firstly with the bulk features before applying any feature selection algorithm; secondly, the classifiers were trained using only the selected features. The model's performance was measured by calculating classification accuracy, confusion matrix and statistical parameters, and model execution (training) time.

2.2.4. Evaluation of Models Performances

For model performance evaluation, confusion matrix related to all traditional machine learning classifiers was extracted. The parameters in the confusion matrix express the actual labels of the hazelnut class and the labels predicted by the classifiers. The instances in the actual class are represented by the columns of the matrix, while the instances in the predicted class are represented by the rows. This can be used to see if the classifier is frequently mislabeling one item as another. A two-class confusion matrix is a two-row, two-column table that enables more detailed analysis than accuracy rate. The confusion matrix and the data entries for two-class classifiers are presented in *Table 1*. We used a confusion matrix in this work because classification accuracy is not a trustable measure for evaluating a classifier's efficiency, and it can generate misleading results when the quantity of samples in different classes varies greatly (Ghosh et al., 2014).

Table 1. Confusion matrix for two class classifier

	Predicted class	
	Positive	**Negative**
Actual class		
Positive	tp	fp
Negative	fn	tn

Where;

'tp' represents the number of positive samples classified as positive, 'fp' is the number of negative samples classified as positive, 'fn' is the number of positive samples classified as negative, and 'tn' is the number of negative samples classified as negative. Also, we evaluated the performance of our traditional models depending on some confusion matrix metrics such as precision, specificity, and sensitivity. The formulas of these measures are presented in *Table 2* (Taheri et al., 2015).

Table 2. Statistics parameters of confusion matrix and related formulas

Measure	**Formula**	**Evaluation focus**
Accuracy	$\frac{\mathrm{nTP\ nTN}}{\mathrm{nTP\ nTN\ nFP\ nFN}}$	Calculate the classifier's general performance.
Recall	$\frac{\mathrm{nTP}}{\mathrm{nTP + nFN}}$	The class arrangement between the data labels and the classifier's positive labels
Precision	$\frac{\mathrm{nTP}}{\mathrm{nTP + nFP}}$	An evaluation of a classifier's capacity to identify incidences of a specific class.
Specificity	$\frac{\mathrm{nTN}}{\mathrm{nTN + nFP}}$	How well a classifier can detect negative labels

3. RESULTS AND DISCUSSION

3.1. Image Segmentation

Image segmentation process is the primary stage of the image processing task. To segment hazelnut images from the background, simple (global) thresholding approach was applied. A threshold value of 50 was determined from the gray-level histogram (*Figure 6*) and the value was able to separate the background from hazelnut images properly. According to the histogram, hazelnut image pixels with intensities greater than 50 demonstrate the hazelnut image, while the pixels with intensities less than the threshold value demonstrate the dark background.

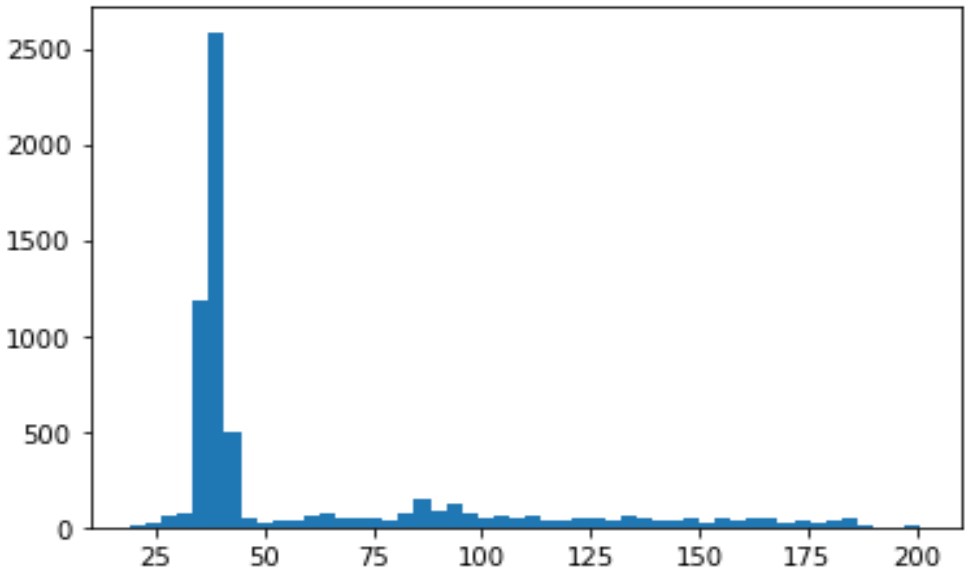

Figure 6. Gray-level histogram of hazelnut image

The applied background removal method was sufficient to get the required RGB hazelnut images as illustrated in *Figure 7*. The simple thresholding technique was efficient in the segmentation process and was applied frequently by researchers for background removal purposes (Senthilkumaran and Vaithegi, 2016).

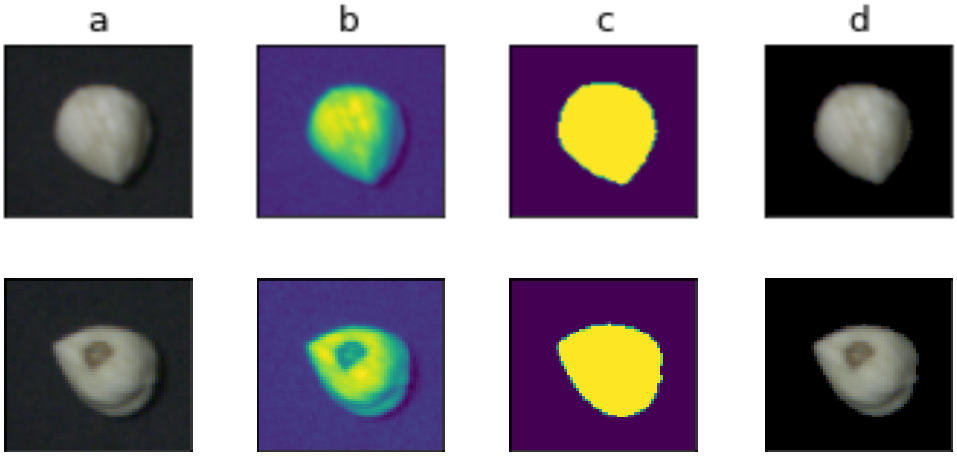

Figure 7. Segmentation steps. (a) RGB image, (b) Gray hazelnut image, (c) Thresholded image, (d) Segmented image

3.2. Feature Extraction and Selection

A total of 24 color features were extracted and saved to an excel file. These features were tested by the classifiers to evaluate their performance in predicting hazelnut classes. The applied feature selection algorithm confirmed that out of 24 features only 7 color features were important and discriminative more than the other features. The confirmed and rejected features are present in *Table 3*.

The majority of the accepted features were displayed in the l*a*b* color space, which reflects the reality described by Saruhan (2010) when researching the symptoms of BMSB infestation on hazelnut kernels after harvesting. In the l*, a*, and b* color spaces, the defection caused by the BMSB appears as discoloration and color changes. The characteristics of red mean, green skewness, and green mean were also found to be effective in classifying hazelnut into BMSB-defected and healthy hazelnut. This may support the theory of, BMSB infestations on hazelnut surfaces appear such little yellowish-green spots (Short, 2010).

Table 3. Confirmed and rejected features by applying BorutaPy

No.	Feature	Status	No.	Feature	Status
1	Red mean	Confirmed	13	l* mean	Rejected
2	Green mean	Confirmed	14	a* mean	Confirmed
3	Blue mean	Rejected	15	b* mean	Rejected
4	Red variance	Rejected	16	l* variance	Confirmed
5	Green variance	Rejected	17	a* variance	Rejected
6	Blue variance	Rejected	18	b* variance	Rejected
7	Red range	Rejected	19	l* range	Rejected
8	Green Range	Rejected	20	a* range	Rejected
9	Blue Range	Rejected	21	b* range	Rejected
10	Red skewness	Rejected	22	l* skewness	Rejected
11	Green skewness	Confirmed	23	a* skewness	Confirmed
12	Blue skewness	Rejected	24	b* skewness	Confirmed

3.3. Machine Learning Models Performance

Using test data, the performance of all trained traditional machine learning classifiers was assessed, as well as the confusion matrix. *Table 4* shows the classifier's performance resulting from training traditional machine learning classifiers with bulk features before any feature selection process.

Table 4. Classification accuracy and training time with bulk features

No.	Classifier	Acc. with bulk features	Training time
1	Support Vector Machine	98.75	0:00:00.007994
2	Logistic Regression	96.25	0:00:00.021987
3	Random Forest	95%	0:00:05.538398
4	Decision Tree	93.75	0:00:16.806870
5	Naïve Bayes	90%	0:00:00.001998

Based on all the extracted feature vectors, the highest classification accuracy (98.75%) was achieved from the Support Vector Machine model. This model can construct a good hazelnut classification system in real-time applications in food industries. Using the same color features, a Support Vector Machine-based computer vision system was developed and applied in real-time to classify pistachio nut kernels, and the same result was obtained (Nouri et al., 2017). Pacheco and López (2019) utilized RGB and l*a*b* color features with the digital image processing techniques for tomato flaws detection purposes. Multiple unsupervised machine learning algorithms were applied to identify the highest classification performance depending on the overall accuracy and confusion matrix metric and performance indices parameters such as precision, sensitivity, and specificity. Classification performances of 98.6, 98.3, 92, and 100% were achieved in the results for accuracy, specificity, precision, and sensitivity, respectively. These results consider very comparable to our results when RGB and l*a*b* color features were used. Acceptable performance was also achieved by training Random Forest and Logistic Regression models with bulk features, with an overall accuracy of 95 % and 96.25%, respectively. The confusion matrix was created to evaluate the effectiveness of each classifier in predicting individual hazelnut classes using all feature vectors as shown in *Figure 8*.

The Decision Tree and Naïve Bayes models had the lowest classification accuracy amongst the classifiers, with 90 % and 93.75 %, respectively. A comparable result was achieved from a Decision Tree-based classifier applied to classify areca nuts into healthy and defective nuts (Akshay and Hegde, 2021). Statistical parameters related to the confusion matrix were also calculated and presented in *Table 5*.

The Support Vector Machine showed the highest accuracy in predicting defective and healthy hazelnut samples when traditional machine learning classifiers were validated. However, the sensitivity of all classifiers (in except of Naïve Bayes classifier) in predicting defected hazelnut samples was very high and reached 100%. When predicting defected hazelnut samples. Individually, the least classification accuracy of defected hazelnut prediction

was achieved from Naïve Bayes classifier. Hence, out of all defected hazelnut samples, 5 samples were falsely predicted as healthy samples which reduced the sensitivity of the model.

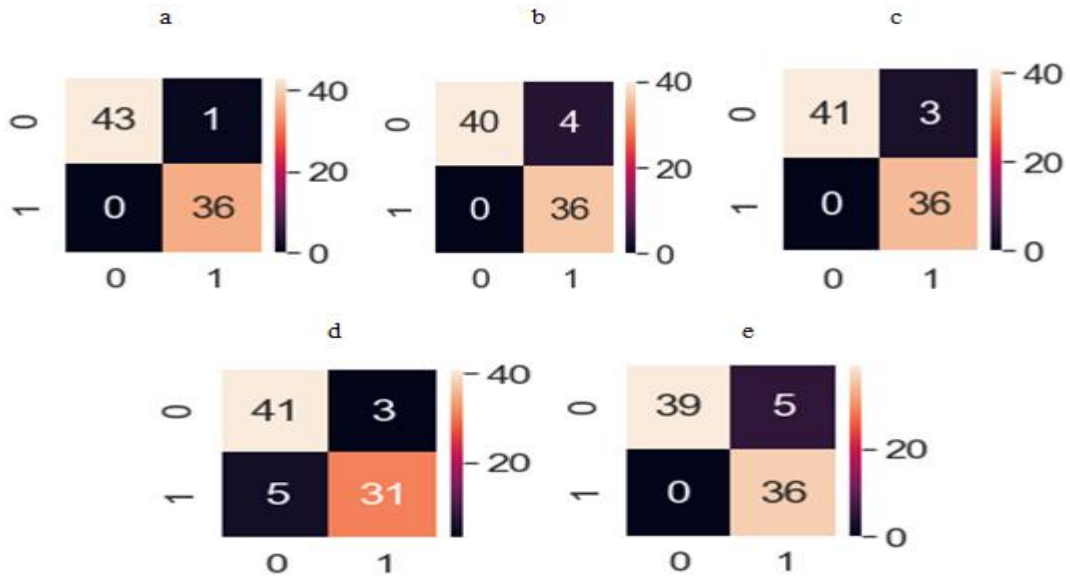

Figure 8. Confusion matrix of traditional classifiers; Support Vector Machine (a), Random Forest (b), Logistic Regression (c), Naïve Bayes (d), and Decision tree (e)

Table 5. Confusion matrix statistical parameters related to defected and healthy healthy classes with bulk features.

NO.	Measure %	Classifiers				
		SVM	RF	LR	NB	DT
1	Recall	100	100	100	89	100
2	Precision	97.7	90.9	93	93	88.6
3	Specificity	97	90	92	91	87.8

The classification accuracy of Random Forest and Naïve Bayes classifiers was improved by using the feature selection technique. That seems to be, the applied feature selection technique reduced the redundant data and minimized the opportunity of making decisions based on noise, resulting in increased accuracy (Balachandran et al., 2018). Furthermore, the Support Vector Machine's classification accuracy with the selected features was reduced to 96.25 %. *Table 6* shows the performance of traditional machine learning classifiers using the selected features.

Table 6. Classification accuracy and training time with selected feature

No.	Classifier	Acc. with bulk features	Training time
1	Random Forest	97.5%	0:00:00.256844
2	Support Vector Machine	96.25%	0:00:00.005997
3	Logistic Regression	96.25%	0:00:00.021984
4	Naïve Bayes	93.75%	0:00:00.001997
5	Decision Tree	93.75%	0:00:00.004996

Since the primary aim of the SVM classifier is to find the best and most appropriate separating hyperplane in an N-dimensional space that perfectly distinguishes the data point, in our case, using all input data (features) may have helped to create a plane with a maximum margin, which maximized the

distance between the data points of the defected and healthy hazelnut classes, resulting in improved classification accuracy. When only selected features were used, all machine learning classifiers performed positively by decreasing model training time. *Figure 9* represents the confusion matrices of the classifiers with the selected features. Color features have been identified as powerful features in multiple classification tasks. Hong et al. (2011) used image processing and machine learning approaches to assess the quality of peanut products using the same color features. Based on pattern recognition, the color features R, G, and B of the damaged area were extracted to identify the damaged peanuts. The recognition task was completed with an accuracy of 80.12%. Furthermore, color features were selected from shape and color combinations to classify white cashew nut kernels using Artificial Neural Networks. from the input cashew kernel images, 24 color features were selected to achieve an accuracy of 88.93% (Narendra and Hareesha, 2016).

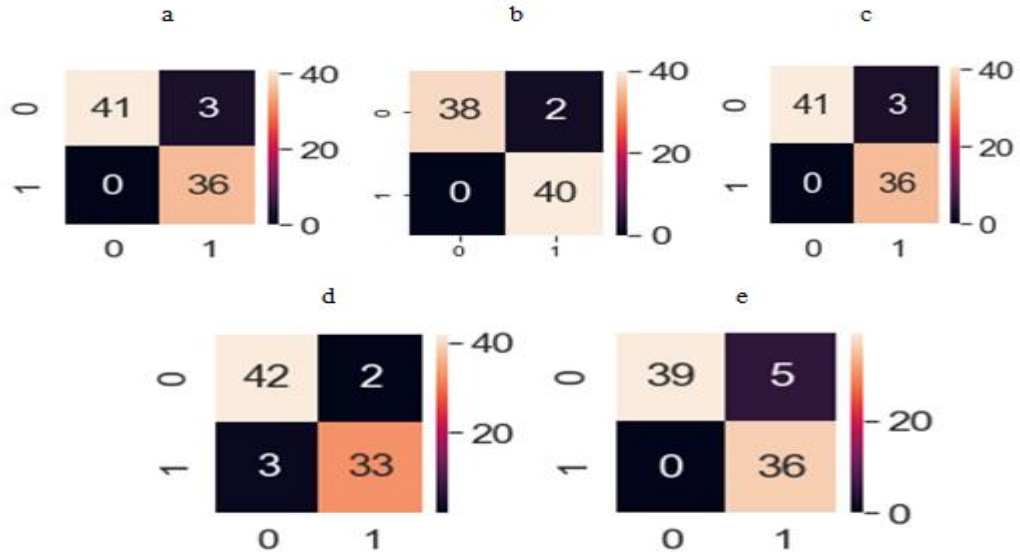

Figure 9. Confusion matrix of traditional classifiers; Support Vector Machine (a), Random Forest (b), Logistic Regression (c), Naïve Bayes (d), and Decision tree (e)

While the accuracy Random Forest and Naïve Bayes models was enhanced by considering only the relevant features, precision rate was also enhanced to be 95% for each of them. However, the statistical parameters of the SVM model were generally decreased as the overall classification accuracy was decreased as in *Table 7*.

Table 7. Confusion matrix statistical parameters related to defected and healthy classes with selected features.

NO.	Measure %	Classifiers				
		SVM	RF	LR	NB	DT
1	Recall	100	100	100	95	100
2	Precision	93	95	93	95	88.6
3	Specificity	92	95	92	94	87.8

4. Conclusion

After testing different background subtraction methods, the applied background subtraction thresholding method was able to extract the hazelnut image from the background perfectly and overcome the difficulties resulting from hazelnut spot color variation. The extracted color moment features represent in mean, variance, range, and skewness in the l*a*b* and RGB split color spaces were able to be describable and distinguishable in classifying healthy and hazelnut that defected with the BMSB. When all extracted moment features were applied for hazelnut classification, the Support Vector Machine classifier performed better than other traditional machine learning algorithms. After testing several feature selection approaches, the BorutaPy feature selector was identified

as the most suitable one, and able to select the most relevant and discriminative features. Only seven features were selected from a total of 24 l*a*b* and RGB split color feature spaces. Using this strategy, data redundancy was also reduced. The majority of the features selected originated from the l*a*b* color spaces. As a result, the changes in hazelnut generated by brown marmorated stink insect infestation have been recognized to appear mostly in this color space after harvesting.

By training traditional machine learning classifiers with the selected futures only, the performances of most of these classifiers were improved. The performance of Random Forest, Naïve Byes, and Logistic Regression models was increased as well as the training time was reduced. In this case, the features selection objective was achieved by improving the learning process of machine learning models and increasing the predictive power of machine learning algorithms by selecting the most important and the most relevant variables and eliminating redundant and irrelevant features. There was no improvement in Decision Tress classifier performance in terms of accuracy, precision, and recall parameters. However, an improvement in processing time was achieved. The performance of the Support Vector Machine model by using only the selected features was decreased due to the margin maximization.

Although machine learning and deep learning models for computer and machine vision are widely used around the world and applied in various field, hazelnut research integrating this technology is very limited, leaving a significant gap in the sector. This gap is appearing in hazelnut processing factories and food industries. In addition, there are still manual and semi-automated hazelnut sorting and classification systems in use in post-harvest activities. These systems consider very poor, time-consuming, and inefficient systems. To overcome this challenge, it can be concluded that using the same technology more investigations are necessary for the hazelnut quality control to facilitate hazelnut processing easier in the food industry and increasing hazelnut quality. Hazelnut infestation with BMSB is a relatively new problem and is under investigation. In particular, hazelnut infestation by the BMSB poses a serious threat to hazelnut quality on a wide scale and negatively impacts national and international marketability and acceptability.

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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Agricultural Production Connectedness and Networks in Türkiye

Türkiye'de Tarımsal Üretim Bağlantılılığı ve Ağları

Türker Açıkgöz[1*]

Abstract

The world's population has been growing rapidly and since the 2006–2008 global food crisis, it has been questioned many times that how the world's growing population will be fed properly. According to reputable international institutions, the world may be insufficient to supply enough food in the near future, and this fact may cause many economic, social, and government problems. In Türkiye, these problems will be realized more harshly than in peer countries for some reasons. Türkiye has one of the highest population growth rates in the world, while it hosts the highest number of refugees in the world. In addition, Türkiye's agriculture sector has been experiencing a harsh downfall recently and the country has been dependent on importing food and agricultural commodities. Therefore, in this paper, I investigate the connectedness and networks of agricultural production in Türkiye by using the connectedness approach of Diebold and Yilmaz (2012, 2014), which is based on the forecast error variance decomposition methodology of generalized vector autoregressive models. I use Türkiye's most produced agricultural commodity data, which are barley, wheat, rye, paddy, lentil, chickpea, and oat. The material consists of annual production data from 1938 to 2019. According to the analysis results, Türkiye's agricultural production has been highly connected. Our findings show that production shocks arising from wheat and barley have spilled over to other commodities. Agricultural production networks and pairwise spillovers also exhibit a similar result that most of the commodities are highly interconnected to wheat and barley production. Besides, pairwise connectedness results show that there are some strong and weak connectivity relations, and these can be used for the decision-making process, risk aversion, and risk-seeking purposes. Our findings have important implications for policymaking for institutions, diversification, and risk management for producers, suppliers, and traders.

Keywords: Agricultural production, Agricultural policymaking, Network analysis, Risk management, Spillover effects

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Öz

Dünya nüfusu hızla artmakta ve 2006-2008 küresel gıda krizinden bu yana dünya nüfusunun nasıl doyurulacağı sorusu defalarca kez sorulmaktadır. Saygın uluslararası kuruluşlara göre, dünya yakın bir gelecekte yeterli gıdayı tedarik etmekte yetersiz kalabilir ve bu durum birçok ekonomik, sosyal ve devlet sorununa neden olabilir. Türkiye'de bu sorunların bazı nedenden dolayı benzer ülkelere göre daha sert bir şekilde gerçekleşeceğine inanılmaktadır. Türkiye, dünyadaki en yüksek nüfus artış oranlarından birine sahipken, dünyanın en fazla mülteciye ev sahipliği yapan ülkesidir. Buna ek olarak, Türkiye'nin tarım sektörü son yıllarda sert bir düşüş yaşamakta ve ülke gıda ve tarımsal emtia ithalatına bağımlı hale gelmektedir. Bu bağlamda, bu çalışmada, Vektör Otoregresif Modellerinin tahmin hata varyans ayrıştırma metodolojisine dayanan Diebold ve Yılmaz'ın (2012, 2014) bağlantılılık yaklaşımı kullanılarak Türkiye'deki tarımsal üretimin bağlantılılık ve ağları araştırılmaktadır. Çalışmada Türkiye'de en çok üretilen yedi tarımsal emtianın verisi kullanılmıştır. Bu ürünler; arpa, buğday, çavdar, pirinç, mercimek, nohut ve yulaf şeklindedir. Araştırmada kullanılan veri seti 1938 yılından 2019 yılına kadarki süreyi kapsayan tarımsal üretim verisidir. Analiz sonuçlarına göre, Türkiye'nin tarımsal üretimi yüksek oranda bağlantılıdır. Araştırmanın bulguları, buğday ve arpa kaynaklı üretim şoklarının diğer tarım ürünlerine de önemli ölçüde sıçradığını göstermektedir. Tarımsal üretim ağları ve ikili yayılmalar, emtiaların çoğunun buğday ve arpa üretimiyle yüksek oranda bağlantılı olduğu konusunda da benzer bir sonuç sergiler. Ayrıca, ikili bağlantılılık sonuçları, bazı güçlü ve zayıf bağlantı ilişkilerinin olduğunu ve bunların karar verme süreci, riskten kaçınma ve risk arama için kullanılabileceğini göstermektedir. Bulgularımızın kurumlar için politika oluşturma, çeşitlendirme ve üreticiler, tedarikçiler ve tüccarlar için risk yönetimi için önemli etkileri vardır.

Anahtar Kelimeler: Tarımsal üretim, Tarımsal politika, Ağ analizi, Risk yönetimi, Yayılma etkileri

1. Introduction

Since the recent global food crisis (from 2006 to mid-2008), the question of "how the world's growing population will be fed" has started to gain popularity and become one of the main questions by many national and international institutions (FAO, 2009; Tian et al., 2021). According to the projections of the United Nations, the world population will reach 9.3 billion by 2050. In this situation, the world has to increase its current global crop production by %70-%100 and this estimation has been made by taking consumption and income growth trends into account (Bruinsma, 2009; Van Wart et al., 2013). Although past and current production of agricultural commodities has been sufficient to support the growing population (Pingali, 2012; Rosa et al., 2018), the world is moving toward the point where this situation may change (Negiş et al., 2017). Thus, policymakers and institutions of national economies should take action and set measures proactively and manage the oncoming risks due to agricultural production and access to food.

The agricultural production has an important place in national economies because of its highly integrated structure with social conflicts and macroeconomic problems (Bozkurt and Kaya, 2021). On the macroeconomic side, its importance is irrefutable for economic growth (Singariya and Naval, 2016; Mohammed, 2020), the labor market (Mellor, 1995; Cristea and Noja, 2019), and sustainable development (Johnston, 1970; Asim and Akbar, 2019), and its interrelation with the real side of the economy (Mohammad, 2020), both industrial and services, makes it one of the most important sectors in nations' economies. For socioeconomics, agricultural production has an important sociological role in societies as it bears and rivets negative impacts of income inequality and poverty (Machethe, 2004; Dhahri and Omri, 2020), and thus, it may cause social conflicts (Crost et al., 2018). Therefore, determining policies on agricultural production can be counted as one of the most crucial ones for policymakers and economic institutions in a nation for the sake of macroeconomic stability, future growth, sustainable development, and social welfare.

In the Republic of Türkiye, I believe that this problem will be realized more harshly than peer nations. There are many indicators that confirm this hypothesis. First, Türkiye's population grows rapidly. According to World Bank (2022) statistics, Türkiye has the second-highest population growth rate (last 10 years average is %1.5) in the European region after Luxembourg. Second, in addition to its swiftly increasing population, Türkiye is the number one country that hosts the highest number of refugees in the world with approximately 3.7 million refugees (UNHCR, 2022). With a high growth rate of population and being a center for immigration, Türkiye has to feed its rapidly growing population and may face many problems for this purpose.

Table 1. Türkiye's Export-Import Data of Agriculture, Forestry and Fishing (Thousands of US$)

Year	Export	Import	Net Export (Export-Import)
2021	7,160,039	12,082,065	-4,922,027
2020	5,956,937	9,834,246	-3,877,309
2019	5,588,545	9,835,392	-4,246,847
2018	5,846,649	9,498,144	-3,651,495
2017	5,579,339	9,374,405	-3,795,066
2016	5,686,894	7,345,239	-1,658,344
2015	5,293,786	7,501,500	-2,207,713
2014	5,712,144	8,948,939	-3,236,795
2013	5,339,324	7,792,640	-2,453,317
Mean	**5,795,962**	**9,134,730**	**-3,338,768**
Standard Deviation	**522,681**	**1,396,901**	**990,223**

Third, Türkiye's agriculture sector has been decaying for a long time. *Table 1* reports export and import statistics of Türkiye's agriculture, forestry, and fishing industries (TURKSTAT, 2022). There is a huge gap between import and export values. According to *table 1*, Türkiye's food demand has been dependent on and fulfilled by imports from other countries more and more every year. This picture has been realized because the demand for food has been increasing owing to the rapidly increasing population and immigration. But the supply side is in an insufficient position and cannot catch the trend. Considering global trends in increasing food prices and global population, exchange rates in Türkiye, and energy prices, this import-based agriculture policy is

unsustainable. Thus, meeting the food demand by importing can cause many problems in the future. In the near future, the dependance on imports for food can reach to a point of no return. Therefore, it is not a sustainable solution to the problem.

Based on the discussion above, I believe that Türkiye will experience a food crisis in the future more severely than many other countries. For this reason, there is a need for research that will be a roadmap for regulating agricultural policy. Our study is important in policymaking.

Speaking of agricultural production, cereals and pulses are among the most important products because they are cheap, have high efficiency in terms of production, and are nutritious since they contain a high level of carbohydrates, protein, and vitamins. They also constitute most of Türkiye's agricultural production (Balkan et al., 2011). Therefore, this study mainly focuses on cereals and pulses, which are mentioned when talking about agricultural production.

This study examines the issue of agricultural production, which can become an important problem in the future. This study analyzes production connectedness and networks between barley, wheat, rye, paddy, lentils, chickpeas and oats, which are the seven most important agricultural products of Türkiye. For this purpose, I use the connectedness analysis of Diebold and Yilmaz (2012; 2014), which investigates connectedness between variables in the time domain. Connectedness and network analysis have gained popularity recently in finance and economics literature. The main purpose of connectedness analysis is to examine how different markets, economies, assets, or macroeconomic and financial variables are connected. Although the connectedness approach has been tested for macroeconomic and financial variables, its use in the agriculture area is quite limited. For instance, Diebold and Yilmaz (2009), Barunik et al., (2016), Zhang (2017), Su (2020) and Polat (2020) have tested equity markets around the world and concluded that connectedness effects exist and spillover effects of shocks are very high. Similar results are obtained by Alter and Beyer (2014), Reboredo et al. (2020), Shahzad et al. (2019) on debt markets, Antonakasis and Kizys (2015), Balli et al. (2019) on commodity markets, and Ferrer et al. (2018), Lovcha and Perez-Labardo (2020) and Toyoshima and Hamori (2018) on oil and energy markets. All these studies confirmed that economic and financial variables are highly connected, and spillover effects of volatility shocks spread rapidly in the system. However, its usage for agricultural economics and agricultural sciences has not yet been popularized. Thus, in the literature review conducted up to this date, I have not found sufficient studies in the agricultural economics literature that investigate connectedness and spillover effects. There is a huge gap on the connectedness and network studies in the literature on agricultural economics. In a way, this study has great value in showing that connectedness and network analysis can be used in the field of agricultural science to analyze interactions between variables, commodities, and countries as well as policy-making on economic and agricultural production.

2. Materials and Methods

2.1. Dataset

Since this study investigates agricultural production, I use production amount data for seven agricultural commodities which are barley, wheat, rye, paddy, lentils, chickpeas, and oats. The dataset was obtained from the Turkish Grain Board (TGB, 2022) website and is publicly available. For the sake of stationary and robust econometric modeling, I use logarithmic returns of the raw data in connectedness analysis. The calculation of return series is given in Equation 1.

$$r_{i,t} = \ln\left(\frac{d_{i,t}}{d_{i,t-1}}\right) \qquad \text{(Eq. 1)}$$

In equation 1, parameters are given as follows: $d_{i,t}$: production amount of i^{th} commodity and $r_{i,t}$: logarithmic returns of production series of i^{th} commodity.

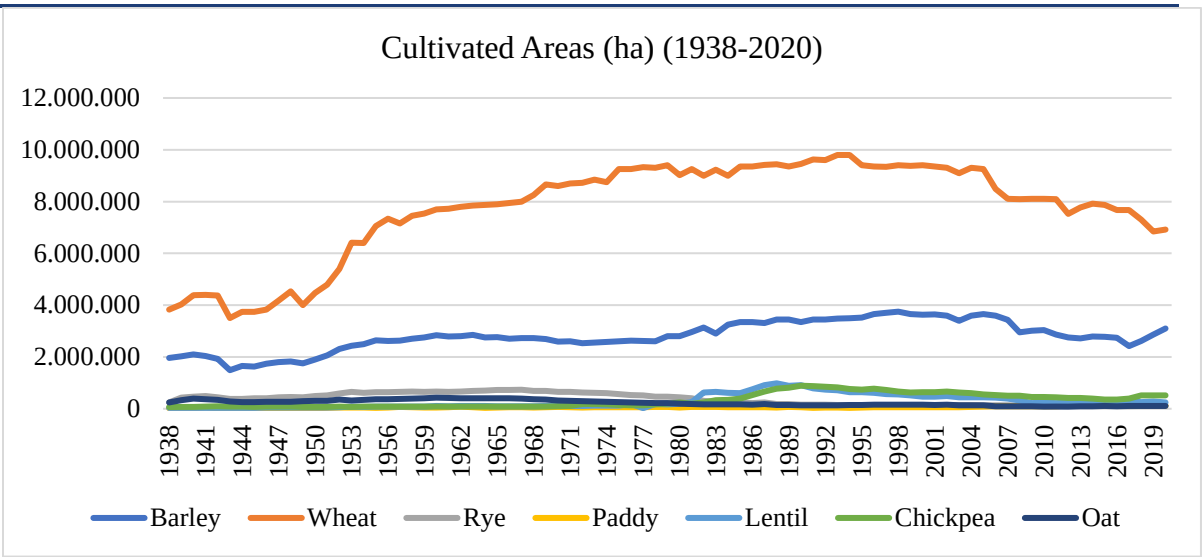

Figure 1. Cultivated areas in Türkiye

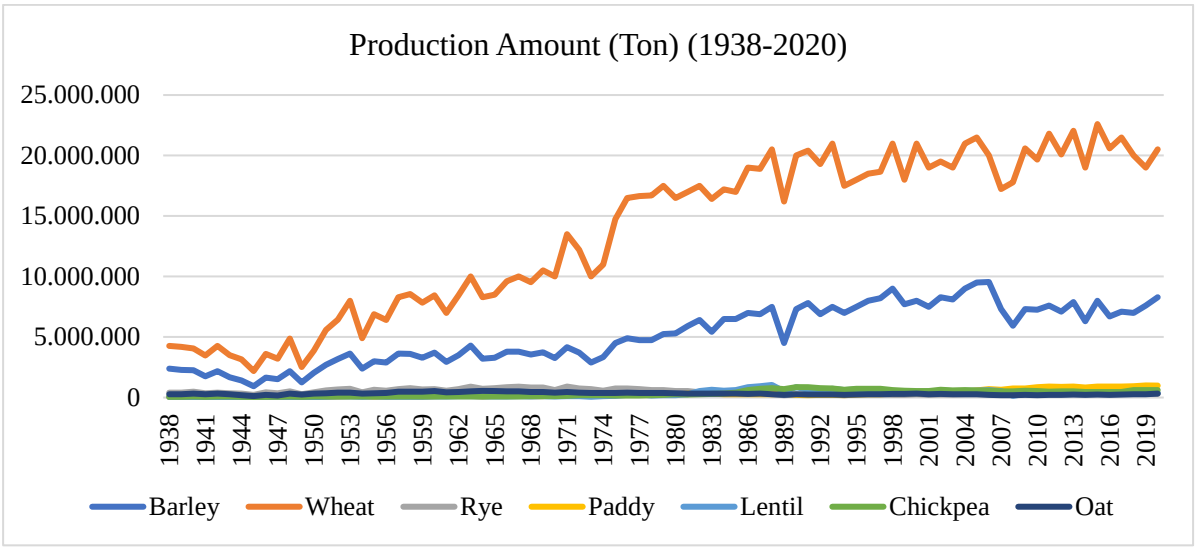

Figure 2. The production number of agricultural commodities

Figures 1 and *2* exhibit cultivated areas (ha) and production amounts (ton) of agricultural commodities. According to *figure 1*, wheat and barley have been dwelling in almost all areas for cultivation. Barley has performed very slow upward trend from 1943 to 2008. Speaking of wheat, it is one of the most important commodities of Türkiye's agricultural policies. Most of the cultivated land has been assigned to the wheat production. Besides, Türkiye's policy on wheat production has been consistent. Türkiye's cultivated land policy has been mostly stable in the sample period for the rest. I may comment that other commodities have not been getting attention from policymakers as their cultivated areas are too low compared to others. *Figure 2* displays characteristics similar to *figure 1*, but with some strict differences. The production amount is more volatile than cultivated areas. Although the behavior of cultivated areas is smoother, the production amount exhibits an exponential increasing and oscillates around the trend. Production amounts increased sharply while cultivated areas shrunk. This may be due to the fact that agricultural technologies have been increasing since the last century, and Türkiye has successfully benefited from it. Therefore, Türkiye's productivity per hectare has been increasing higher than the increase in cultivated areas. Besides, the fact that the production amount has been increasing exponentially while cultivated areas go up smoother supports this view.

Table 2. Descriptive statistics and correlation matrix

Panel A. Descriptive Statistics							
	Barley	**Wheat**	**Rye**	**Paddy**	**Lentil**	**Chickpea**	**Oat**
Mean	0.015	0.019	-0.004	0.037	0.033	0.030	0.002
Median	0.034	0.025	0.000	0.039	0.047	0.009	0.005
Std. Dev.	0.203	0.184	0.212	0.215	0.295	0.153	0.167
Kurtosis	1.082	2.367	1.365	3.305	6.682	1.045	3.905
Skewness	-0.177	-0.356	0.141	-0.606	-1.184	0.262	0.820
Range	1.124	1.162	1.299	1.507	2.240	0.837	1.063
Min.	-0.553	-0.660	-0.627	-0.764	-1.406	-0.383	-0.424
Max.	0.571	0.502	0.671	0.743	0.834	0.454	0.639
Total	1.246	1.567	-0.320	3.052	2.700	2.441	0.135
# of obs.	82	82	82	82	82	82	82
Panel B. Correlation Matrix							
	Barley	Wheat	Rye	Paddy	Lentil	Chickpea	Oat
Barley	1						
Wheat	0.924	1					
Rye	0.842	0.875	1				
Paddy	0.006	0.033	0.009	1			
Lentil	0.565	0.432	0.365	-0.060	1		
Chickpea	0.528	0.480	0.450	-0.021	0.297	1	
Oat	0.771	0.778	0.781	0.099	0.337	0.601	1

Table 2 presents descriptive statistics and the Pearson correlation matrix of the log return series to draw a general picture of the dataset. Panel A in *Table 2* reports that most of the variables have positive means and high standard deviations. For all commodities, maximum and minimum values are relatively high. Passing through panel B, the results suggest a high correlation between wheat-barley (92.4%), wheat-rye (87.5%), and barley-rye (84.2%). There are positive correlations between almost all variables except paddy-lentil and paddy-chickpea. Lastly, correlation results indicate that paddy moves independently and that there is almost no significant correlation with any other commodities.

2.2. Econometric Models

Introduced by Diebold and Yilmaz (2014), connectedness analysis is a variance decomposition method based on a covariance-stationary vector autoregressive model. Using this methodology, I decompose forecast error variance shares of shocks for all variables in the VAR system. Afterwards, in line with the results of the connectedness methodology, I perform network analysis, which uses variance decompositions for interactions. This part of the study first introduces the time domain connectedness methodology of Diebold and Yilmaz (2012) and then discusses the network topology of the variance decomposition.

2.2.1. DY Method

The DY method is based on a covariance stationary *N* variable generalized vector autoregressive (VAR) process with (p) order as in equation 2:

$$y_t = \sum_{i=1}^{p} \Phi_i y_{t-i} + \varepsilon_t \; ; \varepsilon_t \sim (0, \Sigma) \qquad \text{(Eq.2)}$$

As the VAR (p) model has constant covariance, it has the following moving average MA (∞) representation in equation 3:

$$y_t = \sum_{i=0}^{\infty} A_i \varepsilon_{t-i} \qquad \text{(Eq.3)}$$

In equation 3, A_i represents a square coefficient matrix with *N* dimension and it has a recursive process such that $A_i = \Phi_1 A_{i-1} + \Phi_2 A_{i-2} + \ldots + \Phi_p A_{i-p}$. Furthermore, A_0 matrix is compatible with the identity matrix of *N* dimension and has a value of zero where $i=0$. Following Diebold and Yilmaz (2012) using a generalized impulse response function, it is possible to assess the shares of each variable *j* in the *H*-step ahead forecast error variance decomposition of variable *i*

for each *H* step ahead where *H=1,2,3,…* can be calculated as follows in equation 4:

$$\theta_{ij}^{g}(H) = \frac{\sigma_{ii}^{-1} \sum_{h=0}^{H-1} (e_i' A_h \Sigma e_j)^2}{\sum_{h=0}^{H-1} (e_i' A_h \Sigma A_h' e_i)^2} \quad \text{(Eq.4)}$$

where Σ symbolizes the variance-covariance matrix of the error vector, σ_{ii} stands for the standard deviation of the i^{th} error term, and e_i stands for the selection vector that i^{th} component takes 1 and others take zero. Since the shocks to each variable are not orthogonalized, the sum of each row does not equal one. Therefore, each element of the variance decomposition matrix can be normalized as follows in equation 5:

$$\tilde{\theta}_{ij}^{g}(H) = \frac{\theta_{ij}^{g}(H)}{\sum_{j=1}^{N} \theta_{ij}^{g}(H)} \quad \text{(Eq.5)}$$

By utilizing normalized forecast error variance decompositions (spillovers), various connectedness measures are possible. For instance, total connectedness in the system (C(H)) can be calculated as shown in equation 6. The total connectedness index shows the extent to which shocks occur in a variable spillover through the system.

$$C(H) = \frac{\sum_{i,j=1,i\neq j}^{N} \tilde{\theta}_{ij}^{g}(H)}{\sum_{i,j=1}^{N} \tilde{\theta}_{ij}^{g}(H)} = \frac{\sum_{i,j=1,i\neq j}^{N} \tilde{\theta}_{ij}^{g}(H)}{N} \quad \text{(Eq.6)}$$

Volatility shocks from all other variables *j* to the variable *i* due to the shocks arising from all other *j* are named contributions from other spillovers. These directional "FROM" spillovers are calculated as follows in equation 7:

$$C_{i\leftarrow\blacksquare}(H) = \frac{\sum_{j=1,j\neq i}^{N} \tilde{\theta}_{ij}^{g}(H)}{\sum_{i,j=1}^{N} \tilde{\theta}_{ij}^{g}(H)} * 100 = \frac{\sum_{j=1,j\neq i}^{N} \tilde{\theta}_{ij}^{g}(H)}{N} * 100 \quad \text{(Eq.7)}$$

Similarly, shocks arising from variable *i* directed to all other variables *j* due to shocks to *i* are called contributions to others spillovers (directional "TO" spillovers). Directional "TO" spillovers are calculated as in equation 8.

$$C_{\blacksquare\leftarrow i}(H) = \frac{\sum_{j=1,j\neq i}^{N} \tilde{\theta}_{ji}^{g}(H)}{\sum_{i,j=1}^{N} \tilde{\theta}_{ji}^{g}(H)} * 100 = \frac{\sum_{j=1,j\neq i}^{N} \tilde{\theta}_{ji}^{g}(H)}{N} * 100 \quad \text{(Eq.8)}$$

Net spillovers can be estimated by equation 9. It estimates net directional spillovers by subtracting total shocks from all others *j* to *i* and shocks from variable *i* to all others *j*.

$$C_i(H) = C_{\blacksquare\leftarrow i}(H) - C_{i\leftarrow\blacksquare}(H) \quad \text{(Eq.9)}$$

Lastly, net pairwise spillovers can be estimated by equation 10. Net pairwise spillovers are important to understand pairwise dynamics in a system. It measures net directional spillovers between *i* and *j*. In other words, it is the difference between how many shocks are transmitted from variable *j* to *i* and from variable *i* to *j*.

$$Cn_{ij}(H) = C_{i\leftarrow j}(H) - C_{j\leftarrow i}(H) \quad \text{(Eq.10)}$$

2.2.2. Network Topology of Variance Decomposition.

The network topology of this study relies on variance decompositions and directional spillovers in the system. As Diebold and Yilmaz (2014) proved, variance decompositions can determine weighted and directed networks in a system of variables. By using the variance decomposition, it is possible to measure connectedness among variables and describe networks of various economic and financial relationships. In line with the abovementioned methodology of variance decomposition, I draw networks of Türkiye agricultural production. To effectively depict interactions in the network topology, it is preferable to examine ties bigger than a given threshold value (*φ*). The following equation (11) defines this function.

$$\tau_x \coloneqq (\max(\text{directional from spillovers})) * \varphi;\ 0 \leq \varphi < 1 \quad \text{(Eq.11)}$$

In network analysis, node sizes and labels are attributed to the average production amount of each agricultural commodity, while tie thickness is attributed to directional spillovers from commodities *i* to *j*. Arrow and arrowhead sizes are determined by the strength of pairwise directional spillovers. Lastly, for node colors, I use net total directional spillovers. In this process, I select the color green for positive net total directional spillovers and the color red for negatives. Color tones approach dark green as net total directional spillovers for positive ones rise. Color tones approach deep red as they drop for negative ones. To scale color tones, I use the deepest green and red to paint the

highest and lowest values, respectively.

3. Results and Discussion

This section first introduces connectedness analysis results and presents networks afterward. I use a generalized VAR (1) model with six years ahead variance decomposition H=6 since after H=6 analyzes results remain unchanged. For network analysis, I use two different threshold values such that $\varphi_1=0$, $\varphi_2=0.20$. φ_1 displays all ties in the networks but suffers from noise. By using φ_2, I apply a denoising process and eliminated weak ties. *Table 3* presents the connectedness results for production data.

Table 3. Connectedness Matrix of Agricultural Production

	Barley	Wheat	Rye	Paddy	Lentil	Chickpea	Oat	**FROM**
Barley	26.92	23.40	18.05	1.91	8.66	6.32	14.74	73.08
Wheat	22.69	28.37	20.25	2.56	4.96	4.86	16.31	71.61
Rye	19.86	22.44	29.81	1.46	4.44	4.60	17.39	70.21
Paddy	3.41	5.53	2.55	81.70	0.32	0.79	5.69	18.27
Lentil	16.54	8.50	5.62	4.17	57.16	3.55	4.46	42.84
Chickpea	13.07	9.85	8.62	1.06	4.00	45.96	17.44	54.04
Oat	17.17	17.81	17.42	2.42	3.91	9.74	31.53	68.46
TO	92.75	87.57	72.52	13.58	26.32	29.89	76.02	**TSI: 56.94%**
NET	19.67	15.96	2.31	-4.69	-16.52	-24.15	7.56	

Table 3 presents that the total spillover index is %56.94 for agricultural production. These results show that most of the volatility shocks of production amount arise from system-wide shocks. Directional "FROM" spillovers show that volatility shocks in the production of barley, wheat, rye, and oat are mostly due to production shocks arising from all other commodities. Directional "TO" spillovers also display similar results. Production shocks in these four commodities are highly transmitted to others. For the directional pairwise connectedness of commodities, my findings indicate that production shocks between barley, wheat, rye, and oat are transmitted to each other mutually. Therefore, these findings show that from a production amount perspective, these four commodities are highly connected. Lastly, on net spillovers, the findings show that barley and wheat have the highest positive net spillovers in the system. These commodities spread more shocks to others than they receive from others. On the other hand, chickpea and lentil have the highest negative net spillovers.

Table 4. Net Pairwise Connectedness Matrix

	Barley	Wheat	Rye	Paddy	Lentil	Chickpea	Oat
Barley	0.00	0.71	-1.81	-1.50	-7.88	-6.75	-2.43
Wheat	-0.71	0.00	-2.19	-2.97	-3.54	-4.99	-1.50
Rye	1.81	2.19	0.00	-1.09	-1.18	-4.02	-0.03
Paddy	1.50	2.97	1.09	0.00	-3.85	-0.27	3.27
Lentil	7.88	3.54	1.18	3.85	0.00	-0.45	0.55
Chickpea	6.75	4.99	4.02	0.27	0.45	0.00	7.70
Oat	2.43	1.50	0.03	-3.27	-0.55	-7.70	0.00

Table 4 exhibits the net pairwise connectedness results for agricultural commodities. Notice that *table 4* is a skew-symmetric matrix where the transpose of the $[Cn_{ij}]$ component equals minus $[Cn_{ji}]$ and the summation of all component in the matrix equals zero. The net pairwise connectedness matrix measures the pairwise direction of net volatility shocks. Among all commodities, the direction of production shocks is from wheat to others. Production shocks arising from wheat that spills over to others are much higher than the shocks transmitted from others to wheat production. Besides wheat, barley is also an important commodity whose net pairwise volatility shocks are transmitted to others, but wheat. Lastly, in *table 4*, the most important net pairwise connectedness of agricultural production in the system occurs from lentil → barley, chickpea → oat, and chickpea → barley, respectively.

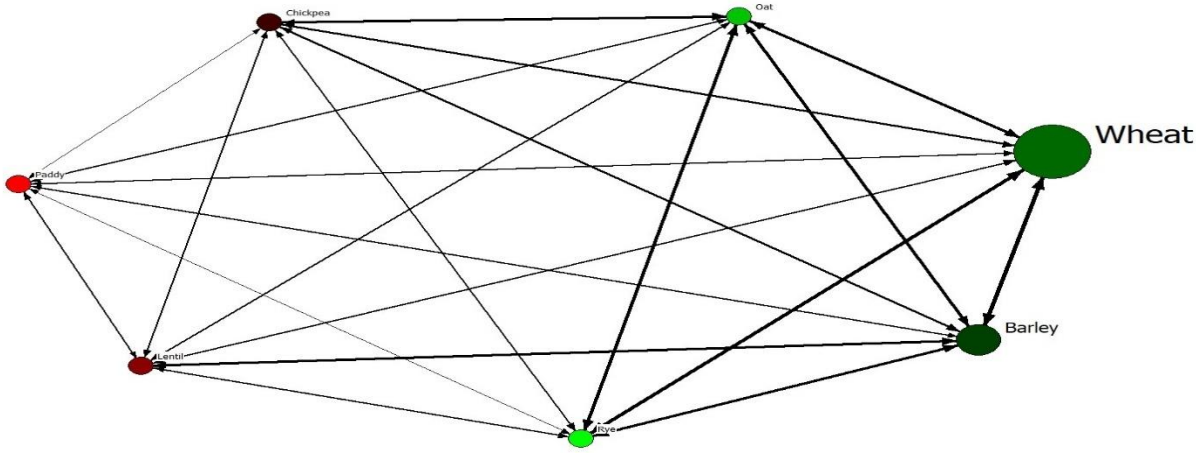

Figure 3.A. Networks of Production Amounts (φ_0=0, τ_x=0)

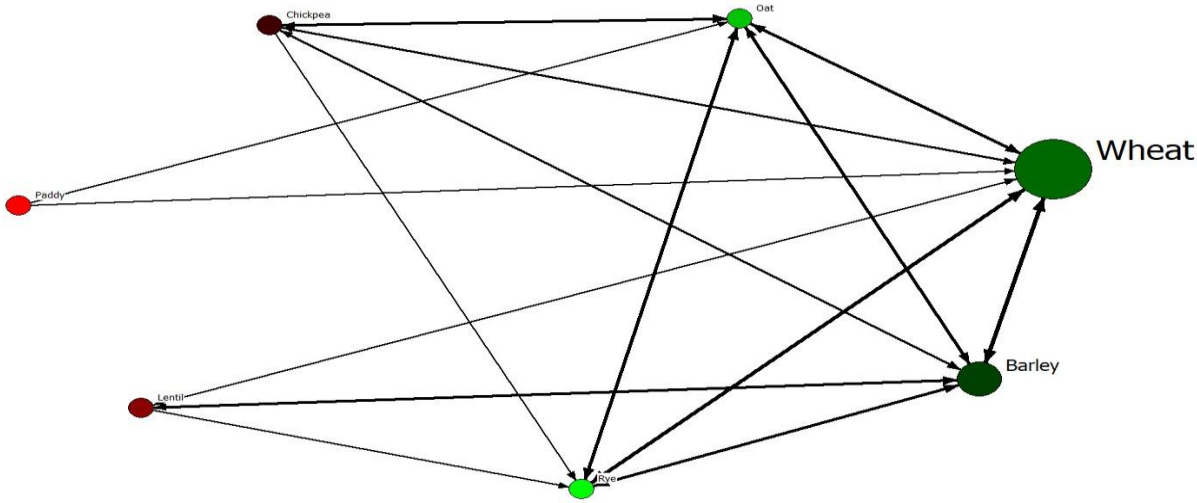

Figure 3.B. Networks of Production Amounts (φ_1=0.20, τ_x=4.68)

From now on, I exhibit network analysis based on variance decompositions. *Figure 3* displays networks for production amounts of seven agricultural commodities. As described in *figure 3*, the agricultural production of four commodities; wheat, barley, rye, and oat, are highly interconnected to each other. Between production amounts of agricultural commodities, many strong ties exist such as wheat-oat, wheat-rye, wheat-barley, barley-oat, barley-rye, and rye-oat. Besides these seven pairwise ties, there are also some one-sided bounds and dependence such as from paddy to oat, paddy to wheat, lentil to barley, chickpea to rye, and chickpea to wheat. Once again, the findings on production networks indicate that the most important actors in the system are wheat and barley. The results show that the most dominant actors in the network, according to the number of ties and strength of links, are barley and wheat.

4. Conclusion

The principal purpose of this article is to analyze the connectedness and networks of agricultural production in Türkiye. According to the worlds acknowledged economic and agricultural institutions, global agricultural production cannot meet the rapidly growing population of the world. Therefore, the scarcity of food will be a non-negligible issue in the near future and some actions must be taken proactively. Having the second-highest population growth rate in Europe and being the world's immigration center, Türkiye has a special role and the effect of this dilemma on Türkiye should be carefully watched.

This paper aims to guide policymakers of agriculture and stakeholders of agricultural production such as producers, suppliers, traders, etc. about how the production of agricultural commodities is interconnected to each other. The

findings of this study indicate a convergence situation in producing agricultural commodities. I found that the production shocks of agricultural commodities have highly spilled over among each other. This convergence situation demonstrates that agricultural commodity production highly interacts with each other. Therefore, when developing policies related to a specific product, the impacts on the production of other products should also be considered. This is because research findings on production networks have shown the existence of highly interconnected networks.

The findings also report that wheat and barley production in Türkiye has been highly integrated, and these two commodities are the most important actors in the agricultural production network of Türkiye. Production shocks arising from wheat and barley have been highly transmitted to others. Thus, the production of these two commodities should be carefully monitored. The policies implemented for these commodities, such as production planning, incentives, and export/import regulations, will have a significant impact on the production of other products. Policy makers should consider the potential effects of the policies applied to these two commodities, especially on oat, rye, and chickpea, and plan accordingly.

The results on pairwise connectedness demonstrate that diversification opportunities and risk management tools exist. For instance, a producer can cultivate a type of commodity that has low connectedness (e.g. paddy or lentil) with others in absolute terms or cultivate two commodities rather than one commodity that almost has no pairwise shock transmissions. Thus, with this perspective, diversification and managing operational risks are possible. Besides, if there is an expectation (market or individual) that the production of a specific commodity will rise in the next period, a risk-seeking producer may take a counter position by connectedness and network analysis results and maximize his/her profit. For example, if there were an expectation that barley production would rise in the next period, one could cultivate lentils and chickpeas to benefit from the decreasing supply of these commodities. Although many other producers would cultivate barley and the supply of other commodities in the market will fall, the ones with the highest net negative pairwise connectedness with barley will be affected more than others. Different scenarios can be constituted for other commodities as well. The recommendations above are also true for parties other than producers, such as governments who would like to direct the production of specific commodities and set agricultural policies, profit-seeking suppliers, traders, business parties both national and cross-border, and so on.

The main limitation of this study is that I only constructed a static analysis that has no time-varying features of agricultural production. But as the theory of economics tells us, relationships and interactions in economic variables are time-varying and change over time. Also, some important global events and crises (such as wars, global financial crises, and global food crises) have important impacts on economic variables. By using a static analysis, I ignore these effects. For further studies, this issue can be overcome with a model capable of predicting time-varying parameters. This can be accomplished using higher frequency time series data (such as monthly or quarterly data). As a result, the evolving structure of network relationships can be examined, enabling a more in-depth analysis.

Another important limitation of this study is that I built an endogenous model and did not consider any exogenous effects. In further studies, considering the influences of other variables such as production in other countries, the real side of the economy, socioeconomic indicators, and so forth, on agricultural production will contribute to the literature on economics. A panel VAR model based on different countries' agricultural productions can be used to examine the impact of inter-country interactions agricultural production. Alternatively, the effect of various variables that are assumed to influence the agricultural production, such as industrial output, inflation, and energy prices, can be studied. Thus, the impact of different economic dimensions on agricultural production in Turkey can be investigated, leading to a better understanding of the subject.

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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Toprakta Farklı Derinliklerde Gömülü *Ipomoea triloba* L. ve *Convolvulus arvensis* L. Tohumlarının Çıkış Derinliği ve Çimlenme Oranlarının Belirlenmesi*

Determination of Emergence Depth and Germination Rates of *Ipomoea triloba* L. and *Convolvulus arvensis* L. Seeds Buried at Different Depths in the Soil*

Mine ÖZKİL[1*], İlhan ÜREMİŞ[2]

Öz

Akdeniz Bölgesi tarım alanlarındaki önemli yabancı ot türlerinden olan *Ipomoea triloba* L. (IPOTR) ve *Convolvulus arvensis* L. (CONAR)'in mücadelesine yönelik olarak tohumların canlılık oranlarının zaman içerisindeki değişiminin belirlenmesi ve çıkış derinliklerinin saptanması çalışmanın amaçlarını oluşturmaktadır. Çalışmada CONAR ve IPOTR tohumlarının optimum çıkış derinliğinin belirlenmesi için, yabancı ot tohumları 30 °C'ye ayarlı iklim odasında, toprak+ torf+ kum karışımı bulunan saksılar içerisine 2, 5, 10, 15 ve 20 cm derinliklerine 25'şer adet konulmuştur. Değerlendirme için 7, 14, 21 ve 28. günlerde toprak yüzeyine çıkan bitkiler sayılmış, çıkış yapan tohumlar dışarı alınmış ve çıkış yüzdesi hesaplanmıştır. CONAR ve IPOTR türlerinin topraktaki yaşam sürelerinin belirlenmesi amacıyla yapılan çalışmada; her iki yabancı ot türü için 10 ve 20 cm derinlikte çukurlar hazırlanmıştır. Toprağa gömülen her torba içerisine 0.25 kg sterilize edilmiş toprağa 300'er adet tohum konulmuştur. Toprağın 10 ve 20 cm derinliğine gömülü tohumlar gömülme tarihini takip eden 6, 12, 18, 24, 30. aylarda tohumların canlılıklarının belirlenmesine yönelik çimlenme denemeleri yapmak üzere tohumlar kullanılmıştır. Her iki türün tohumlarında en iyi çıkışın 2 cm derinlikte gerçekleştiği ve çıkış oranının CONAR için %84.00, IPOTR için %96.80 oranında olduğu belirlenmiştir. CONAR tohumlarının topraktaki yaşam süreleri, toprağın 10 ve 20 cm derinliklerinde, sırasıyla 6. ayda %90.10 ve %87.50 olarak belirlenirken, 30. ayda %15.40 ve %9.50; IPOTR'de ise 6. ayda %93.90 ve %85.20; 30. ayda %10.10 ve %7.60 olarak tespit edilmiştir. Çalışma sonuçlarına göre CONAR ve IPOTR ile mücadelede derin toprak işlemesi yapılarak tohum çimlenmesinin/çıkışının azaltılabileceği ve bu sayede yoğunluğun azaltılıp yeni tohum oluşumunun engellenebileceği değerlendirilmektedir. Ülkemizin pek çok bölgesinde görülen ve ekonomik zarara sebep olan CONAR ve IPOTR ile mücadele yöntemleri içerisinde, toprak işlemenin kullanılabileceği ve üreticilere fayda sağlayacağı düşünülmektedir.

Anahtar Kelimeler: *Ipomoea triloba* L., *Convolvulus arvensis* L., Çıkış derinliği, Çıkış oranı, Yabancı ot

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Abstract

The aim of the study is to determine the change in the viability rates of seeds by time and to determine the depth of emergence for the control of *Ipomoea triloba* (IPOTR) and *Convolvulus arvensis* (CONAR), which are important weed species in the agricultural areas of the Mediterranean region. In order to determine the optimum emergence depth of CONAR and IPOTR seeds in the study, 25 weed seeds were placed at 2, 5, 10, 15 and 20 cm depths in pots with soil + peat + sand mixture in a climate chamber adjusted at 30 °C. For the evaluation, the seedlings that emerged to the soil surface on the 7th, 14th, 21st and 28th days were counted, the emergenced seeds were taken out and the emergence rate percentage was calculated. In the study carried out to determine the life span of CONAR and IPOTR species in the soil; 10 and 20 cm deep pits were prepared for both weed species. 0.25 kg of sterilized soil and 300 pieces of seeds were placed in each bag to be buried in the ground. Seeds buried at 10 and 20 cm depths of the soil were used to conduct germination experiments to determine the viability of the seeds at 6, 12, 18, 24 and 30 months following the burial date. It was determined that the best emergence in the seeds of both species occurred at a depth of 2 cm and the emergence rate was 84.0% for CONAR and 96.8% for IPOTR. The longevity of CONAR seeds at 10 and 20 cm depths of the soil were determined as 90.10%and 87.5% at the 6th month, and 15.40% and 9.50% at the 30th month. The longevity of IPOTR seeds at 10 and 20 cm depths of the soil were 93.90% and 85.20% at the 6th month, while it was determined as 10.10% and 7.60% at the 30th month, respectively. According to our study results, it is thought that seed germination can be reduced by deep tillage to control CONAR and IPOTR, and thus the density can also be reduced and new seed formation can be prevented. It is thought that among the methods of control CONAR and IPOTR, which are seen in many regions of our country and cause economic damage, it will be beneficial for the producers to determine the application method in this direction, where tillage can be used.

Keywords: *Ipomoea triloba*, *Convolvulus arvensis*, Emergence depth, Emergence rate, Weed

1. Giriş

Bitki çeşitliliği açısından ülkemizde 12.000'den fazla bitki taksonu bulunmaktadır (Güner ve ark., 2012), *Magnoliopsida* sınıfının *Solanales* takımı içinde yer alan *Convolvulaceae* familyası; dünyada 58 cins ve 2000 kadar tür içermekte olup, tropikal kuşaktan ılıman kuşağa kadar uzanan oldukça geniş bir alanda yayılış göstermektedir (Staples ve Yang, 1998). Bu bağlamda *Ipomoea triloba* (Pembe çiçekli akşamsefası; IPOTR) ve *Convolvulus arvensis* (Tarla sarmaşığı; CONAR) *Convolvulaceae* familyasına ait önemli yabancı ot türleridir (Mabberley, 1997; Yadav ve ark., 2018).

Ipomoea triloba tohumla çoğalan tek yıllık bir bitkidir (Haselwood ve Motter, 1966). Dünya'da ilk kez 1986 yılında pamuk üretim alanlarında tespit edilen *I. triloba* istilacı bir yabancı ot türüdür (Joel ve Liston, 1986). Ülkemizde ilk olarak Antalya ilinde pamuk üretim alanlarında yaygın olarak bulunduğu saptanan *I. triloba*'nın hızla yayılmaya devam ettiği ve diğer yabancı otları baskıladığı tespit edilmiştir (Yazlık ve ark., 2014; Yazlık ve ark., 2018; Özkil ve Üremiş, 2020). *Ipomoea* türleri istilacı özelliği sayesinde çok hızlı gelişerek kültür bitkisinin büyüme ve gelişmesini engellemektedir. Ülkemizde pamuk, mısır, domates, şekerpancarı, soya fasulyesi ve narenciye gibi pek çok üründe zararlara sebep olarak verimin azalmasına neden olmaktadır (Özkil ve Üremiş, 2020). Ayrıca, pamuk ekim alanlarında elle veya makine ile yapılan hasadı güçleştirmektedir (Özkil ve ark., 2019).

Convolvulus cinsine ait türler dünyadaki en önemli yabancı otlardandır. Bunlardan *C. arvensis* çok yıllık, sürünücü veya tırmanıcı, toprak altı gövdeleri dallanan bir türdür (Davis, 1978; Jacobs, 2007). *C. arvensis* ülkemiz tarımsal üretiminde önemli yeri olan kültür bitkilerinde ekonomik zararlara sebep olmaktadır. Sahip olduğu biyolojik özellikleri nedeniyle mücadelesi oldukça güçtür. İlkbaharda hava sıcaklıklarının artmasıyla birlikte sürgünler toprak yüzeyinde görülmekte, Mayıs ayından itibaren çiçeklenmeler başlamakta ve tohumlar oluşmaktadır. Tohum kabuğu sert olduğundan uzun yıllar canlılığını korumaktadır (Uygur ve ark., 1986; Americanos, 1994). *C. arvensis*'in Avrasya'nın doğal bir türü olduğu (Austin, 2000) ve 44'den fazla ülkede önemli ürün grupları için ciddi bir problem teşkil ettiği bildirilmiştir (Schroeder ve ark., 1993; Americanos, 1994). Tarla sarmaşığının sahip olduğu vejetatif ve generatif özellikleri, ayrıca yüksek rekabetçiliğinden dolayı geleneksel yabancı ot kontrol yöntemleriyle kontrol altına alınması oldukça zordur (Vogelgsang, 1998). Bunun yanı sıra, Tarla sarmaşığı tarım ürünlerinde %20-80 arasında ürün kayıplarına neden olduğu tespit edilmiştir (Lanini ve Miyao, 1987; Black ve ark., 1994).

Dünyada ekonomik öneme sahip olan pamuk, mısır, buğday ve çeltik gibi kültür bitkilerinde zarara neden olan hastalık, zararlı ve yabancı otlardan dolayı oluşan ürün kaybı yaklaşık %67.15 olup, bunun %31.62'si yabancı otlardan kaynaklanmaktadır (Oerke ve ark., 1994). Ülkemiz Dünya tarımsal üretiminde sahip olduğu çeşit ve üretim potansiyeli ile tarımda önemli bir paya sahiptir. Tarımsal üretimde verim ve kalitenin artırılması için bitkisel üretimde çevre koşulları haricinde ürün kayıplarının ana sebeplerinden birisi olan yabancı otlarla etkili bir şekilde mücadele etmek gerekmektedir (Bayat ve ark., 1996; Tepe, 2014; Güncan, 2016; Yazlık ve ark., 2019; Üremiş ve ark., 2020).

Tarımın sürdürülebilirliğinin sağlanması, doğru mücadele yönteminin seçilip uygulanabilmesi için tarım alanlarındaki yabancı ot türlerinin tespiti, yoğunluklarının saptanması, generatif ve vejetatif üreme yeteneği, dormansi, çimlenme sıcaklığı, topraktaki çimlenme derinliği ve toprakta canlı kalma süresi gibi biyolojik karakterlerinin bilinmesi önemli bir yer tutmaktadır (Üstüner, 2002). Çimlenmenin meydana geldiği dönem yabancı ot kontrolü ve kültür bitkisi ile rekabeti açısından önemlidir. Uygun ve etkili yabancı ot kontrol yöntemlerinin geliştirilmesi yabancı ot biyolojisinin ve ekolojisinin iyi bilinmesine dayanmaktadır (Mennan ve ark., 2006; Nakamura ve Hossain, 2009; Talaka ve Rajab, 2013; Tanveer ve ark., 2014). Çevresel faktörlerin tohumların çimlenme ve ortaya çıkma davranışları üzerindeki etkisine ilişkin bilgilerin, yeni alanlara yabancı ot istilasının önlenmesinde ve yönetim uygulamalarının belirlenmesinde ve geliştirilmesinde yardımcı olacağı belirtilmiştir (Peters ve ark., 2000). Yabancı ot kontrolünün geliştirilmesi için, belirli yönetim uygulamalarına ilişkin yabancı ot davranışının ayrıntılı bir şekilde anlaşılmasının çok önemli olduğu, özellikle istilacı yabancı ot türlerinin tohum yaşam süresi ve dormansi gibi biyolojileri hakkında daha fazla bilginin, en sorunlu yabancı ot türlerinin etkisini sınırlamak için çok yararlı olabileceği bildirilmiştir (Vleeshouwers, 1997; Masin ve ark., 2006). Böylece, gelecekteki istilaların daha iyi tahmin edilmesi sağlanarak, yabancı ot yönetimine yardımcı olacaktır.

Ülkemizde Akdeniz Bölgesi tarımsal üretim alanlarında önemli bir sorun olan *I. triloba* ve *C. arvensis*'in mücadelesine yönelik olarak tohumların canlılık oranlarının zaman içerisindeki değişiminin ve çıkış derinliklerinin belirlenmesi bu çalışmanın amaçlarını oluşturmaktadır.

2. Materyal ve Metot

2.1. Toprak derinliğinin I. triloba ve C. arvensis tohumlarının çıkışları üzerine etkilerinin belirlenmesi

IPOTR için 15 dakika H_2SO_4 (%95-98) ve CONAR için 90 dakika H_2SO_4 uygulamaları yapılarak bu tohumların dormansileri kırılmış (Üstüner ve Çakır, 2018; Özkil ve Üremiş, 2019), daha sonra bunların çıkış derinliğini belirlemek için denemeler kurulmuştur. Denemeler iklim odasında saksılarda 07.11.2018 tarihinde kurulmuş, bitkiler tüm deneme boyunca eşit miktarda sulanmıştır. Çalışmada CONAR ve IPOTR tohumlarının optimum çıkış derinliğinin belirlenmesi için, yabancı ot tohumları 30 °C'ye ayarlı iklim odasında, toprak+ torf+ kum (1:1:1) karışımı bulunan saksılar içerisine 2, 5, 10, 15 ve 20 cm derinliklerine 25'şer adet konulmuştur. Denemede kullanılan toprağın sterilizasyonuna yönelik olarak 120 °C'de 30 dakika otoklav edilmiştir (Shuab ve ark., 2014). Değerlendirme için 7, 14, 21 ve 28. günlerde toprak yüzeyine çıkan bitkiler sayılmış, çıkış yapan tohumlar dışarı alınmış ve çıkış yüzdesi hesaplanmıştır (Güncan, 1979).

2.2. I. triloba ve C. arvensis tohumlarının canlılık oranlarının zaman içerisindeki değişiminin belirlenmesi

CONAR ve IPOTR türlerinin topraktaki yaşam sürelerinin belirlenmesi amacıyla yapılan çalışmada; CONAR tohumları Adana'nın Çukurova ilçesindeki ayçiçeği tarlasından Ağustos 2017'de, IPOTR tohumları ise Adana'nın Ceyhan ilçesi pamuk tarlalarından 2017 yılının Eylül ayında toplanmıştır. Denemeler Adana Biyolojik Mücadele Araştırma Enstitüsü'nün deneme bahçesinde tohumların gömüleceği alan temizlenerek parseller oluşturulmuş ve deneme için hazırlanmıştır. Bu parsellerden alınan topraklar 120 °C'de 30 dakika otoklav edilerek sterilizasyon işlemi uygulanmıştır (Shuab ve ark., 2014). Tohumların gömüleceği parselde her iki yabancı ot türü için 10 ve 20 cm derinlikte çukurlar hazırlanmış, çıkan toprak çukurun yanına bırakılmıştır. Toprağa gömülecek her torba içerisine 0.25 kg sterilize edilmiş toprak ve sağlam görünümlü 300'er adet tohum konulmuş ve torbaların ağızları bağlanarak tel süzgeç içerisine konulmuştur. Tohumlar 07.11.2017 tarihinde 10 ve 20 cm derinliğinde hazırlanan çukurlara yerleştirilmiştir.

Toprağın 10 ve 20 cm derinliğine gömülü tohumlar gömülme tarihini takip eden 6, 12, 18, 24, 30. aylarda tohumların canlılıklarının belirlenmesine yönelik çimlenme denemeleri yapmak üzere her derinlikten ve her türden bir torba olmak üzere bulundukları yerden çıkarılmış ve içerisinde toprak + tohum karışımı bulunan torbalar laboratuvara getirilmiştir. Torbalar açıldıktan sonra elde edilen toprak + tohum karışımı 0.18 mm'lik nematod eleğine boşaltılmış, yıkandıktan sonra kalan tohumlar çalışmada kullanılmıştır. Çimlenme ve canlılık oranlarını saptamak amacıyla yapılan denemelerde tabanına iki kat Whatman No 1 filtre kağıtları yerleştirilmiş 9 cm çapındaki Petri kapları kullanılmıştır. Her petriye tohumlar yerleştirildikten sonra 6 ml saf su eklenmiştir. Hazırlanan Petriler 30 °C'ye ayarlı çimlendirme dolaplarına 5 tekerrürlü olarak yerleştirilmiştir. Deneme süresince ihtiyaç oldukça petrilere saf su ilavesi yapılmıştır. Çimlenme oranlarını saptamak amacıyla yapılan bu çalışma iki aşamalı olarak yapılmıştır. Birinci aşamada denemenin kurulduğu günden itibaren 3, 5, 7 ve 14. günlerde sayımlar yapılmış ve çim bitkisi boyu 0.5 cm uzunluğa ulaşanlar çimlenmiş olarak kabul edilerek petri dışına aktarılmıştır. İkinci aşamada tohum kabuğu sert olanlar endosperm (besin dokusu) tabakasına zarar vermeden zımparalanmıştır. Bu aşamada daha önceki 14 güne ilaveten 7 gün daha deneme devam etmiş ve çimlenen tohum sayıları kaydedilmiştir (Üremiş ve Uygur, 2002; Üremiş ve Uygur, 2004).

2.3. Verilerin Değerlendirilmesi

Denemeler tesadüf parselleri deneme desenine göre beş tekerrürlü, iki tekrarlı kurulmuş olup sonuçlara varyans analizi uygulanmıştır. Uygulamalar arasındaki farklar ise Duncan Çoklu Karşılaştırma testi ile belirlenmiştir ($P \leq 0,05$). Yapılan istatistik analizine göre iki tekrarlama arasında istatistikî fark görülmediğinden veriler birleştirilerek kullanılmıştır. Değerlendirmede çimlenme yüzdesi hesaplanmıştır.

3. Araştırma Sonuçları ve Tartışma

3.1. Toprak derinliğinin I. triloba ve C. arvensis tohumlarının çıkışları üzerine etkilerinin belirlenmesi

Çıkış oranları incelendiğinde en iyi çıkış derinliğinin her iki tür için de 2, 5 ve 10 cm derinliklerde olduğu tespit edilmiştir (*Tablo 1*). CONAR için tohum çıkışı 2 cm derinlikte %84.00, 5 cm derinlikte %78.40 ve 10 cm

derinlikte ise %78.00 olarak belirlenmiştir. CONAR tohumlarının çıkış oranı derinlik miktarı arttıkça hızlı bir şekilde düştüğü, 15 ve 20 cm derinlikte çıkış oranlarının sırasıyla %24.80, %3.60 olduğu saptanmıştır. IPOTR için ise 2 cm derinlikte %96.80, 5 ve 10 cm derinlikte ise %93.60 olduğu tespit edilmiştir. IPOTR tohumlarının çıkış oranları derinlik arttıkça azaldığı ancak, CONAR tohumlarına göre çıkış oranının daha yüksek olduğu belirlenmiştir. IPOTR tohumları 15 cm'de %81.60 oranında çıkış yaparken, 20 cm derinlikte bu oran %45.20 olmuştur (*Tablo 1*).

Tablo 1. CONAR ve IPOTR tohumlarının gömülme derinliklerine göre çıkış oranları (%)

Table1. Emergence rates of CONAR and IPOTR seeds by burial depth (%)

Derinlikler (cm)	CONAR* Çıkış Oranları (%)	IPOTR Çıkış Oranları (%)
2	$84.00 \pm 1.98^{a*B**}$	$96.80 \pm 1.55^{aA***}$
5	$78.40 \pm 2.25^{a*B**}$	93.60 ± 1.48^{aA}
10	$78.00 \pm 2.94^{a*B**}$	93.60 ± 2.25^{aA}
15	$24.80 \pm 3.31^{b*B**}$	81.60 ± 3.22^{bA}
20	$3.60 \pm 4.84^{cB**}$	45.20 ± 5.63^{cA}

*Her sütun kendi içerisinde değerlendirilmiş olup, aynı harfi taşıyan uygulamalar arasındaki fark önemsizdir ($P \leq 0.05$).
** Her satır kendi içerisinde değerlendirilmiş olup aynı harfi taşıyan uygulamalar arasındaki fark önemsizdir ($P \leq 0.05$).

CONAR'ın çıkış derinliği ile ilgili Asgharipour (2011)'un sera koşullarında yaptığı bir çalışmada 0.5 ile 6.5 cm arasında 13 farklı derinlik çalışmasında tarla sarmaşığının 6 cm'ye kadar çıkış gösterdiği, en iyi çıkışın 0.5-1.5 cm arasında olduğunu ve derinlik arttıkça tohumların çimlenme yüzdelerinin azaldığı ve 6 cm'den daha derinde çimlenmediğini bildirmiştir. Bu sonuçlardan farklı olarak çalışmamızda 20 cm derinlikte %3.60 oranında çimlenmenin olduğu tespit edilmiştir. Çimlenme oranındaki bu farklılığın sebebinin tohumun fizyolojik özellikleri, kullanılan tohum yaşı, toprak tipi ve sıcaklık vb. nedenlerle olabileceği düşünülmektedir.

Bu çalışmada benzer şekilde IPOTR'nin farklı derinliklerden çıkışıyla ilgili bir çalışmada 10 cm'den fazla olan derinliklerden çimlenme oranının azaldığı tespit edilmiştir (Gaungoo ve ark., 2010). Aynı cinse ait *I. hederacea* türünün farklı derinliklerde çimlenme oranlarının incelendiği çalışmada 1 ile 10 cm derinlikte çimlenme yüzdeleri arasında istatistiksel olarak fark olmadığı bildirilmiştir (Siahmarguee ve ark., 2020). Chauhan ve Abugho (2012) IPOTR'nin farklı derinlikte gömülü tohumlarla yaptığı bir çalışmada toprak derinliği arttıkça çimlenme oranının azaldığı, maksimum çimlenmenin toprak yüzeyinde olduğu bildirilmiştir. Çalışmamızda da toprak derinliği arttıkça çıkış oranının azaldığı belirlenmiştir. Tohumun fizyolojik özellikleri, kullanılan tohum yaşı, toprak tipi ve sıcaklık gibi birçok biyotik ve abiyotik faktörler nedeniyle bu farklılıkların olabileceği düşünülmektedir.

3.2. I. triloba ve C. arvensis tohumlarının canlılık oranlarının zaman içerisindeki değişiminin belirlenmesi

CONAR ve IPOTR türlerinin topraktaki yaşam sürelerinin belirlenmesi amacıyla yapılan bu çalışmada toprağın 10 ve 20 cm derinliğine gömülü tohumlar 6, 12, 18, 24, 30. aylarda çimlenme denemelerinde kullanılmak üzere bulundukları yerden çıkarılarak çimlenme oranları (%) belirlenmiştir.

Şekil 1'de CONAR yabancı ot türünün tohumlarının 6, 12, 18, 24 ve 30. aylardaki çimlenme oranı verilmiştir. CONAR tohumlarının çimlenme oranı 10 cm derinlikte 6. ayda %90.10 olarak belirlenirken, 12. ayda %76.90, 18. ayda %54.60, 24. ayda %30.80 ve 30. ayda %15.40 olarak belirlenmiştir. Çimlenme oranı 20 cm derinlikte 6. ayda %87.50 iken 12. ayda %72.70, 18. ayda %46.00, 24. ayda %20.20 ve 30. ayda %9.50 olarak tespit edilmiştir. Sonuçlara göre ülkemizin pek çok bölgesinde görülen ve ekonomik zarara sebep olan CONAR ile mücadele yöntemleri içerisinde derin toprak işlemenin kullanılabileceği üreticilerin bu doğrultuda uygulama şekli belirlemesine fayda sağlayacağı düşünülmektedir.

IPOTR için, elde edilen sonuçlara göre çimlenme yüzdeleri hesaplanmıştır (*Şekil 2*). Değerlendirme sonucunda 10 cm derinlikte çimlenme oranı 6. ayda %93.90 olarak belirlenirken 12. ayda %74.90, 18. ayda %42.90, 24. ayda %23.30 ve 30. ayda %10.10 olarak saptanmıştır. Çimlenme oranı 20 cm derinlikte 6. ayda %85.20 iken 12. ayda %60.60, 18. ayda %41.80, 24. ayda %16.50 ve 30. ayda %7.60 olarak belirlenmiştir. Her iki derinlikte bulunan tohumların canlılık oranları zamana bağlı olarak azaldığı, özellikle 20 cm derinlikte bulunan tohumların çimlenme oranlarında 10 cm' ye gömülen tohumlara göre daha hızlı canlılığını kaybettiği tespit edilmiştir. Bu

bilgilere göre tek yıllık bir yabancı ot türü olan IPOTR ile mücadelede toprak işlemenin yeni bulaşmaların engellenmesi şartıyla yabancı ot yoğunluğunda azalmaya neden olabileceği düşünülmektedir.

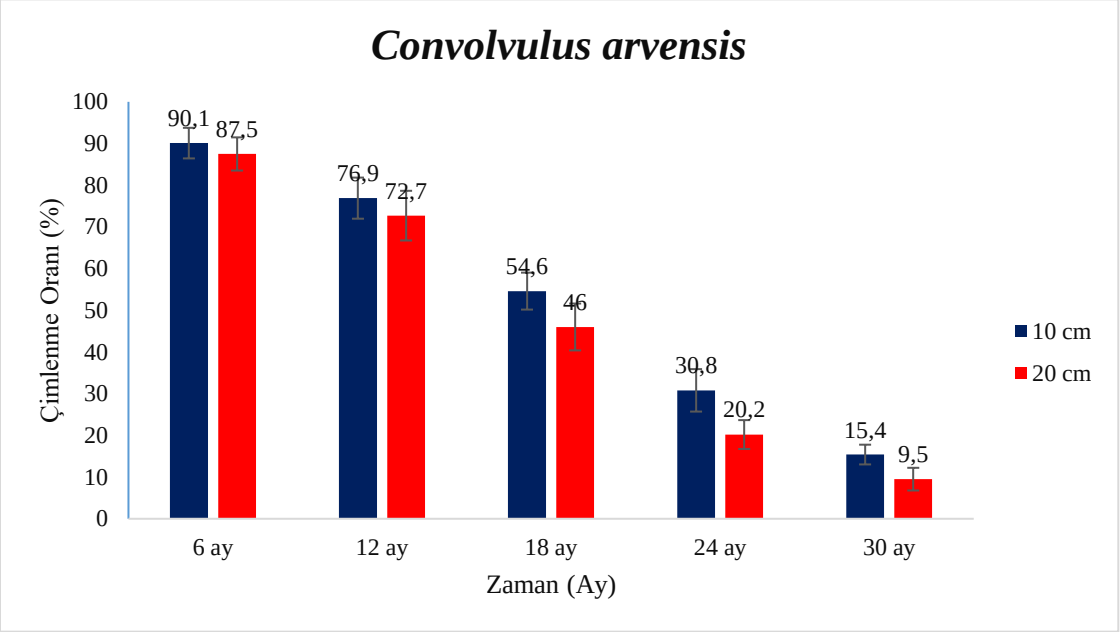

Figure 1. Germination rates of CONAR seeds at 6, 12, 18, 24 and 30 months (%)

Şekil 1. CONAR tohumlarının 6, 12, 18, 24 ve 30. aylardaki çimlenme oranları (%)

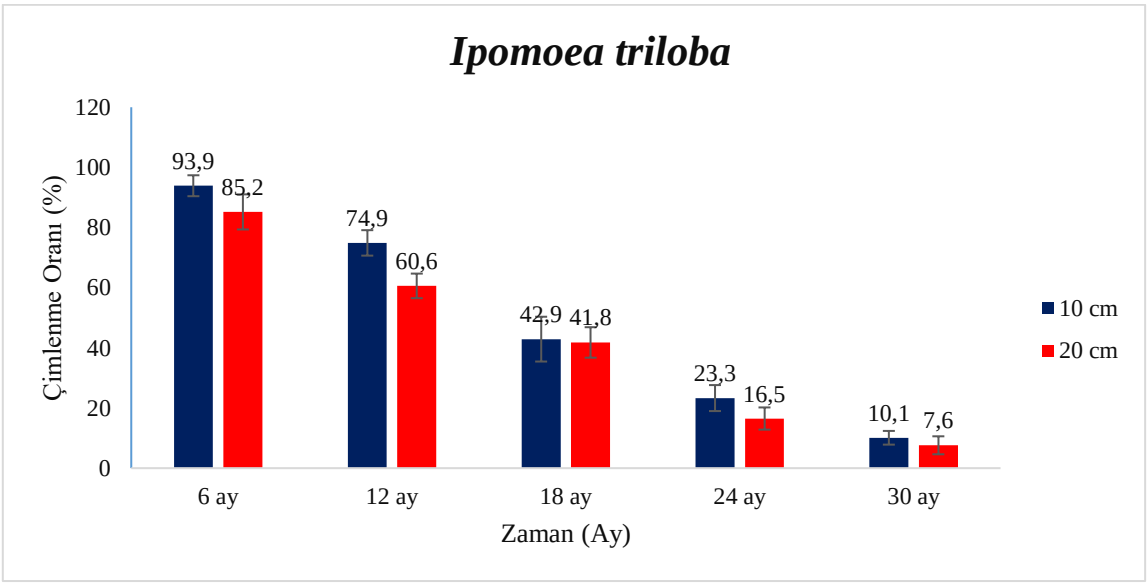

Figure 2. Germination rates of IPOTR seeds at 6, 12, 18, 24 and 30 months (%)

Şekil 2. IPOTR tohumlarının 6, 12, 18, 24 ve 30. aylardaki çimlenme oranları (%)

Toprakta tohum çimlenme sayısındaki düşüş; toprak derinliği, çimlenme, yaşlanma, böcek veya patojenlerin saldırısı gibi çeşitli nedenlerden kaynaklanan ölümlere bağlı olabileceği, tohumların büyük çoğunluğunun (%90'a kadar) bu nedenlerden biri nedeniyle canlılığını kaybettiği bildirilmiştir (Harper, 1977; Cavers, 1983; Cook, 1980). Çalışmamızda da tohumların zamanla canlılıklarını kaybettikleri belirlenmiştir. Toprakta tohum yaşam süreleri ve tohum bankası ile ilgili bilgiler yabancı ot yönetim programlarının oluşturulmasında önemli bir yer almaktadır (Martins ve Silva, 1994; Voll ve ark., 1996). Yapılan bir çalışmada toprağa gömülen tohumlara 24 ay sonra uygulanan çimlendirme denemelerinde geniş yapraklı yabancı otlardan 10 cm derinliğe gömülen *Cirsium arvense*

(L), Scop. *Rumex crispus* L. ve *Cardaria draba* (L) Desv. *Ipomoea hederacea* (L.) Jacq. tohumlarının çimlenme oranları sırasıyla %39.00, %73.00, %21.00, %3.00 olduğu, 20 cm derinliğe gömülen tohumların ise %29.00, %93.00, %28.00, %10.00 olduğu bildirilmiştir. Ayrıca, topraktaki tohum ömrü, uzun ömürlü türlerin hayatta kalması için yıllık veya iki yıllık türlerde olduğu kadar önemli olmadığı, çünkü çok yıllık yabancı otların çoğunda vejetatif yayılma ve tabii ki ilave hayatta kalma mekanizmaları sayesinde yıllar boyunca kalıcılık sağladıkları ifade edilmiştir (Burnside ve ark, 1996). Birçok araştırma göstermiştir ki bazı yabancı ot tohumları toprakta uzun yıllar canlılıklarını koruyabilmektedirler (Uygur ve ark., 1984; Özer ve ark. 1998, Üremiş ve Uygur, 1999; Kaya ve ark., 2010).

4. Sonuç

CONAR ve IPOTR tohumlarının 2, 5, 10, 15 ve 20 cm derinliklerde gömülü tohumlar ile optimum çıkış derinliği belirleme çalışması yapılmıştır. Çalışmamızda çimlenme oranları incelendiğinde en iyi çimlenme derinliğinin her iki tür için de 2, 5 ve 10 cm derinliklerde olduğu tespit edilmiştir. CONAR için tohum çimlenmesi 2 cm derinlikte %84.00, 5 cm derinlikte %78.40 ve 10 cm derinlikte ise %78.00 olarak belirlenmiştir. IPOTR için ise 2 cm derinlikte %96.80, 5 ve 10 cm derinlikte ise %93.60 olduğu tespit edilmiştir. IPOTR tohumlarının çimlenme oranları derinlik arttıkça azaldığı ancak, CONAR tohumlarına göre çimlenme oranının daha yüksek olduğu belirlenmiştir.

CONAR ve IPOTR türlerinin topraktaki yaşam sürelerinin belirlenmesi amacıyla yapılan çalışmada; CONAR tohumlarının çimlenme oranı 10 cm derinlikte 6. ayda %90.10 olarak belirlenirken, 12. ayda %76.90, 18. ayda %54.60, 24. ayda %30.80 ve 30. ayda %15.40 olarak belirlenmiştir. Çimlenme oranı 20 cm derinlikte 6. ayda %87.50 iken 12. ayda %72.70, 18. ayda %46.00, 24. ayda %20.20 ve 30. ayda %9.50 olarak tespit edilmiştir. IPOTR için, elde edilen sonuçlara göre çimlenme yüzdeleri hesaplanmıştır. Değerlendirme sonucunda 10 cm derinlikte çimlenme oranı 6. ayda %93.90 olarak belirlenirken 12. ayda %74.90, 18. ayda %42.90, 24. ayda %23.30 ve 30. ayda %10.10 olarak saptanmıştır. Çimlenme oranı 20 cm derinlikte 6. ayda %85.20 iken 12. ayda %60.60, 18. ayda %41.80, 24. ayda %16.50 ve 30. ayda %7.60 olarak belirlenmiştir.

Yabancı ot zararının azaltılmasında kullanılan en etkili yöntemin bulaşmayı engelleyici tedbirlerin alınması ve yeni tohum oluşumunun engellenmesi olduğu yabancı otlarla mücadelede önemli unsurlardan olduğu bilinmektedir. Çalışma sonuçlarına göre CONAR ve IPOTR ile mücadelede derin toprak işlemesi yapılarak tohum çimlenmesinin azaltılabileceği ve bu sayede yoğunluğun azaltılıp yeni tohum oluşumunun engellenebileceği düşünülmektedir. Ülkemiz için önemli yabancı ot türleri arasında bulunan ve bu çalışmada ele alınan CONAR ve IPOTR'nin toprakta yaşam sürelerini bilmek bunların gelecekte oluşturacağı ekonomik kayıplar hakkında bilgi vermesi yönünden çok önemlidir.

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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Hububat Üreticilerinin Lisanslı Depoculuğa Bakış Açısı: Yozgat İli Yerköy İlçesi Örneği

The Perspective of Grain Producers on Licensed Warehousing: The Example of Yerkoy District of Yozgat Province

Zehra Meliha TENGİZ[1*], Merve AYYILDIZ[2]

Özet

Lisanslı depoculuk sistemi, tarımsal ürünlerin sağlıklı koşullarda depolanmasını ve ticaretinin kolaylaştırılmasını amaçlayan bir sistemdir. Üreticiler açısından kaliteli ürünleri daha iyi fiyatlara satış imkanı sağlamasının yanında sanayiler için istedikleri zaman istenilen kalitede ürünü tedarik edebilme imkanı sunan çok yönlü yapıya sahiptir. Türkiye'de giderek yaygınlaşan lisanslı depoculuk sistemi tarım piyasaları için büyük önem arz etmektedir. Bu çalışmanın amacı lisanslı depoculuk sisteminin temelinde bulunan üreticilerin sistem hakkındaki tutum ve davranışlarının belirlenmesidir. Bu amaç doğrultusunda çalışmanın ana materyalini 2021-2022 üretim döneminde, Yozgat ili Yerköy ilçesinde oransal dağılım yöntemiyle belirlenen 76 hububat üreticisinden sağlanan birincil veriler oluşturmaktadır. Elde edilen bulgulara göre hububat üreticilerinin %55.3'ünün lisanslı depoları tercih ettiği, üretilen hububatın %51.4'ünün, ortalama 18.13 ton buğdayın ve 3.15 ton arpanın lisanslı depolarda depolandığı tespit edilmiştir. Üreticilerin lisanslı depoları tercih etmesinde, peşin ödeme alma imkanı, depoların yakınlığı ve yüksek fiyat ile satış olanağı gibi faktörlerin etkili olduğu belirlenmiştir. Diğer yandan bölgede, kendi deposunu (%19.4) kullanan üreticilerde bulunmakta ve bu üreticilerin ticaret amaçlı depolama yapmadığı, yemlik kullanımı için değerlendirdiği belirlenmiştir. Depolama yapılmayan hububat miktarının da yüksek olduğu (%29.2) dikkat çekmektedir. Bu durum son yıllarda yaşanan rekolte kaybı, ürün fiyatlarındaki aşırı yükselme ve artan maliyetlere bağlı nakit ihtiyacını karşılamak amacıyla üreticilerin doğrudan satışa yönelmesi ile ilgilidir. Araştırma sonucunda, üreticilerin lisanslı depolar hakkında yeterli bilgiye sahip oldukları ve lisanslı depolamayı faydalı buldukları gözlenmiştir. Buna karşın üreticilerin lisanslı depolama yapma oranı düşük seviyededir ve lisanslı depoları kullanan üreticilerin %40.5'inin devlet teşviklerinin kaldırılması durumunda depolara ürün bırakmayacağı sonucuna varılmıştır. Buna göre üreticilerin sisteme dahil olması ve lisanslı depoculuk sisteminin işlevsel bir yapı kazanmasında kısa ve orta vadede geliştirilecek ve iyileştirilecek teşvik ve destek politikalarına ihtiyaç duyduğu söylenebilir.

Anahtar Kelimeler: Lisanslı depoculuk sistemi, Hububat üretimi, Üretici davranışları, Gıda güvencesi, Yozgat ili

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Abstract

Licensed warehouse system is a system that aims to store agricultural products in healthy conditions and facilitate trade. It offers producers the opportunity to sell their quality products at better prices. It has a versatile construction that allows to supply with the desired quality product at any time for industrial. The licensed storage system, which is becoming increasingly widespread in Turkey, is very important for agricultural markets. The purpose of this study is to determine attitudes and behavior of producers on the licensed storage system. This study of cover the cereals producers in Yerköy district of Yozgat province by means of 2021-2022 production periods. For this purpose, a survey of 76 sample the producers were conducted using the Proportional Distribution Method. According to the findings, 55.3% of cereals producers prefer licensed warehouses. 51.4% of the cereals produced, 18.13 tons of average wheat and 3.15 tons of average barley were found to be stored in licensed warehouses. It has been determined that factors such as the possibility of paying in advance, the proximity of warehouses, and the possibility of high prices sales are effective in producers' preference for licensed warehouses. In addition, there are producers who use their own warehouse (19.4%) in the production of cereals. It has been determined that these producers do not store for commercial purposes, but evaluate their products for animal feed use. The study also found that the amount of cereals not stored is high (29.2%). This situation is related to the direct sales of the producers in order to meet the need for cash due to the loss of yield, excessive increase in product prices and increasing costs in recent years. As a result of the research, it was observed that producers have sufficient information about licensed warehouses and find licensed storage useful. In a addition, It has been determined that 40.5% of producers using licensed warehouses will not leave products in warehouses if government grants are removed. In this respect, the licensed warehouse system may be said to require incentive and support policies to be developed in the short and medium for include producers in the system and to gain a functional structure of the system.

Keywords: Licensed warehousing system, Cereals production, Producers’s behavior, Food security, Yozgat province

1. Giriş

Gıda güvencesi, gıda güvenliğini de içine alan, yeterli miktarda gıdanın varlığı, gıdaya fiziksel ve ekonomik ulaşım ve tüm bunlarda istikrarın sağlanmasını kapsayan bir döngüdür (Niyaz ve İnan, 2016). Gıda güvencesinin sağlanabilmesinde gıdanın bulunabilirliğinin, erişilebilirliğinin ve sürdürülebilir gıda yönetiminin bir arada bulunması gerekmektedir (Koca ve Somuncu, 2021). Kuşkusuz gıda güvencesi içerisinde en önemli unsurlardan biri gıdanın bulunabilirliği başka bir ifade ile yeterli miktarda gıdanın mevcut olmasıdır (Ekşi, 2020). Aksi takdirde gıda güvencesinin diğer unsurları sağlanabilse dahi gıda güvencesinden söz edebilmek mümkün olmamaktadır.

Gıda güvencesinin oluşturulmasında gıda arz ve talep dengesinin sağlanması gerekmektedir. Günümüzde değişen ve gelişen dünya konjonktüründe ülkelerin kendi ihtiyaçları olan gıda arzını güvence altına alma gerekliliği önem kazanmaktadır (Koca ve Somuncu, 2021). Bu nedenle çoğu dünya ülkesi arz ve talep dengesizliğinin önlenmesi ve fiyat istikrarını sağlanması için çeşitli çözümler arayışındadır (Ergun ve ark., 2022). Çok sayıda gelişmiş ve gelişmekte olan ülkeler üretimlerinin yanı sıra gıda güvencesini gerekçe göstererek farklı ülkelerden arazi satın alma ve kiralama yoluna gitmekte veya rekolte kayıplarının olduğu dönemlerde ithalat yoluna başvurmakta ve bu yollarla arz edilen ürünlerin sağlıklı koşullarda stoklamasını yapmaktadır (Ceyhan ve ark., 2018; Ataseven ve ark., 2020). Türkiye'de ise özellikle son yıllarda üretim politikaları incelendiğinde ithalat yönlü bir eğilim görülmektedir. Bu sürecin devam etmesi durumunda, üreticilerin tarımdan vazgeçme eğiliminin artacağı ve gıda arz güvencesinde dar boğazlar yaşanacağı öngörülmektedir. Bu bağlamda gıda arzını güvence altına almaya yönelik geliştirilebilecek politikalara ve yasal düzenlemelere ihtiyaç duyulmaktadır (Eştürk ve Ören, 2014).

Pek çok ülkede tarım ürünlerinde arz ve talep dengesizliğini önlenmek ve tarım piyasalarında istikrarı sağlamak amacıyla lisanslı depoculuk sistemi yaygın olarak kullanılmaktadır (Ceyhan ve ark., 2018; Yavuz, 2021; Ergun ve ark., 2022). Lisanslı depoculuk sistemi üretici, sanayici, tüccar ve kamu yararına sağladığı faydalarla çok yönlü bir yapıya sahiptir. Özellikle oluşabilecek fiyat istikrarsızlığının önüne geçen bir sistem olarak kullanımı depolama ile arz esnekliğini arttırmaktadır. Zira tarım ürünlerinin kısa dönemde arz esnekliği sıfıra yakın olduğundan depolama imkanının bulunmadığı durumlarda düşük fiyatlardan ürünlerini satmak zorunda kalmalarına ve gelir düşüklüğüne neden olmaktadır. Depolanan ürünlerin doğrudan lisanslı depolara, tüccarlara, ofise satışı yapılabildiği gibi borsa üzerinden işlem görmesi, üreticiyi satışını istediği zaman o günün fiyatından satma imkanı tanımaktadır. Sanayicilere ve tüccarlar ise stok maliyetine katlanmadan kaliteli ürün ve hammaddeyi zamanında tedarik etme imkanı sağlamaktadır (Tektaş, 2008; Karabaş ve Gürler, 2010; Tosun ve ark., 2014; Kaya, 2018).

Türkiye'de ise lisanslı depoculuk sistemiyle tarım ürünlerinde standardizasyonun geliştirilmesi, depolamada modernizasyona geçilmesi, fiyat istikrarlılığının sağlanması ve pazarlama olanaklarının iyileştirilmesi amaçlanmaktadır. Bu amaç doğrultusunda ilk olarak 2005 yılında 5300 sayılı kanununun kabulüyle lisanslı depoculuk sisteminin ilk adımları atılmıştır. (Kanun, 2005). Ancak yatırım maliyetlerinin yüksek olması, özel sektörün sisteme çekinceli yaklaşması gibi nedenlerden dolayı lisanslı depoculuk sistemi uygulamada uzun bir süre yer bulamamış, 2011 yılı itibariyle lisanslı depolar aktif hale gelmeye başlamıştır (Fırat Kalkınma Ajansı, 2011; Memiş ve Keskin, 2015). Özellikle son beş yıllık süreçte lisanslı depo sayılarında önemli bir artış söz konusudur. Süreç bir bütün olarak değerlendirildiğinde lisanslı depo işletme sayısı 164'e ulaşırken toplam 283 lisanslı depoya kuruluş izni verilmiştir. 2022 yılında bir önceki yıla göre lisans alan lisanslı depo kapasitesi %26 artış göstererek 8.6 milyon tona ulaşmıştır. Gelecek yıllarda mevcut kuruluş izni verilen depolar ile bu rakamın 16.3 milyon tona ulaşması beklenmektedir (Ticaret Bakanlığı, 2022).

Lisanslı depoların kullanımında ve sayısındaki artışta uygulanan teşvik ve yardımlarında rolü büyüktür (Sezal, 2017; Çelik, 2019; Doğan ve Bulut, 2021). Türkiye'de üreticilere nakliye, analiz ve depo kira desteği, Elektronik Ürün Senedi (ELÜS) karşılığında sıfır faizli kredi desteği ve vergi muafiyetleri sağlanmaktadır (Türkiye Ürün İhtisas Borsası [TÜRİB], 2022). Sağlanan destekler 2016 yılından 2020 yılına kadar ELÜS üzerinden gerçekleştirilen işlem hacminde 10.8 kat büyümeyi sağlamıştır (Yiğit, 2021).

Temel beslenmedeki yeri ile gıda güvencesi ve gıda arzında ayrı bir öneme sahip olan hububat (Taşçı ve ark., 2018), üretilmesi ve stratejik bir ürün olması nedeniyle lisanslı depolarda en çok depolanan üründür. Toplam lisanslı depo kapasitesinin %99'unu hububat alanı oluşturmaktadır. Yozgat ili sadece hububatta lisanslı 13 adet depo ile Türkiye'de en fazla lisanslı depoya sahip üçüncü ildir. Çalışmanın yapıldığı ve hububat üretiminin yoğun olarak gerçekleştirildiği Yerköy ilçesinde toplam 92130 ton lisans kapasitesine sahip iki lisanslı depo

bulunmaktadır (Ticaret Bakanlığı, 2022). Lisanslı depolar 2021-2022 sezonunda tam kapasite doluluğa ulaşmıştır (Anonim, 2022).

Hububatın lisanslı depolarda önemli bir orana sahip olması nedeni ile bu çalışmada hububat üretiminin yoğun olarak yapıldığı Yozgat ili Yerköy ilçesindeki üreticilerin lisanslı depolara eğilimi ve sisteme bakış açıları değerlendirilmiştir.

2. Materyal ve Metot

Çalışmanın ana materyalini 2021-2022 üretim döneminde Yozgat ili Yerköy ilçesinde bulunan hububat üreticilerinden anket yoluyla elde edilen veriler oluşturmaktadır. Yerköy ilçesinde 20 dekar üzeri ekilen araziye sahip üreticiler, çalışmanın ana popülasyonunu oluşturmaktadır. Örnekleme amacıyla çalışma alanında bitkisel üretim yapan işletme sayısı Çiftçi Kayıt Sistemi'nden elde edilmiştir. Yerköy ilçesinde toplam 3747 tarım işletmesi bulunmaktadır (Anonim, 2021). Çalışma kapsamında 20 dekar üzerinde 3509 tarım işletmesi, örneklemeye dahil edilmiştir. Söz konusu işletmelerin ortalama arazi varlığı 137.99 da ve standart sapması 258.33 olarak belirlenmiştir. Anket sayısı oransal dağılım yöntemine göre Eşitlik (1) kullanılarak 76 olarak belirlenmiştir (Çiçek ve Erkan, 1996).

$$n = \frac{N[\sum(Nh(Sh)^2)]}{N^2D^2 + \sum[Nh(Sh)^2]} \qquad \text{(Eş.1)}$$

Formülde; n: Örnek hacmi, Nh: h. tabakadaki birim sayısı, Sh: h. tabakanın standart sapması, N: Toplam birim sayısı, D: d/t, d: Ortalamadan belirli bir oran sapma (%5 sapma), t: %99 güven sınırındaki t değerini temsil etmektedir.

Çalışmada işletme sayıları 20-100 da (küçük işletme grubu, 37), 100.01- 250 (orta işletme grubu, 25) ve 250.01 ve üzeri (büyük işletme grubu, 14) olmak üzere üç tabakaya ayrılmıştır. Üreticilerden anket yoluyla elde edilen veriler işletme büyüklüklerine göre çapraz tablolar halinde verilmiştir.

3. Araştırma Sonuçları ve Tartışma

Hububat üreticilerine ait genel bilgiler *Tablo 1*'de verilmiştir. Çalışmada hububat üreticilerinin yaş ortalaması 49.05 olarak belirlenmiştir. Eğitim durumları dikkate alındığında üreticilerin %31.58'i lise mezunu, %28.4'ü üniversite mezunu ve işletmeler ortalamasında 10.50 eğitim yılı bulunmuştur. Ailedeki birey sayısı 4.24, tarımla uğraşan aile bireyi ortalaması 1.79 kişi olarak belirlenmiştir. Üreticilerin yılık tarımsal gelirleri işletme ortalamasında 129.490,13 TL'dir. Tarımsal gelir yanında üreticilerin %84'ü tarım dışı gelire de sahiptir. İşletmeler ortalamasında tarım dışı gelir ise 59.062,50 TL olarak tespit edilmiştir. Üreticilerin ortalama 18.12 yıllık hububat üretim deneyimine sahip olup, işletme büyüklüğü arttıkça deneyim süresinin arttığı görülmektedir.

Tablo 1. Hububat üreticilerine ait genel bilgiler

Table 1. General information about cereals producers

		İşletme Büyüklükleri Grupları			
		Küçük	**Orta**	**Büyük**	**Genel**
Yaş	Yıl	51.46	46.28	47.64	49.05
Eğitim	Yıl	10.00	10.40	12.00	10.50
Hane halkı büyüklüğü	Kişi	4.05	4.68	3.93	4.24
Tarımla uğraşan hane halkı büyüklüğü	Kişi	1.54	1.84	2.36	1.79
Yıllık ortalama tarımsal gelir	₺	52.547,30	118.480,00	352.500,00	129.490,13
Yıllık ortalama tarım dışı gelir	₺	39.625,00	51.600,00	123.333,33	59.062,50
Hububat deneyim süresi	Yıl	16.19	18.44	22.64	18.12

Tablo 2. Hububat üreticilerinin arazi yapısı

Table 2. Cereals producers' land structure

	İşletme Büyüklükleri Grupları			
	Küçük	**Orta**	**Büyük**	**Genel**
İşletme Arazisi (da)	65.13	171.20	382.86	158.55
Ortalama Parsel Sayısı (adet)	4.19	7.52	19.86	8.17
Ortalama Parsel Genişliği (da)	15.55	22.77	19.28	19.40

İncelenen tarım işletmelerinde ortalama arazi büyüklüğü 158.55 da olup, araziler ortalama 8.17 parselden oluşmaktadır. Ortalama parsel genişliği ise 19.40 dekardır (*Tablo 2*).

Yozgat ili Yerköy ilçesinin bitkisel üretim deseni hububat ve şeker pancarı ağırlıklıdır. İncelenen işletmelerde ise kuru tarımın yer aldığı sadece buğday ve arpa ekiminin yapıldığı belirlenmiş farklı bir ürünün ekimine işletmede yer verilmediği görülmüştür.

İşletmelerde hububat üretiminin yanı sıra elde edilen ürünlerin yıl boyunca ihtiyaç duyulduğu zaman kullanımı ve arz fazlasının olduğu hasat döneminde düşük fiyatlardan satılması yerine istenilen fiyatlardan satılabilmesi için depolamaya ihtiyaç duyulmaktadır. Hububat üreticisi işletmelerin ürün depolama bilgileri incelendiğinde %80.26'sının depolama yaptığı, geriye kalanının ise hasat sonrası depolama yapmadan satış yaptığı tespit edilmiştir. İncelenen işletmelerin %25.00'inin kendi depolarını, %55.26'sının ise lisanslı depoları kullandığı belirlenmiştir. İşletme büyüklüğünde ise üreticilerin küçük, orta ve büyük ölçekli işletmelerde sırasıyla %48.6'sının, %60.0'ının ve %64.3'ünün lisanslı depolara ürün teslim ettiği belirlenmiştir. Genel bir ifadeyle işletme ölçeği büyüdükçe lisanslı depolara ürün teslim eden işletme sayısı artmaktadır. Çelik (2019) ve Acıbuca (2021) yapmış oldukları çalışmalarda hububat üreticilerinin lisanslı depoyu tercih etme oranlarını sırasıyla %27.0 ve %36.6 olarak belirlemişlerdir. Araştırma bölgesinde üreticilerin lisanslı depoları tercih etme oranının diğer bölgeler ile kıyaslandığında yüksek olmasına karşın yeterli düzeyde olmadığı ifade edilebilir.

Üreticilerin lisanslı depoyu tercih etme nedenleri satış sonrası ödemenin hemen alınması (%31.96), mesafe olarak yakınlık (%26.80) ve yüksek fiyat (%24.23) iken; yeterince bilgi sahibi olmama (%26.47), daha yüksek fiyattan pazarlama imkanı (%23.53) ve hayvancılıkta yem girdisi olarak kullanılma gibi faktörlerin (%23.53) üreticiler tarafından lisanslı depoların tercih edilmemesinde etkili olduğu sonucuna varılmıştır. İşletme ölçeğinde lisanslı depoların tercihi incelendiğinde küçük ölçekli işletmelerde önem sırasında durum değişiklik göstermemiştir. Orta ölçekli işletmelerde peşin ödeme yapılması (%43.55) önemini korurken, yüksek fiyat (%27.42) mesafeye (%12.90) oranla daha çok önem kazanmıştır. Büyük ölçekli işletmelerde ise yakın mesafe (%35.29) en önemli tercih nedeni olurken, yüksek fiyat (%29.41) ve peşin ödeme yapılması (%23.53) daha az öneme sahiptir. Ceyhan ve ark. (2018) Ankara ve Kırıkkale de yapmış oldukları çalışmada benzer sonuçlara ulaşmış, lisanslı depoculuğun tercih edilmesinin nedenlerini tanıdık ve güvenilir bir yer olması, mesafenin yakınlığı ve yüksek fiyat olarak tespit etmiştir. Kırşehir ilinde gerçekleştirilen çalışmada tercih edilmeme nedeni olarak bilgi sahibi olunmaması (%22), daha yüksek fiyattan pazarlama imkanının olması (%13), sisteme güvenmeme (%8), ürün fiyatında düşme riski (%10) ve teşvikleri yetersiz bulma (%12) sıralanmıştır (Çelik, 2019).

Çalışma bölgesinde bulunan lisanslı depolarda sadece hububat depolaması yapılmaktadır. Depolama durumu ürün bazlı incelendiğinde toplam üretilen buğdayın %55.32'sinin arpanın ise %19.08'inin lisanslı depolara aktarıldığı tespit edilmiştir. Arpanın ağırlıklı olarak hayvan beslemede kullanımı depolama oranının düşük olmasında etkili olduğu söylenebilir. İşletme ortalamasında hububat depolama miktarları ise işletme büyüklük gruplarına göre *Tablo 3*'te verilmiştir. Lisanslı depolara bırakılan ortalama buğday ve arpa miktarları sırasıyla 18.13 ve 3.15 tondur. Lisanslı depolara bırakılan ürün miktarındaki artışla beraber ölçek içerisindeki bırakan sayısı da artış göstermektedir. Büyük ölçekli işletmelerde hayvancılığa da büyük oranda yer verilmesi sonucu üretilen arpanın lisanslı depolara teslim edilmeyerek kendi depolarında stoklandığı belirlenmiştir. Ayrıca işletmelerde hasattan hemen sonra depolama yapılmadan direkt satışların (%19.74) da gerçekleştiği belirlenmiştir. Hasat sonrası hemen satış yapanların depolama yapmama nedeninin ise, acil nakit ihtiyacından kaynaklandığı tespit edilmiştir.

Tablo 3. Üretim miktarının depolama durumuna göre dağılımı

Table 3. Distribution of production quantity according to storage condition

		İşletme Büyüklükleri Grupları							
		Küçük		Orta		Büyük		Genel	
		Ton	%	Ton	%	Ton	%	Ton	%
Buğday	Kendi deposu	2.41	17.80	6.03	19.83	11.86	13.50	5.34	16.29
	Lisanslı depo	6.38	47.12	15.68	51.56	53.57	60.99	18.13	55.31
	Depolama yok	4.75	35.08	8.70	28.61	22.41	25.51	9.30	28.37
	Toplam	13.54	100.00	30.41	100.00	87.84	100.00	32.78	100.00
Arpa	Kendi deposu	1.74	44.85	1.88	14.71	6.68	48.90	2.70	31.36
	Lisanslı depo	2.09	53.87	6.48	50.70	-	-	3.15	36.59
	Depolama yok	0.04	1.03	4.42	34.59	6.99	51.17	2.76	32.06
	Toplam	3.88	100.00	12.78	100.00	13.66	100.00	8.61	100.00

Çalışmada lisanslı depoları kullananların bıraktıkları ürün miktarındaki değişimde incelenmiş ve *Tablo 4*'te verilmiştir. Lisanslı depolara ürün bırakanların çoğunluğunun (%61.9) bıraktıkları ürün miktarında değişimin olmadığı görülmüştür. Bırakılan üründe artış olduğunu belirten işletmelerin artışın nedeni olarak ekim alanındaki artış, lisanslı depoların temiz ve üründe zaiyat olmadan geri alınabilmesi öncelikle sıralanmış, bırakılan üründe azalmanın nedenleri arasında ise kuraklık ve maliyetlerdeki artışından dolayı kullanılan girdinin azaltılması nedeniyle ürün verimindeki kayıplar belirtilmiştir.

Tablo 4. Üreticilerin lisanslı depolara bıraktıkları ürün miktarında değişim

Table 4. Change in the amount of products stored in licensed warehouses

	İşletme Büyüklükleri Grupları							
	Küçük		Orta		Büyük		Genel	
	Frekans	%	Frekans	%	Frekans	%	Frekans	%
Arttı	4	22.22	1	6.67	1	11.11	6	14.29
Azaldı	5	27.78	2	13.33	3	33.33	10	23.81
Değişmedi	9	50.00	12	80.00	5	55.56	26	61.90
Toplam	18	100.00	15	100.00	9	100.00	42	100.00

Üreticiler lisanslı depolara teslim ettikleri ürünü doğrudan lisanslı depolara satabildiği gibi aynı zamanda borsa ve Toprak Mahsulleri Ofisi (TMO) aracılığıyla da satış gerçekleştirebilmektedirler. Üreticiler borsalar aracılığıyla daha yüksek fiyattan ürününü satma şansına sahipken, alım garantisi ve güvenilirliği uzantısında ise depolanan ürünlerini TMO'ya satmaktadırlar. Araştırma bölgesinde işletmelerin %45'i aynı zamanda borsa üzerinden de işlem yapmaktadır. Borsa da işlem yapan işletmeler ölçek bazında değerlendirdiğinde, işletme büyüklüğüne bağlı olarak artış göstermektedir. Küçük, orta ve büyük ölçekli işletmelerin sırasıyla %16.7'si, %53.3'ü ve %88.9'u borsa da işlem yapmaktadır. Borsayı tercih etme nedenleri arasında yüksek fiyat verilmesi ve peşin ödeme yapılması gelmiştir. İşletmelerin daha çok TMO (%96)'ya satış yapmak aracılığıyla ürünü lisanslı depolarda beklettikleri tespit edilmiştir. Türkiye'de TMO tahıl ticareti başta olmak üzere tahıl piyasasındaki fiyatların belirlenmesi, ilanı ve ürün alımları yolu ile söz sahibidir (Konyalı ve Gaytancıoğlu, 2007; Keleş, 2019). TMO'yu seçimlerinde öncelikle devlet kuruluşu olmasından dolayı satış kanalı olarak tercih ettiklerini belirtmişlerdir. Ayrıca ödemelerin peşin yapılıyor olması, mesafenin yakınlığı ve her kalitede ürünün alınıyor olması bu kararlarında etkili olmaktadır. Nitekim TMO'nun buğdayda alım fiyatını açıklaması üreticilerin ürünü doğrudan veya depolarda bekleterek ofise satmalarını sağlamaktadır. Bu durum hasat döneminde hububatta arz fazlası nedeniyle oluşabilecek fiyat düşüşlerinin önlenmesi ve piyasanın dengelenmesi ile üreticilerin daha iyi koşullarda ürünlerini pazarlayabilmesi sağlanmaktadır. 2021 yılı için TMO'nun buğday da ton başına açıkladığı alım fiyatı 2 bin 250 TL iken, 6 bin 450 TL'ye çıkarılmıştır (TMO, 2022). 2021 sezonunda buğdayını bekleten veya yatırım yapanların ürünleri yüksek fiyatlardan satılmıştır. Ayrıca buğdayını ofise satan üreticiye 1000 TL'lik de prim desteği verilmiştir. TMO tarafından yapılan müdahale alım fiyatları ile işletmelerin hasat dönemine oranla daha yüksek fiyatlarla satış söz konusu olmuştur.

Çalışmada lisanslı depolara ürün bırakan işletmelerin yüksek bir kısmı (%90.5) hasat dönemine göre daha yüksek fiyatlardan satış yaptıklarını belirtmişlerdir. Ceyhan ve ark. (2018) çalışmasında lisanslı depolara ürün bırakan üreticilerin hasat dönemine oranla hasattan 8 ay sonra yaklaşık %35 daha yüksek fiyatla ürün sattıklarını belirtmiştir.

Lisanslı depoların yaygınlaşması ve daha etkin hale gelmesinde üretici görüşleri önem arz etmektedir. Bu bağlamda üreticilerin lisanslı depoculuğa yönelik bakış açıları likert ölçeğinde değerlendirilmiş ve *Tablo 5*'te sunulmuştur. Lisanslı depoculuk sistemine bakış açıları kullanan ve kullanmayan hububat üreticilerine göre işletme büyüklük grupları arasında belirgin farklılıklar olmadığı için karşılaştırma yapılmamıştır. Lisanslı depo kullanan üreticiler sistemin üreticiye güvenli ve temiz bir depo imkanı sağladığını, ürününü ihtiyacı olduğu zaman kullanabilme, depolama süresi boyunca kalitenin korunabildiği ve hasat döneminde düşük fiyattansa depolama ile ürün senedi alarak yüksek fiyattan satabilme imkanının olduğunu ifade etmişlerdir. Lisanslı depoların kuruluş amaçlarından biri olan fiyat istikrarının korumasında yeterlilik yargısına üreticiler katılım sağlamıştır. Nitekim Kırşehir ilinde gerçekleştirilen benzer bir çalışmada üreticilerin lisanslı depoların fiyatlama politikasından memnun (%80) oldukları belirlenmiştir (Doğan ve Bulut, 2021). Sistem ile ilgili önyargılardan biriside sınıflamanın doğru yapılmıyor olduğu düşüncesidir (Ceyhan ve ark., 2018). Lisanslı depoculuk sistemini kullanan işletmelerin sınıflandırmanın doğru yapıldığı görüşü hakimken (3.86), bu oran sistemi kullanmayan işletmelerde daha düşüktür (3.77). Aydın ilinde gerçekleştirilen çalışmada ise üreticilerin standartlara uygun sınıflama yapılma durumuna katılım düzeyi daha yüksek (3.89) bulunmuştur (Güney Ege Kalkınma Ajansı [GEKA], 2014). Ancak ürünlerin kalitesine göre sınıflandırılması Ticaret Bakanlığı kontrolünde ve bakanlık tarafından belgelendirilen yetkili sınıflandırıcılar tarafından gerçekleştirilmektedir (Kanun, 2005). Depolama sistemini kullanmayan üreticilerin ifadelere genel katılımda kararsız oldukları görülmektedir. Bu durum lisanslı depoculuk sisteminin yeteri kadar bilinmediğinden ve yaygınlaşmamasından kaynaklandığı düşünülmektedir. Nitekim üreticilerin %44.7'si lisanslı depoların yaygınlaşmadığı görüşündedir. Genel olarak lisanslı depoculuk sistemine bakış açıları değerlendirildiğinde hububat üreticilerin sistemin sağlamış olduğu avantajları bilmeleri sonucunda depo kullanımının artacağı söylenebilir. Halihazırda sistemin içinde olan üreticilerin avantajlar konusunda bilgi düzeylerinin yüksek olduğu, kullanmayanların ise avantajlar konusunda kısmen bilgi sahibi olduğu görülmektedir. Kırşehir ilinde gerçekleştirilen çalışmada üreticilerin %35'inin lisanslı depoların avantajları hakkında bilgi sahibi olduğu saptanmıştır (Çelik, 2019).

Lisanslı depoculuk sistemi içerisine giren işletmeler için bazı devlet teşvikleri söz konusudur. Bu nedenle teşvikler konusunda üreticilerin bilgi düzeyi ve sisteme dahil olma üzerindeki etkilerinin incelenmesine gerek duyulmuştur. Lisanslı depoları kullanan üreticilerin çoğunluğunun, kullanmayan üreticilerin ise yaklaşık yarısının teşvikleri bildiği söylenebilir. Lisanslı depoculuk sistemini kullanan işletmelerin; sistemin devlet güvencesi altında olduğu (%92.9), %2 stopaj muafiyeti (%92.9), bağkur muafiyeti (%88.1), depo kira+nakliye+analiz desteği (%90.5), faizsiz kredi imkanı (%66.7) ve kalite kaybına uğramadan geri çekebilme imkanını (%83.3) bildiği belirlenmiştir. Sistemi kullanmayan işletmelerin ise teşvikleri kısmen bildiği ve sırasıyla bilme durumlarındaki dağılımın %55.9, %44.1, %52.9, %4.2, %23.5 ve %44.1 olduğu görülmüştür. Bu durum lisanslı depo kullanan ve kullanmayan ayırmaksızın teşvikler ile ilgili yeteri kadar işletmelerde bilgi seviyesine ulaşılmadığını göstermektedir. Doğan ve Bulut (2021) üreticilerin en fazla önem verdikleri teşvik/destekleme araçlarını %2 stopaj indirimi, devlet güvencesinin olması ve kira+nakliye+analiz desteği olarak belirtmiştir. Çelik (2019) ise devlet güvencesinde olması, birçok desteğin veriliyor olması ve muafiyetlerin olması olarak sıralamıştır.

Teşvik ve desteklemelerin varlığı birçok konuda üreticilerin faaliyetlere katılımına teşvik etmektedir. Üreticilerin destekleme ve teşviklere verdiği önem "teşviklerin lisanslı depoculuk kullanımını arttırması" yargısı ile de desteklenmektedir (*Tablo 6*). Sistemi kullanan işletmelerin %54.8'i, kullanmayanların ise %58.8'i teşviklerin depo kullanımını arttıracağı görüşündedir. Bu durumda sistemi kullanan işletmelerin destek ve teşviklerin kaldırılması sonucu depolama kararlarını sorgulayacağı, sistemi kullanmayan işletmelerin ise teşvik ve desteklemelerin arttırıcı yönde yeniden düzenlenmesi sonucu sisteme dahil olabileceği söylenebilir. Nitekim lisanslı depolara ürün bırakan işletmelerin %59.52'si devlet desteklemelerinin kaldırılması sonucunda ürün teslim etmeye devam edeceklerini belirtmiştir. Ürün bırakmaya devam edeceğini belirten üreticiler borsa kanalıyla Türkiye'nin her yerine ürün satılabilmesi, ihtiyaç halinde hiçbir kayıp olmadan ürününü teslim alabilmesi ve hasat sonrası hemen ürününü depolama imkanının olmasından dolayı lisanslı depolara ürün bırakmaya devam edeceklerini belirtmiştir. Desteklemelerin kaldırılması sonucu ürün bırakmayacak (%40.48) üreticiler ise

bırakmama nedenleri arasında depo kirasının alınacak olması, ürünün direkt tarladan zahireciye satılabilme imkanı ve devlet desteğinin kalkması durumunda ürünün fiyatlandırmasına güven duymayacaklarını belirtmiştir.

Tablo 5. Üreticilerin lisanslı depoculuk sistemine bakış açıları

Table 5. Producers' perspectives towards licensed warehousing system

Farkındalık durumu	Lisanslı Depo Kullanan						Lisanslı Depo Kullanmayan					
	1	2	3	4	5	Ort. Puan	1	2	3	4	5	Ort. Puan
Güvenli ve temiz bir depo imkanı sağlar.	2.4	-	14.3	33.3	50.0	4.29	-	2.9	47.1	35.3	14.7	3.62
Ürünü istediği zamanda kullanma imkanı vermektedir.	-	-	11.9	35.7	52.4	4.40	-	2.9	44.1	41.2	11.8	3.62
Depolama karşılığında alınan ürün senedi vasıtasıyla kredi imkanını kolaylaştırır.	-	-	14.3	42.9	42.9	4.29	2.9	14.7	47.1	23.5	11.8	3.26
Kolay ve uygun fiyatla pazarlama imkanı sağlamaktadır.	-	4.8	11.9	31.0	52.4	4.31	-	8.8	44.1	35.3	11.8	3.38
Türkiye de lisanslı depo sistemi tam olarak anlaşılmıştır.	16.7	-	11.9	38.1	33.3	3.71	20.6	-	26.5	17.6	35.3	3.47
Lisanslı depolar fiyat istikrarını sağlamada yeterlidir.	11.9	-	14.3	52.4	21.4	3.71	5.9	-	52.9	14.7	26.5	3.56
Devlet teşvikleri lisanslı depo kullanımının artmasını sağlar.	9.5	19.0	16.7	31.0	23.8	3.40	-	-	41.2	35.3	23.5	3.82
Mevcut lisanslı depolar bölge kapasitesi için yeterlidir.	9.5		16.7	47.6	26.2	3.81	8.8	35.3	35.3	35.3	20.6	3.59
Lisanslı depolarda hasattan sonra depolanan ürünün satışa kadar geçen sürede kalitesi korunmaktadır.	9.5	-	21.4	47.6	21.4	3.71	11.8	-	44.1	20.6	23.5	3.44
Lisanslı depolarda analizler uzman kişiler tarafından doğru sınıflandırma yapılmaktadır.	9.5	-	19.0	38.1	33.3	3.86	2.9	-	44.1	23.5	29.4	3.77
Lisanslı depolarda depolama masrafları düşük olması karın artmasını sağlamaktadır.	14.3	-	16.7	31.0	38.1	3.76	23.5	-	35.3	11.8	29.4	3.24

Likert ölçeğinde değerlendirilmiştir. (Kesinlikle katılmıyorum-1, Katılmıyorum-2, Ne katılıyorum ne katılmıyorum-3, Katılıyorum-4, Kesinlikle katılıyorum-5)

4. Sonuç

Yozgat ili Yerköy ilçesinde gerçekleştirilen çalışmada üreticilerin %55.26'sı lisanslı depoları tercih etmektedir. Tüm işletme gruplarında lisanslı depolara ürün bırakan üreticiler yaklaşık eşit pay almaktadır. Üretilen hububatın %51.4'ünün, ortalama 18.13 ton buğday ve 3.15 ton arpanın lisanslı depolarda depolandığı tespit edilmiştir. Lisanslı depoları tercih eden üreticilerin depolama tercihlerinin TMO'ya satış işleminde kullandıkları ve tamamına yakınının TMO'ya satış yaptığı görülmektedir. Bu durum TMO'nun fiyat açıklamasından ve devlet kuruluşu olması kaynaklı güven duyulmasının bir sonucudur.

Lisanslı depoculuk sistemini kullanan üreticilerin lisanslı depoların sağlamış olduğu avantajlara yönelik farkındalığın oluştuğu, kullanmayan üreticilerin ise yeteri kadar bilgi sahibi olmaması nedeniyle ön yargılı oldukları belirlenmiştir. Bunun için bölgede üreticilere lisanslı depoculuğun sağlayacağı avantajları ile ilgili bilgilendirmelerin yapılması lisanslı depo tercihinin artması yönünde faydalı olacaktır.

Üreticilerin depolamada lisanslı depoları tercih etmeye devam edecekleri söylenebilir. Bu noktada desteklemelerin lisanslı depolara ürün bırakmaya devam etme üzerinde etkisinin de yüksek olduğu ifade edilebilir. Bu durumun en temel sebebinin yüksek üretim maliyetlerinden kaynaklandığı düşünülmektedir. Depolanan ürünlerin devlet güvencesi altında olması, sağlanan destek ve muafiyetler ile ürünlerin pazarlanması sonucunda kazanç sağlanabilmekte aksi takdirde yüksek üretim maliyetleri karşısında elde edilen gelir yetersiz kalabilmektedir. Sistemin ürün-fiyat mekanizmasının oluşturulması üzerinde etki yaratması beklenmektedir. Lisanslı depoculuk sistemi içinde yer alan işletmelerde sistemin fiyat oluşumuna destek sağladığı, diğer işletmelerin ise bu görüşe yeteri kadar katılmadığı görülmüştür. Ayrıca üreticilerde desteklemelerin kalkması sonucunda lisanslı depolarda fiyatlandırmanın doğru belirlenmeyeceği görüşü de vardır. Lisanslı depolara ürün bırakmayan hububat üreticilerinde ise yaklaşık yarısının teşviklere yönelik bilgi sahibi olduğu ve teşviklerin lisanslı depo kullanımını arttıracağı (%58.8) görüşündedir. Bu durum lisanslı depolara ürün bırakmayan üreticilerin teşviklerde yapılacak düzenlemeler ile depolama tercihlerinde değişmenin olabileceğini göstermektedir.

Diğer sektörlerde olduğu gibi tarım sektöründe de üreticiler kendi yararına olan oluşumları desteklemektedir. Ancak bu tür oluşumların yaygın olarak kullanımı, sistemin yararına yönelik bilgi düzeyinin arttırılması ile mümkün olabilmektedir. Lisanslı depoculuk sistemini kullanmayan üreticilerin sistem konusunda yeteri kadar bilgi sahibi olmadığı görülmektedir. Diğer önemli konu ise fiyat istikrarını sağlama noktasında üreticilerin acil nakit ihtiyacından doğan doğrudan satışlar sistemin istenilen seviye gelmediğini göstermektedir. Bu durumun önüne geçilmesinde tarımsal destek politikalarının uygulanması önem taşırken, depolama tercihlerinin lisanslı depolara yönelik değişimin sağlanması noktasında referans fiyatların belirlenerek üreticilerin fiyat düşüşlerinden korunması önerilmektedir.

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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Huş Ağacında Zarar Yapan *Nematus* (=*Croesus*) *septentrionalis* (L., 1758) (Hymenoptera: Tenthredinidae)'in Biyolojisi*

Biology of the *Nematus* (=*Croesus*) *septentrionalis* (L., 1758) (Hymenoptera: Tenthredinidae) Making Damage to *Betula pendula* RothTree*

Ahmet Buğra AYKAÇ[1*], Önder ÇALMAŞUR[2]

Öz

Huş ağacı, *Betula pendula* Roth. Dünyanın birçok bölgesinde yayılışa sahip olan ve 40 kadar türü bulunan önemli bir süs ve orman bitkisidir. Türkiye'de ise Kuzey Anadolu ve Doğu Anadolu bölgelerinde sıkça rastlanmaktadır. Huş ağacında zarar yaptığı bilinen birçok zararlı böcek türü bulunmaktadır. Bu çalışmaya konu olan zararlı türe ait örnekler Erzurum Merkez, Atatürk Üniversitesi Yerleşkesi, Merkez Teke deresi ve Aziziye yerleşkesinden alınmıştır. Çalışmanın amacı, *Nematus (=Croesus) septentrionalis*'in (L.,1758) huş ağaçlarında biyolojisini ve verdiği zararı belirlemektir. Örnekler 20 Haziran ile 20 Eylül 2019 ve 2020 tarihleri arasında toplanmıştır. Zararlının ekolojisini belirlemek için 2018 yılında bir ön durum değerlendirme çalışması yapılmıştır. Böceğin ergin örneği güneşli günlerde huş ağaçlarından, larvalar ise yedikleri dalların yaprakları ile birlikte budama makası yardımı ile kesilerek toplanmıştır. Pupalar toprağın 4 parmak (10-15 cm) derinliğe kadar çapa yardımıyla kazılmasıyla elde edilmiştir. Toplanan erginler kavanozlara yerleştirilmiş ve etil asetat ile öldürülmüştür. Ölü numuneler küçük bir karton kutuya aktarılarak, kutunun üzerine numunenin alındığı yer, ebadı ve alındığı tarih yazılmış ve laboratuvara getirilmiş, ardından bantlanarak etiketlenmiştir. Larvalar besleneceği yapraklarla birlikte dip kısmında toprak olacak şekilde desikatöre konulmuş, ardından su ile nemlendirilmiş emici pamuk zemine bırakılmış ve larvaları beslemek ve olgunlaştırmak için gün aşırı taze yapraklar laboratuvara getirilmiştir. Araştırma sonucunda *Nematus (=Croesus) septentrionalis*'in yumurtlama zamanı, larva evresi ve nesil sayısı doğal yaşam koşullarında belirlenmiştir. Bulaşıklık oranını elde etmek için hasarlı ağaç sayısı belirlenirken ayrıca yapraklardaki yumurta ve larva sayısını tespit etmek için rastgele seçilen ağaçların dallarındaki hasarlı yapraklar sayılmıştır. Bu çalışmada *Nematus (=Croesus) septentrionalis*'in biyolojisi ve huş ağaçlarına verdiği zarar incelenmiştir. Bu konu üzerinde çalışan gelecekteki araştırmacılar için faydalı bilgiler ortaya çıkarılmıştır. Erzurum koşullarında zararlının yılda bir nesil verdiği tespit edilmiştir.

Anahtar Kelimeler: *Nematus* (=*Croesus*) *septentrionalis*, Tenthredinidae, Hymenoptera, *Betula pendula*, Erzurum, Türkiye

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Abstract

Birch tree *Betula pendula* Roth. is an important ornamental and forest plant, which has a distribution in many parts of the world and has about 40 species. In Turkey, it is frequently encountered in North Anatolia and East Anatolia regions. There are many harmful insect species known to damage birch trees. Samples of the pest species that are the subject of this study were taken from Erzurum Center, Atatürk University Campus, Merkez Teke Stream and Aziziye Campus. The aim of the study is to determine the biology and damage of *Nematus* (=*Croesus*) *septentrionalis* (L., 1758) on birch trees. Samples were collected between 20 June and 20 September 2019 and 2020. A preliminary assessment study was conducted in 2018 to determine the pest's ecology. The adult specimen of the insect was collected from birch trees on sunny days. The larvae were collected by cutting with the help of pruning shears together with the leaves of the branches they ate. Pupae were obtained by digging the soil to a depth of 4 fingers (10-15 cm) with the help of a hoe. The collected adults were placed in jars and killed with ethyl acetate. Dead samples were transferred to a small cardboard box, brought to the laboratory, taped and labeled with the location, size and date of sample collection written on the box. The larvae were placed in a desiccator with the soil at the bottom together with the leaves they will feed on, absorbent cotton moistened with water was left on the ground and fresh leaves were brought to the laboratory every other day to feed and mature the larvae. As a result of the research, spawning time, larval stage and number of generations of *Nematus* (=*Croesus*) *septentrionalis* were determined under natural living conditions. While the number of damaged trees was determined to obtain the parasitism ratio, the damaged leaves on the branches of randomly selected trees were also counted to determine the number of eggs and larvae on the leaves. In this study, the biology of *Nematus* (=*Croesus*) *septentrionalis* and its damage to birch trees were investigated. Useful information has been uncovered for future researchers working on this topic. It was determined that the pest gave one generation per year in Erzurum conditions.

Keywords: *Nematus* (=*Croesus*) *septentrionalis*, Tenthredinidae, Hymenoptera, *Betula pendula*, Erzurum, Turkey

1. Giriş

Betula pendula Roth. (huş ağacı) pek çok ülkede doğal yayılışa sahip olan ve 40 kadar türü bulunan bir bitkidir. Ülkemizde ise Doğu Anadolu ve Kuzey Anadolu'da çokça bulunmaktadır (Yaltırık, 1993; Aksoy, 2014). *B pendula* diğer türlerden ayrı olarak fazlaca park bahçe ve yol kenarlarında yaygın olarak görülen bir süs bitkisidir. Boyları 20 m'ye kadar uzayan beyaz renkli, ince ve kâğıt gibi soyulan karakteristik gövdeye sahip ağaç türüdür. Genç dallar ince ve hassas olup aşağı doğru sarkmaktadır. Yaprak şekli baklava dilimi ile benzerlik göstermektedir. Erkek çiçek toplulukları, yaz mevsiminde ortaya çıkar; kışı açıkta geçirirler, ilkbaharda gelişimlerini tamamlarlar ve aşağı doğru sarkıktırlar (Jato ve ark., 2007; Piotrowska, 2008). Yurdumuzun yüksek kesimlerinde yayılış gösteren huş türlerinin yapılan incelemeler sonucunda 5000 yıl önce Doğu Anadolu Bölgesi'nde günümüze kıyasla daha geniş alanlarda yayılım gösterdikleri anlaşılmaktadır (Tanrıverdi, 1977). Ancak huş ormanları herhangi bir koruma tedbirinin alınmaması nedeniyle sürekli tahrip edilmişlerdir. Ülkemizde aşırı yapılaşma ve hatalı arazi kullanımları sonucunda birçok tarım alanları ve ormanlar yok edilmiştir. Bu sebeple bitki varlıklarının yaşam alanları zamanla azalmıştır. Bu sebep ile huşların yayılışı her geçen yıl azalmış, bugün korunmaya alınan bölgelerde nadir olarak kalmıştır (Tanrıverdi, 1977). Ekolojik etmenlerin dışında huş ağaçlarının azalmasında zararlı böceklerin payı da oldukça fazladır (Çanakçıoğlu, 1993). Huş ağaçlarında zararlı böcek türleri; Coleoptera [*Phyllodecta vitellinae* (L.), *Plagiodera versicolara* (Laich.), *Altica quercetorum quercetorum* (Foud.), *Crepidodera aurea* (Geoff.), *Apoderus coryli* L., *Deporaus betulae* (L.), *Rhagium inquisitor* (L.), *Scolytus intricatus* (Ratz.), *Agrilus viridis* (L.), *Hylecoetus dermestoides* (L.), *Prionus coriarius* (L.), *Rhagium bifasciatum* (Fabr.), *Melolontha melolontha* (L.)], Lepidoptera [*Arctia caja* (L.), *Autographa gamma* (L.), *Cerura vinula* (L.), *Phalera bucephala* (L.), *Leucoma salicis* (L.), *Collotois pennaria* (L.), *Deuteronomos quercaria* (Hbn.), *Erannis defoliaria* (Cl.), *Erannis marginaria* (Fabr.) ve *Phigalia pilosaria* (Schiff.),] ve Hymenoptera; [*Nematus* (=*Croesus*) *septentrionalis* (L.), *Vespa crabro* (L.), *Xiphydria camelus* (L.) ve *Pseudoclavellaria amerinae* (L.)] takımında toplanmaktadır Huş zararlıları içerisinde önemli bir yere sahip olan Symphyta alttakımı (Hymenoptera) 6 üstfamilyaya ait ve 14 familyadan oluşmaktadır (Gauld ve Bolton, 1988). Tenthredinoidea üstfamilyasına ait Tenthredinidae familyası gerek habitat gerekse de görünüş bakımından oldukça fazla çeşitlilik gösteren bir gruptur. Diğer tüm Symphyta familyasına kıyasla daha fazla tür sayısına sahiptir (Gauld ve Bolton, 1988). Selandriinae, Atelozinae, Nematinae, Heterarthrinae, Blennocampinae, Allantinae ve Tenthredininae olmak üzere yedi altfamilyadan oluşur (Taeger ve ark., 2018). En büyük altfamilya Tenthredininae'dir. Tenthredinidae (Testereli arılar) türlerinin ekvatordan vejetasyonun olabildiği en kuzeydeki noktalara kadar olan her kesimde görüldüğü, kuzey yarımkürenin ılıman iklime sahip bölgelerinde 6000'den fazla tür içerdiği, Kuzey Amerika' da 800, Kanada'da ise 600'e yakın türünün bulunduğu bildirilmiştir (Goulet ve Huber, 1993). Avrupa kıtasında yedi altfamilyaya bağlı yaklaşık 900 tenthredinid türünün bulunduğu belirtilmiştir (Gauld ve Bolton, 1988). Bazı ülkeler, Tenthredinidae tür sayısı bakımından ele alındığında; Arnavutluk' da 131, Almanya'da 696, İsviçre'de 537, Rusya'da 827, Romanya'da 496, İtalya'da 399, Bulgaristan'da 326, Yugoslavya'da 285 ve Yunanistan' da 114 türünün tespit edildiği bildirilmektedir (Liston, 1995). Hymenoptera takımında ekonomik bakımdan önemli zararlı türleri içeren en büyük familya durumundaki tenthredinid erginleri, çiçeklerdeki balözü (nektar) ile beslenmekte olup, larvaların birçoğu bitkilerin yapraklarını doğrudan yemek, bazıları sürgün ve meyvelerde galeri açarak, kimileri (özellikle Nematinae) gal oluşturarak, az sayıdaki bazı türler de yaprakları bükmek suretiyle zararlı oldukları kayıt altına alınmıştır (Gauld ve Bolton, 1988; Goulet ve Huber, 1993).

Tenthredininae familyası içerisinde *Nematus* (=*Croesus*) *septentrionalis* (L.) huş ağaçlarında başlıca potansiyel zararlı durumundadır. Zararlının larvaları *B. pendula* yaprakları ile beslenmektedir. Larvalar yeşilimsi sık siyah lekeli bir görünüme sahiptir. İlk dönemlerinde yaprak kenarına dizilmiş vaziyette beslenirken son dönemlerine doğru dağılarak tek tek beslenmekte ve yoğun popülasyon olduğunda zararın fazla olmasından dolayı ağaç tamamen çıplak bir görünüm almaktadır. Atatürk Üniversitesi Kampüsü'nde bulunan huş ağaçları üzerinde yoğun olduğunda belirli yıllarda birçok huş ağacının kurumasına neden olduğu gözlemlenmiştir. Bazı yıllar, özellikle 2002'de Erzurum'da birçok yerde popülasyon çok artmıştır. *Nematus* (=*Croesus*) *septentrionalis*'in konukçuları, *Alnus, Betula, Salix, Populus, Fraxinus, Sorbus* ve *Corylus* cinslerine bağlı, *Alnus glutinosa, A. incana, Betula alba, B. pubescens, B. pendula, Carpinus betulus, Corylus avellana, Fraxinus excelsior* ve *Salix pentandra* olarak kaydedilniştir (Liston 1995; Taeger ve Blank, 1998; Lacourt, 1999).Ülkemizde Tenthredinidae üzerinde yapılmış sistematik kapsamlı bir takım çalışmaların mevcut oluşu göz önüne alınarak (Çalmaşur, 2002; 2006; 2007; 2011; 2019; 2020), biyolojik çeşitliliğin fazla olduğu, Kuzeydoğu Anadolu Bölgesi'nde Tenthredinidae familyasına ait türlerin her birinin biyolojik çalışmalarının eksikliği ve önemli bir bitki olan huş ağaçları üzerinde zarara sebebiyet

vermesinden dolayı *N.* (=*Croesus*) *septentrionalis*'in biyolojisinin ve zararının ortaya koyulması amacı ile bu çalışma yapılmıştır.

2. Materyal ve Metot

2.1. Materyal

Çalışmanın örnek materyallerini, Erzurum Merkez (Atatürk Üniversitesi Kampüsü) ve Aziziye ilçesindeki tarım ve orman ağaçlarını kapsayan bölgelerden toplanan Tenthredinidae (Hymenoptera, Symphyta) familyasından *Nematus* (=*Croesus*) *septentrionalis*'e ait larva ve erginler oluşturmaktadır. Örneklemeler için 33-35 cm çaplı tül atrap, öldürme şişeleri, etil asetat, % 70'lik alkol, ephendorf tüpleri, yumuşak uçlu fırçalar, şeffaf polietilen torbalar, kese kâğıtları, plastik veya cam kavanozlar, kültür kapları ve diğer laboratuvar ekipmanları kullanılmıştır.

2.2. Yöntem

2.2.1. Çalışma alanının özellikleri

Erzurum Türkiye'nin rakımı yüksek (1900 m) soğuk illerindendir. Yaz mevsimi kurak ve sıcak, kış mevsimi ise yağışlı ve sert geçmektedir. Genelde sert karasal bir iklim hâkimdir. Yıllık ortalama yağış miktarı 453 mm'dir. En fazla yağışı ilkbahar ve yaz aylarında, en az ise kış aylarında almaktadır. Kışın yağışlar genel olarak kar şeklindedir ve kar yağışlı olan gün sayısı 50, karın yerde kalma süresi de 114 gün kadar olmaktadır. Ortalama yıllık sıcak 6.1°C'dir (Anonim, 2019).

2.2.2. Örneklerin toplanması

2019-2020 yılında, 20 Haziran–20 Eylül tarihleri arasında Erzurum Merkez, Atatürk Üniversitesi Kampüsü, Aziziye ilçe arazilerinden toplanmıştır. Zararlının biyolojisinin belirlenebilmesi için, 2018 yılında bilgilenmek amacı ile pilot bir çalışma yapılmıştır. Ergin örnekler, güneşli günlerde huş ağacı (*Betula pendula*) yaprakları üzerinden atrap kullanımına ek olarak elle de toplanmıştır. Yaprak üzerinde beslenen larvalar beslendikleri yaprak dalının sap kısımlarının budama makası yardımıyla kesilmesiyle alınmıştır. Pupalar huş ağacının kök ve taç izdüşümünden kazma yardımıyla 10-15 cm derinliğinde kazılarak alınmıştır. Huş ağacı üzerinden toplanan zararlı türün erginleri cam kavanoza konulmuş ve etil asetat kullanılarak öldürülmüştür. Öldürülen erginler, küçük kutulara aktarılmış, kutuların üzerine ergin örneğin toplandığı yer, rakım ve toplandığı tarih yazılmış, laboratuvar ortamında iğnelenme işlemi yapılmıştır. Taban kısmına toprak koyulan desikatöre beslendiği yapraklar ile birlikte larvalar alınmıştır, desikatör içerisindeki nemi sağlamak için su kullanılarak ıslatılmış pamuk konulmuş ve iki günde bir huş ağacının taze yaprakları ile larvaların beslenmesi sağlanmıştır. Pupalar şeffaf kutu içerisinde taban kısmına 10-15 cm toprak kalınlığı oluşturularak toprak yüzeyine konulmuş, üzeri 5-10 cm olacak şekilde toprak ile kapatılmış, ıslatılan pamuk ile nemlendirme sağlanmış ve şeffaf kutu üzerine havalandırma delikleri açılarak direkt güneşe maruz kalmayacak şekilde oda sıcaklığında muhafaza edilerek gözlenmiştir. Çalışma bölgesindeki şartlar göz önüne alınarak yumurta bırakma zamanları, yumurtanın açılma süresi, larva süresi ve nesil sayısı tespit edilmiştir ve bu tespitlere ilave olarak, bulaşıklık oranının belirlenmesi için larvalar tarafından zarara uğramış ağaçlar sayılmış ve zararlının bulunduğu ağaçlardan rastgele seçilen bir dalda bulunan zarar görmüş yaprak sayısı belirlenmiş ve yaprağa bırakılan yumurta ve larva sayıları da tespit edilmiştir.

2.2.3. Örneklerin değerlendirilmesi

Laboratuvara getirilen *N. septentrionalis* erginleri iğnenin yaklaşık 1/3'ü üstte kalacak şekilde ve alt kısıma yer, tarih, rakım ve toplayıcı ismini belirterek etiket bilgileri yazılarak iğnelenmiş, stereo mikroskop altında ergin bireyler gözlemlenerek cinsiyet ayrımı yapılmıştır. Dişi bireylerin yumurta bıraktığı yapraklar incelenmiş, yapraktaki yumurtaların sayımı ile ölçümü yapılmıştır. Yumurtadan çıkan larvalar besleme kafesinde kültüre alınmış, daha sonra gelişen larvalardan dönemine göre örnekler alınarak içerisinde %70'lik alkol bulunan cam tüplere aktarılmıştır. Leica EZ4 mikroskop altında dönemsel olarak alınan larva örneklerinin boy uzunluğu ile baş çapı ölçülmüş ve fotoğrafları çekilmiştir. Kültüre alınan larvaların daha sonra toprak içerisine geçerek pupa olmaları sağlanmıştır.

2.2.4. Erzurum ili 2019-2020 yılları iklim verileri

Böceklerin yaşam döngüleri için çok önemli bir etkiye sahip olan iklim değerleri (özellikle sıcaklık, nem ve yağış) Erzurum Meteoroloji 12. Bölge Müdürlüğü verilerine bakılarak elde edilmiştir (*Şekil 1*).

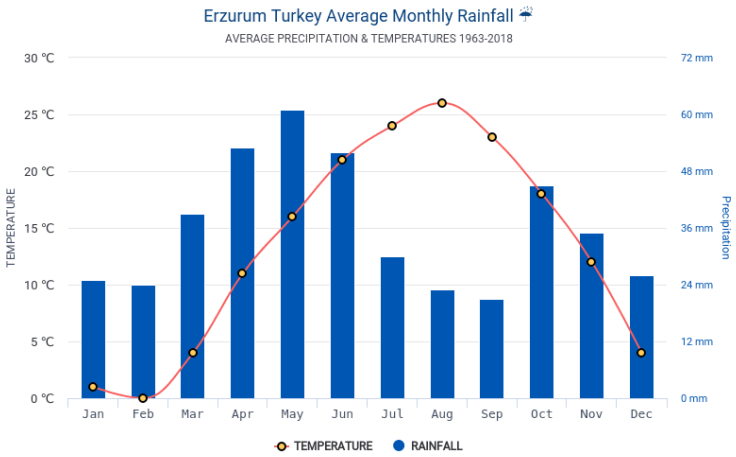


Figure 1. Annual climate values of Erzurum province

Şekil 1. Erzurum ili yıllık iklim değerleri

3. Araştırma Sonuçları ve Tartışma

3.1. Nematus (=*Croesus*) *septentrionalis Linnaeus türünün sistematikteki yeri*

Takım: Hymenoptera Linnaeus, 1758

Alttakım: Symphyta Emery, 1886

Üstfamiya: Tenthredinoidea Latreille, 1802

Familya: Tenthredinidae Latreille, 1802

Cins: Nematus (=*Croesus*) Leach, 1817

Tür: Nematus (=*Croesus*) *septentrionalis* Linnaeus, 1758

3.2. Nematus (=*Croesus*) *septentrionalis Linnaeus'un morfolojisi*

Erginler 8-10 mm boyunda, baş ve thorax siyahtır. Abdomenin basal 2 segmenti ile apikal 2 veya 3 segmenti siyah olup geri kalanı kısmı kırmızımsı kahverengidir. Kanatlar genelde saydam ancak ön kanadın ucu bulutludur. Larvaların uzunlukları 22 mm kadar ulaşabilmektedir. Larvaların baş kısmı parlak siyah vücutları sarımsı yeşil renktedir (Alford, 2016) (*Şekil 2*).

Figure 2. Nematus septentrionalis adult (a) and larvae (b)

Şekil 2. Nematus septentrionalis ergini (a) ve larvaları (b)

3.2.1 Ergin

Erginler 8-10 mm boyunda, baş kısmı ve thorax siyahtır. Abdomenin basal 2 segmenti ile apikal 2 veya 3 segmenti siyah renkli ve geri kalanı kırmızımsı kahverengidir. Kanatlar genelde saydam ancak ön kanadın ucu bulutludur (*Şekil 3*).

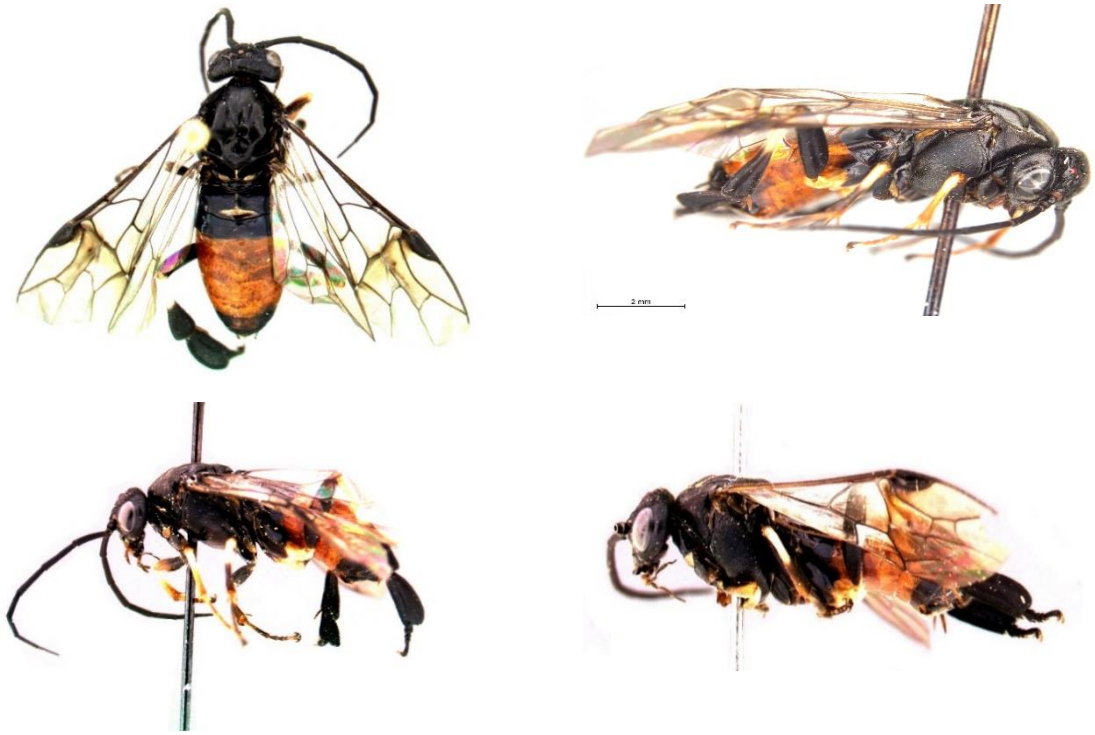


Figure 3. Adult of Nematus (=Croesus) septentrionalis

Şekil 3. Nematus (=Croesus) septentrionalis'in ergini

3.2.2 Yumurta

1.60-1.65 mm boyunda ve 0.55-0.65 mm eninde, beyazımsı renkte, su damlasına benzer silindirik şeklinde ve yaprak arka yüzeyinin ana damar ve yan kol damarları üzerine sıra şeklinde dizilerek bırakılmaktadır (*Şekil 4*).

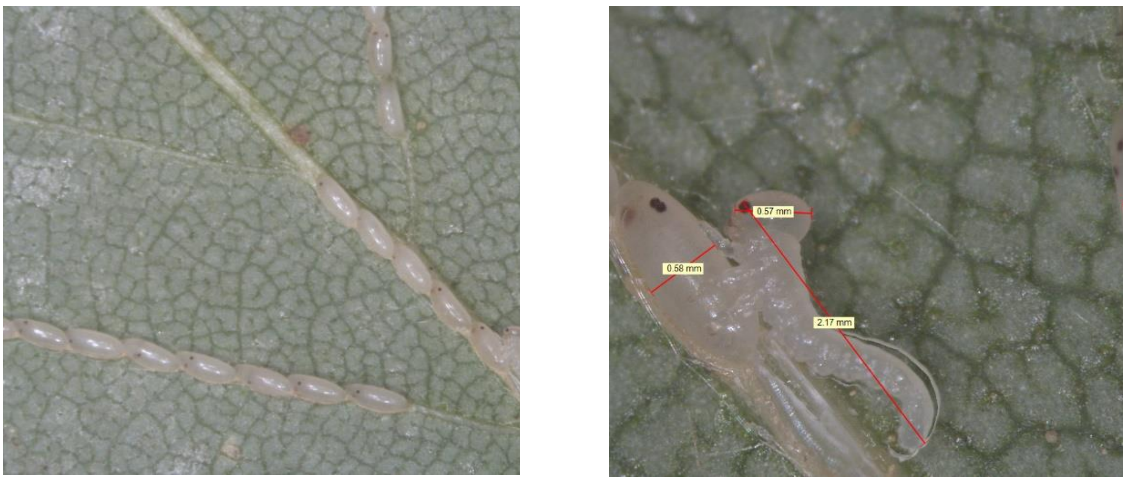
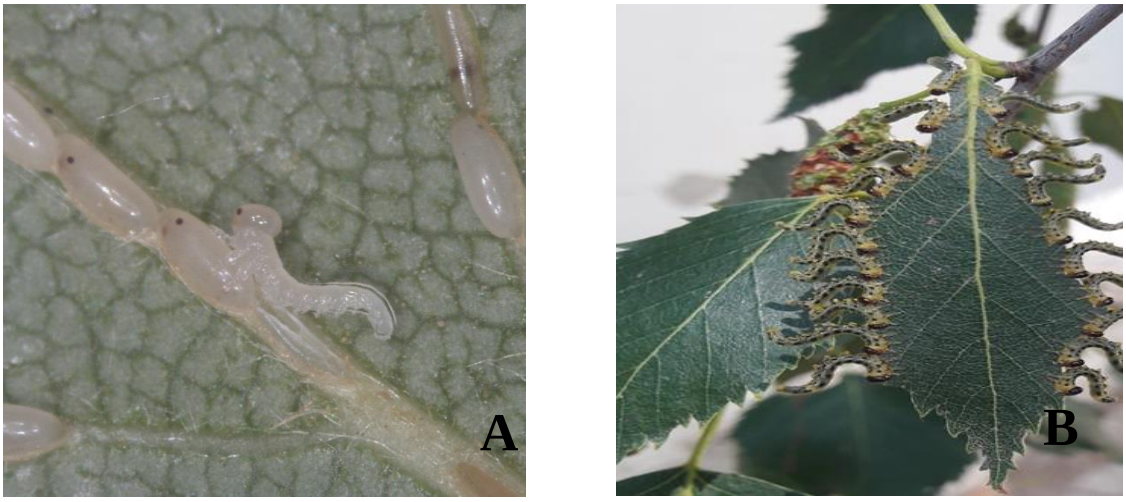


Figure 4. Egg and 1st instar larva of Nematus (=Croesus) septentrionalis
Şekil 4. Nematus (=Croesus) septentrionalis'in yumurtası ve 1. dönem larvası

3.2.3 Larva

Vücut yeşilimsi, dorsal ve lateral kısmı kesik şerit şeklinde siyah lekeli ve ventralde bacak kaide çevresi siyah lekeli, baş bölgesi geniş siyah, vücut arkaya doğru daralarak inceleşir ve üzeri sümüksü bir tabaka ile kaplıdır. Gövde uzunluğu 1. dönem larvada min 4.69 mm, max 5.68 mm; 2. dönem larvada min 4.83 mm max 6.92 mm; 3. dönem larvada min 14.88 mm max 18.15 mm ve 4. dönem larvada: min 20.97 mm max 23.94 mm boyundadır (*Şekil 5; 6*).



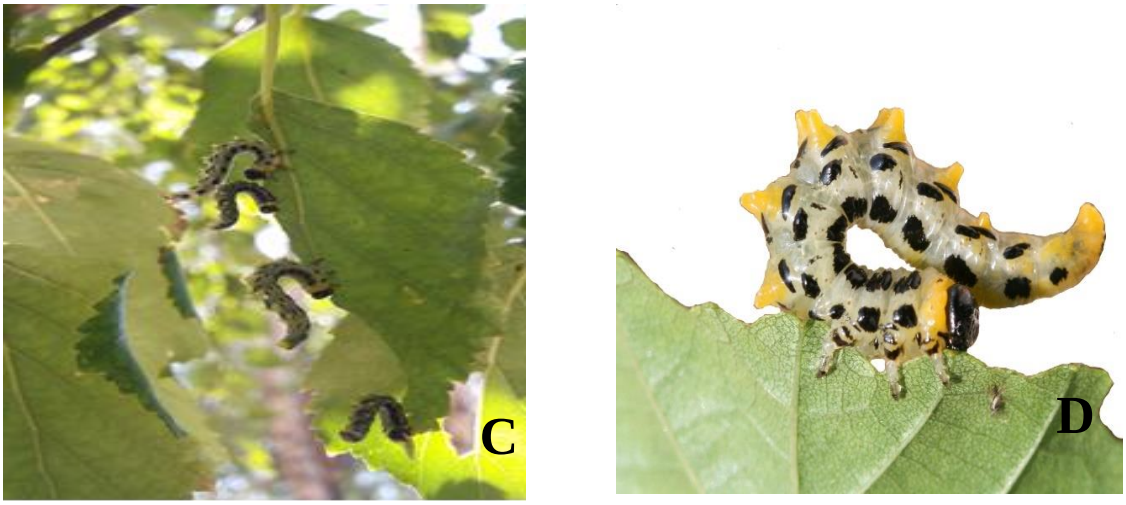


Figure 5. Nematus (=Croesus) septentrionalis larvae. A) 1st Term, B) 2nd Term, C) 3rd Term, D) 4th Term

Şekil 5. Nematus (=Croesus) septentrionalis larvaları. A) 1. Dönem, B) 2. Dönem, C) 3. Dönem, D) 4. Dönem

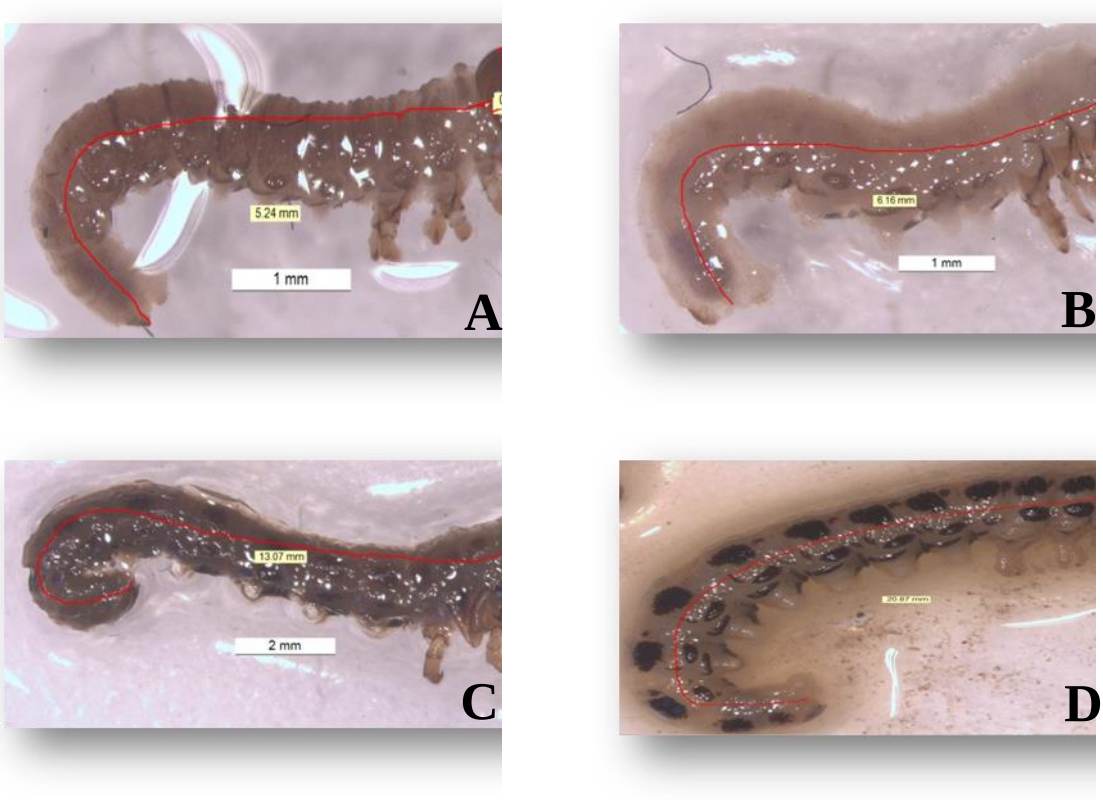


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Figure 6. Larvae of Nematus (=Croesus) septentrionalis. A) 1st Term, B) 2nd Term, C) 3rd Term, D) 4th Term

Şekil 6. Nematus (=Croesus) septentrionalis'in larvaları. A) 1. Dönem, B) 2. Dönem, C) 3. Dönem, D) 4. Dönem

3.2.4 Pupa

Larva genellikle, 10-15 cm (Dört parmak kalınlığı) derinliğindeki toprağa geçer, ince bir koza örer ve pupa olur. Ördüğü koza uzunca, silindirik şekilde ve içerisindeki pupa serbest halde bulunmaktadır (*Şekil 7*).

Figure 7. Pupa of Nematus (=Croesus) septentrionalis

Şekil 7. Nematus (=Croesus) septentrionalis'in pupası

3.3. Nematus (=*Croesus*) *septentrionalis Linnaeus'un biyolojisi ve zararı*

Zararlı türün biyolojisini tespit etmek için, 2018 yılında bir pilot çalışma yapılmıştır. *N. septentrionalis* kışı ördükleri koza içerisinde larva olarak geçirmiş, erginleri ise Erzurum ilinde Haziran ve Temmuz aylarında görülmüştür. Yumurtalarını konukçusunun yaprak damarları içerisine bırakmakta ve 5-14 gün içerisinde yumurtaların açılımı meydana gelmektedir. Larvalar yaprak kenarı boyunca thorax bacakları üzerinde vücudun geri kalan kısmı yay şeklinde kıvrım alarak toplu gruplar halinde beslenmekte, son dönem larvalar dağılarak soliter oburca beslenmesi pupa oluncaya kadar devam etmektedir. Toprak yüzeyine inen son dönem larvaları ördükleri koza içerisinde pupa olmaktadırlar. Pupalar toprak yüzeyine yakın (10-15 cm) derinlikte bulunmaktadırlar. Bu şekilde *N. septentrionalis* yılda 1 nesil vermektedir. Zarar boyutu yönünden larvalar yaprakların kenarlarından başlayarak oburca beslenmekte ve nihayetinde bitkiyi yapraksız bırakabilmektedir. Özellikle, genç ağaçların bu böceğin zararından etkilenip yapraksız kalmaktadır (*Şekil 8*). Huş ağacının yapraklarının larvalar tarafından tüketilmesinden dolayı fotosentez engellenerek bitki gelişiminin olumsuz etkilendiği gözlemlenmiştir.

Yapılan önceki çalışmalarda zararlının kış şartlarını kokon içerisinde larva döneminde geçirdiği ve erginlerinin ilk olarak mayıs haziran aylarında görülmeye başladıkları tespit edilmiştir. Yumurtalarını ise konukçu bitkinin yaprak damar içlerine bırakmaktadırlar. Yumurtalar yaklaşık 2 hafta içinde açılmaktadır. Genç larvalar yaprak kenarlarında pupa oluncaya kadar oburca ve toplu halde beslenmektedir. Pupalar toprak yüzeyine çok yakın yerlerde bulunmaktadır. Haziran sonu temmuz başı aylarında 2. dönem larvalar görülmekte ve en yoğun popülasyon yazın son aylarında görülmekle birlikte zararlı yılda 2 döl vermektedir (Ioachim ve Bobarnac, 1996; Alford, 2012).

Bulaşık ağaçlardan rastgele seçilen bir daldaki zarar görmüş ve sağlam yapraklar sayıldığında; bir dalda (10–28) 19 adet yaprağın (5-23) 14 adetinin (%73.68) zarar gördüğü saptanmıştır. Ayrıca laboratuvarda bir larvanın tükettiği yaprak sayısının (1-6) 3.5 adet olduğu belirlenmiştir. Çalışma sonucunda *N. septentrionalis*'in huş ağacında önemli derecede zarar yaptığı ve yoğunluğun oldukça fazla olduğu görülmüştür. Zararlı ilk çıktığı dönemde huş ağaçlarını tercih ettiği ve yapraklara beslenmek için yöneldiği de tespit edilmiştir.

N. septentrionalis kışı toprağın 10-15 cm derinliğinde, prepupa döneminde ördüğü ince bir koza içinde geçirmektedir. *N. septentrionalis*'in 2019 yılında Erzurum'da ergin çıkışı, 27 Haziran-5 Temmuz tarihinde görülmüştür. Ergin dişiler, yumurtayı çıkışı takip eden birkaç gün içerisinde huş ağacı yaprak ana damarları ve yan damarları içerisine ovipozitorü ile açtığı yara içerisine yerleştirmektedir. Bir yaprak üzerinde 30-45 adet yumurta bıraktığı tespit edilmiştir. Ortalama her bir ana damar üzerine 6-9, yan damar üzerine 7-12 adet yumurta bulunmaktadır. Yumurtalar 10-20 Temmuz tarihlerinde açılmıştır. Zararlının ilk dönem larvaları bulundukları yaprak üzerinde topluca beslendikten sonra diğer yapraklara geçerek beslenmelerine devam etmektedirler. Bu oburca beslenmesinin sonucu olarak yaprağın sadece ana damarları ve yan damarlarını bırakmaktadır (*Şekil 9*).

Figure 8. Damage of birch Nematus septentrionalis larvae

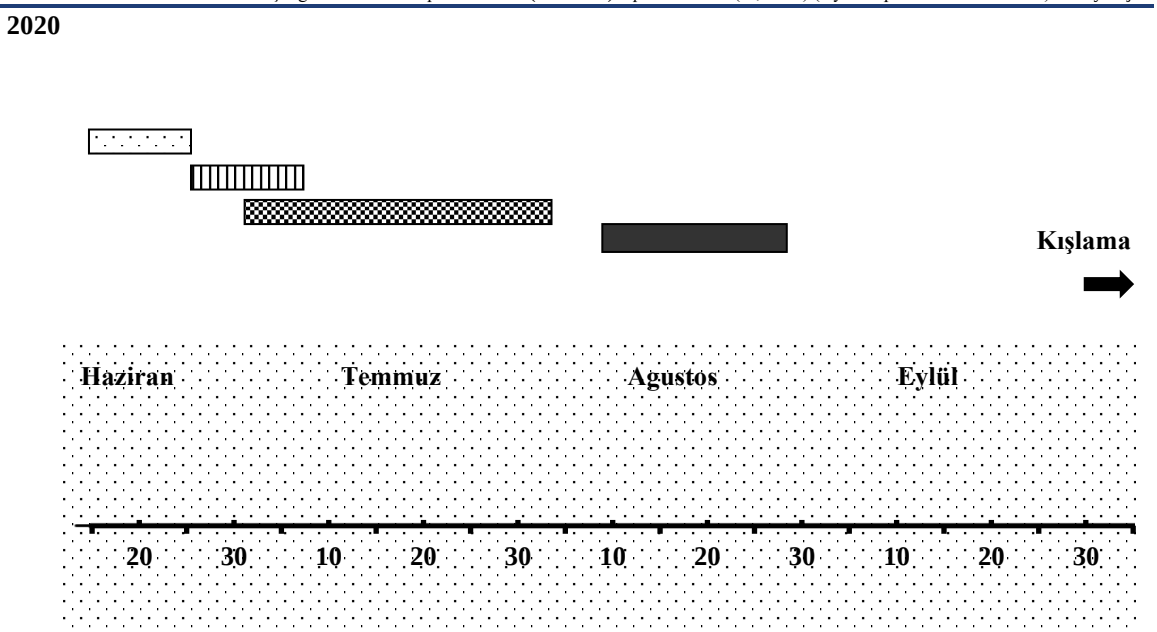
Şekil 8. Huşta Nematus septentrionalis larvasının zararı

Biyolojik dönemini tamamlayan larva 30 Ağustos-20 Eylül 2019 tarihinde toprağa geçerek pupa olmuştur. 2020'de ise 20-28 Haziran tarihinde erginler çıkış göstermiştir. Ergin dişiler, yumurtayı huş yapraklarının ana damarları ve yan damarları boyunca doku içerisine yerleştirmektedir. 2020 yılında bir yaprak üzerinde 20–30 adet yumurta sayılmıştır. Ortalama her bir ana damar üzerine 10-12, yan damar üzerine ise 8-13 adet yumurta tespit edilmiştir. Yumurtalar 5-13 Temmuz tarihlerinde açılmış, çıkan larvalar bulunduğu yaprağın önce üst epidermisi ile daha sonra diğer kısımları ile oburca beslenerek yaprağın sadece ana damarları ve yan damarlarını bırakmıştır. Biyolojik dönemini tamamlayan larva 25 Ağustos-15 Eylül 2020 tarihinde toprağa geçerek pupa olmuştur. Larvaların toprak altına geçme işlemi yaprakların dökülmeye başlamasına kadar devam etmiş, *N. septentrionalis'*in iki yıl süresince Erzurum şartlarında yılda 1 nesil verdiği de tespit edilmiştir. Zarar görmüş ağaçlarda çıplak bir görüntünün oluşması yanı sıra yapraklar elek gibi bir görüntü alarak zamanla kurumuş ve dökülmüştür (*Şekil 8*). Bulaşıklığın yüksek olduğu ağaçlarda tamamen kurumuş, çalışma bölgesindeki ağaçlarda zararlının oldukça yoğun olduğu tespit edilmiştir (*Tablo 1; 2*).

Zararlı türün larvası ile bulaşık ağaçlardan rastgele seçilen bir daldaki zarar görmüş ve sağlam yapraklar sayıldığında; bir dalda (10-28) 19 adet yaprağın (5-23) 14 adetinin (%73.68) zarar gördüğü saptanmıştır. Ayrıca laboratuvarda bir larvanın tükettiği yaprak sayısının (1-6) 3.5 adet olduğu belirlenmiştir. Çalışma sonucunda *N. septentrionalis'in* huş ağacında önemli derecede zarar yaptığı ve yoğunluğunun fazla olduğu görülmüştür. Zararlı ilk çıktığı dönemde huş ağaçlarını tercih ettiği ve yapraklara beslenmek için yöneldiği de tespitlerimiz arasındadır.

Figure 9. Damage caused by N. (=Croesus) septentrionalis larvae on the tree where it is dense

Şekil 9. N. (=Croesus) septentrionalis larvasının yoğun olduğu ağaçta meydana getirdiği zarar



Figüre 10. Life cycle of N. septentrionalis

Şekil 10. N. septentrionalis'in yaşam döngüsü

Tablo 1. N. septentrionalis'in yaşam döngüsü

Table 1. Life cycle of N. septentrionalis

2019 Yılı	27 Haziran- 5 Temmuz	10-20 Temmuz	10 Temmuz - 30 Agustos	30 Agustos -20 Eylül
Ergin Çıkışı	+	-	-	-
Yumurta Dönemi	-	+	-	-
Larva Dönemi	-	-	+	-
Pupa Dönemi	-	-	-	+

Tablo 2. N. septentrionalis'in yaşam döngüsü

Table 2. Life cycle of N. Septentrionalis

2020 Yılı	20-28 Haziran	5-13 Temmuz	5 Temmuz - 25 Ağustos	25 Agustos -15 Eylül
Ergin Çıkışı	+	-	-	-
Yumurta Dönemi	-	+	-	-
Larva Dönemi	-	-	+	-
Pupa Dönemi	-	-	-	+

3.4. Doğal düşman tespiti

2019 yılının Nisan ve Mayıs aylarında toprak altından elde edilen pupalardan 23 Mayıs 2019 tarihinde çıkış yapan *Netelia fuscicornis* Holmgren, 1860 (Hymenoptera; Ichneumonidae) pupa parazitoiti olarak elde edilmiştir (*Şekil 11*). Daha önce yapılan çalışmalarda bu parazitoitin, *Acronicta megacephala* Schiffermüller, 1776 (Lepidoptera)*; Acronicta psi* Linnaeus, 1758 (Lepidoptera); *Agrotis exclamationsi* Linnaeus, 1758 (Lepidoptera); *Agrotis segetum* Denis & Schiffermüller, 1775 (Lepidoptera)*; Anarta myrtilli* Linnaeus, 1761 (Lepidoptera); *Cerura vinula* Linnaeus, 1758 (Lepidoptera); *Cucullia asteris* Denis & Schiffermüller, 1775 (Lepidoptera); *Leucania obsoleta* Hübner, 1803 (Lepidoptera); *Lithostege farinata* Hufnagel, 1767 (Lepidoptera) türlerinden elde

edildiği tespit edilmiştir (Yu ve ark., 2016). *N. septentrionalis'*in bu parazitoit için yeni konukçu durumunda olduğu bu çalışma ile tespit edilmiştir.

Figure 11. Netelia fuscicornis (Holmogren, 1860) parasitoid from N. septentrionalis pupae

Şekil 11. N. septentrionalis pupalarından elde edilen Netelia fuscicornis (Holmogren, 1860) parazitoiti

3.5. Nematus (=*Croesus*) *septentrionalis Linnaeus'un yayılışı*

Asya'nın bazı bölgelerinde ve Avrupa'da karşılaşılan *N. septentrionalis* (Alford, 2012), Romanya ve Polonya'da fındık alanlarında görülmekte ve İngiltere'de yaban fındıkları üzerinde zararı tespit edilmiştir (Ioachim ve Bobarnac, 1996; Hill, 2012; Sadej ve ark., 2012). Zararlı tür yaygın ve ciddi bir problem olarak peyzaj ağaçlarında ve çalı formundaki bitkilerde, özellikle akçaağaç, huş ağacı, söğüt, kavak, üvez ve fındıkta zarar oluşturmaktadır (Alford, 2012). Ülkemiz faunası için ilk kaydı Benson (1968) tarafından yapılmış olup daha sonra farklı peyzaj bitkilerinde zarar oluşturduğu tespit edilmiştir (Çanakçıoğlu ve Mol, 1998; Çalmaşur ve Özbek, 2004). Bu tür 2015-2016 yılları arasında yapılan çalışmalar sonucuna göre Marmara bölgesi fındık bahçelerinde yeni bir zararlı tür olarak ortaya çıkmıştır (Tuncer ve ark., 2020).

4. Sonuç

Süs bitkilerinde ve orman ağaçlarında zararlı olan türler ile mücadelede en fazla kullanılan metot kimyasal mücadeledir. Ancak, kimyasal maddelerin insan, hayvan ve çevre sağlığı açısından dezavantajlarının olması, araştırmacıları zararlı ile mücadelede kimyasal yöntemlere alternatif olan çevre dostu metotları kullanmaya yöneltmiştir (Kuca ve Yağdı, 2020; Kotan ve Tozlu, 2021). Yapılan araştırmalara göre zararlı böcek popülasyonlarını EZE (ekonomik zarar eşiği) altında tutabilmek için özellikle biyolojik mücadele çalışmalarına büyük önem verildiği görülmektedir. Biyolojik mücadele çalışmalarında istenilen sonuçları elde edilebilmesi için hedef alınan türün biyo-ekolojik özellikleri ile birlikte doğal düşmanlarının da tespit edilmesi önemlidir Yapılan 2 yıllık survey çalışması sonucunda *N. septentrionalis*'in biyolojisi ve zararı takip edilmiş ve çok önemli bir biyolojik veri elde edilmiştir. Aynı zamanda zararlının doğal düşmanı da larva-pupa parazitoiti olarak tespit edilmiştir. Bu biyolojik çalışma, bölgedeki diğer zararlıların biyolojilerinin de ortaya konması gerekliliğine destek sağlayacak, daha fazla parazitoit türün belirlenerek biyolojik mücadelede kullanılma olanaklarının araştırılması fikrine ışık tutacaktır.

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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Ozmotik Dehidrasyon ve Mikrodalga Kurutma ile Birlikte Limon Halkalarının Kurutma Koşullarının Optimizasyonu*

Optimizing Drying Conditions of Lemon Rings Combined with Microwave Drying and Osmotic Dehydration*

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Öz

Kurutma süresini kısaltmak, ürün kalite özelliklerini iyileştirmek için ozmotik dehidrasyon ön işlemi ile birlikte birçok kurutma tekniği beraber kullanılmış olup, ozmotik kurutma ile mikrodalga kurutmanın birlikte kullanılması da bu yöntemlerden biridir. Ozmotik dehidrasyon ve mikrodalga kurutma işleminin birlikte kullanılarak kurutma yapılması mikrodalga kurutmanın olumsuz etkilerinin azaltılmasına yardımcı olur. Bu çalışmada, limon halkaların ozmotik dehidrasyon ön kurutma işlemi uygulanmasından sonra mikrodalga fırında kurutma koşullarının optimum seviyeleri Yanıt Yüzey Yöntemi ile belirlenmiştir. Yanıt Yüzey Yöntemin de kurutma parametreleri olarak ozmotik dehidrasyon süresi, çözelti derişimi, mikrodalga kurutma süresi ve mikrodalga gücü seçilmiştir. Optimize edilecek yanıtlar ise nem kaybı, çapsal büzülme oranı ve *b* renk değeri değişimi olarak belirlenmiştir. Dört faktör üç seviye için Box-Behnken tasarımına göre belirlenen 29 deney yapılmış ve yanıtlar alınmıştır. Dört faktörlü üç seviyeli Box-Benchken deneysel tasarım yöntemi, A ozmotik dehidrasyon süresi (60-180 dk), B çözelti derişimi (% 10-20 (w:v)), C mikrodalga kurutucuda kurutma süresi (2-6 dk) ve D mikrodalga güç seviyesi (100-300 W) aralığında uygulanmıştır. Elde edilen veriler ANOVA ile analiz edilmiş ve en yüksek R^2 değerlerini veren nem kaybı için 0,9853, çapsal büzülme oranı için 0,9861 ve *b* renk değeri değişimi için 0,9770 ile kuadratik modellerin uygun olduğu belirlenmiştir. Nem kaybı üzerine C, D, CD, A^2, C^2 ve D^2 değişkenleri, çapsal büzülme oranı üzerine A, C, D, D^2 değişkenleri ve *b* renk değeri değişimi üzerine ise A, C, D, A^2 değişkenlerinin etkisinin önemli olduğu görülmüştür. Kurutma parametrelerinin optimum değişken seviyelerinden olan ozmotik dehidrasyon süresi 120 dk, çözelti derişimi %15, mikrodalga kurutma süresi 4 dk ve mikrodalga güç seviyesi 200 W olarak bulunmuştur.

Anahtar Kelimeler: Limon, Kurutma, Ozmotik dehidrasyon, Mikrodalga kurutma, YYM, Box-Behnken

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Abstract

In order to shorten the drying time and improve product quality characteristics, with together osmotic dehydration pretreatment and many drying techniques have been used, and the combination of microwave drying and osmotic drying is one of these methods. Drying by using microwave drying and osmotic dehydration process together helps to reduce the negative effects of microwave drying. In this study, microwave oven optimum drying conditions were determined by Response Surface Method after osmotic dehydration pre-drying apply of lemon rings. In the Response Surface Method, drying parameters were chosen as osmotic dehydration time, solution concentration, microwave drying time and microwave power. The responses to be optimized were determined as moisture loss, diametrical shrinkage rate and b color value change. For four factor and three levels, 29 experiments determined according to Box-Behnken design were conducted and the answers were received. Box-Benchken experimental design method with four factors and three levels, A osmotic dehydration drying time (60-180 min), B solution concentration (10-20% (w:v)) was applied in the range of drying time (2-6 min) in C microwave dryer and drying power (100-300 W) in D microwave dryer. The obtained data were analyzed by ANOVA and it was determined that the quadratic models were suitable with 0.9853 for moisture loss, 0.9861 for diametrical shrinkage ratio and 0.9770 for b color value change, which gave the highest R^2 values. It was observed that the effects of C, D, CD, A^2, C^2 and D^2 variables on the moisture loss, A, C, D, D^2 variables on diametrical shrinkage rate and A, C, D, A^2 variables on b color value change. Drying parameters were found to be optimum variable levels, osmotic dehydration time was 120 min, solution concentration was 15%, microwave drying time was 4 min and microwave drying power was 200 W.

Keywords: Lemon, Drying, Osmotic dehydration, Microwave drying, RSM, Box-Behnken

1. Giriş

Gıda ürünlerinin kurutulması ile uzun süre bozulmadan korunabilen ürünler elde edilirken nem kaybı ile hacimde meydana gelen azalma sebebiyle taşıma, depolama ve paketleme maliyetleri azalır (Ertekin ve Yaldız, 1998; Güngör ve Özbalta, 1997; EİE, 1999). Hem tüketimden sonra arta kalan yaş meyve ve sebzenin değerlendirilmesinde, hem de katma değeri yüksek kuru ürün elde edilmesinde birçok kurutucu kullanılmaktadır. Kurutucular, ısı transferine göre kondüktif, konvektif ve radyant tipi olarak sınıflandırılmaktadır. Kondüktif tip kurutuculardа ısı doğrudan ürüne ulaşır. Kurutulacak ürün plaka veya metal bir duvarla kurutulmaktadır. Silindirik kurutucular, drum kurutucular, buhar-borulu döner kurutucular, vakumlu döner kurutucular titreşimli tepsili kurutucular ve vakumlu tepsili kurutucular kondüktif kurutuculardır. Konvetif kurutuculardа sıcak hava veya sıcak gazlar kurutulacak ürünün yüzeyine temas etmesiyle üründen nem uzaklaştırılır. Kurutma odaları, pnomatik kurutucu, spray kurutucu, döner kurutucu, akışkan yataklı kurutucu ve tünel tipi kurutucular bu tip kurutuculardır. Radyant kurutucular, dielektrik, kızılötesi, ultraviyole veya mikrodalga kurutucular olarak bilinmektedir. Bu kurutuculardа ısı, radyo, kızıl ötesi, mor ötesi ve mikrodalga frekanslarında alternatif akımlı elektrik alanına konmuş ürün içinde üretilir. Alanda su moleküllerinin titreşmesiyle su buharlaşarak kurutma gerçekleşir (Güngör ve Özbalta, 1997; EİE, 1999). Radyant kurutuculardan olan mikrodalga kurutucu da düşük sıcaklıklarda kurutma işlemi gerçekleştiği için ürün dokusu zarar görmez ve kaliteli kuru ürün elde edilir. Mikrodalga kurutmada fırın boşluğuna gönderilen mikrodalga ışınım ürün tarafından absorbe edilip, ürün içerisindeki su moleküllerini titreştirir ve ürün içerisinde ısı meydana gelir. Mikrodalga kurutmada diğer kurutma yöntemlerinin aksine ısı içten dışa doğru iletilir. Mikrodalga kurutma yönteminde ürünün iç kısımlarına yüksek ısı iletimi ile enerji kazanımı sağlanırken, bu yöntemin temiz ürün eldesi, işlem kontrolü kolaylığı, kurutmanın hızlı gerçekleşmesi gibi avantajları da vardır. Gıda içindeki su molekülleri, diğer yöntemlerle yapılan ısıtma işlemlerinden daha kısa sürede, daha homojen ısı dağılımı ile uzaklaştırılır. Mikrodalga kurutucular konvansiyonel kurutuculardan daha az yer kaplar ve bu kurutucuların işçiliği azdır. Buna karşın mikrodalga kurutma yönteminin ürünler üzerinde düzensiz ısı dağılımı, tekstürel zararlar, yüksek yatırım maliyeti ve mikrodalga ışınlarının ürünler üzerinde etkisinin sınırlı olması gibi olumsuz etkileri de vardır (Schiffmann, 1986; Datta ve Davidson, 2000; Maskan, 2000; Alibaş, 2012; Polatcı ve Taşova, 2017;). En düşük ekonomik girdiyle kaliteli ürün elde etmek ve düşük enerji tüketimi ile kurutma süreçlerinin iyileştirilmesi için kurutma yöntemleri birlikte kullanılmaktadır (Karacabey ve ark., 2020). Mikrodalga kurutmanın olumsuz yönlerini ortadan kaldırmak için mikrodalga kurutma ile ozmotik dehidrasyon beraber kullanılabilir. Son yıllarda, ozmoz yoluyla sağlanan kısmi dehidrasyon, işlem süresini kısaltmasını sağlar böylece enerji tüketimini sınırlamanın ve duyusal özellikleri iyileştirmenin bir yolu olarak mikrodalga kurutmadan önce yaygın olarak kullanılmaktadır. Ozmotik ön kurutma işleminin kurutma prosesini geliştirmede kısmen etkili olduğu belirlenmiştir (Al-Harahsheh ve ark., 2009). Ozmotik dehidrasyon proses değişkenlerine bağlı olarak gerçekleşen renk ve doku değişikliklerini engeller (Moreno ve ark., 2000). Ozmotik dehidrasyon, genellikle kurutmadan önce kullanılan kimyasal bir ön işlemdir. Kısmi bir dehidrasyon işlemi olarak ozmoz, eş zamanlı bir çözücü ve çözünen difüzyon işlemi olarak kabul edilebilir. Bu işlemde eş zamanlı olarak katı madde kazanımı ve bir nem kaybı meydana gelir. Kurutma öncesi uygulanan ozmotik dehidrasyon işleminde nem kaybına bağlı olarak kurutucu yükü azalır, kurutucu potansiyeli artar ve kurutma süresi kısalır (Prosapio ve Norton, 2017).

Yaş meyve ve sebze ihracatımızın yarısını yaklaşık narenciye ürünleri oluşturmaktadır. 2021 yılında gerçekleşen turunçgil üretiminin %34'ü mandalina, %32'si portakal, %29'u limon ve %5'i greyfurttur. Son yıllarda turunçgiller üretiminde mandalina ve limon türlerine ağırlık verilmiştir. Turunçgil üretimdeki en fazla artış %54 ile limonda olmuştur. Türkiye 1,4 milyon ton limon üretimi ile dördüncü sıra yer almaktadır. Türkiye limon üretiminin %93'ü Akdeniz Bölgesinde ve %7'si Ege Bölgesinde gerçekleştirilmiştir. Mersin ili, limon üretiminin %55'i karşılayarak limon üretiminde Türkiye de ilk sırada yer almakta olup, özellikle narenciye üretiminde ülke ekonomisine önemli katkısı olan illerden biridir (Anonim, 2020; Aygören, 2022). Ülkemizde Mersin ilinin batısındaki Erdemli-Silifke yöresinde yetiştirilen Lamas çeşidi limonu orta boyuttaki meyvesi, silindirik, belirgin memeli, boyun halkalı, kabuğu sarı renkli, koku-tat bakımından zengin, sulu bir limondur (Alkaç, 2019). Bu sebeple Lamas türü limon dilimlerinin kurutulması bölgenin tarım ekonomisine ve kırsal gelişime katkıda bulunacaktır. Limon, asidik olup, ince dokuya sahip, açık sarı renkli ve limonen isimli ana aroma verici bileşiği içeren bir meyvedir. Tüketicinin ilk algıladığı ve etkilendiği özellik renk olduğu için kurutma da incelenen en önemli parametrelerdendir (Chen ve ark., 2005). Bu çalışmada limon halkaları ozmotik dehidrasyon destekli mikrodalga kurutma yöntemi ile kurutulmuştur. Limon halka kalınlığı, çözelti derişimi, ozmotik dehidrasyon

süresi, mikrodalga güç seviyesi ve mikrodalga kuruma süresi gibi değişkenlerin nem kaybı, çapsal büzülme oranı ve *b* renk değişimi üzerine etkileri belirlenmiştir. Kurutma deneyleri, Yanıt Yüzey Yöntemi (YYM) ile tasarlanmıştır. Bu çalışmada Box- Behnken deneysel tasarım yöntemi ile 29 adet gibi az sayıda deneyle optimum sonuca ulaşılmıştır. Deney sonuçlarıyla uyumlu matematiksel modeller elde edilmiş ve bu modelin ANOVA istatistiksel analizi yapılmıştır.

2. Materyal ve Metot

Ozmotik dehidrasyon destekli mikrodalga kurutma işlemi için ozmotik dehidrasyon işleminden sonra limon halkaları çözeltiden süzülerek çıkarılmış ve daha sonra mikrodalga fırına dizilerek kurutulmuştur. Mikrodalga kurutma işlemi, Samsung marka Ms23J5133At/tr model solo mikrodalga fırında kesikli olarak gerçekleştirilmiştir. Fırın; 489*275*338 mm boyutunda olup 23 L kapasiteye ve 288 mm döner tablaya sahiptir. 6 farklı güç seviyesi bulunmakta ve maksimum çıkış gücü 800 W'dır. Denemeler için Mersin'in Erdemli ilçesinde yetişen Lamas cinsi limon kullanılmıştır. Ozmotik dehidrasyon işlemi için sodyum klorür çözeltisi kullanılmış olup, işlem oda sıcaklığında yapılmış, halka kalınlığı 10 mm ve katı/çözelti oranı (örnek miktarının ozmotik çözeltiye olan oranı) 1/10 olarak sabit alınmıştır. Deneylerde kurutma işlemi öncesi ve sonrası ölçümler, üç örnek için yapılmış ve ortalaması alınmıştır.

Mikrodalga kurutucudaki kurutma işlemin deneysel tasarımı için Design Expert 13.0.trial kullanılmıştır. Deney tasarımında, dört değişken üç seviye için yanıt yüzey yönteminin Box-Behnken tasarımına göre deneyler gerçekleştirilmiştir. 17 deney gerekli model noktası, 2 deney ek model noktası, 5 deney eksiklik noktası ve 5 deney tekrar olmak üzere toplam 29 deneme içeren bir tasarım oluşturulmuştur (Nurkhoeriyati ve ark., 2021). *Tablo 1*'de bağımsız değişkenlerden olan A ozmotik dehidrasyon süresini (60-180 dk), B çözelti derişimi (10-20 % w:v), C mikrodalga kurutucuda kurutma süresi (2-6 dk) ve D mikrodalga kurutucuda kurutma gücü (100-300 W) ifade etmektedir. Giriş değişkenlerinin seviyelerinin kod değerleri -1, 0, 1 olarak *Tablo 1*' de verilmiştir.

Tablo 1. Değişkenlerin gerçek ve kod değerleri

Table 1. Codes and actual levels of the input variables

Bağımsız Değişkenler	**Semboller**	**Kod Seviyeleri**		
		-1	0	1
Ozmotik Dehidrasyon Kurutma Süresi (dk)	A	60	120	180
Çözelti Derişimi (%w:v)	B	10	15	20
Mikrodalga Kurutucudaki Kurutma Süresi (dk)	C	2	4	6
Mikrodalga Kurutucunun Kurutma Gücü (W)	D	100	200	300

Yanıt olarak (çıkış değişkenleri) uzaklaştırılan nem kaybı (Y_{NK}), çapsal büzülme oranı (Y_{BO}) ve *b* renk değişimi (Y_B) seçilmiştir. Nem kaybı Eşitlik 1'deki gibi kurutmadan önce ve sonra analitik terazi ile ağırlığı ölçülerek hesaplanmıştır (Darıcı, 2012; Aboud, 2013; Pandya ve Yadav, 2014). Bu eşitlikte yer alan M_0 kurutma işlemine tabi tutulan taze limon halkalarının ortalama ağırlığı (g) ve M_t kurutma sonrası limon halkalarının ortalama ağırlığını (g) ifade etmektedir.

$$\text{Nem Kaybı} = \frac{M_0 - M_t}{M_0} \qquad \text{(Eş.1)}$$

Çapsal büzülme oranı kurutmadan önceki ve sonraki çap ölçümlerinden yararlanılarak Eşitlik 2 ile hesaplanmıştır. Eşitlik 2'de yer alan D_0 kurutma öncesi taze limon halkalarının ortalama çapı (mm) ve D_t ise kurutma sonrası limon halkalarının ortalama çapını (mm) ifade etmektedir (Darıcı, 2012; Aboud, 2013; Pandya ve Yadav, 2014).

$$\text{Çapsal büzülme oranı} = \frac{D_0 - D_t}{D_0} \qquad \text{(Eş.2)}$$

Limon dilim kalınlığı dijital kumpasla ölçülmüş olup, deneylerde dilim kalınlığı 10 mm olarak sabit alınmıştır. Limon halkaları beyaz-sarı renklerindedir. Bu yüzden *b* değerlerinin renk analizinde önemi daha fazladır. Çünkü sarı rengi *b* pozitif renk değeri ifade etmektedir (Şahin ve ark., 2012). Kurutma öncesi ve sonrası limon halkalarının *b* değerleri ColorMeter marka Pro model renk analiz ölçüm cihazı ile belirlenmiştir. *b* renk değişimi Eşitlik 3

yardımıyla hesaplanmıştır. b_0 taze limon halkalarına ait ortalama değerlerdir ve b kurutma periyodu sonrası ölçülen ortalama renk parametresini göstermektedir.

$$\frac{\Delta b}{b_0} = \frac{b_0 - b}{b_0} \quad \text{(Eş. 3)}$$

3. Araştırma Sonuçları ve Tartışma

YYM, kurutma alanında yaygın olarak kullanılan deneysel tasarım yöntemlerinden biri olup, deneme setlerinden türetilen basit ampirik modelleri kullanarak optimuma ulaşır (Koç ve Ertekin, 2010). YYM'de bir faktörün etkisinin veya diğer faktörlerle etkileşiminin yanıt değişkeni üzerine etkisini model regresyon analizi belirler. YYM'de en çok kullanılan deneysel tasarımlar, Merkezi Bileşik Tasarımı (CCD) ve Box-Behnken tasarımıdır. Box-Behnken tasarımında, merkezi bileşik tasarımlara kıyasla daha az sayıda deney yapıldığı için daha ekonomik bir tasarım yöntemidir (Özcan ve Samanlı, 2017; Serin ve ark., 2019). Box Behnken tasarımları 3^k faktöriyel tasarımlarına bir alternatiftir. Tasarım 2^k faktöriyel ve tamamlanmamış blok tasarımlarının kombinasyonundan oluşmuştur. Box- Behnken tasarımları küresel bir tasarım olmasından dolayı her faktör minimum, orta ve maksimum seviyelerinden oluşmak üzere sadece 3 düzeyde incelenir (Özden, 2020). Box Behnken tasarımının en önemli avantajları, deneyin sayısının daha az olması ile zaman ve paradan tasarruf sağlaması ve faktör limitlerinin kolayca ayarlanabilmesidir (Deveci ve ark., 2019).

Tablo 2. Box-Behnken deney koşulları ve alınan yanıtlar

Table 2. Box-Behnken experimental conditions and responses

Deney No	A	B	C	D	Y_{NK}	Y_{BO}	Y_B
1	60	10	4	200	0.1232	0.0589	0.0485
2	180	10	4	200	0.1566	0.0771	0.0729
3	60	20	4	200	0.1387	0.0634	0.0591
4	180	20	4	200	0.1532	0.0702	0.0649
5	120	15	2	100	0.0959	0.0412	0.0278
6	120	15	6	100	0.1549	0.0743	0.0695
7	120	15	2	300	0.2455	0.0984	0.0837
8	120	15	6	300	0.4466	0.1612	0.1362
9	60	15	4	100	0.0804	0.0399	0.0245
10	180	15	4	100	0.1412	0.0678	0.0616
11	60	15	4	300	0.2603	0.1098	0.0937
12	180	15	4	300	0.3292	0.1269	0.1178
13	120	10	2	200	0.1163	0.0478	0.0365
14	120	20	2	200	0.1359	0.0617	0.0552
15	120	10	6	200	0.2548	0.1035	0.0862
16	120	20	6	200	0.2755	0.1122	0.0989
17	60	15	2	200	0.1219	0.0498	0.0403
18	180	15	2	200	0.1229	0.0514	0.0437
19	60	15	6	200	0.2159	0.0962	0.0814
20	180	15	6	200	0.2584	0.1065	0.0894
21	120	10	4	100	0.1085	0.0445	0.0324
22	120	20	4	100	0.1282	0.0598	0.0512
23	120	10	4	300	0.2892	0.1147	0.1028
24	120	20	4	300	0.3251	0.1218	0.1139
25	120	15	4	200	0.1869	0.081	0.0785
26	120	15	4	200	0.1841	0.0808	0.0781
27	120	15	4	200	0.1863	0.0799	0.0794
28	120	15	4	200	0.1848	0.0804	0.0791
29	120	15	4	200	0.1854	0.0801	0.0786

Tablo 2' de dört faktör üç seviye için Box-Behnken tasarımına göre belirlenen 29 deneye ait çalışma koşulları ve deneyler sonucunda alınan yanıtlar verilmiştir. YYM dizaynına göre yapılan deneylerin sonuçlarına göre Y_{NK} 0.0804-0.4466 arasında, Y_{BO} 0.0399-0.1612 arasında ve Y_B 0.0245-0.1362 arasında değişmektedir. 9. deney koşulları; nem kaybı, *b* renk değişimi ve büzülme oranı için en düşük değerleri veren deney koşullarını sağlamıştır. Bu deneyde ozmotik dehidrasyon süresi 60 dk, çözelti derişimi %15, mikrodalga kurutma süresi 4 dk ve mikrodalga gücü 100 W olarak saptanmıştır. En düşük nem kaybı, çapsal büzülme oranı ve *b* renk değişimi sırasıyla 0.0804, 0.0399 ve 0.0245. Nem kaybı, çapsal büzülme oranı ve *b* renk değişimi en yüksek gözlendiği deney ise 8. deneydir. Bu deney koşullarında, ozmotik dehidrasyon süresi 120 dk, çözelti derişimi % 15, mikrodalga kurutma süresi 6 dk ve mikrodalga gücü 300W tır. En yüksek yanıtların elde edildiği 8. deneydeki koşullarda; nem kaybı, çapsal büzülme oranı ve *b* renk değişimi sırasıyla 0.4466, 0.1612 ve 0.1362 olarak belirlenmiştir.

Lineer, 2FI, kuadratik ve kübik modeller içerisinden en yüksek uyumu (R^2) veren model kuadratik denklem olarak seçilmiştir (R^2 değerleri nem kaybı için 0.9853, çapsal büzülme oranı için 0,9861 ve *b* renk değişimi için ise 0.9770). *Tablo 3*'te kurutma işlemi için önerilen kuadratik modellerin varyans analizi (ANOVA) tablosu yer almaktadır. ANOVA tablosunda da görüldüğü üzere modelin serbestlik derecesi (df), F değeri ve düşük olasılık değeri (P<0.0001) yanıtlar için modellerin anlamlı olduğunu ifade etmektedir (Bilen ve ark., 2018).

Tablo 3. YYM için regresyon parametrelerinin ANOVA değeri

Table 3. ANOVA value of regression parameters for RSM

Yanıt	Regrasyon	df	R^2	F	Pr > F
Nem Kaybı	Lineer	4	0.8577	36.17	< 0.0001
	2FI	6	0.8860	0.7433	0.6221
	Kuadratik	4	0.9853	23.69	< 0.0001
	Kübik	8	0.9979	4.54	0.0409
	Artık	6			
	Toplam	29			
Çapsal Büzülme Oranı	Lineer	4	0.9065	58.16	< 0.0001
	2FI	6	0.9198	0.4987	0.8011
	Kuadratik	4	0.9861	16.62	< 0.0001
	Kübik	8	0.9978	4.10	0.0512
	Artık	6			
	Toplam	29			
b Renk Değişimi	Lineer	4	0.9108	61.24	< 0.0001
	Çapraz çarpım	6	0.9195	0.3237	0.9159
	Kuadratik	4	0.9770	8.75	0.0009
	Kübik	8	0.9989	14.28	0.0022
	Artık	6			
	Toplam	29			

Yanıt yüzey yönteminde yer alan giriş değişkenleri ve yanıtlar arasında uygun bir matematiksel bağıntı kurulmuş ve bu model kullanılarak optimizasyon yapılmıştır. Eşitlik 4' te verilen denklemde, i ve j doğrusal ve ikinci dereceden katsayılar; b regresyon katsayısı, k optimize edilen faktörleri ve e hatayı tanımlamaktadır. Kurutma koşulları ve yanıtlar arasındaki ilişkiyi tanımlayan kod değerlerine göre matematiksel modeller Y_{NK} için eşitlik 5, Y_{BO} için eşitlik 6 ve Y_B için eşitlik 7 ile verilmiştir.

$$y = b_0 + \sum_{i=1}^{k} b_i X_i + \sum_{i=1}^{k} b_{ij} X_i^2 + \sum_{i<j}^{k} \sum_{j}^{k} b_{ij} X_i X_j + e \qquad \text{(Eş. 4)}$$

Tablo 2'de verilen koşullarda yapılan deneyler sonucunda alınan yanıtlara göre kurutma koşulları ve yanıtlar arasındaki ilişkiyi tanımlayan kod değerlerine göre matematiksel modeller aşağıdaki eşitliklerle verilmiştir.

$$Y_{NK} = 0.1855 + 0.0184\text{A} + 0.0090\text{B} + 0.0640\text{C} + 0.0989\text{D} - 0.0047\text{AB} + 0.0104\text{AC} + 0.0020\text{AD} + 0.0003\text{BC} + 0.0040\text{BD} + 0.0355\text{CD} - 0.0249A^2 - 0.0120B^2 + 0.0179C^2 + 0.0379D^2 \qquad \text{(Eş. 5)}$$

$$Y_{BO} = 0.0804 + 0.0068\text{A} + 0.0035\text{B} + 0.0253\text{C} + 0.0338\text{D} - 0.028\text{AB} + 0.0022\text{AC} - 0.0027\text{AD} - 0.0013\text{BC} - 0.0020\text{BD} + 0.0074\text{CD} - 0.0071A^2 - 0.0049B^2 + 0.0037C^2 + 0.0107D^2 \quad \text{(Eş. 6)}$$

$$Y_B = 0.0787 + 0.0086\text{A} + 0.0053\text{B} + 0.0229\text{C} + 0.0318\text{D} - 0.0046\text{AB} + 0.0011\text{AC} - 0.0033\text{AD} - 0.0015\text{BC} - 0.0019\text{BD} + 0.0027\text{CD} - 0.0101A^2 - 0.0071B^2 - 0.0038C^2 + 0.0045D^2 \quad \text{(Eş. 7)}$$

Bu modellerde görüldüğü üzere, mikrodalga kurutma süresi ve mikrodalga gücün, nem kaybı üzerine etkisinin daha fazla ve olumlu yönde olduğu belirlenmiştir. Kurutma devam ettikçe numune içindeki nem içeriği azalır, böylece mikrodalga gücünün emilimi azalır ve kurutma sonuna doğru nem kaybı düşer (Al-Harahsheh ve ark., 2009). Çapsal büzülme oranı ve *b* renk değişimi mikrodalga kurutma süresi, mikrodalga gücü ve ozmotik dehidrasyon süresinin artması ile birlikte artmıştır.

Kurutulmuş ürünün görünür hacmi ve gözenekliliği, mikrodalga gücündeki artışla azalma eğilimindedir (Pereira ve ark., 2007). Azadbakht ve ark. (2018), portakal halkalarının mikrodalgada kurutulması üzerine ozmotik dehidrasyon ön işleminin etkisini incelemek üzerine yaptıkları çalışmada ozmotik dehidrasyon çözeltisi olarak NaCl kullanmışlar ve ozmoz süresinin artışına bağlı olarak nem kaybının ve kuru madde miktarının artması ile enerji verimliliği önemli ölçüde arttığını belirlemişlerdir. Bu sonuç, numunenin daldırılması sırasında sodyum ve klorür iyonlarının sarı dokuya nüfuz etmesinden kaynaklanmaktadır. Ayrıca NaCl kristalleri daldırılan ürünün hücresel kısımlarına nüfuz ettikçe hücreler uyarılmış ve büzülme azalmıştır. Dokuda sodyum klorür bulunması, sudan daha iyi mikrodalga ısı karakteristiğine sahip iyonik bir çözelti olan ürünün dokusunda su artışına ve üründe ısı emiliminin artmasına neden olmuştur. Ayrıca ozmoz süresinin artmasıyla birlikte üründe sıcaklık emilimi de artmış ve üründen daha hızlı su uzaklaşmıştır. Sonuç olarak, kurutma süresinde azalma ve mikrodalga kurutucunun enerji verimliliğinde bir artış meydana gelmiştir. Dehidrasyon hızı mikrodalga güç seviyesi ile artar. Sonuçta kurutma esnasında yüksek mikrodalga gücünde örnek içerisinde daha fazla ısı oluştuğundan kütle transferi artar. Mikrodalga fırında daha az sürede yüksek basınç ve derişim değişimi ile gıdadan sınıra sıvı akışı artar (Ghanem ve ark., 2012).

Ozmotik dehidrasyon proses değişkenlerine bağlı olarak gerçekleşen renk değişikliklerini engeller (Moreno ve ark., 2000). Limon halkaları açık sarı renginde olup, sarılığı gösteren *b* değeri kurutmanın optimizasyonunda yanıt olarak seçilmiştir. Daha yüksek *b* değeri kurutulan ürünün sarı renk tonunun daha fazla olduğunu gösterir. Mikrodalga gücündeki artışla limon halkalarının rengi kahverengileşme sebebiyle sarıdan kırmızıya doğru kayar (Darvishi ve ark., 2014). Kurutma sırasında kahverengileşme reaksiyonu nedeniyle sarılık sıcaklık ile artarken aydınlık azalır (Salehi ve Kashaninejad, 2018). *b* değerindeki düşüş, sarı rengi veren flavonoidlerin ve karotenoid pigmentlerin yıkımına ve ısıl işlem sonrası kabuklarda kalan farklı karotenoidlerin miktarlarına bağlıdır. Kurutma sırasında renk bozulmasının başlıca nedenleri, enzimatik olmayan esmerleşme, karotenoid kaybı, pigment bozulması ve L-askorbik asit oksidasyonudur. Bitki hücrelerindeki yarı geçirgen zar, çevreden tam bir izolasyon sağlamayabilir ve ozmotik dehidrasyon işlemi sırasında mineraller, vitaminler ve pigmentler çözeltiye geçerek kısmi renk kaybına neden olabilir (Ghanem ve ark., 2012).

Y_{NK}, Y_{BO} ve Y_B yanıtları için oluşturulan modelin ANOVA sonucunda, P değeri alması modelin önemini ortaya koymaktadır. ANOVA, P değerinin 0.0001'den küçük olduğu yerlerde modelin anlamlı olduğunu ileri sürmektedir. Katsayıların her birinin önemini kontrol etmek için bir araç olarak kullanılan P değerleri, değişkenler arasındaki etkileşim şeklini göstermektedir. Değişkenlerin her bir yanıt üzerindeki etkisi ANOVA tabloları ile verilmiştir. P değerleri, bağımsız değişkenler arasında ortak etkileşimli modelleri tanımlamak için gerekli olan katsayıların önemini vurgulamaktadır. P değeri 0.0001'den küçük olması modelin önemli olduğunu göstermektedir. *Tablo 4*'te P<0.0001'deki yanıtlar için istatistiksel olarak önemli olan bazı değişkenler bulunmaktadır. Her katsayının ve etkileşimlerin önemi *Tablo 4*'te gösterilmiştir. Nem kaybı üzerine C, D, CD, A^2, C^2 ve D^2 değişkenlerinin, büzülme oranı üzerine A, C, D, D^2 değişkenleri ve *b* değişimi üzerine ise A, C, D, A^2 değişkenlerinin etkisinin önemli olduğu görülmüştür.

YYM programı tarafından belirlenen deney koşullarında yapılan deneyler sonucunda elde edilen yanıtlar değerlendirilerek arzu edilebilirlik değeri 1 olan on deney seti belirlenmiştir. Bu çözümlerin arasından maliyet kıstasları çerçevesinde seçim yapılmıştır. Çalışmanın endüstriyel uygulama safhasındaki en büyük maliyetin enerji olacağı öngörülerek en düşük mikrodalga güç seviyesi ve mikrodalga süresi değerleri seçilmiştir. Buna göre optimum değişken seviyeleri olarak belirlenmiş olan, ozmotik dehidrasyon kurutma süresi 120 dk, çözelti derişimi

% 15, mikrodalga kurutma süresi 4 dk ve mikrodalga kurutma gücü 200 W bulunmuştur. Limon halkalarının ozmotik dehidrasyon işleminin oda sıcaklığında yapıldığı koşullarda ozmotik dehidrasyon kurutma süresi 120 dk ve çözelti derişimi %15 olarak bulunmuştur. Limon halkalarının ozmotik dehidrasyonu için optimum koşulları belirlemek amacıyla Deepika ve Sutar (2017) tarafından yapılan çalışmada, 30°C'de ozmotik dehidrasyon süresi 180 dk ve çözelti konsantrasyonu %20 olarak bulunmuştur. Çalışmada, düşük konsantrasyonlarda, tuz kazanımı ile birlikte daha yüksek nem kaybı elde etmek için gereken sürenin 4 saatten fazla olduğu gözlenmiştir. Ayrıca, düşük konsantrasyonda kütle transferini hızlandırmak için, işlem maliyetini artıran çalkalama işleminin gerekli olduğu belirtilmiştir.

Tablo 4. Yanıt modelleri için ANOVA

Table 4. ANOVA for responses

Faktör	Kareler Toplamı	df	Kareler Ortalaması	F-değeri	p>F
Y_{NK}					
Model	0.1971	14	0.0141	67.14	< 0.0001
A	0.0041	1	0.0041	19.43	0.0006
B	0.0010	1	0.0010	4.64	0.0492
C	0.0491	1	0.0491	234.26	< 0.0001
D	0.1174	1	0.1174	559.85	< 0.0001
AB	0.0001	1	0.0001	0.4260	0.5246
AC	0.0004	1	0.0004	2.05	0.1738
AD	0.0000	1	0.0000	0.0782	0.7838
BC	3.025E-07	1	3.025E-07	0.0014	0.9702
BD	0.0001	1	0.0001	0.3129	0.5847
CD	0.0050	1	0.0050	24.08	0.0002
A^2	0.0040	1	0.0040	19.25	0.0006
B^2	0.0009	1	0.0009	4.48	0.0528
C^2	0.0021	1	0.0021	9.89	0.0072
D^2	0.0093	1	0.0093	44.55	< 0.0001
Artık	0.0029	14	0.0002		
Model Uyumsuzluğu	0.0029	10	0.0003	231.63	< 0.0001
Hata	5.060E-06	4	1.265E-06		
Toplam	0.2000	28			
R^2	0.9853				
Y_{BO}					
Model	0.0240	14	0.0017	70.70	< 0.0001
A	0.0006	1	0.0006	23.03	0.0003
B	0.0002	1	0.0002	6.23	0.0256
C	0.0077	1	0.0077	316.52	< 0.0001
D	0.0137	1	0.0137	564.09	< 0.0001
AB	0.0000	1	0.0000	1.34	0.2666
AC	0.0000	1	0.0000	0.7797	0.3921
AD	0.0000	1	0.0000	1.20	0.2915
BC	6.760E-06	1	6.760E-06	0.2786	0.6059
BD	0.0000	1	0.0000	0.6927	0.4192
CD	0.0002	1	0.0002	9.09	0.0093
A^2	0.0003	1	0.0003	13.50	0.0025
B^2	0.0002	1	0.0002	6.40	0.0240

Tablo 4. (devamı)

Table 4. (continuance)

C^2	0.0001	1	0.0001	3.62	0.0779
D^2	0.0007	1	0.0007	30.56	< 0.0001
Artık	0.0003	14	0.0000		
Model Uyumsuzluğu	0.0003	10	0.0000	159.10	< 0.0001
Hata	8.520E-07	4	2.130E-07		
Toplam	0.0244	28			
R^2	0.9861				
Y_B					
Model	0.0210	14	0.0015	42.44	< 0.0001
A	0.0009	1	0.0009	24.89	0.0002
B	0.0003	1	0.0003	9.62	0.0078
C	0.0063	1	0.0063	177.33	< 0.0001
D	0.0121	1	0.0121	342.06	< 0.0001
AB	0.0001	1	0.0001	2.44	0.1403
AC	5.290E-06	1	5.290E-06	0.1495	0.7048
AD	0.0000	1	0.0000	1.19	0.2930
BC	9.000E-06	1	9.000E-06	0.2544	0.6219
BD	0.0000	1	0.0000	0.4189	0.5280
CD	0.0000	1	0.0000	0.8241	0.3793
A^2	0.0007	1	0.0007	18.88	0.0007
B^2	0.0003	1	0.0003	9.14	0.0091
C^2	0.0001	1	0.0001	2.61	0.1284
D^2	0.0001	1	0.0001	3.73	0.0738
Artık	0.0005	14	0.0000		
Model Uyumsuzluğu	0.0005	10	0.0000	187.95	< 0.0001
Hata	1.052E-06	4	2.630E-07		
Toplam	0.0215	28			
R^2	0.9770				

Mikrodalga kurutma koşulları altında nem oranına karşı ozmotik ön işlemin kuruma hızına etkisi, aynı zamanda, bağlı olmayan suya sahip dokuda sodyum klorürün varlığı, suya kıyasla daha iyi mikrodalga ısıtma özelliklerine sahip iyonik bir çözelti oluşturur. Bunun nedeni, mikrodalga enerjisinin hacimsel absorpsiyonu ile orantılı olan bu çözeltinin daha yüksek kayıp faktörüdür. Bu, ozmotik ön-işlem yapılmayanla karşılaştırıldığında yüksek bir kuruma hızına yol açar. Bununla birlikte, kurutma işleminin sonuna doğru, daha yüksek ozmotik çözelti konsantrasyonuyla işlenen numuneler için daha düşük kuruma hızıyla bu durum tersine dönmüştür. Bunun nedeni, ozmotik maddenin bağlayıcı etkisidir ve bu durum kurutma işleminin sonuna doğru, suyun uzaklaştırılmasının daha zor olmasına neden olmaktadır. Artan mikrodalga çıkış gücü ve ozmotik konsantrasyon ile kurutma işleminin süresi azalmıştır (Al-Harahsheh ve ark., 2009).

4. Sonuç

Limon halkalarının ozmotik dehidrasyon ve mikrodalga ile birlikte kurutma işleminde seçilen dört değişken ve üç seviye için YYM nin Box-Behnken deneysel tasarım yöntemin göre 29 deney yapılmıştır. Bu deney sonuçlarına göre her bir yanıt için en yüksek uyumu veren modelin R^2 değerleri nem kaybı için 0.9853, çapsal büzülme oranı için 0.9861 ve *b* renk değişimi için ise 0.9770 değerlerini veren kuadratik model olduğu görülmüştür. Bu modellere ait ANOVA tablosu incelendiğinde mikrodalga gücü ve mikrodalga süresinin nem kaybı, büzülme oranı ve *b* değişimi için önemli olduğu görülmüştür. Ayrıca çapsal büzülme oranı ve *b* değişimi için ozmotik dehidrasyon

süresinin de etkili olduğu belirlenmiştir. Arzu edilebilirliği maksimum yapan değişkenlerin optimum seviyelerinin ozmotik dehidrasyon süresi için 120 dk, çözelti derişimi için % 15, mikrodalga kurutma süresi için 4 dk ve mikrodalga kurutma gücü için 200 W olduğu belirlenmiştir.

Teşekkür

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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Evaluation of Wild Annual Sunflower Species for Some Morphological, Phenological, and Agronomic Characters under Field Conditions*

Tek Yıllık Yabani Ayçiçeği Türlerinin Tarla Koşullarında Bazı Morfolojik, Fenolojik ve Agronomik Karakterleri Açısından Değerlendirilmesi*

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Abstract

New gene sources are needed for adaptation to climatic changes, resistance to the regeneration of diseases and pests, and achieving high heterosis in sunflower breeding. Wild species are the most important gene sources for sunflower breeding studies. For breeding studies, it is necessary to know the morphological, Phenological, and agronomic characteristics of these genotypes in field conditions. The aim of this research was to determine these components of annual wild sunflower (*Helianthus*) species under field conditions in the 2012 and 2013 growing seasons for new gene sources. In this research, *H. agrestis*, *H. annuus* (4 different genotypes), *H. anomalus*, *H. argophyllus*, *H. bolanderi*, *H. debilis* (*ssp. debilis, ssp. cucumerifolius, ssp. silvestris, ssp. tardiflorus* and *ssp. vestitus subspecies), H. deserticola, H. exilis, H. neglectus, H. niveus (ssp. niveus, ssp. canescens and ssp. tephrodes subspecies) H. petiolaris (ssp. petiolaris* (2 different genotypes) and *ssp. fallax subspecies), H. porteri*, and *H. praecox (ssp. praecox* (2 different genotypes), *ssp. hirtus, and ssp. runyani subspecies)* were used as material. In this study, determined characters on annual wild sunflower genotypes were plant height, primary branches number, secondary branches number per primary branches, plant spreading diameter, the number of days from planting to first flowering, the number of days from planting to 50 % flowering, the number of days from planting to the end of flowering, the number of days of the flowering period, main stem diameter, head diameter, 1000 seeds weight, and seed yield. Year factor had a significant effect on these characters except plant height. Genotype had a significant effect on all characters in both years except seed width in 2013. In both years, the highest values for seed yield, 100 seed weight, head diameter, and main stem diameter were obtained in wild *H. annuus* genotypes while *H. argophyllus* had the highest values for plant height and primary branches number, and the highest days numbers from planting to first and 50% flowering. In the first and second growing seasons; values of the genotypes changed between 61.33 and 325.67 cm for plant height, between 0.73 and 101.20 g for thousand seed weight, between 97 and 223 days for the time from planting to 50% flowering, between 50 and 171 days for the flowering period, between 5.0 and 800.70 units for the number of plant heads, between 1.57 and 233.20 g for plant grain yields.

Keywords: *Helianthus*, Flowering period, Plant height, Seed yield, Seed weight

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Öz

Ayçiçeği ıslahında; iklim değişikliklerine uyum, yenilenen hastalık ve zararlılara dayanıklılık ve yüksek heterosisi yakalamak için yeni gen kaynaklarına gereksinim duyulmaktadır. Yabani türler ayçiçeği ıslahı çalışmaları için en önemli gen kaynaklarıdır. Islah çalışmaları için bu genotiplerin tarla koşullarındaki morfolojik, Fenolojik ve agronomik özelliklerinin bilinmesi gereklidir. Bu araştırmanın amacı; tek yıllık yabani ayçiçeği (*Helianthus*) türlerinin bu komponentlerini 2012 ve 2013 yetiştirme sezonlarında tarla koşullarında yeni genetik kaynağı olarak belirlemektir. Araştırmada; *H. agrestis, H. annuus* (4 farklı genotip), *H. anomalus, H. argophyllus, H. bolanderi, H. debilis (ssp. debilis, ssp. cucumerifolius, ssp. silvestris, ssp. tardiflorus ve ssp. vestitus alttürleri)*, *H. deserticola, H. exilis, H. neglectus, H. niveus (ssp. niveus, ssp. canescens ve ssp. tephrodes* alt türleri) *H. petiolaris* (*ssp. petiolaris* (2 farklı genotip) ve *ssp. fallax* alt türleri), *H. porteri*, ve *H. praecox* (*ssp. praecox* (2 farklı genotip), *ssp. hirtus*, ve *ssp. runyani* alttürleri) materyal olarak kullanılmıştır. Çalışmada tek yıllık yabani ayçiçeği genotipleri üzerinde incelenen karakterler; bitki boyu, birincil yan dal sayısı, birincil yan dala düşen ikincil dal sayısı, bitki yayılma çapı, ekimden ilk çiçeklenmeye kadar olan gün sayısı, ekimden %50 çiçeklenmeye kadar olan gün sayısı, ekimden çiçeklenme sonuna kadar olan gün sayısı, çiçeklenme periyodu gün sayısı, ana sap çapı, tabla çapı, bin dane ağırlığı ve tane verimi unsurlarıdır. Yıl faktörü, bitki boyu haricinde incelenen tüm karakterler üzerinde önemli etkiye sahip olmuştur. Genotip, 2013 yılındaki tohum haricinde her iki yılda incelenen tüm karakterler üzerinde istatistiki önemli etkiye sahip olmuştur. Her iki yılda, *H. argophyllus* genotipinde en yüksek bitki boyu, en fazla birincil dal sayısı ve en yüksek ekimden % 50 çiçeklenmeye kadar olan gün sayısı değerleri elde edilirken, *H. annuus* genotiplerinde en yüksek tohum verimleri, en yüksek 1000 tane ağırlıkları, en yüksek tabla çapları ve en yüksek sap çapı değerleri belirlenmiştir. Yabani tek yıllık ayçiçeği türlerine ait genotiplerin morfolojik ve agronomik karakterlerine ait her iki yetiştirme sezonundaki değerlerde; bitki boyları 61.33 ve 325.67 cm, bin tane ağırlığı 0.73 ve 101.20 g, ekimden % 50 çiçeklenmeye kadar olan süre 97 ve 223 gün, çiçeklenme periyodu 50 ve 171 gün, bitki tabla sayısı 5.0 ve 800.70 adet ve bitki tane verimleri 1.57 ve 233.20 g arasında değişmiştir.

Anahtar kelimeler: *Helianthus*, Çiçeklenme periyodu, Bitki boyu, Tane verimi, Tane ağırlığı

1. Introduction

Sunflower (*Helianthus annuus* L.) is the fourth crop for contributes to world vegetable oil production after palm, soybean, and rapeseed. It is grown in many regions of the world and adapted to different agroecological conditions due to its genetic structure with high adaptability. Cultivated sunflower belongs to the genus *Helianthus*, a member of the Asteraceae family consisting of 53 species and 19 subspecies, including 14 annual and 39 perennials (Seiler et al., 2017). Sunflower is thought to have been domesticated 3000–5000 years ago by Native Americans who primarily used it as a source of edible seed (Heiser, 1951). It was introduced to Europe in the early 16th century. The first oilseed cultivars were developed and grown at an industrial scale in Russia (Gavrilova and Anisimova, 2017). The later, breeding efforts have transitioned sunflower from primarily open-pollinated varieties into hybrid cultivars. Hybrid production refers to the establishment of novel cultivars that are reproductively isolated from their parental species and genetically stabilized (Rieseberg, 2006; Rauf, 2019).

Interspecific hybridization has been extensively applied in sunflower breeding. Wild species are adapted to a wide range of habitats and possess considerable variability for most biotic and abiotic traits (Seiler et al., 2017). Wild genotypes have been undeniably beneficial to modern agriculture dating back 100 yr, providing plant breeders with a broad pool of potentially useful genetic resources (Hajjar and Hodgkin, 2007). Wild sunflower species have been used as sources of desirable genes for a number of characteristics. With hybrid cultivar breeding, the importance of wild sunflower species has increased even more to capture heterosis and resistance to disease, pests, stress, and herbicide. Many traits dealing with morphology, architecture, and disease resistance have been transferred from *Helianthus* species to sunflower (Onemli and Gucer, 2010c: Qi et al., 2019). The genetic research on the development of new CMS - restorers of fertility have contributed to enriching diversity and increasing heterosis in sunflower (Atlagić et al., 2006; Seiler, 2007; Nooryazdan et al., 2010; Onemli and Gucer, 2010b; Whitney et al., 2010; Seiler et al., 2017). Wild species are a potentially important source of abiotic tolerance; therefore, it may be desirable to introgress drought, heat, and salinity tolerant genes from wild relatives (Onemli and Gucer, 2010a; Seiler et al., 2017; Hernández et al., 2018). They also contain considerable variability for biotic stress such as disease, orobanche, and insect pest resistance (Vear, 2016; Seiler et al., 2017; Talukder et al., 2019; Fernández-Aparicio, 2022). The increase in sunflower production has been largely connected to the inclusion of wild Helianthus species in the improvement work on sunflower (De Haro, 1991; Perez et al., 2007; Nooryazdan et al., 2010; Onemli, 2012a; 2012b; Seiler et al., 2017). Although interest in using wild species in breeding programs has increased, the limited genetic variability in cultivated sunflower has slowed the future improvement of the crop, and has placed the crop in a vulnerable position should any major shifts of disease races or pests occur.

Evaluations of wild species have provided information about useful genes for future sunflower improvement. However, there are still numerous genes in wild sunflower species yet to be identified and introgressed into cultivated sunflower. Plant breeders need more detailed information about wild genotypes. The understanding of wild *Helianthus* species will increase the number of useful genes available from wild *Helianthus* species, making it possible to transfer cultivated sunflower (Hernández et al., 2019). In addition, it is also important for the arrangement of hybridization programs such as flowering calendars. In the present study, we focus on the evaluation of wild annual *Helianthus* species for their morphological, phenological, and agronomic characteristics in field conditions to determine useful features for future sunflower breeding.

2. Materials and Methods

2.1. Plant materials

In this research, twenty-seven wild annual *Helianthus* species and subspecies listed in *Table 1* getting from the USDA-ARS North Central Regional Plant Introduction Station-Iowa State University were used as materials. In this research annual wild sunflower (*Helianthus*) species; *H. agrestis*, *H. annuus* (4 different genotypes), *H. anomalus*, *H. argophyllus*, *H. bolanderi*, *H. debilis* (*ssp. debilis, ssp. cucumerifolius, ssp. silvestris, ssp. tardiflorus* and *ssp. vestitus subspecies)*, *H. deserticola*, *H. exilis*, *H. neglectus*, *H. niveus (ssp. niveus, ssp. canescens and ssp. tephrodes subspecies) H. petiolaris (ssp. petiolaris* (2 different genotype) and *ssp. fallax subspecies)*, *H. porteri,* and *H. praecox (ssp. praecox* (2 different genotypes), *ssp. hirtus, and ssp. runyani subspecies)* were planted under field conditions in the 2012 and 2013 sunflower growing seasons. Wild sunflower genotypes are origins of USA except *H. annuus* Ames 29348 and *H. niveus subsp. tephrodes*.

Table 1: Genotypes and origins of annual wild Helianthus species and subspecies

Genotype	*Helianthus species and subspecies*	Origins
1	*H. agrestis*	USA, Florida
2	*H. annuus* Ames 4114	USA, North Dakota
3	*H. annuus* Ames 7111	USA, California
4	*H. annuus* Ames 29273	USA, Texas
5	*H. annuus* Ames 29348	Australia, South Australia
6	*H. anomalus* S.F. Blake	USA, Utah
7	*H. argophyllus* Torr.& A. Gray	USA, Texas
8	*H. bolanderi* A. Gray	USA, California
9	*H. debilis* Nutt. subsp. *cucumerifolius* (Torr. & A.Gray)Heiser	USA, Texas
10	*H. debilis* Nutt. subsp. *debilis*	USA, Florida
11	*H. debilis* Nutt. subsp. *silvestris* Heiser	USA, Texas
12	*H. debilis* Nutt. subsp. *tardiflorus* Heiser	USA, Florida
13	*H. debilis* Nutt. subsp. *vestitus* (E. Watson) Heiser	USA, Florida
14	*H. deserticola* Heiser	USA, Nevada
15	*H. exilis* A. Gray	USA, California
16	*H. neglectus* Heiser	USA, New Mexico
17	*H. niveus* (Benth.) Brandegee	USA, Arizona
18	*H. niveus* (Benth.) Brandegee subsp. *canescens* (A. Gray) Heiser	USA, Utah
19	*H. niveus* (Benth.) Brandegee subsp. *tephrodes* (A. Gray) Heiser	Mexico
20	*H. petiolaris* Nutt.	USA, South Dakota
21	*H. petiolaris* Nutt. subsp. *fallax* Heiser	USA, New Mexico
22	*H. petiolaris* Nutt. subsp. *petiolaris*	USA, Oklahoma
23	*H. porteri* (A. Gray) Pruski	USA, Georgia
24	*H. praecox* Engelm. & A. Gray	USA, Texas
25	*H. praecox* Engelm. & A. Gray subsp. *hirtus* (Heiser) Heiser	USA, Texas
26	*H. praecox* Engelm. & A. Gray subsp. *praecox*	USA, Texas
27	*H. praecox* Engelm. & A. Gray subsp. *runyonii* (Heiser) Heiser	USA, Texas

2.2. Meteorological data and field soil properties in the Experimental location

The experiments were carried out in the Research area of the Field Crops Department of the Faculty of Agriculture at Namık Kemal University in Süleymanpaşa, Tekirdağ, Turkey (40º59´N, 27º33´E, elevation 3 m) on soil with clay loam and low organic matter content (*Table 2*).

Table 2. Soil properties of the experimental field

Soil Depth cm	PH (Sat)	Salt EC µS/cm (Sat)	SOM %	SW (Sat) %	P_2O_5 kg/ha	Lime %	Cu ppm	Fe ppm	Mn ppm	Ca ppm	K ppm	Mg ppm	Zn ppm
0-20	7.78	866	1.37	42	108.3	1.82	0.75	3.81	8.83	6076	210	241	0.15
30-60	7.82	720	1.18	43	72.6	3.71	0.67	3.62	6.60	6055	151	247	0.10
60-90	7.85	631	0.92	43	55.9	8.06	0.62	3.62	7.08	5911	125	263	0.09

SOM: Soil Organic matter, SW: Soil Water Content, Sat: Saturation

Climatic data during growing periods of wild *Helianthus* ssp. in 2012 and 2013 are given in *Table 3*. Generally, the values of rainfall, relative humidity, and temperature in the vegetative growth period and flowering duration of wild sunflower genotypes in the first year of field conditions were higher than in 2013 except for June rainfall and May temperature.

Table 3. Climatic data during growing periods of wild annual Helianthus genotypes in 2012 and 2013

Month	Year	Total Precip (mm)	Rainy day (day)	Sun. per day (hour)	Relat. Humi. (%)	Humi of Soil (%)	Aver. Air Temp. (^{0}C)	Max. Air Temp. (^{0}C)	Min. Air Temp. (^{0}C)	Aver.Soil Temp. (^{0}C)
March	2012	18.0	8	6.3	81.8	22.0	7.9	12.3	3.6	1.6
	2013	52.8	8	4.5	98.5	24.1	9.6	13.5	5.9	4.8
April	2012	61.4	10	7.4	82.4	24.4	14.1	19.3	9.6	8.7
	2013	16.0	6	6.7	84.8	23.5	13.5	17.7	9.4	8.0
May	2012	62.4	13	7.1	91.2	25.6	18.1	22.5	14.2	13.5
	2013	8.0	2	9.4	69.7	20.7	19.5	23.8	15.1	14.6
June	2012	0.2	1	10.9	78.2	23.6	24.1	28.4	18.9	18.4
	2013	35.0	10	8.4	68.7	18.5	22.4	26.7	18.1	17.5
July	2012	6.0	2	10.6	68.7	16.1	27.0	31.5	22.1	21.2
	2013	0.0	0	10.5	61.4	15.5	24.7	28.8	20.0	19.5
August	2012	7.8	2	10.3	62.7	13.8	26.0	31.1	20.9	19.9
	2013	0.2	1	9.6	62.3	13.3	25.9	30.1	21.7	20.7
September	2012	8.4	3	8.1	73.6	12.9	22.2	26.6	18.1	17.3
	2013	10.2	3	8.4	61.4	12.4	21.6	25.6	16.9	15.8
October	2012	51.0	7	6.5	87.3	17.6	19.2	23.5	15.1	14..0
	2013	96.4	5	6.5	76.2	21.2	14.3	17.9	10.4	9.3
November	2012	24.8	5	3.4	97.0	24.3	13.7	16.9	10.7	10.0
	2013	36.6	6	3.6	79.0	21.3	12.6	15.9	9.6	7.9
December	2012	184.6	17	2.6	97.3	25.1	6.4	9.7	3.1	2.6
	2013	2.4	3	2.7	74.1	20.6	6.2	9.7	3.0	1.7

Precip: Precipitation, Sun: Sunshine, Temp: Temperature Humi: Humidity Aver.: Average

2.3. Experimental design and treatments

In the first year, seeds of wild sunflower were sown into multiple pots in the glasshouse on March 13, 2012, and their seedlings were planted into fields on April 25, 2012. In the second year, the sowing time of seeds into multiple pots and planting time of seedlings on the field were March 12, 2013, and May 17, 2013, respectively. Each experiment was laid out in a Randomized Complete Block Design (RCBD) having four replications and genotypes belonging to different wild annual *Helianthus* species and subspecies. Plot length was kept at 5m in both years. The distance between the rows and between the plants in the rows was 1 m for each. Irrigation was applied for the seedlings to stay alive and hold on the soil during the planting time of seedlings in both years. Weeds were cleaned by mechanical hoeing.

Morphological, agronomic, and Phenological characters such as plant height, primary branches number, secondary branches number per primary branches, plant spreading diameter, the number of days from planting to first flowering, the number of days from planting to 50 % flowering, the number of days from planting to the end of flowering, the number of days of the flowering period, main stem diameter, head diameter, 1000 seeds weight, and seed yield were determined on wild genotypes.

The beginning and ending dates of seed harvest of annual *Helianthus* species are given in *Table 4*. Seed harvest dates of wild annual sunflower genotypes. Harvest times of annual *Helianthus* species were changed from July 15 to December 10 depending on year and genotype.

We could not get enough seeds to calculate yield during harvest from *H. agrestis*, *H. anomalus*, H. *deserticola*, *H. niveus*, *H. niveus subsp. canescens*, *H. niveus subsp. tephrodes*, *H. petiolaris subsp. fallax*, *H. petiolaris subsp. petiolaris*, *H. porteri* in both years, and *H. exilis* in the second year due to plant drying and pollination problems depending on climatic conditions although they had plant emergence, plant development, and flowering. Therefore, some agronomic characters were not evaluated for these genotypes.

2.4. Statistical analysis

Statistical analysis was performed according to standard procedures for a randomized complete block design (RCBD) including replication, year, and genotype factors. The SAS System was used to generate the analysis of variance (ANOVA) for determining treatment effects on the dependent variables (SAS Institute, 1997). Mean comparisons in each year were based on F-Protected Least Significance Differences (LSD) at $P \leq 0.05$.

Table 4. Seed harvest dates of annual Helianthus species in 2012 and 2013

	Harvest duration	
Helianthus species and subspecies	**2012 Start-End**	**2013 Start-End**
H. annuus Ames 4114	July 20 - August 31	July 30 - August 01
H. annuus Ames 7111	August 05 – October 31	August 25 – November 20
H. annuus Ames 29273	August 05 – October 31	September 05 – November 30
H. annuus Ames 29348	August 01 – October 31	August 20 – October 31
H. argophyllus	November 05 - November 20	November 05 - November 20
H. bolanderi	August 1 – October 31	August 20 – November 20
H. debilis ssp. *cucumerifolius*	July 15 - November 08	August 20 - November 20
H. debilis ssp. *debilis*	August 05 – November 30	September 01 – December 10
H. debilis ssp. *silvestris*	August 05 – September 30	August 20 – November 30
H. debilis ssp. *tardiflorus*	August 01 – September 30	August 20 – November 25
H. debilis ssp. *vestitus*	August 05 – November 30	August 10 – December 10
H. exilis	August 25 – September 30	-
H. neglectus	July 20 - September 30	August 10 - November 30
H. petiolaris	August 05 – November 08	August 15 - November 05
H. praecox	July 15 – October 31	August 01 – December 10
H. praecox ssp. *hirtus*	July 15 – October 31	August 01 – December 10
H. praecox ssp. *praecox*	July 15 – October 31	August 01 – December 10
H. praecox ssp. *runyonii*	July 25 – October 20	August 01 – December 10

3. Results and Discussion

According to the analysis of variance in Table 5, year, genotype and year x genotype interaction factors for seed yield, 1000 seeds weight, head diameter, primary branches number, plant spreading diameter, main stem diameter, number of days from planting to first flowering, number of days from planting to 50% flowering, number of days from planting to the end of flowering and number of days of the flowering period were statistically significant at $P < 0.01$. Plant height was affected significantly by genotype and year x genotype interaction factors. The reason for the CV values higher than 10 % was due to the inhomogeneity of the genetic material. The mean comparisons for genotypes were analyzed separately on the basis of years.

Table 6, shows mean comparisons for seed yield, 1000 seed weight, and head diameter of wild annual *Helianthus* species and years. Seed yield per decare of wild sunflower genotypes in 2012 and 2013 ranged between 3.00 and 32.00 kg, and between 1.68 and 249.75 kg, respectively. In the first year, the seed yield of genotypes was very low. We think that the reason for the low yield was the fertility problems experienced in pollination and forming seeds in flowers due to the high air temperature. Hernández et al. (2018) also indicated negative effects of heat stress on seed setting of wild sunflower germplasm. Pollen development has been shown to be highly sensitive to elevated temperatures while the development of the female gametophyte as well as sporophytic tissues might also be disturbed under mild or severe heat stress conditions (Mesihovic et al., 2016). The material was brought from Iowa State, USA. Genotypes grown there at low temperatures were more affected by the high-temperature conditions in the Thrace region of Turkey. In the second year, this effect decreased with the decrease in regional temperatures. *H. annuus* Ames 4114 had the highest seed yield in the first year. This genotype was followed by *H. argophyllus*. In 2013, H. annuus Ames 29348 had the highest seed yield while *H. annuus* Ames 29273, *H.debilis* ssp.*cucumerifolius*, *H. bolanderi*, *H. neglectus*, *H. annuus* Ames 7111 and *H. praecox* ssp. *Runyonii* were in the second-highest seed yield group.

Table 5. Analysis of variance of some seed yield and yield components

Variation sources	Seed yield	1000 seeds weight	Head diameter	Plant height	1. branches number	Spreading diameter
Replication	1084.00	8.94	1.21	111.11	6.22	653.95
Y (Year)	47175.65**	116.48**	33.67**	978.98	657.66**	17368.25**
G (Genotype)	5837.03**	2409.99**	45.43**	26543.64**	384.13**	5910.17**
Y*G	5050.21**	92.71**	13.57**	1920.42**	36.49**	3166.62**
C.V. (%)	94.10	36.52	37.13	16.53	19.19	17.81
Variation sources	**Main stem diameter**	**NDFP^{+} to first flowering**	**NDFP to 50% flowering**	**NDFP to the end of flowering**	**Flowering period days**	
Replication	0.88	32.91	100.70	163.12	135.46	
Y (Year)	10.94**	10360.63**	40480.63**	19298.13**	1400.82**	
G (Genotype)	5.51**	3275.71**	4879.93**	3643.19**	5612.44**	
Y*G	2.27**	125.56**	645.04**	1032.44**	1060.68**	
C.V. (%)	22.00	4.77	4.71	3.63	9.21	
Variation sources	**2. stem diameter 2012**	**Head number 2013**	**Seed number per head 2013**	**Seed yield per plant 2013**	**Seed length 2013**	**Seed width 2013**
Replication	0.26	156295.43	5588.88	1937.05	0.30	0.32
G (Genotype)	1.14**	127995.46**	23260.77**	9305.93**	13.08**	3.41

* and ** : Significant differences are shown at P < 0.05 and P < 0.01, respectively, NDFP^{+}: Number of days from planting

Table 6. Mean comparisons for seed yield, 1000 seed weight, and head diameter

Genotype number		Seed yield (kg da^{-1})		1000 seeds weight (g.)		Head diameter (cm)	
	Helianthus species / subspecies	**2012**	**2013**	**2012**	**2013**	**2012**	**2013**
2	*H. annuus* Ames 4114	32.00a	17.21c	101.20a	69.20a	18.33a	6.40a
3	*H. annuus* Ames 7111	8.00fg	55.34bc	12.50b	10.13b	6.47b	3.23c
4	*H. annuus* Ames 29273	12.00de	112.28b	4.40d	6.50bcd	5.77b	4.10b
5	*H. annuus* Ames 29348	22.00c	249.75a	12.80b	9.20bc	7.57b	4.40b
7	*H. argophyllus*	24.00b	16.60c	9.00c	6.53bcd	2.30c	3.20b
8	*H. bolanderi*	13.00d	61.03bc	3.20e	5.60bcd	2.23c	2.33d
9	*H.debilis* ssp.*cucumerifolius*	7.00gh	109.65b	1.30ij	2.86bcd	1.90c	2.23de
10	*H. debilis* ssp. *debilis*	6.00h	1.68c	1.40hij	0.73d	1.13e	1.13h
11	*H. debilis* ssp. *silvestris*	3.00i	16.50c	1.60ghi	1.03d	1.70c	1.77efg
12	*H. debilis* ssp. *tardiflorus*	7.00gh	13.77c	1.10j	1.33d	1.37c	1.63fgh
13	*H. debilis* ssp. *vestitus*	3.00i	17.40c	1.20ij	0.90d	1.37c	1.30gh
15	*H. exilis*	9.00f	-	1.90fg	-	1.83c	-
16	*H. neglectus*	3.00i	60.29bc	1.40hij	2.93bcd	2.20c	2.10def
20	*H. petiolaris*	11.00e	35.17c	4.60d	3.77bcd	2.50c	2.30de
24	*H. praecox*	11.00e	25.43c	1.80gh	1.90cd	2.30c	1.83defg
25	*H. praecox* ssp. *hirtus*	3.00i	31.99c	1.40hij	1.37d	1.63c	1.87def
26	*H. praecox* ssp. *praecox*	3.00i	34.18c	1.80gh	1.43d	1.90c	1.60fgh
27	*H. praecox* ssp. *runyonii*	3.00i	44.10bc	2.30f	1.23d	2.17c	1.87def
	Means for year	10.07B	53.08A	9.59A	7.45B	3.70A	2.55B
	LSD (p<0.05) for genotype	1.61	69.55	0.47	7.32	2.56	0.54
	LSD (p<0.05) for years	11.75		1.23		0.46	

*: Within each column for genotype and line for the year in each character, means followed by the same letters are not significantly different at P ≤ 0.05.

1000 seed weight of wild annual sunflower genotypes ranged from 1.10 to 101.20 g in 2012 although it was between 0.73 and 69.20 g in the second growing season. In the first year, the seed weight was higher than in the second year. Despite the fertilization and seed setting problems due to high temperature, the development of the formed grains was better in the first year. *H. annuus* Ames 4114 gave the highest seed weights in both years. This genotype was followed by *H. annuus* Ames 29348 and *H. annuus* Ames 7111 in 2012, and by *H. annuus* Ames 7111, *H. annuus* Ames 29348, *H. argophyllus*, *H. annuus* Ames 29273, *H. bolanderi*, *H. petiolaris*, *H. neglectus* and *H. debilis ssp. cucumerifolius* in 2013.

Head diameter according to genotypes ranged between 1.13 and 18.33 cm in 2012, and between 1.13 and 6.40 in 2013. In the first year, the head diameter was measured higher than in the second year. In this result, it is thought that the fact that the precipitation in April and May in the first year was much higher than in the second year had a positive effect on the head development. *H. annuus* Ames 4114 had the highest head diameter in both growing seasons. The second highest head diameter group was created by *H. annuus* Ames 29348, H. annuus Ames 7111and *H. annuus* Ames 29273 in 2012, and by *H. annuus* Ames 29348, *H. annuus* Ames 29273, and *H. argophyllus*.

Mean comparisons for plant height, primary branches number, plant spreading diameter, and main stem diameter are given in *Table 7*.

Table 7. Mean comparisons for plant height, primary branches number, plant spreading diameter, and main stem diameter

Geno. No.	Plant height (cm)		Primary branches number (No)		Plant spreading diameter (cm)		Main stem diameter (cm)	
	2012	2013	2012	2013	2012	2013	2012	2013
2[+]	138.33ef	79.33j	5.00i	5.00h	100.67gh	37.67g	4.17b	1.27 fg
3	187.67ed	165.33efg	20.67cd	21.33defg	210.00bc	143.67bcde	4.03b	2.29cde
4	251.00b	234.67b	27.33b	27.67bcd	274.00a	157.33abc	6.00a	3.17ab
5	166.33cde	228.00bc	19.67cde	27.00bcd	123.33efgh	129.00cdef	3.47bc	3.86a
7	325.67a	305.00a	45.67a	39.33a	156.00de	125.67cdef	3.73bc	2.90c
8	194.33c	198.67cd	22.00bcd	28.00bc	175.00cd	159.67abc	2.50def	3.15abc
9	118.67fgh	192.67de	14.00efgh	27.33bcd	152.67def	192.33a	1.90efgh	2.81bc
10	80.33hi	61.33j	8.33hi	17.33fg	147.00def	95.67f	1.30gh	0.95g
11	145.00def	89.67ij	17.00def	19.33efg	155.00de	112.33def	1.90efgh	1.58efg
12	108.00fghi	136.67gh	25.33bc	31.00b	150.00def	156.0 abcd	2.17defg	2.30bcde
13	71.00i	78.33j	13.67efgh	15.00g	143.33defg	151.7abcde	1.00h	1.39fg
15	105.67fghi	-	25.00bc	-	93.67h	-	1.77fgh	-
16	188.00cd	171.67def	18.00de	26.67bcd	150.00def	183.00ab	5.30a	2.42bcde
20	197.67c	155.67fg	20.00cde	25.33bcde	220.00b	112.00ef	2.80cde	1.79efg
24	102.33fghi	91.0ij	11.33fghi	22.67cdef	176.00bcd	126.33cdef	2.00efg	1.86def
25	84.00ghi	89.0ij	10.00ghi	19.33efg	110.00fgh	135.33cdef	1.60fgh	1.75efg
26	81.67hi	62.67j	15.67defg	19.67efg	139.0defgh	133.33cdef	2.00efg	1.57efg
27	130.67efg	121.0hi	16.0defg	24.00cde	170.0cd	157.33abc	3.00cd	2.68bcd
Means	151.22A	145.02A	18.22 B	23.29A	161.88A	135.78B	2.88A	2.22B
LSD1	46.88	32.18	6.64	6.66	44.54	43.69	0.94	0.88
LSD2	9.68		1.58		10.48		0.22	

[+]: *Helianthus* species, subspecies, and genotype names in Table 1

Mean: Mean for two growing seasons LSD1: LSD for genotype at p<0.05 LSD2: LSD for the year at p<0.05, Geno.: Genotype

*: Within each column for genotype and line for the year in each character, means followed by the same letters are not significantly different at $P \leq 0.05$.

Plant height for the genotype ranged between 71.0 and 325.67 cm in 2012, and between 61.33 and 305.00 cm in 2013. There was no difference between years for plant height. *H. argophyllus* had the highest plant heights in both years. This genotype was followed by *H. annuus* Ames 29273 in the first year, by *H. annuus* Ames 29273

and *H. annuus* Ames 29348 in the second year. *H. debilis ssp. debilis*, *H. debilis* ssp. *vestitus*, *H. praecox*, *H. praecox ssp. hirtus* and *H. praecox ssp. praecox* were in the shortest plant height group in both years. In addition, *H. exilis* and *H. debilis* ssp. *tardiflorus* were in the shortest plant height group in the first growing season while in the second year, *H. debilis* ssp. *silvestris* and *H. annuus* Ames 4114 in this shortest plant height group.

Primary branches number of wild sunflower genotypes ranged between 5.00 and 45.67 cm in 2012, and between 5.00 and 39.33 cm in 2013. In both years, *H. argophyllus* had the highest primary branches number while *H. annuus* Ames 4114 created the lowest primary branches number group. The means of primary branches number of genotypes in the second year was significantly higher than in the first year. We think that this was due to the very low rainfall in the first year in June when branching was at its peak.

Plant spreading diameter of wild annual sunflower genotypes ranged between 93.67 and 274.00 cm in the first year, and between 95.67 and 192.33 cm in the second year. The highest plant spreading diameter was measured in *H. annuus* Ames 29273 in 2012 while the highest plant spreading group had *H.debilis* ssp.*cucumerifolius*, *H. neglectus*, *H. bolanderi*, *H. praecox* ssp. *runyonii*, *H. debilis ssp. tardiflorus*,. *H debilis ssp. vestitus* genotypes. The mean of the first-year plant spreading diameter was statistically higher than the second year.

The main stem diameter of genotypes ranged from 1.00 to 6.00 cm in 2012, and from 0.95 to 3.86 in 2013. In the first year, the highest main stem diameter was measured in *H. annuus* Ames 29273 and *H. neglectus* while it was obtained in *H. annuus* Ames 29348, *H. annuus* Ames 29273 and *H. bolanderi* in the second year. There was a statistically significant difference between years for this character, and the main stem diameter in 2012 was higher than in 2013.

Table 8. Mean comparisons for the number of days from planting to first flowering, number of days from planting to 50% flowering and number of days from planting to the end of flowering, number of days of the flowering period

	Number of days from planting to first flowering (No)		Number of days from planting to 50% flowering (No)		Number of days from planting to the end of flowering (No)		Number of days of the flowering period (No)	
Geno.	**2012**	**2013**	**2012**	**2013**	**2012**	**2013**	**2012**	**2013**
2+	95.00h	105.33g	107.00j	122.00f	173.33k	146.00f	78.33m	40.67g
3	109.33cd	129.67cd	113.33gh	160.67cde	232.00fg	250.00cde	121.67i	120.33ef
4	108.00d	138.00bc	112.33b	161.67cde	236.67d	264.00abc	128.33gh	126.00ef
5	103.00e	126.00cd	114.00g	164.67cd	230.67gh	237.67e	127.67h	116.67f
7	202.00a	202.33a	221.00a	223.00a	252.00b	254.00cde	50.00o	51.67g
8	102.00ef	122.67de	107.00j	162.67cde	232.33fg	256.00bcde	130.33g	133.33def
9	91.00i	125.67cd	97.00l	158.00cde	241.67c	258.3abcde	150.67c	132.67def
10	108.33d	144.00b	158.00c	216.33a	265.00a	278.33a	156.67b	134.33def
11	111.00c	128.00cd	151.00d	169.67bc	202.00j	265.67abcd	91.00l	137.67cdef
12	101.67ef	130.00cd	141.67f	150.33de	203.00j	260.67abcd	101.33k	130.67def
13	103.00e	121.00def	144.00e	216.33a	266.67a	278.67a	162.67a	157.67abcd
15	129.00b	-	159.33c	-	202.00j	-	73.00n	-
16	98.00g	122.67de	143.33e	165.00cd	202.00j	265.33abc	104.00j	142.67bcde
20	108.00d	126.33cd	164.67b	185.67b	242.67c	239.33de	134.67f	113.00f
24	91.00i	112.00efg	97.00l	149.33de	233.67ef	276.33ab	142.67d	164.33abc
25	91.67i	110.00efg	97.33l	146.67e	229.67h	278.33a	138.00e	168.33ab
26	94.00h	108.00fg	101.67k	151.67de	235.33de	279.00a	141.33d	171.00a
27	101.00f	109.33fg	110.00i	154.00cde	221.00i	278.67a	120.00i	169.33ab
Mean	106.96B	127.12A	128.26B	168.10A	229.33B	256.84A	122.31B	129.73A
LSD1	1.93	13.09	1.52	16.06	2.26	22.24	2.63	27.09
LSD2	2.21		2.76		3.78		4.59	

+: Helianthus species, subspecies, and genotype names in *Table 1*
Mean: Mean for two growing seasons, Geno.: Genotype
LSD1: LSD for genotype at p<0.05 LSD2: LSD for year at p<0.05

Mean comparisons for the number of days from planting to first flowering, number of days from planting to 50% flowering, and number of days from planting to the end of flowering, number of days of the flowering period are given in *Table 8*.

The beginning of flowering, the end of flowering, and the flowering period are the most important Phenological characters for sunflower breeding studies with wild sunflowers. Because in hybridization studies, sowing time should be arranged between the parents in order to obtain pollen from the wild at the appropriate time.

The number of days from planting to first flowering ranged from 91.00 to 202.00 in 2012, and from 105.33 to 202.33 in 2013. In the first year, the earliest flowering was observed in *H.debilis* ssp.*cucumerifolius*, *H. praecox,* and *H. praecox ssp. hirtus* while *H. annuus* Ames 4114 and four H. praecox subspecies had the earliest flowering I the second year. In both years, *H. argophyllus* had the latest first flowering. The beginning of this wild annual sunflower species was later than the nearest genotype about 2.5 months in 2012 and 2 months in 2013. In the second year, the beginning of the flowering of genotypes was delayed as statistically significant due to climatic conditions.

The results of the number of days from planting to 50% flowering was similar to beginning of flower. H. *debilis* ssp. *cucumerifolius*, *H. praecox,* and *H. praecox ssp. hirtus* reached earliest to 50% flowering in 2012 while *H. annuus* Ames 4114 had earliest 50% flowering in 2013. *H. argophyllus* had the latest 50% flowering in both years while *H. debilis* ssp. *silvestris* and *H. debilis* ssp. *vestitus* were in the same latest 50% flowering group in 2013. The number of days from planting to 50% flowering in the second year was higher than in the first year.

The number of days from planting to the end of flowering ranged from 173.33 to 266.67 in 2012, and from 146.00 to 278.67 in 2013. The flowering of the first year was completed earlier than the second year. In both years, *H. annuus* Ames 4114 reached the earliest to the end of flowering.

The flowering period of wild sunflower genotypes ranged from 50.0 to 162.67 days in 2012, and from 40.67 to 171.0 days in 2013. In the first year, *H. argophyllus* had the shortest flowering period while *H. debilis ssp. vestitus* had the highest days number for flowering period. In the second year, *H. argophyllus* and *H. annuus* Ames 4114 had the shortest flowering period while the longest flowering was observed in *H. debilis ssp. vestitus* and all *H. praecox* subspecies. Four *H. praecox* genotypes had longer flowering in 2013 according to the first year.

Mean comparisons for the secondary stem diameter, head number per plant, seed number per head, seed yield per plant, seed length, and seed width are given in *Table 9*. Stem diameter was observed in the first year while head number per plant, seed number per head, seed yield per plant, seed length, and seed width were taken in the second year. The main stem diameter of genotypes ranged from 0.60 to 2.33 cm. The highest main stem diameters were measured in *H. annuus* Ames 4114, *H. annuus* Ames 29273, *H. neglectus*, *H. annuus* Ames 29348, *H. argophyllus* and *H. praecox ssp. runyonii*. Head number per plant ranged between 5.0 and 800.70. *H.debilis ssp.cucumerifolius*, *H. neglectus*, *H. praecox ssp. praecox, H. praecox*, *H. bolanderi*, *H. debilis ssp. tardiflorus* and *H. praecox ssp. runyonii* were in the highest head number per plant group while *H. annuus* Ames 4114 had the lowest head number per plant. Seed number per head ranged from 48.67 to 401.00. *H. annuus* Ames 29348 had the highest seed number per plant. Seed yield per plant was observed between 16.07 and 233.20 g. *H. annuus* Ames 29348 had the highest seed yield per plant while the lowest seed yield per plant was observed in *H. annuus* Ames 4114. Seed length ranged from 2.98 to 12.25 mm while seed width was between 1.35 to 5.80 mm. *H. annuus* Ames 4114 highest seed sizes.

It was not possible to measure some features in some species not included in previous results. The characters measured in these species are given in *Table 10*. But, statistical analyzes were not made on these data. It was not possible to obtain grains because these genotypes (*H. agrestis*, H. *anomalus*, *H. deserticola*, *H. debilis subsp. tardiflorus,* all *H. niveus species*, *H. porteri* and two *H. petiolaris* species) had bad germination, growth problems or drying out due to climatic conditions.

Table 9. Mean comparisons for the secondary stem diameter, head number per plant, seed number per head, seed yield per plant, seed length, and seed width

Helianthus species/subspecies	**Secondary stem diameter (cm) 2012**	**Head number per plant (No) 2013**	**Seed number per head (No) 2013**	**Seed yield per plant (g.) 2013**	**Seed length (mm) 2013**	**Seed width (mm) 2013**
H. annuus Ames 4114	2.33a	5.00e	48.67e	16.07e	12.25a	5.80a
H. annuus Ames 7111	1.50bc	117.00cde	177.33c	51.67bc	5.72bc	2.73bc
H. annuus Ames 29273	2.27a	203.70bcde	286.33b	104.84b	4.64cdefg	2.26bc
H. annuus Ames 29348	1.93ab	223.70bcde	401.00a	233.20a	6.44b	3.13bc
H. argophyllus	1.93ab	228.00bcde	177.00c	15.50c	5.10bcde	1.71c
H. bolanderi	1.10cd	479.00abcd	107.67cde	56.99bc	4.97cdef	2.16bc
H.debilis ssp.*cucumerifolius*	0.83d	800.70a	140.00cde	102.38b	3.64fgh	2.10bc
H. debilis ssp. *debilis*	0.73d	83.70de	54.67e	15.57c	4.14defgh	1.83bc
H. debilis ssp. *silvestris*	0.80d	221.30bcde	130.00cde	15.41c	2.98h	1.35c
H. debilis ssp. *tardiflorus*	0.90d	417.00abcde	68.00e	12.85c	4.32defg	3.76b
H. debilis ssp. *vestitus*	0.63d	217.70bcde	78.00de	16.25c	3.75efgh	3.05bc
H. exilis	0.90d	-	-	-	-	-
H. neglectus	2.20a	593.30ab	86.67cde	56.30bc	4.21defgh	2.14bc
H. petiolaris	1.60bc	245.00bcde	117.67cde	32.84c	5.30bcd	2.65bc
H. praecox	0.60d	501.70abc	98.67cde	23.75c	4.00defgh	2.27bc
H. praecox ssp. *hirtus*	0.87d	297.70bcde	133.00cde	28.93c	3.90efgh	1.53c
H. praecox ssp. *praecox*	0.97d	536.70ab	122.00cde	32.00c	3.53gh	1.63c
H. praecox ssp. *runyonii*	1.90ab	418.30abcde	163.33cd	41.18bc	4.02defgh	1.92bc
LSD ($p<0.05$) for genotype	0.53	413.35	94.50	64.97	1.38	1.94

The phylogenetic classification studies on annual wild sunflower species showed that *H. annuus*, *H. argophyllus*, *H. bolanderi* and *H. exilis* were in one of the branches while *H. niveus subsp niveus*, *H. niveus subsp. tephrodes*, *H. niveus subsp. canescens*, *H. praecox*, *H. debilis*, *H. neglectus* and *H. petiolaris* were in the second other branches. In these studies, *H anomalous*, *H. deserticola* and *H. paradoxus* were stated between *H. annuus* and *H. petiolaris* (Rieseberg, 2006). Jocković et al. (2020) studied the pericarp features of wild *Helianthus* L. species as a potential source for improvement of the technical and technological properties of cultivated. They found that the achene length of wild perennial species changed from 3.2 to 6.0 mm, while the achene width was between 1.2 and 2.5 mm. Presotto et al. (2019) indicated that the number of branches and tertiary head diameter could be direct selection criteria for wild sunflower genotypes under stress conditions. Flowering and self-pollination are among the most important characters in sunflower hybrid breeding (Onemli, 2005a; 2005b). The results found in this study are in agreement with previous studies.

4. Conclusion

This study presented data on some phenological and agronomic characteristics of annual wild sunflower species in field conditions containing very valuable preliminary information for plant breeders. The information we have obtained for flowering date adjustments in hybridization studies and important yield components includes very valuable findings for future breeding studies. We had significant problems obtaining seeds from some species. This was due to the difficulty of adapting these species to the local field conditions. Because the gene center of these species is in special climatic regions such as the desert. Studies should be continued, and precautions should be taken for these species which are difficult to adapt to field conditions.

Table 10. Mean comparisons for some characters observed in other species.

Helianthus species/subspecies	**Plant spreading diameter (cm) 2012/2013**	**Plant height (cm) 2012/2013**	**Head diameter (cm) 2012/2013**	**Main stem diameter (cm) 2012/2013**	**NDFP+ to first flowering 2012/2013**	**NDFP to 50% flowering 2012/2013**
H. agrestis	-/104	-/106	-/1.20	-/1.14	-/223	-/244
H. anomalus	-/82	-/78	-/1.40	-/1.35	-/117	-/153
H. debilis subsp. tardiflorus	-/256	-	-	-	-/125	-/160
H. deserticola	-	-	-	-	-/108	-
H. exilis	-/101	-/102	-/1.50	-/1.72	-/132	-/162
H. niveus	28/18	77/50	0.70/0.50	0.70/0.63	108/129	142/174
H. petiolaris subsp. *fallax*	-/187	-/208	-/3.90	-/4.06	-/127	-/169
H. petiolaris subsp. *petiolaris*	-/107	-/124	-/2.80	-/1.63	-/111	-/161
H. porteri	125/111	78/51	-/1.20	1.80/1.14	-/214	-/227
Helianthus species/subspecies	**NDFP to the end of flowering (No) 2012/2013**	**Flowering period days (No) 2012/2013**	**Seed number per head (No) 2013**	**1000 seeds weight (g) 2013**	**Seed yield per plant (g) 2013**	**Seed length/ width (mm) 2013**
H. agrestis	192/280	84/57	50	3.40	-	4.93/1.50
H. anomalus	-/207	-/90	16	0.90	-	5.33/1.13
H. debilis subsp. tardiflorus	-/273	-/35	-	-	-	-
H. deserticola	-	-	-	-	-	-
H. exilis	-/198	-/30	49	1.70	6.20	2.50/1.16
H. niveus	-/232	-/42	28	1.00	-	3.76/1.30
H. petiolaris subsp. *fallax*	-/224	-/42	212	5.30	-	4.60/2.43
H. petiolaris subsp. *petiolaris*	-/217	-/50	182	2.10	--	4.16/5.10
H. porteri	-/251	-/13	23	3.50	2.74	3.40/1.80

NDFP+: Number of days from planting

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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

The Physical, Chemical, Sensory Properties and Aromatic Organic Substance Profile of Kefir Added Citrus Fruits in Different Proportions

Farklı Oranlarda Turunçgil İlave Edilen Kefirin Fiziksel, Kimyasal, Duyusal ve Organik Madde Profili

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Abstract

This study aims to increase the functionality of plain kefir by adding citrus fruits. Dry matter ratios of kefir samples ranged from 11.04 % to 11.75 %. The addition of fruit to kefir reduced the milk-fat ratios. The pH values of kefir samples ranged from 3.37 to 4.08 depending on fruit concentration. pH values also ranged from 3.37 to 4.08 depending on fruit concentration. Kefir samples containing grapefruit (37.5 %) had the lowest pH value (3.37) among the kefir samples. The viscosity of kefir samples at 20 rpm and 50 rpm at sliding speed ranged from 0.42 Pa.s to 2.88 Pa.s and from 0.31 to 1.60 Pa.s, respectively. The addition of fruit to plain kefir was reduced its viscosity. DPPH* of samples was between 1.21 and 38.93 % DPPH of samples with citrus fruit were statistically ($p<0.01$) higher than that of plain kefir samples. While adding orange to plain kefir samples reduced the amount of ethanol, adding grapefruit increased its amount, conversely. Plain kefir samples had higher acetic acid, butanoic acid, hexanoic acid, octanoic acid, n-decanoic acid, benzoic acid, benzaldehyde, benzaldehyde (2,5 bis), silanediol dimethyl, and benzyl alcohol ratios than that of orange, mandarin and grapefruit samples. However, the d-limonene, 1-methyl benzene and benzene 2-ethyl-1,3-dimethyl ratios of kefir samples containing orange, mandarin and grapefruit increased significantly compared to plain kefir. Panelists preferred orange (23 % and 37.5 %) and mandarin (37.5 %) kefir samples more than the others. Panelists gave lower scores to grapefruit-added samples than the other kefir samples.

Keywords: Kefir, Citrus fruits, Aromatic organic matter, Viscosity, Sensory quality

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Öz

Bu çalışmanın amacı, kefirin işlevselliğinin artırılmak için turunçgil ilave edilmiştir. Kefir örneklerinin kuru madde oranları 11.04 % ile 11.75 % arasında değişmektedir. Meyve eklenmesi, kefirin süt yağı oranlarını azaltmıştır. Kefir örneklerinin pH değerleri, meyve konsantrasyonuna bağlı olarak 3.37 ile 4.08 arasında değişmektedir. Kefir örnekleri içinde greyfurt bulunanlar (%37.5), en düşük pH değerine (3.37) sahip olan kefir örnekleri arasında yer almıştır. Kefir örneklerinin 20 rpm ve 50 rpm'deki kayma hızında viskozitesi sırasıyla 0.42 Pa.s ile 2.88 Pa.s ve 0.31 ile 1.60 Pa.s arasında değişmektedir. Meyve eklennesi, kefirin viskozitesini azaltmıştır. Örneklerin DPPH* değerleri %1.21 ile %38.93 arasında değişmektedir. Turunçgiller içeren örneklerin DPPH değerleri istatistiksel olarak ($p<0.01$) kefir örneklerinden daha yüksektir. Portakal eklenen kefir örnekleri etanol miktarını azaltırken, greyfurt eklenmiş olan kefir örnekleri etanol miktarını artırmıştır. Düz kefir örnekleri, portakal, mandalina ve greyfurt örneklerinden daha yüksek asetik asit, butanoik asit, heksanoik asit, oktanoik asit, n-dekanoik asit, benzoik asit, benzaldehit, benzaldehit (2,5 bis), silanediyol dimetil ve benzil alkol oranlarına sahip olduğu görülmüştür. Bununla birlikte, portakal, mandalina ve greyfurt içeren kefir örneklerinin d-limonen, 1-metil benzen ve benzen 2-etil-1,3-dimetil oranları kefire göre önemli ölçüde artmıştır. Panelistler diğerlerine oranla daha çok portakal (%23 ve %37.5) ve mandalina (%37.5) kefir örneklerini tercih etmiştir. Panelistlerin greyfurt eklenmiş örnekleri diğer kefir örneklerine göre daha düşük puan vermiştir.

Anahtar Kelimeler: Kefir, Citrus meyveleri, Aromatik organik madde, Viskozite, Sensör kalitesi

1. Introduction

The word “kefir” is associated with something enjoyable that gives pleasure in Turkish language. This milk product is made by soaking kefir grains in fresh milk and then fermenting it with alcohol and acid (Moltiva et al., 2013).

This product is known by different names such as Kefir, Kiapur, Kanapon, Kopi or Kipi in different parts of the World (Arslan, 2015). The origin of kefir is believed to be in the Caucasus (Özden, 2008). Kefir production has been popular in eastern and central Europe since the 19th century (Russia, Germany, Poland, Slovakia, Denmark, Switzerland, Norway and Hungary) (Karatepe et al., 2012). Kefir contains lactic acid, CO_2 and a small amount of ethanol as well as aromatic substances such as acetaldehyde, acetone and diacetyl, that give kefir its organoleptic properties (Arslan, 2005). The quality and sensory properties of kefir are affected by the type of milk consumed, type and ratios of microorganisms present in the kefir grain, incubation period, storage temperature and its duration (Yaygın, 1996).

Fruit juice is contains sugars, antioxidants, carotenoids, vitamins and polyphenols which are important for human health (Noğay, 2019). Since the fruit sugar present in fruit kefir is used by the kefir microbiota, causing their number to increase, the functional level of fruit kefir as a functional food increases. Citrus fruits have beneficial effects on health due to their components such as ascorbic acid, folic acid, dietary fiber, pectin, potassium, magnesium, carotenoids and flavonoids. Citrus flavonoids such as Naringin and hesperidin that are prominent components in citrus fruits have beneficial effects on hyperglycemia, hyperlipidemia, hypertension, inflammation and weight control. In a study conducted by Kök-Taş et al. (2013) kefir was produced with the addition of 10 % plum and 7.5 % molasses. They found that the total antioxidant content of control, and added kefir samples containing plum or molasses were 13.30 µmol mL^{-1}, 16.80 µmol mL^{-1} and 17.35 µmol mL^{-1} respectively. The control sample and kefir containing dried tangerine, orange or lemon peels had total phenolic content of 945.70 mg mL^{-1}, 2535.80 mg mL^{-1} and 2357.60 mg mL^{-1}, respectively. Dry matter, ash, oil content, pH values and titratable acidity were in the range of 8.64-10.38 %, 0.74-0.79 %, 2.50-3.10 %, 4.15-4.33 and 0.57-0.74 %, respectively. Harmankaya et al. (2019) found that apricot kefir had the highest acidity (0.73%) at the end of the incubation stage, and strawberry kefir and apricot kefir had the lowest pH (5.80). Apricot kefir had the highest acidity at the end of the storage period (+4 °C).

The objective of this study was to determine the quality characteristics of kefir with various citrus fruits added to increase its nutritional value and consumer acceptability Due to the fact that fruit kefir can attract the attention of children and all other age groups and improve the health of consumers, this study was conducted to produce functional kefir in order to improve dietary diversity. In this study evaluates the effect of citrus juice on the physical, chemical and sensory quality of kefir, and it has attempted to determine whether kefir could be combined with citrus juices. In this study, Kefirs containing citrus juices was compared to plain kefir in terms of antioxidant capacity and aroma component differences.

2. Materials and Methods

2.1. Production of plain and fruit kefir

Citrus juices (orange, tangerine and grapefruit) were obtained in a hygienic condition. Plain kefir was made with milk and powdered kefir grain (home kefir grain(vivo)). 1 g of powdered kefir grain was added to 1 kg of milk at 22-25 ^{o}C and incubated for 1 day at the same temperature. The plain kefir was then refrigerated (4 ^{o}C±2 ^{o}C) for 1 day. Citrus kefir samples were made by combining 400 g plain kefir with 120 g (23 %) and 240 g (37.5 %) citrus juice (orange, tangerine and grapefruit). These kefir samples were kept in the refrigerator (4±2 ^{o}C) and subjected to microbiological, physical and chemical analyses.

2.2. Phsical, chemical and biochemical analysis

Dry matter, ash and fat content were determined according to the methods of the Kurt et al. (2012). pH was measured using with a pH meter(Seven Compact pH/Ionmeter S220; Mettler Toledo, Switzerland) (Kurt et al., 2012). The color analyses were done by measuring L* (brightness, 0: black, 100: white), a* (+: red, −: green) and b* (+: yellow, −: blue) values were determined using a chroma meter (CR-300; Konica Minolta, Japan, (Karshenas

et al., 2018). Mix viscosity was measured at 4°C using a viscometer (Model DV-II; Brookfield EngineeringLaboratories, USA) at 20 and 50 rpm (Soukoulis et al., 2014).

The antioxidant activity was analyzed according to DPPH* radical scavenging activity. DPPH* radical scavenging activity was determined, According to the methods modified by Binici et al. (2021). Briefly, DPPH* solution was prepared by dissolving 39 mg of DPPH* in 100 mL ethyl alchol. Sample extracts were mixed with 0.5 mL of the DPPH* solution and adjusted to a final volume of 3 mL with ethyl alchol. After 30 minutes in the dark, the absorbance value was measured at 517 nm. DPPH* values are calculated as a percentage. DPPH*% radical inhibition was calculated as follows: Flavor and aroma compounds were determined according to the modified methods of Grabarczyk and Korolczuk (2010). Briefly, 20 g was diluted from each sample with made up 30 mL of distilled water. Then, HCl was added until the pH was 2.5 and mixed for 1 hour. Samples were centrifuged (10 min, 4000 rpm) and defatted with hexane.

2.3. Sensory analysis

Acording to Nelson and Trout (1951), kefir samples were placed in special 150 mL odor-free containers with glass lids and presented to the panelists in a randomly coded manner at regular intervals. While the panelists were performing sensory analysis, water was placed in 100 ml glass bottle containers to clean their mouths before moving on to the other sample. Sensory evaluations were made by considering color and appearance, texture and fluency, taste and aroma, and general acceptability. Sensory evaluation was performed in a spaced seating arrangement in a room at an appropriate temperature (20±2°C). The 8 experts in the Department of Food Engineering were selected as panelists. Each panelist was experienced, trained and informed about sensory analysis methodology.

2.4. Statistical analysis

The data was analyzed using ANOVA procedures using SPSS (Statistical Software 10.0 for Windows, SPSS). Significant differences between parameters were calculated using the Duncan comparison test at ($p<0.05$) (Pripp, 2013).

3. Results and Discussion

3.1. The results of the dry matter and pH analysis of milk and fruit juices

The results of the drymatter and pH analysis of milk and fruit juices are shown in *Table 1*.

Table 1. Dry matter and pH analysis of milk and fruit juices

Samples	Dry Matter (%)	pH
Milk	12.70	6.75
Orange juice	9.18	3.54
Mandarin juice	10.96	3.20
Grapefruit juice	9.18	2.88

Dry matter and pH values of milk samples were found to be 12.70 % and 6.75 %, respectively. Sahin et al. (2014) found that the pH of the milk was between 6.55 and 6.57. Önal et al. (2021) found that the dry matter ratio of cow milk was between 12.35-13.50 %. Our results were similar to the findings of those studies.

The results of some physical and chemical analyses of fruit kefir samples are shown in *Table 2*.

The dry matter ratios of kefir samples ranged from 11.04 % to11.75 %. Kök-Taş et al. (2013) determined that the dry matter content of the control kefir sample was 11.91 % which is consistent with our findings. The addition of fruit juice reduced the fat ratio of samples (*Table 2*). In our study, pH values ranged from 3.37 to 4.08 depending on fruit concentration with lower pH values found when compared to the related study. Kefir samples containing grapefruit 37,5 % had the lowest pH value (3.37) compared to the other. In line with our findings, Harmankaya et al. (2019) determined that the pH level was lower in all fruit kefirs when compared to plain kefir. Dinç (2008) determined the pH level of plain kefir to be 4.26 which was higher than that of samples of our plain kefir. Uslu

(2010) determined the pH level of plain kefir to be 4.73 while fruit kefirs had an average pH of 4.65. The pH of plain and fruit kefir samples in this study was lower than that of Uslu (2010). It can stem from a difference in kefir grains, incubation periods and fruits at different acidity. The pH degree found by Yilmaz et al. (2006), Güzel-Seydim et al. (2005) and Öner et al. (2010) were higher than our findings. Garrote et al. (2001) found that the pH levels of plain kefir samples were between 3.5 and 4.0, which is similar to our results. In a study conducted by Al and Yıldız (2018), 3 different fruits (gojibery, blueberry and banana) were used, and the fruits used in the production of fruit kefir. They reported that the pH of blueberry kefir samples was 4.60 which was higher than our results.

Table 2. Some phsical and chemical analysis of fruit kefir

Kefir Samples	Dry Matter (%)	Milk Fat (%)	pH	Viscosity (Pa. s)20 RPM	Viscosity (Pa. s)50 RPM	DPPH* (%)
Plain (Control)	11.25±0.45^{a}	3.20±0.14^{a}	4.08±0.03^{a}	2.88±0.04^{a}	1.60±0.0^{a}	1.28±0.10^{e}
Orange (23%)	11.34±0.78^{a}	2.60±0.14^{b}	3.90±0.33^{a}	0.95±0.04^{c}	0.64±0.03^{c}	32.59±0.10^{c}
Orange (37.5%)	11.04±0.37^{a}	2.33±0.18^{b}	3.82±0.01^{a}	0.52±0.04^{d}	0.27±0.01^{e}	32.86±0.10^{c}
Mandarin (23%)	11.75±0.21^{a}	2.50±0.14^{b}	3.78±0.20^{a}	0.99±0.03^{c}	0.70±0.01^{c}	18.69±0.29^{d}
Mandarin (37.5%)	11.73±0.23^{a}	2.35±0.07^{b}	3.68±0.04^{a}	0.52±0.03^{d}	0.35±0.04^{d}	38.26±0.10^{b}
Grapefruit (23%)	11.73±0.31^{a}	2.55±0.28^{b}	3.60±0.28^{a}	1.31±0.03^{b}	0.88±0.03^{b}	38.67±0.02^{ab}
Grapefruit (37.5%)	11.05±0.23^{a}	2.30±0.14^{b}	3.37±0.18^{a}	0.42±0.04^{e}	0.31±0.03^{de}	38.93±0.48^{a}
Sig.	ns	*	ns	**	**	**

Note: Data are the average of two replicates, ^{a,b,c,d} means shown with different letters are statistically different from each other, *: $p<0.05$ **: $p<0.01$

The viscosity of kefir samples at 20 rpm and 50 rpm ranged from 0.42 Pa.s to 2.88 Pa.s and from 0.31 and to 1.60 Pa.s, respectively. The addition of fruit to plain kefir reduced its viscosity. Kök-Taş et al. (2013) who made kefir samples found that the viscosity of samples was between 0.225 Pa.s and 0.315 Pa.s. DPPH* % of samples ranged from 1.28 to 38.93. The addition of citrus fruits to kefir caused their antioxidant activity to increase significantly ($p< 0.01$). Randazzo et al. (2016) made the fruit kefir by adding apple, grape, kivifruit, pomegranate, prickly pear and quince. They reported that the DPPH* values of the samples ranged from 34.21 % to 94.70 %. These values were higher than the findings of Randazzo et al. (2016) and our study results. The DPPH* radical scavenging activities of 6 kefir samples collected from the markets were between 58.35 %-94.08 %. The findings of Taşkın (2011) were higher than that of our study results.

The aromatic matter amounts of plain and fruit kefir samples are shown in *Table 3* and continuation of *Table 3*. The ethanol ratio of kefir samples ranged from 0.89 % to 5.67 % (*Table 3*). As the orange amount added to kefir samples increased, the ethanol amount decreased, but the grapefruit addition caused the ethanol level to increase. Randazzo et al. (2016) reported that samples of apple, grape, kiwifruit, pomegranate, prickly pear, and quince kefir contain ethanol at 2.67 %, 4.44 %, 1.03 %, 4.96 %, 2.31 % and 4.51 % ratios, respectively. The results of Randazzo et al. (2016) were consistent with our study results.

The acetic acid, butanoic acid, hexanoic acid, octanoic acid, n-decanoic acid, benzoic acid, benzaldehyde, benzaldehyde (2,5 bis), silanediol dimethyl and benzyl alcohol ratio of plain kefir samples were higher than orange, mandarin and grapefruit. The benzaldehyde content of orange juice was low as 6.3 µg L^{-1} (Erdoğan, 2019), so the benzaldehyde content of the orange kefir samples was also very low. The benzaldehyde ratio decreased as the amount of fruit added to kefir increased (*Table 3*). However, the d-limonene, 1-methyl benzene and benzene 2-ethyl-1,3-dimethyl ratios of kefir samples containing orange, mandarin and grapefruit increased at a significantly higher level than that of plain kefir (*Table 3*). Because a large amount (90.4 %) of the terpene compounds in orange juice is composed of DL-limonene (Erdoğan, 2019), the kefir samples containing citrus fruit had D- limonene at a

higher ratio. Randazzo et al. (2016) found that the acetic acid ratio of fruit kefir samples ranged from 3.34 % to 9.77 %. But, we found that the acetic acid ratio of fruit kefir samples ranged between 1.81 % and 6.69 %. Randazzo et al. (2016) discovered that the benzyl alcohol ratio of fruit kefir samples was below a detectable level, which was consistent with our findings. Randazzo et al. (2016) found that the benzaldehyde ratio of various fruit kefir samples ranged from 1.57 % to11.69 % which was higher than our research findings. The organoleptic analysis results of kefir samples are shown in *Table 4*.

Table 3. Aromatic organic matter ratios of plain and citrus fruit kefir samples

Kefir Samples	Ethanol (%)	Acetic acid (%)	Butanoic acid (%)	Hexanoic acid (%)	Octanoic acid (%)	n-decanoic acid (%)	Benzoic acid (%)
Plain (Control)	4.35	14.69	5.79	14.08	12.59	5.25	3.18
Orange (23%)	1.12	3.96	1.20	3.11	2.98	1.33	0.83
Orange (37.5%)	0.89	1.81	0.62	1.51	1.56	0.74	0.50
Mandarin (23%)	2.40	4.70	1.60	3.80	3.55	1.43	1.19
Mandarin (37.5%)	4.40	3.43	1.41	3.47	3.29	1.62	1.03
Grapefruit (23%)	5.67	6.69	2.36	5.57	5.25	2.23	1.93
Grapefruit (37.5%)	5.01	3.72	1.12	2.83	2.82	1.26	0.97

Table 3. (Continued)

Kefir Samples	Benzaldehyde (%)	Benzaldehyde (2,5 bis) (%)	Silanediol dimethyl (%)	Benzyl alcohol (%)	d-limonene (%)	1-methyl benzene (%)	Eugenol % (%)	Benzene 2-ethyl-1,3-dimethyl (%)
Plain (Control)	1.34	3.32	2.07	1.36	nd	nd	nd	nd
Orange (23%)	0.47	0.99	0.90	nd	38.17	3.84	1.82	2.47
Orange (37.5%)	0.44	0.54	0.41	nd	49.30	2.61	nd	2.69
Mandarin (23%)	0.40	0.99	0.84	nd	39.64	2.77	nd	7.10
Mandarin (37.5%)	0.69	1.01	0.64	nd	38.41	4.13	nd	3.15
Grapefruit (23%)	0.71	1.48	0.73	nd	28.34	2.32	nd	3.52
Grapefruit (37.5%)	0.83	0.92	1.18	nd	39.57	2.77	nd	4.88

Table 4. Sensory analysis results of Kefir samples

Kefir Samples	Color and apperance	Texture and fluency	Taste and aroma	General acceptability
Plain (Control)	8.20	7.30	7.15	7.20
Orange (23%)	8.25	7.20	8.10	8.30
Orange (37.5%)	8.10	7.15	8.45	8.80
Mandarin (23%)	7.05	6.10	7.15	7.10
Mandarin (37.5%)	8.05	7.15	8.10	8.50
Grapefruit (23%)	6.90	6.15	6.05	6.20
Grapefruit (37.5%)	6.70	5.10	6.05	6.05

Panelists favored the orange (23 % and 37.5 %) and mandarin (37.5 %) kefir samples. Panelists gave lower score to grapefruit kefir samples than the others. Generally, all samples were found to have poor texture and fluency scores (*Table 4*). Harmankaya et al. (2019) determined that panelists favored banana and plain kefir more compared to the other samples. In this investigation, plain samples had greater texture and fluency scores than the other samples (*Table 4*). Kök-Taş et al. (2014) found that fruit kefir samples had higher sensory scores than plain kefir sample. The similar results were obtained for orange samples in this study too.

4. Conclusions

The addition of citrus fruit to plain kefir reduced the viscosity of kefir samples. Considering that the viscosity of kefir is an important quality factor, some stabilizers can be added to kefirs containing fruits to increase their viscosity. As orange was added to kefir samples, the ethanol level decreased, but the grapefruit addition caused the ethanol level to increase. The addition of citrus fruit enhanced the aromatic organic matter content, but it caused a decrease in the aromatic organic matter ratios of plain kefir. Considering the ideal aroma level of fruit and kefir, the fruit ratio must be adjusted. As a result, 23 % orange juice and mandarin juice can be added to plain kefir. The addition of 37.5 % citrus fruit juice significantly reduced the viscosity, so this state was not considered as a good result.

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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Germination and Seedling Properties of *Lotus corniculatus* L. Under Simulated Drought Stress

Simüle Edilmiş Kuraklık Stresi Altında *Lotus corniculatus* L.'nin Çimlenme ve Fide Özellikleri

Ramazan BEYAZ[1*]

Abstract

Drought is an important abiotic stress factor that reduces agricultural production and yield in many crops, including forage crops, in agricultural areas around the world. *Lotus corniculatus* L. is the agriculturally crucial perennial legume forage crop that can tolerate moderate drought. However, studies to determine the responses of *L. corniculatus* to drought are limited. Therefore, this study was carried out to determine the seed germination and early seedling growth properties of *L. corniculatus* at different PEG_{6000} induced-drought treatments under *in vitro* conditions. In order to do this, *L. corniculatus* (cv. 'AC Langille') seeds were planted in MS (Murashige and Skoog/Gamborg) medium containing 0%, 4%, and 8% (w/v) PEG_{6000} for 14 days. In this study, germination percentage, mean germination time, germination rate index (speed of germination), shoot and root length, root to shoot length ratio, shoot and root fresh weight, shoot and root dry weight, shoot and root dry matter ratio, root shoot dry matter ratio, shoot and root water content and seedling vigor index parameters were measured. Our results showed that increasing drought levels resulted in an overall significant reduction in germination and seedling growth parameters except shortened mean germination time (especially, 4% PEG_{6000} treatment) and increased shoot and root dry matter ratio at higher (especially, 8% PEG_{6000} treatment) drought levels. When important growth parameters such as length, fresh and dry weight, dry matter ratio and water content, which show the development of root and shoot organs, are evaluated together, it has been determined that the root is negatively affected by drought stress at a higher rate. Based on these data, it can be concluded that the *L. corniculatus* will suffer a high yield loss under the drought stress at the osmotic potential (-1.03 bar) created by 8% PEG_{6000} treatment.

Keywords: *Lotus corniculatus* L., Drought stress, Germination, Seedling properties, *in vitro*

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Öz

Kuraklık, dünyadaki tarım alanlarında yem bitkileri de dahil olmak üzere birçok üründe tarımsal üretimi ve verimi azaltan önemli bir abiyotik stres faktörüdür. *Lotus corniculatus* L., orta derecede kuraklığı tolere edebilen, tarımsal açıdan çok önemli bir çok yıllık baklagil yem bitkisidir. Bununla birlikte, *L. corniculatus*'un kuraklığa verdiği tepkileri belirlemeye yönelik çalışmalar sınırlıdır. Bu nedenle bu çalışma, *L. corniculatus*'un farklı PEG_{6000} kaynaklı kuraklık uygulamalarında *in vitro* koşullarda tohum çimlenmesi ve erken fide büyüme özelliklerini belirlemek amacıyla yapılmıştır. Bunu yapmak için *L. corniculatus* (cv. 'AC Langille') tohumları, 14 gün boyunca %0, %4 ve %8 (w/v) PEG_{6000} içeren MS (Murashige ve Skoog/Gamborg) ortamına ekilmiştir. Bu çalışmada çimlenme yüzdesi, ortalama çimlenme süresi, çimlenme oranı indeksi (çimlenme hızı), sürgün ve kök uzunluğu, kök sürgün uzunluğu oranı, sürgün ve kök yaş ağırlığı, sürgün ve kök kuru ağırlığı, sürgün ve kök kuru madde oranı, kök sürgün kuru madde oranı, sürgün ve kök su içeriği ve fide canlılık indeksi parametreleri ölçülmüştür. Sonuçlar, artan kuraklık seviyelerinin, kısalan ortalama çimlenme süresi (özellikle %4 PEG_{6000} uygulamasında) ve daha yüksek sürgün ve kök kuru madde oranı (özellikle, %8 PEG_{6000} uygulamasında) dışında çimlenme ve fide büyüme parametrelerinde genel olarak önemli bir azalmaya yol açtığını göstermiştir. Kök ve sürgün organlarının gelişimini gösteren uzunluk, yaş ve kuru ağırlık, kuru madde oranı ve su içeriği gibi önemli büyüme parametreleri birlikte değerlendirildiğinde, kökün kuraklık stresinden daha yüksek oranda olumsuz etkilendiği tespit edilmiştir. Bu verilere dayanarak, *L. corniculatus*'un %8 PEG_{6000} uygulaması ile oluşturulan ozmotik potansiyeldeki (-1.03 bar) kuraklık stresi altında yüksek verim kaybına uğrayacağı sonucuna varılabilir.

Anahtar Kelimeler: *Lotus corniculatus* L., Kuraklık stresi, Çimlenme, Fide özellikleri, *in vitro*

1. Introduction

Plants are subject to a variety of biotic and abiotic pressures that may limit their ability to operate to their highest level and jeopardize their survival (Soltanbeigi, 2019; Ahmed et al., 2022). Drought stress is one of the most detrimental abiotic stresses that affect and lower agricultural productivity globally (Yousefi et al., 2020). Cells' equilibrium is impacted by water stress because there is less water available in their cytoplasm. Osmotic and oxidative stress are brought on by a cell's lack of water and harm physiological, biochemical, and molecular functions (Martínez-Santos et al., 2021). The tactics used by plants to deal with water shortages include drought tolerance, drought avoidance, and drought escape (Luo et al., 2020). Potentially, the most important times for water stress in plants are germination and early seedling growth stages (Li et al., 2013). Hence, the understanding of drought impacts on germination and early seedling growth stage is critical for crop production and productivity. Recent studies have examined how plants respond to drought stress in terms of seed germination and seedling growth, particularly those involving agricultural crops (Tong et al., 2021; Ahmed et al., 2022; Beyaz, 2022).

The ability to manipulate the parameters and homogeneity of the culture makes in vitro tissue culture techniques an effective tool for investigating the responses of plants to drought stress (Murshed et al., 2015; Martínez-Santos et al., 2021). PEG is mostly used to extract information from plants relating to drought stress. (Meher et al., 2018). Because PEG cannot penetrate through the plant cell wall and has a higher molecular weight than the other osmotic chemicals utilized (especially PEG_{6000}). As a result, it is frequently utilized in germination and drought research to control osmotic potential (Badr et al., 2020).

In contrast to other common forage legumes like *Medicago sativa* (alfalfa) and/or *Trifolium repens* (white clover), the Lotus genus, native to the Mediterranean basin, is extensively distributed around the world. Its species are suited to many different types of environmental stress and soil conditions. It is well known that *L. corniculatus* L. can grow in soils that are acidic, salinous, low in fertility, and poorly drained (Striker et al., 2005). Compared to other forages, it has been found to improve the output of meat and milk (Hunt et al., 2014). Despite all of these significant traits, the majority of *L. corniculatus* L. is reportedly vulnerable to drought (Bao et al., 2014; Ünlüsoy et al., 2022). However, there are limited reports on the effects of drought on seed germination and early growth stage of *Lotus corniculatus*. Therefore, this study was conducted to examine the germination and early growth stage responses of *Lotus corniculatus* L. to PEG_{6000}-induced drought stress under *in vitro* conditions.

2. Materials and Methods

2.1. Plant Material

In this study, the seeds of the *Lotus corniculatus* L. cultivar 'AC Langille', harvested in 2012 and supplied by Utah State University, Plants, Soils and Climate Department, were used as plant material. A cultivar of birdsfoot trefoil (*Lotus corniculatus* L.) called 'AC Langille' was created by Agriculture and Agri-Food Canada's Nappan Research Farm. Two cycles of mass selection for winter hardiness and one cycle of mass selection for seedling vigor were used to generate it. The initial material was six unique germplasms chosen from the cultivar 'Leo' and made available by the University of Guelph's Crop Science Department (Papadopoulos et al., 1998).

2.2. Plant Tissue Culture and Drought Treatments

The growth medium, standard Murashige and Skoog/Gamborg (Plant Media, USA) (Gamborg et al. 1968), contained 3% sucrose (Research Product International, USA) and 0.25% phytagel (Plant Media, USA). Before autoclaving at 121 ºC, 7.25 psia for 20 minutes, the pH of the medium was set to 5.7. *Lotus corniculatus* seeds were surface sterilized in 50% commercial bleach (Clorox-USA, containing 8.25% sodium hypochlorite) in which 1 drop of Tween-20 (Acros Organics) was added for 20 minutes and then rinsed 3 times with distilled water. For drought stress, seeds were sown on standard MS medium with 4% and 8% w/v PEG_{6000}. According to Michel and Kaufmann (1973), the osmotic potential created by 4% w/v PEG_{6000} is -0.36 bar, and the osmotic potential created by 8% w/v PEG_{6000} is -1.03 bar. Germination of seeds and subsequent seedling growth were carried out at 25±1 °C under white fluorescent lamps at an intensity of 30 μmol m^{-2} s^{-1} (PAR) in a photoperiod of 16 h light and 8 h dark.

2.3. Germination Experiment and Morphological Observations

When the growing radicle lengthened to 2 mm, the seed was considered germinated. Starting on the 9th day, the number of germinations was noted every 24 hours (ISTA, 2003). Mean germination time (MGT) was calculated according to Ellis and Roberts (1980).

$MGT = \sum Dn/\sum D$, where n is the number of freshly germinated seeds on day D, and D is the number of days since the start of the experiment. (Eq. 1)

The percentage of seeds that germinated after being exposed to drought stress were estimated using the equation:

$$\text{Germination percentage (GP)} = (\text{Number of germinating seeds}/\text{ Total number of seeds}) \times 100$$

(Al-Enezi et al., 2012) (Eq. 2)

Morphological observations (shoot and root length -cm-; fresh and dry weight -mg per plant-) were made on the seedlings that developed 14 days after the start of the experiment (*Figure 1.*). Dry weights were calculated after samples were dried in an oven (VWR Scientific Inc., USA) at 70°C for 48 hours (Beyaz et al., 2011). Water content (WC), dry matter (%) (DM) and vigor index (VI) were calculated according to the following formulas, respectively:

$$\text{Water content (WC)} = (\text{fresh weight} - \text{dry weight})/\text{fresh weight} \times 100$$ (Zheng et al., 2008) (Eq. 3)

$$\text{Dry matter (DM)} = (\text{dry weight}/\text{fresh weight}) \times 100$$ (Bres et al., 2022) (Eq. 4)

$$\text{Vigor index (VI)} = (\text{average root length} + \text{average hypocotyl length}) \text{ x germination percentage (GP)}$$

(Abdul-Baki and Anderson, 1973) (Eq. 5)

Germination rate was expressed as the germination rate index (GRI) according to Maguire (1962):

$$\text{GRI} = \Sigma \text{ No of Germinated Seeds}/\Sigma \text{ No of Days}$$ (Eq. 6)

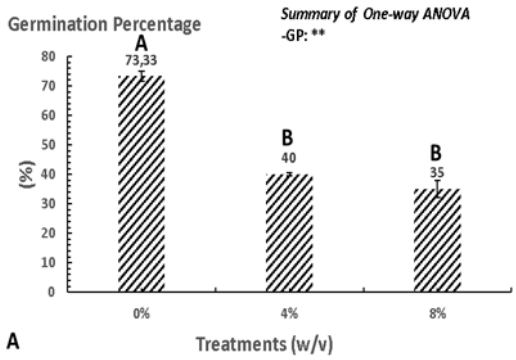
Figure 1. 14-days-old L. corniculatus (cv. 'AC Langille') seedlings at different PEG_{6000} treatments under in vitro conditions. A) 0% PEG_{6000}; B) 4% PEG_{6000}; E) 8% (w/v) PEG_{6000}

2.4. Statistical Analysis

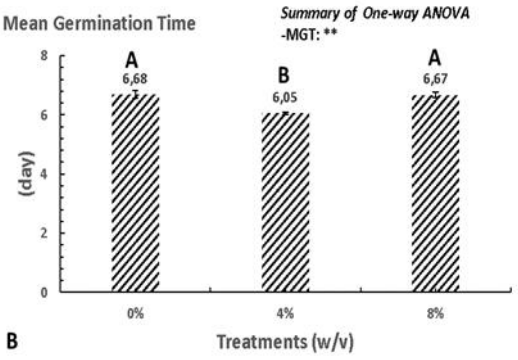
The data collected were analyzed using a one-way Analysis of Variance (ANOVA) in the statistical software package SPSS 22. This analysis aimed to evaluate whether the treatments had a significant impact on the observed parameters. The statistical significance of the means was assessed using the Duncan Multiple Range Test (DMRT) at a significance level of $P<0.05$. Before statistical analysis, the data in percentages were transformed using Arcsine transformation (Snedecor and Cochran 1967).

3. Results and Discussion

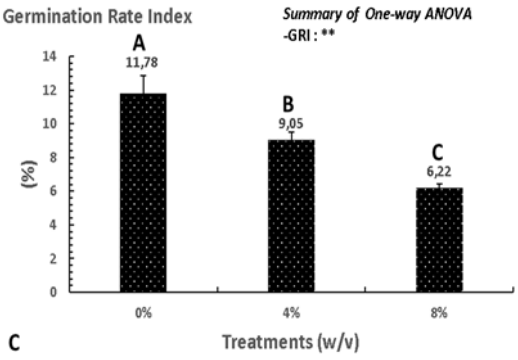
Lotus corniculatus cv. AC Langille's germination percentage, mean germination time, and germination rate index (speed of germination) were all significantly ($P<0.01$) impacted by PEG-induced drought stress (*Figure 2A-2B-2C*). The control had the highest percentage of seeds that germinated (73.33%), whereas the 8% PEG_{6000} treatment had the lowest percentage of seeds that germinated (35.00%). The 4% PEG_{6000} treatment had the fastest mean germination time (6.05 days), which was followed by the 8% PEG_{6000} treatment (6.67 days), and the control (6.68 days). The highest germination rate index (speed of germination) value was obtained with 11.78% in the control group, and the lowest value with 6.22% was obtained in 8% PEG_{6000} treatment. Overall, germination percentage was reduced by 45.45% and 52.27% under 4% PEG_{6000} and 8% PEG_{6000} treatments, respectively. The mean germination time was shortened by 9.43% and 0.14% under 4% PEG_{6000} and 8% PEG_{6000} treatment treatments, respectively. As compared to control, germination rate index (speed of germination) was reduced by 23.17% under 4% PEG_{6000}, and 47.19% under 8% PEG_{6000} treatments. The PEG_{6000}-induced drought conditions also had an impact on shoot and root length (*Figure 2D*). Under control, 4% PEG_{6000}, and 8% PEG_{6000} treatments, the shoot length was observed at 2.22cm, 2.10cm, and 1.16cm, respectively. Root lenght was recorded at 4.92cm, 3.50cm and 2cm under control, 4% PEG_{6000} and 8% PEG_{6000} treatments, respectively. The root to shoot ratio recorded 2.21%, 1.97% and 1.73% in control, 4% PEG and 8% PEG treatments, respectively (*Figure 2E*). Shoot lenght was reduced by 5.40% under 4% PEG_{6000} treatment, and 47.74% under 8% PEG_{6000} treatment, in comparison with control. For root lenght, 28.86% and 59.34% reduction was noticed under 4% PEG_{6000} and 8% PEG_{6000}treatments, respectively, as compared with control. The root to shoot ratio was reduced by 10.85% under 4% PEG_{6000} treatment, and 20.81% under 8% PEG_{6000} treatment, in comparison with control. Under conditions of drought brought on by the PEG_{6000} treatments, the shoot fresh weight, root fresh weight, the shoot dry weight, and root dry weight of *L. corniculatus* seedlings was considerably impacted (*Figure 2F-2G*). The control group recorded the highest fresh weight of shoot (0.043 mg) and root (0.072 mg), whereas 8% PEG_{6000} treatment yielded the lowest fresh weight of shoot (0.014 mg) and root (0.010 mg). Similar to this, the control group had the highest dry weights of the shoot (0.0046 mg) and root (0.0028 mg), while 8% PEG_{6000} treatment had the lowest dry weights of the shoot (0.0033 mg) and root (0.0019 mg). The highest reduction in fresh weight of shoot and root by 67.44% and 86.11%, and dry weight of shoot and root by 28.26% and 32.14%, respectively, was seen under 8% PEG_{6000} treatment, on average, compared to control. Shoot and root dry matter ratio was also influenced ($P<0.01$) by the drought treatments (*Figure 2H*). The highest dry matter of shoot (23.27%) and root (19.28%) was obtained in 8% PEG_{6000} while the lowest dry matter of shoot (10.67%) and root (4.16%) was measured under control. Overall, drought stress increased the shoot dry matter ratio by 40.48% and 118.08% while the root dry matter ratio by 90.86 % and 363.46% under 4% PEG_{6000} and 8% PEG_{6000} treatments, respectively. Under conditions of drought brought on by the PEG treatments, the root to shoot dry matter ratio of *L. corniculatus* seedlings was considerably ($P<0.01$) impacted (*Figure 2I*). In terms of root to shoot dry matter ratio, 8% PEG_{6000} had the highest value (0.82%), followed by 4% PEG_{6000} with a value of 0.54% and control with a value of 0.38%. The root to shoot dry matter ratio increased gradually from 42.10% under 4% PEG_{6000} to 118.75% under 8% PEG_{6000} treatment. Water content of shoot and root was also influenced ($P<0.01$) by the drought treatments (*Figure 2J*). The highest water content of shoot (89.32%) and root (95.83%) was obtained in control while the lowest dry matter of shoot (76.72%) and root (80.71%) was measured under 8% PEG_{6000}. When treated with 4% PEG_{6000} and 8% PEG_{6000}, respectively, water contents of shoot and root showed reductions of 4.83% and 3.94% and 14.10% and 15.77%. Similarly, PEG treatment had an impact on *L. corniculatus*'s seedling vigor index (*Figure 2K*). PEG_{6000} treatments caused a significant ($P<0.01$) reduction of seedling vigor index. The highest seedling vigor index value was obtained from the control group with 525.6, followed by 196 with 4% PEG_{6000} treatment and 127.22 with 8% PEG_{6000} treatment, respectively. Under treatments with 4% PEG_{6000} and 8% PEG_{6000}, it dropped by 67.70% and 75.79%, respectively.


Germination Percentage
Summary of One-way ANOVA
-GP: **
A
73,33
B
40
B
35
(%)
0%
4%
8%
Treatments (w/v)

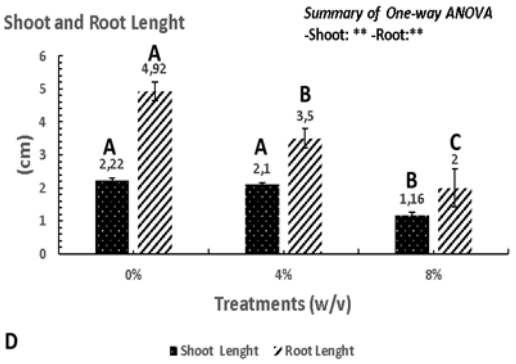
A


Mean Germination Time
Summary of One-way ANOVA
-MGT: **
A
6,68
B
6,05
A
6,67
(day)
0%
4%
8%
Treatments (w/v)

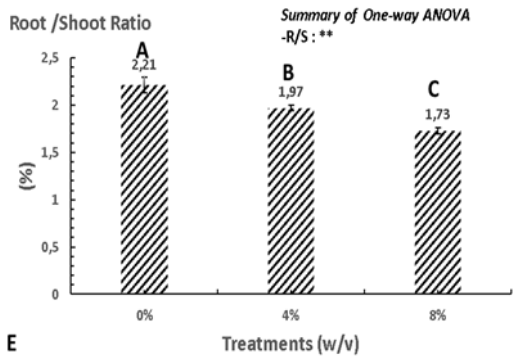
B


Germination Rate Index
Summary of One-way ANOVA
-GRI : **
A
11,78
B
9,05
C
6,22
(%)
0%
4%
8%
Treatments (w/v)

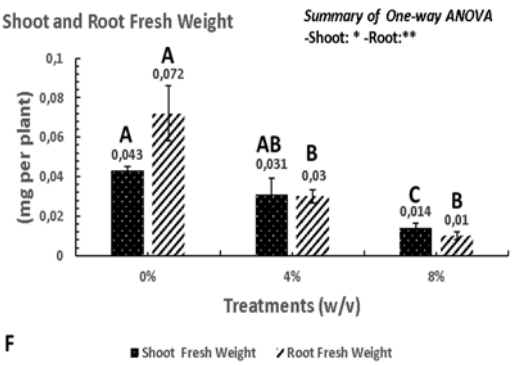
C


Shoot and Root Lenght
Summary of One-way ANOVA
-Shoot: ** -Root:**
A
2,22
A
4,92
A
2,1
B
3,5
B
1,16
C
2
(cm)
0%
4%
8%
Treatments (w/v)
Shoot Lenght
Root Lenght

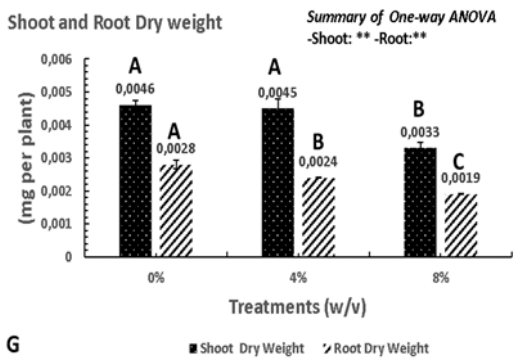
D


Root /Shoot Ratio
Summary of One-way ANOVA
-R/S : **
A
2,21
B
1,97
C
1,73
(%)
0%
4%
8%
Treatments (w/v)

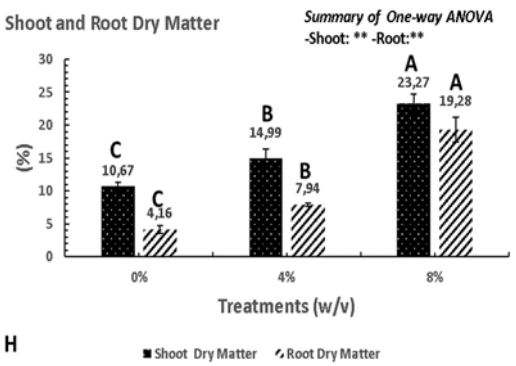
E


Shoot and Root Fresh Weight
Summary of One-way ANOVA
-Shoot: * -Root:**
A
0,043
A
0,072
AB
0,031
B
0,03
C
0,014
B
0,01
(mg per plant)
0%
4%
8%
Treatments (w/v)
Shoot Fresh Weight
Root Fresh Weight

F


Shoot and Root Dry weight
Summary of One-way ANOVA
-Shoot: ** -Root:**
A
0,0046
A
0,0028
A
0,0045
B
0,0024
B
0,0033
C
0,0019
(mg per plant)
0%
4%
8%
Treatments (w/v)
Shoot Dry Weight
Root Dry Weight

G


Shoot and Root Dry Matter
Summary of One-way ANOVA
-Shoot: ** -Root:**
C
10,67
C
4,16
B
14,99
B
7,94
A
23,27
A
19,28
(%)
0%
4%
8%
Treatments (w/v)
Shoot Dry Matter
Root Dry Matter

H

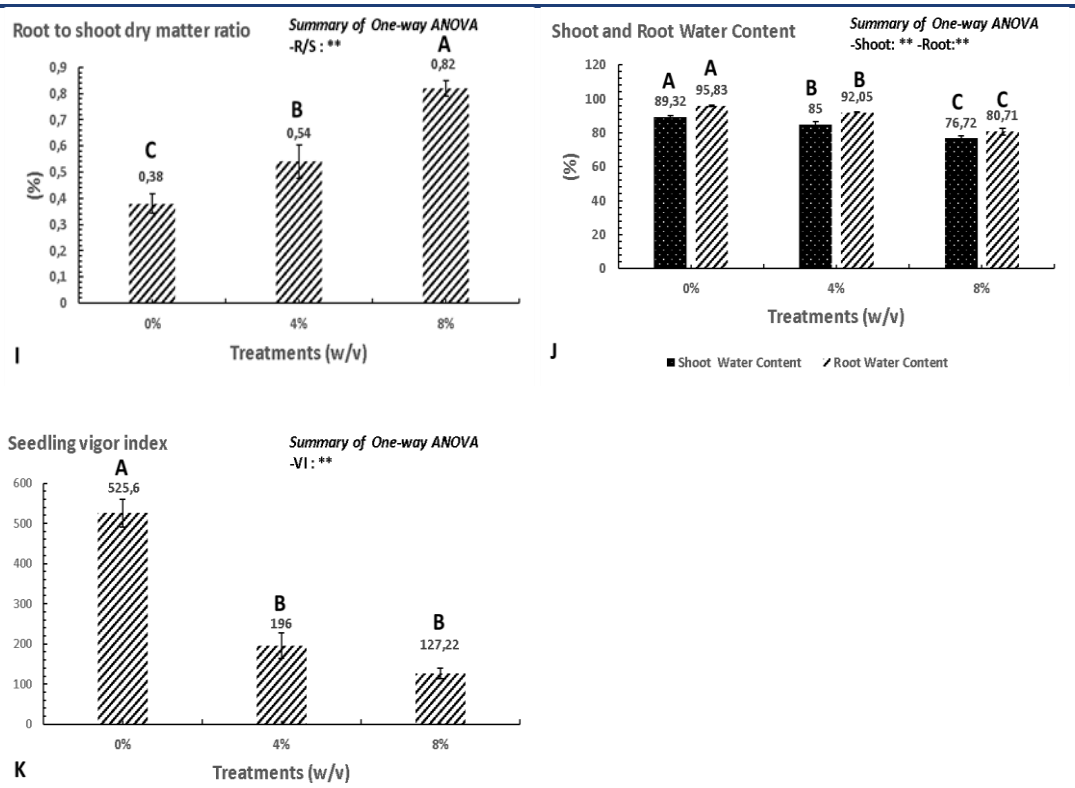


Figure 2. The effect of different levels (0% PEG_{6000}, 4% PEG_{6000}, and 8% PEG_{6000}) of the simulated drought stress on germination percentage, mean germination time, germination rate index, shoot and root length, root shoot length ratio, shoot and root fresh weight, shoot and root dry matter, root shoot dry matter ratio, shoot and root water content, and seedling vigor index of 14-days-old L. corniculatus seedling. **: P<0.01; *: P<0.05.

A significant abiotic factor that significantly reduces crop productivity in the world's arid and semi-arid regions is drought stress. Drought or a water shortage affects germination, which in turn lowers seedling emergence and stops plants from growing further. Successful crop establishment in semi-arid regions mostly depends on good and quick seed germination, which is closely related to seeds' ability to even sprout in the presence of water shortages (Khan et al., 2019). The results of this investigation showed that the growing drought stress steadily hindered (52.27% compared to control with 8% PEG_{6000} treatment) *L. corniculatus* seed germination (*Figure 2A*). Water stress may cause a decrease in the percentage of seeds that germinate because it makes water less permeable through the seed coat and causes seeds to absorb less water initiall (Turk et al., 2004; Bahrami et al., 2012). Similar to the results of this study, it was reported that germination percentage decreased due to increasing drought stress (PEG_{6000}-induced) in other legumes such as sainfoin, alfalfa and white clover (Wang et al., 2009; Hamidi and Safarnejad, 2010; Li et al., 2013; Demiroğlu-Topçu and Özkan, 2016; Bıçakçı et al., 2020; Tong et al., 2021). However, our results highlighted that drought treatments (especially, 4% $PEG6_{000}$) resulted in shortening of mean germination time (*Figure 2B*). Slow germination and slow establishment are a serious problem for *L. corniculatus*. Therefore, researchers try to increase fast of germination of *L. corniculatus* with using some priming techniques. Using PEG_{6000} is one of these priming techniques. Aydınoğlu (2019) reported that the negative effect of drought stress on germination in some forage crops can be reduced by PEG_{6000} priming. However, Jia et al. (2020) noted that a low concentration PEG solution can be employed as an osmotic regulator to control the level and status of water absorption in cells. This can stabilize water absorption in seeds, which will increase their germination and tidy rate. Therefore, it can be interpreted that the concentration of 4% PEG_{6000} treatment makes a positive contribution to the seed germination rate of *L. corniculatus* due to the osmotic potential it creates. However, the mechanism that allows this situation needs to be clarified with more detailed studies (such as physiological, biochemical and molecular). Drought stress influenced the germination rate index (speed of germination) of *L. corniculatus*. The germination rate index (speed of germination) decreased most significantly due to increased PEG_{6000} treatments (*Figure 2C*). As the concentration of the PEG solution increased, the germination of *L.*

corniculatus seeds was also inhibited, according to the study's findings. This could be because low water potential or high osmotic pressure made it difficult for seeds to absorb water during their initial stages of germination, which prevented seeds from germinating normally (Lamia et al., 2012). This show that the negative osmotic effect of PEG on germination, which is in agreement with earlier studies in white clover (Hou and Ma, 2022), sesame (Ahmed et al., 2022), and desert plants (Yousefi et al., 2020). Shoot and root lenght, root shoot ratio, shoot and root fresh weight, shoot and root dry weight parameters are important seedling growth characteristics of seedling establishment. Drought exposure resulted in lower root length, shoot length, root shoot ratio, root and shoot fresh and dry weight at all tested stress levels (*Figure 2D-2E-2F-2G*). Similar findings were made in earlier studies by Ahmed et al. (2022), who found that lower osmotic potential during drought conditions had a negative impact on seedling characteristics of sesame. When lenght, fersh and dry weight were evaluated together for shoot and root organs, it was determined that the highest decrease for lenght and dry weight was in the root under drought stress, however, the highest decrease in fresh weight was in the shoot. It can be interpreted that the further decrease in the lenght and dry weight parameters of the root organ may have occurred because it is the first and harder organ to be exposed to the stress factor. However, the fact that the decrease in fresh weight is less can be interpreted as the fact that the root organ has a higher water content compared to the shoot (*Figure 2J*). Furthermore, the variation in length and fresh and dry weight of shoots and roots could be caused by two factors: (1) impaired cell division and elongation, which eventually slowed down plant growth; and (2) damage from dehydration to the meristem cells of the root and shoot, which interfered with cell division and elongation (Ahmed et al., 2022). A functional balance of growth is provided by the biomass partitioning of roots and shoot growth. Root growth depends on the availability of photosynthates from the shoot, but shoot growth depends on the water absorbed from the root. When under drought stress, stored photosynthates are transferred to the root, allowing it to grow and expand in search of soil water when shoot expansion becomes constrained. The name "Water Spenders" refers to plants that have adapted morphologically to avoid drought stress by increasing root depth, root to shoot ratio, and maintaining water potential above that of the environment (Farooq et al., 2009; Farooq et al., 2012; Bodner et al., 2015; Dissanayake et al., 2019). Therefore, it can be seen as an adaptation developed by this plant so that it can withstand the stress factor more strongly. These findings support earlier research by Hamidi and Safarnejad (2010), Demiroğlu-Topçu and Özkan (2016), Beyaz (2022) and Ahmed et al., (2022) who found significant decreases in growth paramaters of alfalfa, sainfoin, common vetch and sesame under PEG_{6000} induced drought stress. The partitioning of dry matter (DM) in plants is closely correlated with crop productivity during drought stress (Rauf and Sadaqat, 2007). PEG_{6000} induced-drought stress significantly increased dry matter ratio of shoot and root of *L. corniculatus* seedlings (*Figure 2H*). The results revealed that this increase was greater in the root (363.46%) than in the shoot (118.08%) at the highest PEG_{6000} (8%) treatment. However, this increase is indicated by root shoot dry matter ratio (*Figure 2I*). Similar to the research findings, Beyaz (2022) reported that in vitro PEG_{6000} drought treatment (10%) caused an increase in dry matter ratio in both shoot and root organs of common vetch, and this increase was more in the root. A decrease in water content and disruption to cellular membranes, which aid the body in coping with abiotic stresses such as drought, are the primary impacts of stress (Zhou et al., 2012). According to the results of the research, drought treatments caused a decrease in both shoot and root organ water content (*Figure 2J*). This decrease was greater at the root (15.77%) under highest PEG treatment, in comparison with control. Meher et al. (2018) stated that with an increase in PEG_{6000} concentrations (5%, 10%, 15%, and 20%), relative water content (RWC) dramatically decreased in both leaves and roots of peanut. Seed vigor, a crucial indicator of seed quality, assesses the likelihood of rapid and uniform plant emergence (Yousef et al., 2020). Drought stress has an inhibiting influence on the seedling vigor index in the current investigation (*Figure 2K*). In this study, the calculation of seedling vigor index was made by considering seed germination, shoot and root length. Therefore, the sharp decrease in these parameters caused by drought stress also affected seedling vigor index. A very significant loss of 75.79% occurred in seedling vigor index of *L. corniculatus* cultivar AC Langille with 8% PEG_{6000} treatment. The findings of present study are in agreement with the results of Ahmed et al. (2022), Badr et al. (2020), Yousef et al. (2020), and Hou and Ma (2022) who reported the PEG_{6000}-induced drought stress caused a decrease in seedling vigor index.

4. Conclusions

In nutshell, PEG_{6000} induced-drought stress significantly inhibited germination and seedling growth of *Lotus corniculatus* cultivar ˈAC Langilleˈ. When seed germination parameters (germination percentage+germination rate

index) were evaluated cumulatively and their means were calculated, 8% PEG_{6000} treatment caused a 49.73% decrease. On the other hand, in the 4% PEG_{6000} treatment, a 9.43% shortening of the mean germination time occurred, most likely due to the low dose effect. When the above-ground parameters (lenght+fresh and dry weight+ water content of shoot) were evaluated cumulatively and their means were calculated, 8% PEG_{6000} treatment caused a decrease of 37.69%. However, when the below-ground parameters (lenght+fresh and dry weight+water content of root) were evaluated cumulatively and their means were calculated, 8% PEG_{6000} treatment caused a decrease of 47.37%. On the other hand, the above-ground dry matter ratio increased by 118.08% and the below-ground dry matter ratio increased by 363.46%. Additionally, *L. corniculatus* has lost 75.79% of its seedling vigor index under 8% PEG_{6000} treatment. Therefore, in the light of these data, it is seen that the *L. corniculatus* will suffer a high yield loss under the drought stress at the osmotic potential (-1.03 bar) created by 8% PEG_{6000} treatment. In future studies, it is recommended that the germination and initial seedling growth stage of salt stress, which is defined as a sister to drought stress in nature, should be tested to determine the *L. corniculatus* responses, which are stated to be sensitive to salinity.

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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

InDel Variations of *PRL* and *GHR* Genes Associated with Litter Size in Pirlak sheep breed*

Pırlak Koyun Irkında Bir Batında Doğan Yavru Sayısı ile Ilişkili *PRL* ve *BHR* Genlerindeki inDel Varyasyonlar*

Sude ATAY[1], Kadriye Gul YURDAGUL[2], Umit BILGINER[3], Taki KARSLI[4], Eymen DEMIR[5*]

Abstract

Numerous molecular genotyping methods are available to analyse local livestock populations at molecular level in which traditional Polymerase Chain Reaction (PCR) guided by specific oligonucleotides is a fast and cost-effective method to investigate single genes. Until today, many genes which are of major effects on litter size have been reported in sheep. Genetic variations in these genes shaping the expression profile at DNA level may lead to differences in litter size among the sheep breeds. This is the first attempt to investigate insertion/deletion (inDel) variations in Prolactin (*PRL*) intron 2 and Growth Hormone Receptor (*GHR*) intron 3 and intron 4 genes in Pirlak sheep breed via traditional PCR technique. A total of 100 unrelated animals sampled from representative herds reared in Antalya were genotyped based on absence/presence of 23 base pairs (bp) length inDel in which three genotypes (II, ID, and DD) were detected in all loci. I and D allele frequency were 0.421 and 0.579, respectively in terms of *PRL*-intron 2 locus. I / D allele frequencies were found as 0.599 / 0.401 and 0.372 / 0.628 in *GHR* intron 3 and intron 4, respectively. The lowest II (0.181) and DD (0.177) genotype frequencies were detected in *GHR*-intron 4 and *GHR*-intron 3 loci, respectively. The lowest (0.177 for DD) and highest (0.448 for ID) genotype frequencies were detected in *GHR* intron 3 locus across the population. Significant deviation from Hardy-Weinberg Equilibrium (HWE) was detected only in *PRL*-intron 2 locus. The results of the present study confirm that Pirlak breed conserves sufficient genetic variation in *PRL* and *GHR* gene regions which could be utilized in selection strategies in order to increase litter size in the future.

Keywords: Deletion, Insertion, Litter size, Multiple birth, PCR

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Öz

Çiftlik hayvanı populasyonlarının moleküler seviyede analiz edilmesine olanak sağlayan çok sayıda moleküler genotipleme yöntemleri bulunmakla birlikte özgün oligonükleotidlerle yönlendirilmiş geleneksel Polimeraz Zincir Reaksiyonu (PZR) tekli genlerin incelenmesinde kullanılan hızlı ve uygun maliyetli bir tekniktir. Günümüze kadar koyunlarda bir batında doğan yavru sayısı üzerine major etkileri olan çok sayıda gen bildirilmiştir. DNA seviyesinde ekspresyon profilini şekillendiren bu genlerdeki varyasyonlar koyun ırklarında bir batında doğan yavru sayısında farklılıkların görülmesine neden olabilmektedir. Bu çalışma Pırlak koyun ırkında Prolaktin (*PRL*) intron 2 ve Büyüme Hormonu Reseptörü (*BHR*) intron 3 ve intron 4 gen bölgelerindeki insersiyon/delesyon (inDel) varyasyonlarının geleneksel PZR yöntemiyle incelendiği ilk araştırmadır. Antalya'da yetiştirilen, ırkı temsil eden ve birbiriyle akraba olmayan toplam 100 hayvan 23 baz çifti (bç) uzunluğundaki inDel'in varlığı/yokluğuna göre genotiplendirilmiş ve bütün varyasyonlar bakımından üç farklı genotip (II, ID ve DD) tespit edilmiştir. *PRL*-intron 2 lokusunda I ve D allel frekansı sırasıyla 0.421 ve 0.579 olarak bulunmuştur. *BHR*-intron 3 ve intron 4 lokuslarındaki I / D allel frekansları sırasıyla 0.599 / 0.401 ve 0.372 / 0.628 olarak bulunmuştur. En düşük II (0.181) ve DD (0.177) genotip frekansı sırasıyla *BHR*-intron 4 ve *BHR*-intron 3 lokusunda belirlenmiştir. Populasyon seviyesinde en düşük (DD için 0.177) ve en yüksek (ID için 0.448) genotip frekasnları *BHR* intron 3 lokusunda tespit edilmiştir. Sadece *PRL*-intron 2 lokusunda Hardy-Weinberg (HWD) dengesinden önemli sapma tespit edilmiştir. Bu çalışmanın sonuçları Pırlak ırkının *PRL* ve *BHR* gen bölgeleri bakımından yeterli seviyede genetik varyasyona sahip olduğunu ve bunun gelecekte bir batında doğan yavru sayısını arttırmak için yapılacak seleksiyon stratejilerinde kullanılabileceğini göstermiştir.

Anahtar Kelimeler: Delesyon, Insersiyon, Bir batında doğan yavru sayısı, Çoklu doğum, PZR

1. Introduction

Small ruminant breeding is of significant potential to effectively utilise the lands which are not suitable for crop production. Since the meadows and pastures of Türkiye are more suitable for small ruminants rather than cattle (Şişman et al., 2009), sheep rearing is commonly practiced by smallholder farmers (Ertuğrul et al., 2009). Official data reported in 2023 indicates that approximately 45 million sheep most of which are native breeds and their crossbreeds are reared in Türkiye (Anonymous, 2023). Sheep rearing plays an important role in meeting demands for beef in Türkiye (Kocaman and Günal, 2007). Therefore, commercial companies prefer to rear sheep breeds famous for multiple birth traits as well as conducting selection practices in order to increase litter size. In this regard, Pirlak is increasingly preferred in Türkiye due to its advantages characteristics such as high adaptation ability, milk yield, as well as litter size. As highlighted by the General Directorate of Agricultural Research and Policies of Türkiye (GDARP) in 2009, possessing thin-tailed phenotype, Pirlak is reared for dual purposes mainly in Burdur, Isparta and Antalya provinces (Özçelik and Bayram, 2012; Çelikeloğlu et al., 2018). There are several studies in the literature indicating that Pirlak is derived from crossbreeding studies between Daglic and Kivircik breeds (Koyuncu et al., 2005; Özçelik and Bayram 2012; Çelikeloğlu et al., 2018).

Being one of the most important reproductive traits, litter size is of the importance for farm animals such as sheep to survive as well as making contribution to incomes of farmers (Karsli et al., 2012; Tao et al., 2021). Advances in feeding techniques, biotechnology, and molecular genetics have created several alternatives for farmers to improve litter size trait in local sheep breeds. For example, Koyuncu and Canbolat (2009), revealed that energy supplementation at pre-mating (21-day) could increase litter size in Kivircik sheep breed. Similarly, Cam and Kuran (2004), studied the effects of single injection of hCG and GnRH at post-mating (12-day) on litter size in Karayaka and Karayayaka x Sakiz F2 crossbreds in which more twins birth was reported in test group than control group. Although feeding and biotechnological applications could increase litter size in a given sheep population, it may be neither practical nor affordable for smallholder farmers. Multiple births supported by feeding and biotechnological applications are not inherited to the next generations and should be repeated for each mating season due to the nature of quantitative traits including litter size. Indeed, as highlighted by Karsli and Balcioglu (2010), litter size is a complex trait which is influenced by numerous environmental factors and controlled by polygenes. This complexity has been forcing scientists to detect genomic regions having minor and major effects on litter size in sheep. Indeed, numerous genomic regions have been reported to influence litter size in sheep (Karsli and Balcioglu et al., 2010; Karsli et al., 2012). Bone Morphogenetic Protein Receptor IB (*BMPR-IB*) region containing Fecundity Booroola (*FecB*) mutation was reported to be associated with litter size in sheep for the first time (Davis et al., 1982). Since then, scientific efforts making use of developing molecular genotyping methods have yielded numerous gene regions such as Growth Differentiation Factor 9 (*GDF9*) (Hanrahan et al., 2004), *BMP15* (Bodin et al., 2007), Histone Cell Cycle Regulator (*HIRA*) (Zhou et al., 2018), Thyroid Stimulating Hormone Receptor (*TSHR*) (Tao et al., 2021), Neurotrophic Receptor Tyrosine Kinase 2 (*NTRK2*) (Esmaeili-Fard et al., 2021), etc., which are of minor and/or major effects on litter size in sheep. Among litter size-associated genes, *PRL* which is located on chromosome 20 with five exons and four introns in the sheep genome, is known to encode prolactin hormone (Al-Thuwaini, 2021). Being an anterior pituitary peptide hormone, *PRL* plays a key role in many endocrine activities to maintain reproduction in mammals (Ran et al., 2011). The *GHR* gene, on the other hand, encodes a protein called growth hormone receptor which affects follicular growth by stimulating Insulin-like Growth Factor 1 (*IGF-1*) gene (Ghiasi and Abdollahi-Arpanahi, 2021). Genetic variations in reproduction-related genes may lead to different amino acid syntheses as well as affect expression profiles at DNA level. Of these genetic variations, insertion and deletion define the acquisition and loss of different numbers of nucleotides in a given sequence, respectively. InDels are of potential to cause different variations across the genome by causing small frameshift mutations, radically altering genes, changing the binding and splicing sites of the genes as well as disrupting other genomic regions (Narzisi and Schatz, 2015).

Variations in the genomic regions related to litter size allow farmers to improve selection strategies in which animals with desired genotype are used for mating programs (Demir et al., 2022). Also known as Marker Assisted Selection (MAS), this kind of selection is of possibility to increase the frequency of the desired genotype by which litter size are increased from one generation to another. Indeed, a recent study showed that the variations caused by inDel in *PRL* and *GHR* genes are responsible for litter size in Australian White sheep breed (Akhatayeva et al., 2020). Moreover, the authors stressed that these variations could be utilized to improve reproductive traits in sheep

breeds via MAS. Hence, this study aims to detect inDel variations in *PRL* and *GHR* genes in Pirlak sheep breed for the first time. In this regard, the objective of the current study is to assess for the first time genetic variations of Pirlak sheep in terms of *PRL* and *GHR* genes which were previously reported to be associated with litter size in different sheep breeds. Additionally, in case of the detection of advantages genotypes, it is aimed to evaluate the usefulness of these genes in further MAS studies to increase litter size in Pirlak sheep.

2. Materials and Methods

2.1. Sample Collection and DNA Extraction

A total of 100 female animals belonging to Pirlak breed were randomly chosen from five representative herds reared in Antalya province. Blood samples were collected from jugular vein into vacutainer tubes containing EDTA solution as an anticoagulant and stored at -20 °C till DNA extraction was performed. DNA was extracted from whole blood samples via a salting-out method reported by Miller et al. (1988). DNA quality and quantity were checked by both 1% agarose gel electrophoresis and spectrophotometer (NanoDrop-SD 1000). DNA concentration was optimised at 50 ng μL^{-1} for PCR amplification.

2.1. PCR Amplification and Genotyping

In this study, a total of three primer sets (*Table 1*) were used to amplify *PRL* intron 2 *GHR* intron 3, and intron 4 regions.

Table 1. An overview of primer sequence of PRL and GHR gene polymorphisms

Gene	Locus	InDel Type	Primer Sequence (5'-3')	Tm (°C)	Reference
PRL	Intron 2	Insertion	F: GGGAAGGGAAGAGAAACAGAGG R: GCTTGTAGGGTGGAACACTGA	60-59.9	Akhatayeva et al., 2020
GHR	Intron 3	Deletion	F: TGCTGTATGGCCCCTCTAGTA R: CTAAAGAGTTTCCCCAGTCCCC	59.8-60	
GHR	Intron 4	Deletion	F: GCTTCTTGCCCAACCCAATG R: CTGGGCAGTGGAGGAGAAAG	60	

PRL: Prolactin; ***GHR:*** Growth hormone receptor; **F:** Forward primer; **R:** Reverse primer; **Tm:** Annealing temperature.

As reported by Akhatayeva et al. (2020), the genetic variations in these genomic regions occurred due to a 23 bp length of inDel variations. Therefore, traditional PCR was preferred to genotype the individuals. PCR was performed in 50 µL reaction volume with 50 ng template DNA, 5 µL 10X reaction buffer, 0.6 mM dNTPs, 2,5 mM $MgCl_2$,10 pM of each primer, 1 U of Taq DNA polymerase (GeNet Bio, Korea) and 31.25 µL nuclease-free water. PCR amplification was carried out in initial denaturation at 94 °C for 10 min, followed by 31 cycles at 94 °C for 40 s, at 60 °C for 40 s and at 72 °C for 40 s. The final extension was applied at 72 °C for 10 min. All PCR products were separated on 3.5% agarose gel in order to genotype individuals as follows II, ID and DD based on the presence or absence of the PCR fragments.

2.2. Statistical Analysis

GenAlEx software (Peakall and Smouse, 2012) was used to calculate allele and genotype frequencies and to test HWE via chi-square ($\chi 2$) approach.

3. Results and Discussion

While some samples (5 samples in *PRL*-intron 2 locus, 4 samples in *GHR*-intron 3 locus, and 6 samples in *GHR*-intron 4 locus) were removed from the analyses due to failures that occurred in DNA isolation and PCR amplification stages, three genomic regions belonging to *PRL* (intron 2) and *GHR* (intron 3 and 4) turned out to be polymorphic yielding three genotypes such as II, ID and DD in Pirlak breed (*Figure 1*). I allele frequency varied between 0.372 (*GHR*-intron 4) and 0.599 (*GHR*-intron 3), whereas D allele frequency ranged from 0.401 (*GHR*-intron 3) to 0.628 (*GHR*-intron 4) (*Table 2*). Comparatively II genotype frequency was low in terms of *PRL*-intron 2 (0.232) and *GHR*-intron 4 (0.181), DD genotype was detected at lowest frequency in *GHR*-intron 3 polymorphisms. *GHR* gene region was at the HWE, whereas a significant deviation from HWE was detected in *PRL* polymorphism (*Table 2*). Selection

practices on multiple birth traits over generations could significantly change allele frequencies leading to deviation from HWE in Pirlak population.

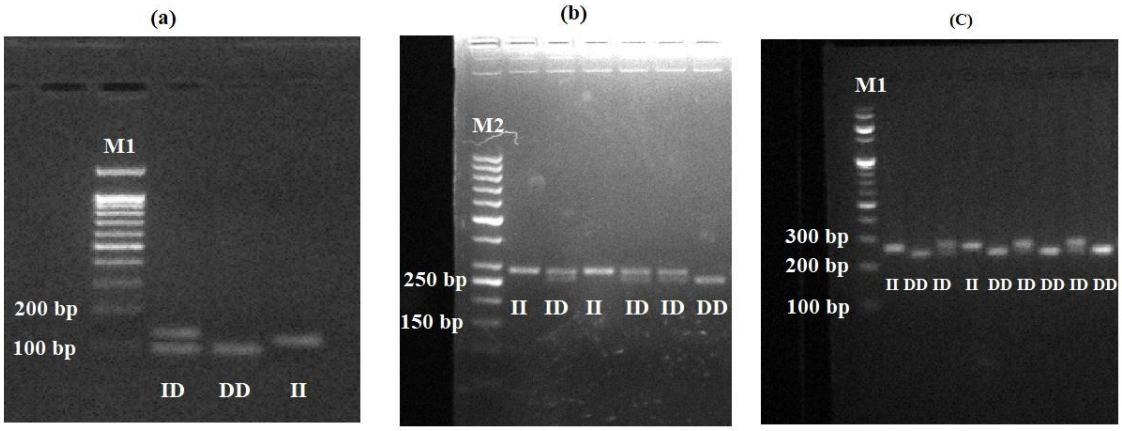

Figure 1. Agarose gel image of (a) PRL-intron 2 (M1: 100 bp ladder, ID: 87 and 110 bp, DD: 87 bp and II: 110 bp), (b) GHR-intron 3 (M2: 50 bp ladder, ID: 260 and 283 bp, DD: 260 bp and II: 283 bp) and (c) GHR-intron 4 ((M1: 100 bp ladder, ID: 238 and 261 bp, DD: 238 bp and II: 261 bp) polymorphisms.

Table 2. Allele and genotype frequency of PRL and GHR indels in Pirlak

Gene	Region	n	Allele Frequency		Genotype Frequency			χ^2
			I	D	II	ID	DD	
PRL	Intron 2	95	0.421	0.579	0.232	0.379	0.389	*
GHR	Intron 3	96	0.599	0.401	0.375	0.448	0.177	ns
GHR	Intron 4	94	0.372	0.628	0.181	0.383	0.436	ns

PRL: Prolactin; ***GHR:*** Growth hormone receptor; **n:** Number of samples; *: Significant deviation from HWE ($p<0.05$), **ns:** Non-significant deviation from HWE.

The same InDel variation of *PRL* intron 2 locus was also studied in Australian White and Luxi Blackhead sheep breeds (Akhatayeva et al., 2020; Mao et al., 2021). Akhatayeva et al. (2020), highlighted that animals with the DD genotype showed superior values in terms of the first parity litter size. In this study, the DD genotype frequency in Pirlak sheep (0.389) was higher than the values reported in Australian White (0.107) and Luxi Blackhead (0.326) sheep breeds (Akhatayeva et al., 2020; Mao et al., 2021). On the contrary, Akhatayeva et al. (2020), confirmed that animals with the II genotype showed superior values of first parity litter size in terms of the *GHR* intron 3 and intron 4 loci. The II genotype frequencies for *GHR* intron 3 and intron 4 were lower in Pirlak sheep (0.375-0.181) compared to Australian White breed (0.378-0.287). Unfortunately, no previous studies focusing on revealing genetic variations of *PRL* and *GHR* genes in Pirlak breed are available in the literature. Moreover, while few similar studies in other native Turkish sheep breeds were available in terms of the *PRL* gene, no studies were detected for the *GHR* gene. For example, Ozmen and Kul (2016) investigated Single Nucleotide Polymorphisms (SNPs) in *PRL* intron 2 across three native sheep breeds namely Sakiz, Akkaraman, and Awassi. The authors detected two alleles (A and B) and three genotypes (AA, AB, and BB) in which A was the most frequent allele (0.77) in Sakiz, whereas the frequency of B allele was higher in Akkaraman (0.85) and Awassi sheep (0.77). Ozmen et al., (2011) sequenced *PRL* receptor intron 1 and exon 10 loci in three native Turkish sheep breeds (Chios, White Karaman, and Awassi) in which 6 and 7 different haplotypes were reported for intron 1 and exon 10, respectively. The authors highlighted that White Karaman and Awassi were similar to each other in terms of *PRL* receptor intron 1 and exon 10, whereas Chios had unique variations.

Except for *PRL* and *GHR*, numerous genes (*AA-NAT*, *GDF9*, *PRLR*, *BMP15*, *BMPR-1B*, *PRL*) were previously studied in other native Turkish sheep breeds rather than Pirlak sheep (Karsli and Balcioglu, 2010; Karsli et al., 2011; Ozmen et al., 2011; Öner et al., 2014; Ozmen and Kul, 2016; Al-Anbari et al., 2018; Kirikci et al., 2021). Both the present research and previous studies evidenced that native Turkish sheep breeds conserve a considerable genetic variation in terms of litter size-related genes. On the other hand, the number of studies is still scarce, and more scientific

studies focusing on different genes associated with reproduction traits are required in Pirlak as well as other native Turkish cattle breeds in order to obtain deeper results which could be used to improve selection strategies.

4. Conclusions

Genetic variations, which were previously reported to be associated with litter size in sheep and caused by 23 bp length of inDel in *PRL* and *GHR* genes, were assessed in Pirlak breed for the first time. These gene regions were highly polymorphic conserving all the possible genotypes including II, ID and DD. Priority should be given to conserve of these variations not only for animals to survive but also for farmers to improve selection strategies in terms of reproductive traits such as litter size. However, Pirlak sheep breed has been neglected so far, since molecular studies are very scarce in the literature. We highly recommend further studies focusing on the other genetic variations in the related gene regions in Pirlak breed.

Ethical Statement

This study was approved by the Akdeniz University Animal Experiments Local Ethics Committee (Protocol No. 1391/2022.01.003).

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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Fertility Characteristics of Soils in Different Stream Beds under Transitional Climate Conditions

Geçiş İklimi Koşulları Altında Farklı Akarsu Yataklarında Yer Alan Toprakların Verimlilik Özellikleri

Bahadır ATMACA[1*]

Abstract

This study was conducted to identify and evaluate some of the fertility characteristics of soils in stream beds in Şebinkarahisar district of the Giresun province of Türkiye, which has a transitional climate between semi-arid and humid climate zones. To this end, a total of 48 soil samples, surface (0-30 cm) and subsurface (30-60/61/62/65 cm), were collected from 24 different sampling points on various stream beds. The textures of the surface and subsurface soils taken were determined as CL, SL, SCL, L, C, and LS. pH values of surface soils ranged from 5.84 to 7.98, and the pH values of subsurface soils ranged from 6.06 to 8.05. Lime contents of the soils without salinity problem ranged from 0.00% to 38.30% for surface soils, and from 0.00% to 37.90% for subsurface soils. Organic matter contents varied between 0.32% and 4.16% for surface soils, and between 0.14% and 2.16% for subsurface soils. While the soils were poor in total nitrogen, phosphorus, zinc and manganese, it was determined that the calcium and copper contents were at sufficient levels. Although deficiencies were detected in some soils for potassium, magnesium and iron, they were generally determined to be at sufficient levels. To deal with deficiencies of macro and micro plant nutrients, including deficiency of organic matter, a fertilization planning is recommended that includes barnyard manure, poultry manure, green manure, compost, vermicompost, and various organic fertilizers containing macro and micro elements. Within the scope of the research, climate classifications were also made according to Thornthwaite, De Martonne-Gottman and Erinç methods by had used 48 years (1965-2012) climate data of Şebinkarahisar district. Plant species and varieties to be grown in the study area should be selected from among those suitable to the transitional climate conditions prevailing in the region. It will also be useful to analyze the stream waters in the areas where soil sampling is done.

Keywords: Soil fertility, Stream bed, Transitional climate, Şebinkarahisar, Giresun

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Öz

Bu çalışma, Türkiye'nin Giresun ilinde, yarı kurak iklim ile nemli iklim arasında bir geçiş ikliminin görüldüğü Şebinkarahisar ilçesinde yer alan akarsu yataklarındaki toprakların bazı verimlilik özelliklerinin belirlenmesi ve değerlendirilmesi amacıyla yapılmıştır. Bu amaçla çeşitli akarsu yataklarının bulunduğu 24 farklı örnekleme noktasından yüzey (0-30 cm) ve yüzey altı (30-60/61/62/65 cm) olmak üzere toplamda 48 adet toprak örneği alınmıştır. Alınan yüzey ve yüzey altı toprakların bünyeleri CL, SL, SCL, L, C, ve LS olarak belirlenmiştir. Yüzey topraklarının pH değerleri 5.84 - 7.98 aralığında değişirken; yüzey altı topraklarının da pH değerleri 6.06 - 8.05 aralığında değişmektedir. Tuzluluk problemi bulunmayan toprakların kireç kapsamları yüzey topraklarında 0.00 ile %38.30 arasında; yüzey altı topraklarında ise 0.00 ile %37.90 arasında değişmektedir. Organik madde içerikleri yüzey topraklarında %0.32 - %4.16 arasında; yüzey altı topraklarında ise %0.14 - %2.16 arasında değişmektedir. Topraklar toplam azot, fosfor, çinko ve manganca fakir iken, kalsiyum ve bakır içeriklerinin yeterli düzeylerde olduğu belirlenmiştir. Potasyum, magnezyum ve demir için bazı topraklarda noksanlıklar saptanmasına rağmen, genellikle yeterli düzeylerde oldukları belirlenmiştir. Organik madde eksikliği de dâhil olacak şekilde belirlenen makro ve mikro besin elementleri eksiklikleri için ahır gübresi, kanatlı hayvan gübreleri, yeşil gübre, kompost, solucan gübresi ve çeşitli makro ve mikro elementli organik gübreleri içeren bir gübreleme planlaması yapılmasının verimlilik için gerekli olduğu düşünülmektedir. Araştırma kapsamında Şebinkarahisar ilçesinin 48 yıllık (1965-2012) iklim verilerinden faydalanılarak, Thornthwaite, De Martonne-Gottman ve Erinç yöntemlerine göre iklim sınıflandırmaları da yapılmıştır. Çalışma alanında yetiştirilebilecek bitki tür ve çeşitleri yörede etkili olan geçiş iklimi koşullarına uygun olanlar arasından seçilmelidir. Toprak örneklemesi yapılan alanlardaki akarsu sularının da analiz edilmesi faydalı olacaktır.

Anahtar Kelimeler: Toprak verimliliği, Akarsu yatağı, Geçiş iklimi, Şebinkarahisar, Giresun

1. Introduction

Soil and water resources are of great importance for living things to survive. These resources, which are among the most important factors of agricultural production, must be protected and their sustainability must be ensured. The success of agricultural activities to be carried out in different regions and under different climatic conditions depends on the availability of clean, healthy and suitable natural resources. Soils in stream beds are very valuable for agriculture. Determining and evaluating the some characteristics of these soils will make an important contribution to various agricultural applications.

Multiple climate conditions prevail in different parts of Türkiye because of differences in elevation and the presence of mountain ranges that act as barriers to rain bearing winds. With the exception of a few places, however, almost all regions of Türkiye suffer from drought and water scarcity in summer. Topography and climate characteristics, along with the presence of different sorts of natural vegetation in places with different climate conditions, means Türkiye has a wide variety of soils, which is also reflected in plant nutrients these soils have (Ülgen and Yurtsever, 1984).

Türkiye is rich in terms of the number of streams it has, with a majority of the streams originating in the country draining into the surrounding seas, and some draining into lakes (closed basins) (Başgelen, 2010). The diversity of drainage types in Türkiye is one of the main characteristics of its drainage network. This is a natural consequence of the fact that Türkiye consists of different parts with diverse characteristics in terms of structure, lithology, geological evolution, and tectonic slope. New tectonic movements, climate change, and karstification add to this diversity. One of the findings of studies on drainage types in Türkiye is that stream networks in large areas are usually young in terms of valley evolution (Erinç, 2012a).

Throughout history, civilizations have emerged and developed in river valleys. This was because rivers provided drinking and domestic water for settlement areas, irrigation water for agricultural areas, and later, energy and industrial water (Yanmaz and Usul, 2006). Stream is the general name given to bodies of water that flow within a bed and its banks. This flow can be constant or intermittent. A stream bed emerges when rainwater is collected along a specific line and then starts flowing (Erinç, 2012a). Initially, surface flow is a wide and thin body of water moving downslope, called sheet flow. This fluctuating and shallow surface flow eventually develops into rivulets of water, which in turn form small channels called brooklets. Brooklets combine to form brooks, and brooks combine to form creeks, streams and rivers. What separates streamflow from surface runoff is that streamflow takes places within a stream bed with defined boundaries (Lutgens et al., 2017).

Agricultural production plays an important role in the development of a society. To grow in a healthy environment, plants need more than a good quality soil. They also need the presence of a reliable source of water. Hydrological characteristics of a given locality depends on its climate, topography, and geology. Climate-related or hydro-meteorological factors are solar radiation, temperature, atmospheric pressure, humidity, and wind. These factors are important because they directly effect key components of the hydrological cycle such as precipitation, evaporation, and transpiration. In hydrology, the term precipitation refers to all sorts of water that comes backs to Earth from the atmosphere. Rain, snow, hail, fog, sleet, and dew are the main forms of precipitation. Precipitation is the source of all usable water on land, and is vital for the humankind. Streams are usually considered to be a reliable source in water supply projects (Yanmaz and Usul, 2006). The effect of precipitation that falls on any given area, in other words the degree of humidity or aridity, is not simply a function of the amount of monthly or annual precipitation. Many other factors including temperature, precipitation regime, evaporation conditions, and soil characteristics also play an important role (Erinç, 2012b).

Knowledge of climate characteristics allows identifying the boundaries between different climate zones, which is important for sustainable use of local resources and providing guidance for land use plans (Çolak and Memişoğlu, 2021). In addition to climate conditions, soil formation and characteristics depend on factors such as decomposition, landforms, particularly the slope of the land, vegetation, main material, and time. Air temperature and precipitation are the most important climate elements that affect soil formation. However, there are other factors beside temperature and precipitation that are required for soil formation. The distribution of air temperature and precipitation throughout the year is also very important (Atalay, 2014). Topography, which is related to climate conditions as well, has indirect effects on soil through temperature and precipitation (Sağlam et al., 1993). Leaving the time factor aside, climate has the most important direct and indirect effect on pedogenesis and soil character

(Erinç, 2012a). Local soils are mainly formed under the influence of climate and vegetation. Characteristics of the parent material are very important for plant attachment, rejuvenation, and development. There is a close relationship between the nutrient needs and root systems of plants on the one hand, and the parent material on the other (Saya and Güney, 2014). The climate factor, because it controls plant development, affects plant nutrition and contents. The factor of precipitation or water, in particular, dissolves the elements in soil, helping a plant make use these elements. Similarly, temperature affects development, and as a result, the nutrient content of the plant as well (Karaçal, 2008). In natural resource management, culture, pasture and forest plants that are resistant to drought and the region should be determined together with climate data and soil structure, and zoning plans should be associated with production planning (Cangir and Boyraz, 2008).

In semi-arid regions, streams have a bigger influence on landforming. However, this effect is also more prominent at specific times. In terms of climate and topography, it is difficult to draw strict boundaries between humid and semi-arid regions. In humid regions, streams are the main factor in landforming. In semi-arid regions, on the other hand, local water table levels play an important role, but the main factor is sea level. In this respect, semi-arid regions are similar to humid regions. Humid regions, on the other hand, are distinguished from semi-arid and arid regions in that they are covered, in areas where original vegetation is not destroyed, by forests or permanently green, uninterrupted grassland (Erinç, 2012b). Bahrami and Ghahraman (2019) argued that, because their study area was located in a semi-arid climate with limited soil and water resources, a proper understanding of geomorphological controls on soil development and fertility would help planners and managers with better management regarding irrigation, agriculture, and soil preservation. Chidozie et al. (2019) found that indiscriminate land use in tropical Nigeria, without sufficient attention paid to land and soil evaluation, caused serious ecologic problems and deterioration of land resources.

Today, in addition to the necessity of increasing soil fertility, ensuring and maintaining continuity in yield is also of great importance. This situation can only be achieved by determining the existing physical, chemical and biological properties of the soils and conscious fertilization studies to be carried out in line with these properties (Bellitürk, 2011).

This study aimed to examine the fertility of soils in stream beds and evaluate their agriculture potential in a locality with a transitional climate between semi-arid and humid climate zones. To this end, some of the fertility characteristics of the collected soil samples were analyzed, followed by an evaluation of data and recommendations. There were two main reasons for selecting the district of Şebinkarahisar as the study area: The district has a transitional climate between semi-arid and humid climate zones, and a variety of stream and soil resources.

2. Materials and Methods

2.1. Study area and soil sampling

Şebinkarahisar district of the province of Giresun is in the Eastern Black Sea part of the Black Sea region of Türkiye. The district center is located on the southern outskirts of Giresun Mountains and the northern slopes of Avutmuş Creek Valley (Yürüdür, 1998). Soil sampling sites on stream beds in the study area were identified using the relevant sections of the 1/25.000 scale standard topographical maps obtained from Anonymous (2019), and carrying out fields observations and investigations. Coordinates and altitudes of the sampling points were identified using a GPS device with a sensitivity of 1 to 3 meters (Magellan Explorist 610). A total of 48 surface (0-30 cm) and subsurface (30-60/61/62/65 cm) soil samples were collected from 24 sampling points on different stream beds. Soil samples were collected between September 28, 2019 and November 2, 2019. Samples were collected from uncultivated land, orchards, and cultivated land. The altitudes of the soil sampling points in the study area ranged from 848 meters to 1714 meters. Location and stream/soil sampling maps (*Figure 1* and *Figure 2*) were created in ArcGIS - ArcMap 10.3 software, using the World Geodetic System (WGS) 1984 Datum. For the stream/soil sampling map, a hydrology analysis was conducted using the Digital Elevation Model (DEM) data (SRTM 1 Arc-Second Global/~30 meters) obtained from USGS (2021). For accurate naming of the stream, the relevant sections of the 1/25.000 scale standard topographical map was used, along with the statements of land owners and Anonymous (2021). Information about soil sampling points and streams is presented in *Table 1*.

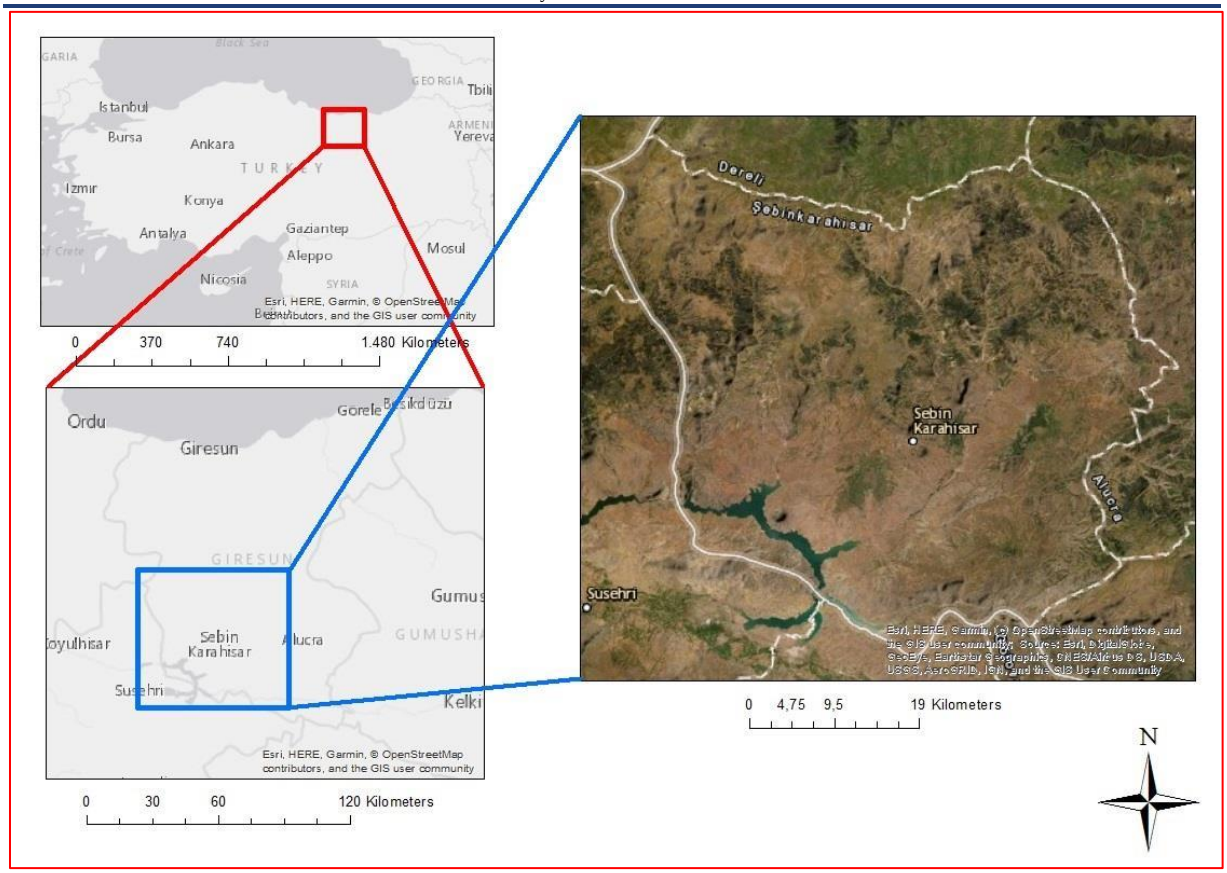

Figure 1. Location map of Şebinkarahisar district

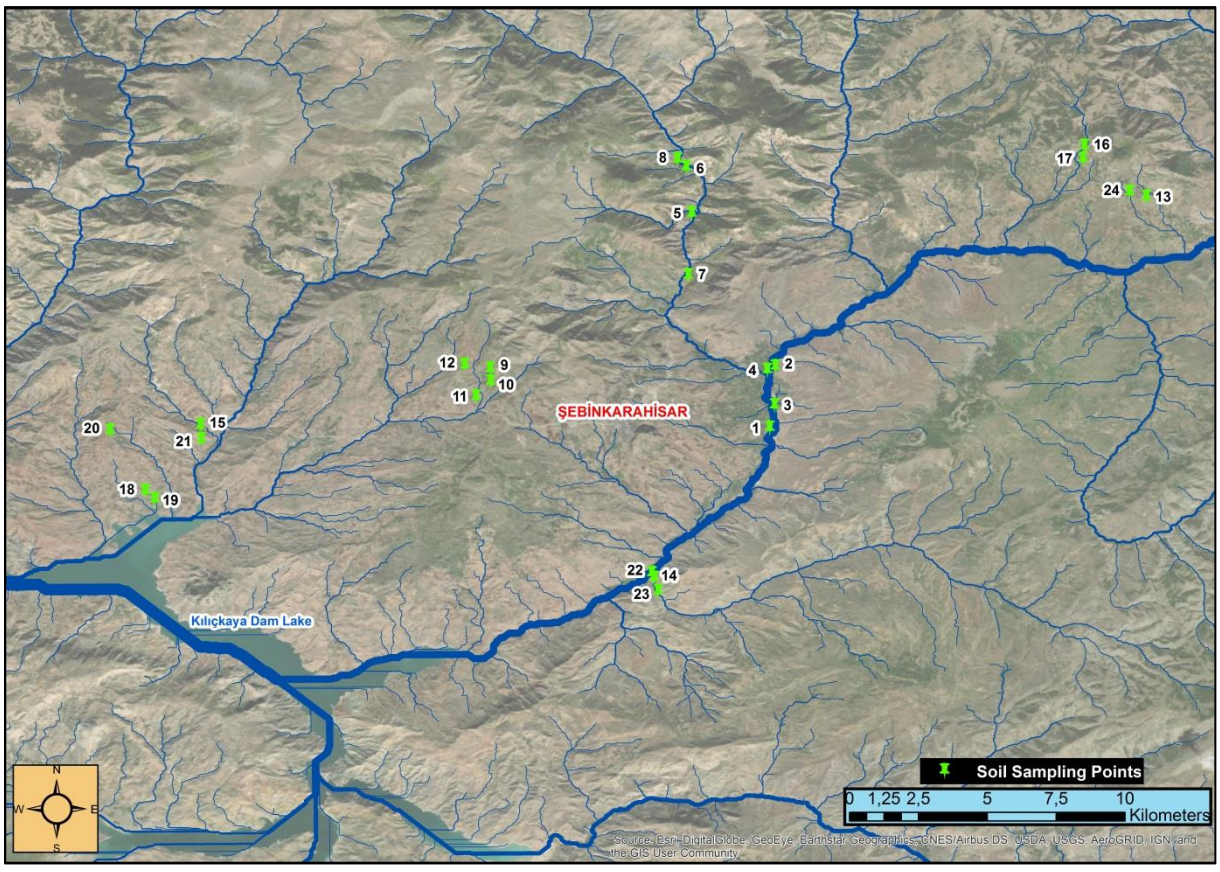

Figure 2. Stream/soil sampling map of the research area

Table 1. Information about the soil sampling points and streams in the study area

Soil sample	Coordinates	Stream name (Stream bed)	Stream
1	40°17'10"N 38°28'15"E	Avutmuş Creek	Active
2	40°18'22"N 38°28'22"E	Avutmuş Creek	Active
3	40°17'36"N 38°28'21"E	Avutmuş Creek	Active
4	40°18'18"N 38°28'12"E	Avutmuş Creek	Active
5	40°21'22"N 38°26'43"E	Asarcık Stream	Active
6	40°22'17"N 38°26'37"E	Asarcık Stream	Active
7	40°20'09"N 38°26'39"E	Asarcık Stream	Active
8	40°22'26"N 38°26'27"E	Asarcık Stream	Active
9	40°18'19"N 38°22'47"E	Bayhasan Stream	Active
10	40°18'04"N 38°22'47"E	Bayhasan Stream	Active
11	40°17'47"N 38°22'30"E	Bayhasan Stream	Active
12	40°18'24"N 38°22'16"E	Kepçeli Stream	Active
13	40°21'42"N 38°35'38"E	Derin Stream	Active
14	40°14'13"N 38°25'59"E	Soğulcuk Stream	Active
15	40°17'13"N 38°17'06"E	Acı Stream	Active
16	40°22'42"N 38°34'25"E	Püsküllü Stream	Active
17	40°22'26"N 38°34'23"E	Püsküllü Stream	Active
18	40°15'55"N 38°16'01"E	Çatalkaya Stream	Intermittent
19	40°15'46"N 38°16'12"E	Çatalkaya Stream	Intermittent
20	40°17'06"N 38°15'20"E	Derin Stream	Intermittent
21	40°16'56"N 38°17'07"E	Yedikardeş Stream	Active
22	40°14'19"N 38°25'56"E	Avutmuş Creek	Active
23	40°13'57"N 38°26'04"E	Soğulcuk Stream	Active
24	40°21'47"N 38°35'18"E	Derin Stream	Active

2.2. Geology and climate

Şebinkarahisar district is located in the southern zone of the eastern belt of Pontides tectonic unit (Ketin, 1966; Altan, 2010; Sarı, 2013). Geological descriptions of the soil sampling points in the study area were taken from the relevant sections of the 1/25.000 scale geological map obtained from the General Directorate of Mineral Research and Exploration, and shown in *Table 2* (Yılmaz, 1984; Akbaş, 1991; Sevin, 1991).

Şebinkarahisar has a transitional climate between semi-arid Central Anatolia climate and humid Black Sea climate (Yürüdür, 1998). Şebinkarahisar has an average temperature of 9.0ºC, a maximum temperature of 39.6ºC, and a minimum temperature of -23.5ºC, and much lower temperatures in winter months compared to Giresun (Kurdoğlu et al., 2017). The average annual precipitation in the district in 584.3 mm, and annual average temperature is 9.1ºC. Data from the Şebinkarahisar station were plugged in the de Martonne formula, and its humid temperate climate was found to display characteristics of a transitional zone between maritime and continental climates (Uzun et al., 2013).

Using the Thornthwaite (1948) method, researchers found the following about the climate of the Şebinkarahisar district: From 1989 to 2008, the climate index was C_2 B'_1 s_2 b'_3 (Subhumid), and it had the sub-climate type 1st degree Mesothermal with strong water deficit in summer, close to marine effects (Aydemir, 2010). From 1981 to 2010, the climate index was C_2 B'_1 s_2 b'_3 (Subhumid), and it had the sub-climate type 1st degree Mesothermal with strong water deficit in summer (Bölük, 2016a). Based on long term annual averages, the climate index of the Şebinkarahisar district was found to be C_2 B'_1 s_2 b'_3 (Çiçek, 1995). Using the De Martonne-Gottman (De Martonne, 1942) method, researchers found the following about the climate of the Şebinkarahisar district: For the period 1989-2008, the aridity index was 16.77 mm, and the climate type of the district approximated that of semi-arid areas (Aydemir, 2010). For the period 1981-2010, the aridity index was 17.13, and the climate type of the district was between semi-arid and humid (Bölük, 2016b). According to the Erinç method, precipitation effectiveness index for the period 1981-2010 was 38.99, and the climate was classified as semi-humid (Bölük, 2016c).

The present study presents average climate data for Şebinkarahisar for the 48 years from 1965 to 2012 (Anonymous, 2020) in *Table 3*, and climate classification of the district on the basis of Thornthwaite (1948), De Martonne-Gottman (De Martonne, 1942; Baltas, 2007) and Erinç (Erinç, 1965; Erinç, 1984) methods in *Table 4*. The average annual temperature in Şebinkarahisar is 9.0°C, the average annual precipitation is 583.5 mm, and the annual average relative humidity is 61.05%.

Table 2. Geological descriptions of soil sampling points

Geological descriptions	Soil sampling points
Oligocene-Lower Miocene, conglomerate-sandstone-mudstone, continental, sedimentary rock	7,9,10,11,12,14,15, 18,19,20,21,22,23
Upper Cretaceous-Paleocene, andesite, shelf, volcanic rock	13,16,17,24
Quaternary, alluvial, continental, sedimentary rock	1,2,3,4
Campanian-Maastrichtian, igneous-sedimentary rock, slope, sedimentary rock	6,8
Senonian-Paleocene, granitoid, deep rock	5

Table 3. Average climate data for Şebinkarahisar for the 48 years (1965 to 2012)

Parameter (Average)	Months												Annual
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	
T	-2.4	-1.5	2.8	8.7	12.8	16.2	19.4	19.7	16.2	11.2	4.8	0.1	9.0
P	50.9	48.8	59.2	86.0	71.2	44.1	15.5	11.8	23.8	54.4	61.8	56.0	583.5
RH	68.8	66.9	63.1	59.1	59.5	57.4	54.8	54.0	54.5	59.9	65.4	69.2	61.05
T_{DMT}	1.6	2.9	7.8	14.2	19.0	23.0	27.1	27.8	23.9	17.3	9.6	3.9	14.84

T: Temperature (°C), P: Precipitation (mm), RH: Relative Humidity (%), T_{DMT}: Daily Maximum Temperature (°C)

Table 4. Climate classifications of Şebinkarahisar district

Şebinkarahisar (Latitude: 40.2872 Longitude: 38.4193 Elevation: 1364.0 m) Climate period (1965 - 2012 / 48 Years)		
Method and classification	**Climate indice**	**Climate feature**
Thornthwaite (1948)	C_2 B'$_1$ s$_2$ b'$_3$ (Moist subhumid)	First Mesothermal, large summer water deficiency, summer concentration 54.57%
De Martonne-Gottman (De Martonne, 1942; Baltas, 2007)	Aridity Index 17.74	Semi Arid - Humid
Erinç (Erinç, 1965; Erinç, 1984)	Precipitation Efficiency Index 39.32	Semi Humid

2.3. Methods

In this study, soil sampling points in Şebinkarahisar district, which has a transitional climate between semi-arid and humid climate zones, were determined by considering the geographical locations and topographic characteristics of the stream beds. Disturbed surface (0-30 cm) and subsurface (30-60/61/62/65 cm) soil samples were collected from the sampling points identified. Soil samples were taken using a spade shovel, a small shovel, a earth auger machine and a tape measure. The samples taken were placed in plastic bags and the characteristics of the sampling area were noted. Afterwards, soil samples brought to the laboratory were laid on newspapers and left to dry. Samples were dried out, and then, prepared for analysis using the necessary materials. The textures of the soils were determined using the hydrometer method (Bouyoucos, 1951). Texture triangle was used to name texture classes (Anonymous, 1993). Soil reaction (pH) was measured using a pH meter in the saturation mud prepared (Richards, 1954). Results of the pH analysis were classified on the basis of the values reported by Kellogg (1952). Total salt contents (%) of the soils were calculated on the basis of the values measured using an electrical conductivity meter in the saturation mud (Richards, 1954). Total salt contents were interpreted on the basis of the values reported by Korkut (1983). Lime contents (%) were measured volumetrically, using the calcimetric method (Kacar, 2016). Organic matter (%) was identified using the Walkley-Black method (Müftüoğlu et al., 2014). Lime contents were classified on the basis of Güçdemir (2008). Organic matter contents were classified according to Ülgen and Yurtsever (1984). Total nitrogen (%) was measured using the Kjeldahl method (Sağlam, 2008). Phosphorus content was measured using the Olsen method (Olsen et al., 1954) and a spectrophotometer; and potassium, calcium, and magnesium contents were measured using an ICP instrument (Müftüoğlu et al., 2014) after extraction with ammonium acetate (Jackson, 1958). Total nitrogen, phosphorus, potassium, calcium, and magnesium contents were interpreted on the basis of the values reported by Anonymous (1990). Fe, Cu, Zn and Mn were extracted with DTPA, and then measured using an ICP instrument (Lindsay and Norvell, 1978). Fe contents were evaluated according to Lindsay and Norvell (1978) Cu contents according to Follett (1969), and Zn and Mn contents according to Anonymous (1990). IBM SPSS Statistics 22 software was used to calculate some of the descriptive statistics and correlation coefficients for soil analysis results.

3. Results and Discussion

In the study, the investigated surface soils (0-30 cm) were classified as CL (nine soil samples), SL (seven soil samples), SCL (four soil samples), L (three soil samples), and C (one soil sample) in terms of texture. Textures of the subsurface soils (30-60/61/62/65 cm) were classified as CL (eight soil samples), as SL (five soil samples), as SCL (five soil samples), as L (three soil samples), as C (two soil samples), and as LS (one soil sample) (Anonymous, 1993). A great majority of the surface soil samples (0-30 cm) collected from orchards around the western parts of the Avutmuş Creek, which is in Şebinkarahisar district of the Giresun province of Türkiye, were classified as sandy clay loam and clay loam in terms of texture (Atmaca and Nalbant, 2018). Textures of surface and subsurface soil samples collected from a stream terrace in Kahta district of the Adıyaman Province of Türkiye were classified as clay loam (CL) and sandy clay loam (SCL) (Çelik and Akça, 2017). In the humid tropical southeastern part of Nigeria, textures of soil samples (0-20) were classified as SL, SCL, and S (Chidozie et al., 2019). Texture classes found in these studies are similar to the texture classes found in the present study. On the other hand, Sowiński et al. (2020) found SiL, SL, and Si texture classes in all horizons of the 20 soil samples they collected from the genetic horizons of 6 soil layers in the soil catena in a hilly valley of Lier River in Buskerud region of Southern Norway, and, Atmaca and Boyraz Erdem

(2016) found that many of the soils in stream beds in the Central district of the province of Tekirdağ were classified as clay, findings that are at variance with the findings of the present study.

Soil in high precipitation areas shows acid reaction, and soil in arid and semi-arid areas with little precipitation shows alkaline reactions (Atalay, 2014). Some of the chemical properties of the soils were analyzed, and the results are reported in *Table 5*. pH values of surface soils ranged from 5.84 to 7.98, and the pH values of subsurface soils ranged from 6.06 to 8.05. Surface and subsurface soils from sampling points 1, 2, 3, 4 (Avutmuş Creek bed), 7 (Asarcık Stream bed), 9, 10, 11 (Bayhasan Stream bed), 12 (Kepçeli Stream bed) and 21 (Yedikardeş Stream bed) were found to be slightly alkaline, and surface and subsurface soils from sampling points 5 (Asarcık Stream bed), 13 and 24 (Derin Stream active bed), 16 (Püsküllü Stream bed) were found to be neutral. Surface and subsurface soils from sampling point 17 (Püsküllü Stream bed) were found to be slightly acidic, and surface and subsurface soils from sampling points 18, 19 (Çatalkaya Stream bed), and 20 (Derin Stream intermittent bed) were found to be alkaline. On the other hand, surface soils from sampling points 14 and 23 (Soğulcuk Stream bed), and 22 (Avutmuş Creek bed) were found to be slightly alkaline, and subsurface soils from the same sampling points were found to be alkaline. Surface soils from the sampling points 6 (Asarcık Stream bed) and 15 (Acı Stream bed) were found to be moderately acidic and alkaline, respectively, and subsurface soils from the same sampling points were found to be slightly acidic and slightly alkaline. Surface soil from the sampling point 8 (Asarcık Stream bed) were neutral, and subsurface soil from the same sampling point were slightly acidic (Kellogg, 1952). Overall, soil reaction results show that soils in the study area, which has a transitional climate between semi-arid and humid climate zones, are similar to soils of semi-arid regions. Some of the soil samples showed acidic reaction, but they were few in number. Because the total salt contents (%) of all the samples from the study area were lower than the reference value of 0.15% reported by Korkut (1983), all of the soils were classified as non-saline. In similar studies, Atmaca and Nalbant (2018) classified as non-saline all of the surface soil samples they collected from around the western parts of the Avutmuş Creek, and reported that most of the soils had neutral soil reaction. Atmaca and Boyraz Erdem (2016) reported that surface soil samples collected from stream beds in the Central district of the province of Tekirdağ in Türkiye usually had neutral pH values, and did not have a salinity problem.

Lime contents of the surface soils from the study area ranged from 0.00% to 38.30%, and lime contents of subsurface soils ranged from 0.00% to 37.90%. Using the threshold values reported by Güçdemir (2008), surface and subsurface soils from sampling points 5, 6, 8 (Asarcık Stream bed), 13 (Derin Stream active bed), and, 16 and 17 (Püsküllü Stream) were classified as lime-free, along with the surface soil from sampling point 24 (Derin Stream active bed). Surface and subsurface soils from sampling points 1 (Avutmuş Creek bed) and 10 (Bayhasan Stream bed) were classified as moderately limey, those from sampling points 11 (Bayhasan Stream bed) and 18 (Çatalkaya Stream bed) were classified as limey, those from sampling points 2 (Avutmuş Creek bed), 15 (Acı Stream bed), 19 (Çatalkaya Stream bed) and 21 (Yedikardeş Stream bed) were classified as highly limey, and those from sampling points 14 and 23 (Soğulcuk Stream bed), and 20 (Derin Stream intermittent bed) were classified as extremely limey. In terms of the lime contents of their surface/subsurface soils, sampling point 4 (Avutmuş Creek bed) was classified as somewhat/slightly limey; sampling point 7 (Asarcık Stream bed) as moderately/highly limey; sampling point 3 (Avutmuş Creek bed) as limey/extremely limey; sampling point 9 (Bayhasan Stream bed) as highly limey/limey; sampling point 12 (Kepçeli Stream bed) as limey/highly limey; and sampling point 22 (Avutmuş Creek bed) as extremely/highly limey. Subsurface soil from the sampling point 24 (Derin Stream active bed) was also classified as slightly limey. One important factor behind the diversity in the lime contents of the soil samples, ranging from non-limey to extremely limey, is that Şebinkarahisar has a transitional climate.

Organic matter contents of the samples varied between 0.32% and 4.16% for surface soils, and between 0.14% and 2.16% for subsurface soils. According to threshold values reported by Ülgen and Yurtsever (1984); surface soils from sampling points 10, 17, and 22 (Bayhasan Stream bed; Püsküllü Stream bed; Avutmuş Creek bed) and subsurface soil from sampling point 6 (Asarcık Stream bed) were found to contain moderate amount organic matter. Surface soils from sampling points 5 and 6 (Asarcık Stream bed) had a high and good amounts of organic matter. The rest of the surface and subsurface soils on the all stream beds had very little or little amounts of organic matter. Ülgen and Yurtsever (1984) argue that soils in Türkiye are usually deficient in organic matter because of a failure to good practice crop rotation, and because the hot and dry climate makes it difficult or impossible for organic matter to accumulate. Atmaca and Boyraz Erdem (2016) found that soils in stream beds in Tekirdağ usually had low lime and organic matter

contents. Atmaca and Nalbant (2018) found that organic matter contents varied between 0.76% and 3.87% around the western part of the Avutmuş Creek in Şebinkarahisar, and lime contents varied between 0.00% and 14.83%.

Bahrami and Ghahraman (2019) found that the soil reaction ranged from 7.66 to 8.81 in 36 soil samples collected from the surfaces (0-30 cm) of three alluvial fans of different ages (relict, old, and young) in Western Sabzevar in Northeast Iran. Chidozie et al. (2019) studied contrasting land use and similar lithology (coastal plain sands) in eight local government areas of Imo State in Nigeria, with an elevation of 91 m above sea level and covering 5530 km^2. They collected surface soil samples (0-20 cm) from all land use types. The authors reported that the hydrology of the study area was governed by Imo River and other rivers such as Nworie, Ogochie, Otamiri, Oramirukpa, and Mba rivers. They found that pH (1:2.5 H_2O) values of the soil samples varied between 4.58 and 6.46 (slightly acidic), and their organic matter contents varied between 0.69% and 2.65%. pH results of study in Northeast Iran and pH and organic matter results of study in Nigeria are different from the findings of the present study regarding pH and organic matter contents of surface soils. Çelik and Akça (2017) reported that elevation from sea level was 637-668 m in their study area in the Kahta district (of Adıyaman), the climate of the district was classified as Csa according to the Köppen-Geiger classification, average annual temperature was 16.6°C, and average annual precipitation was 584 mm. The 14 disturbed soil samples they collected from 7 points on a stream terrace in Research and Application Field of Vocational School of Kahta of Adıyaman University, from depths of 0-30 cm and 30-60 cm, had pH values between 7.60 and 7.89, organic matter contents ranging from 2.02% to 3.11%, and lime contents ranging from 0.08% to 7.47%. They reported that the soils did not have a salinity problem. Çelik and Akça's (2017) findings regarding pH values and lime and organic matter contents are different from those of the present study, but their finding regarding salinity is similar. Mehdi et al. (2002) found that in the surface horizons of Shahdara, Sultanpur and Lyallpur alluvial soil series in Pakistan, $CaCO_3$ levels varied between 1.1% and 13%, organic matter contents between 0.50% and 1.00%, pHs values between 7.8 and 8.1, and ECe between 0.74 and 0.86 dsm^{-1}. At various depths of subsurface horizons, on the other hand, $CaCO_3$ levels varied between 1.1% and 14.0%, organic matter contents between 0.16% and 0.60%, pHs values between 7.9 and 8.2, and ECe values between 0.34 and 9.50 dsm^{-1}. Climate characteristics were classified as semi-arid and semi-humid subtropical continental in the Shahdara series, and semi-arid subtropical continental in Sultanpur and Lyallpur series. Looking at the values reported by Mehdi et al. (2002), all of their findings regarding organic matter, pHs, $CaCO_3$ and ECe results of surface and subsurface soils are different from those of the present study, except for the finding that the surface ve some subsurface soils did not have a salinity problem.

Findings from an analysis of the macro element contents of the study soils are reported in *Table 6*. Total nitrogen, phosphorus, potassium, calcium, and magnesium contents of the soil samples were classified on the basis of the threshold values reported by Anonymous (1990). Total nitrogen contents varied between 0.02% and 0.21% for surface soils, and between 0.01% and 0.11% for subsurface soils. 37.5% of the surface soils were classified as having very little total nitrogen, 41.67% as having little total nitrogen, 12.5% as having sufficient total nitrogen, and 8.33% as having a large amount of total nitrogen. Of the subsurface soil samples, 19 had very little total nitrogen, 4 had little total nitrogen, and 1 had sufficient total nitrogen. Surface and subsurface soils from sampling point 6 on the bed of Asarcık Stream had a large amount and sufficient total nitrogen. Surface soils from sampling points 5 (Asarcık Stream bed), 10 (Bayhasan Stream bed), 17 (Püsküllü Stream bed), and 22 (Avutmuş Creek bed) had sufficient or a large amounts of total nitrogen. The rest of the soil samples on the stream beds had very little or little amounts of total nitrogen.

Phosphorus contents varied between 0.08 ppm and 80.80 ppm for surface soils, and between 0.01 ppm and 48.10 ppm for subsurface soils. Sampling points 9 (Bayhasan Stream bed), 12 (Kepçeli Stream bed), and 19 (Çatalkaya Stream bed) had very little phosphorus, sampling points 1 and 3 (Avutmuş Creek bed), 10 (Bayhasan Stream bed), and 16 (Püsküllü Stream bed) had little phosphorus, and sampling points 4 (Avutmuş Creek bed) and 8 (Asarcık Stream bed) had sufficient phosphorus in their surface and subsurface soils. Sampling points 2 (Avutmuş Creek bed), 13 and 24 (Derin Stream active bed), and 17 (Püsküllü Stream bed), on the other hand, had sufficient phosphorus in their surface soils, and little phosphorus in their subsurface soils. Sampling points 7 (Asarcık Stream bed), 11 (Bayhasan Stream bed), 15 (Acı Stream bed), 18 (Çatalkaya Stream bed), 20 (Derin Stream intermittent bed), 21 (Yedikardeş Stream bed), 14 and 23 (Soğulcuk Stream bed) had little phosphorus in their surface soils, and very little in their subsurface soils. Sampling point 5 (Asarcık Stream bed) had a very large amount phosphorus in its surface soil and a large amount in its subsurface soil; sampling point 6 (Asarcık Stream

bed) had a large amount phosphorus in its surface soil and sufficient in its subsurface soil; and sampling point 22 (Avutmuş Creek bed) had sufficient phosphorus in its surface soil and very little in its subsurface soil.

Table 5. Some of the chemical properties of the research soils

Sample no.	Depth (cm)	pH	Total salt (%)	Lime (%)	OM* (%)	Sample no.	Depth (cm)	pH	Total salt (%)	Lime (%)	OM* (%)
1	0-30	7.53	0.03	4.75	1.10	13	0-30	6.77	0.03	0.00	1.60
	30-60	7.66	0.03	4.91	0.68		30-60	6.97	0.02	0.00	0.82
2	0-30	7.68	0.04	16.17	1.18	14	0-30	7.69	0.02	31.14	0.90
	30-61	7.75	0.04	18.34	0.46		30-60	7.92	0.01	37.90	0.32
3	0-30	7.70	0.01	12.15	0.59	15	0-30	7.89	0.02	24.38	0.40
	30-60	7.68	0.03	26.71	1.04		30-60	7.70	0.01	24.62	0.14
4	0-30	7.54	0.02	3.62	1.49	16	0-30	6.94	0.02	0.00	0.63
	30-60	7.40	0.01	0.16	0.76		30-60	6.98	0.02	0.00	0.46
5	0-30	6.62	0.02	0.00	4.16	17	0-30	6.19	0.01	0.00	2.02
	30-61	6.97	0.01	0.00	1.79		30-61	6.30	0.01	0.00	0.91
6	0-30	5.84	0.01	0.00	3.89	18	0-30	7.91	0.02	13.04	1.23
	30-61	6.06	0.01	0.00	2.16		30-60	7.97	0.02	12.95	0.88
7	0-30	7.36	0.03	5.79	0.34	19	0-30	7.88	0.02	16.74	0.63
	30-61	7.69	0.02	18.34	0.32		30-60	8.02	0.02	19.07	0.32
8	0-30	6.92	0.01	0.00	1.40	20	0-30	7.98	0.01	38.30	0.60
	30-62	6.17	0.01	0.00	0.37		30-61	7.99	0.02	32.02	0.14
9	0-30	7.46	0.05	17.06	0.59	21	0-30	7.78	0.02	23.42	0.83
	30-61	7.67	0.04	10.14	0.37		30-61	7.66	0.02	24.22	0.66
10	0-30	7.56	0.03	7.40	2.16	22	0-30	7.50	0.03	27.12	2.28
	30-62	7.68	0.03	5.71	0.54		30-65	7.87	0.02	21.16	0.32
11	0-30	7.54	0.05	11.18	0.93	23	0-30	7.78	0.03	31.14	1.17
	30-60	7.69	0.04	13.76	0.48		30-60	8.05	0.02	33.47	0.43
12	0-30	7.59	0.03	12.95	0.32	24	0-30	6.84	0.02	0.00	1.77
	30-60	7.69	0.02	16.82	0.34		30-61	7.17	0.02	0.48	0.91

* Organic Matter

Potassium contents of the soils varied between 67.47 ppm and 626.92 ppm for surface soils, and 57.61 ppm and 368.48 ppm for subsurface soils. Sampling points 1, 2, 3, 4 (Avutmuş Creek bed), 6, 7, 8 (Asarcık Stream bed), 9 (Bayhasan Stream bed), 12 (Kepçeli Stream bed), 13 and 24 (Derin Stream active bed), 18 (Çatalkaya Stream bed), 20 (Derin Stream intermittent bed), and 23 (Soğulcuk Stream bed) had sufficient potassium, and sampling point 5 (Asarcık Stream bed) had a large amount of potassium in their surface and subsurface soils. Sampling points 10 and 11 (Bayhasan Stream bed), 21 (Yedikardeş Stream bed), and 22 (Avutmuş Creek bed), on the other hand, had a large amount of potassium in their surface soils, and sufficient potassium in their subsurface soils. Sampling points 16 and 17 (Püsküllü Stream bed) were found to have little potassium in their surface and subsurface soils. Sampling points 14, 15, and 19 (Soğulcuk, Acı and Çatalkaya Stream beds) had sufficient potassium in their surface soils, but little potassium in their subsurface soils.

Bahrami and Ghahraman (2019) found that total nitrogen varied between 0.012% and 0.084%, available phosphorus varied between 0.1 and 19 ppm, and available potassium varied between 52 and 280 ppm in the surface soils they collected. Chidozie et al. (2019) reported that total nitrogen varied between 0.01% and 0.06%, and available phosphorus varied between 0.72 ppm and 6.77 ppm in the surface soil samples they collected in Nigeria. Findings reported by Bahrami and Ghahraman (2019) and Chidozie et al. (2019) regarding surface soils are different from the findings of the present study. In similar studies; Atmaca and Nalbant (2018) reported that surface soils around the western parts of the Avutmuş Creek usually had sufficient total nitrogen and potassium, but some of the soils were deficient in phosphorus. Atmaca and Boyraz Erdem (2016) reported that available phosphorus and exchangeable potassium contents of soil samples collected from stream beds in the Central district of the

province of Tekirdağ were usually sufficient. Çelik and Akça (2017) found that, on average, the soil samples they collected from a stream terrace in Kahta, Adıyaman had low levels of total nitrogen and available phosphorus, and high levels of available potassium.

Calcium contents of surface and subsurface soils from all sampling points varied between 1587.79 ppm and 9648.03 ppm. Surface and subsurface soils from sampling points 6 and 8 (Asarcık Stream bed), and surface soil from sampling point 5 (Asarcık Stream bed) had sufficient calcium. The rest of the surface and subsurface soils from sampling points on the stream beds had a large amount of calcium.

Magnesium contents varied between 168.01 and 821.66 ppm for surface soils, and between 154.29 and 829.02 ppm for subsurface soils. 20 of the surface soils were classified as having sufficient magnesium, and 4 as having a large amount of magnesium. 16 of the subsurface soil samples were found to have sufficient magnesium, and 7 were found to have a large amount of magnesium. Subsurface soil from the sampling point 7 on the bed of Asarcık Stream, on the other hand, was found to contain little magnesium.

Sowiński et al. (2020) found that in the surface horizons of soil samples from the Buskerud region of Southern Norway, total Ca content varied between 3.08 and 7.14 g kg^{-1}, total Mg varied between 3.27 and 4.86 g kg^{-1} and total K varied between 2.85 - 5.12 g kg^{-1}; whereas in subsurface horizons, total Ca varied between 3.18 and 5.97 g kg^{-1}, total Mg varied between 3.66 and 5.46 g kg^{-1} and total K varied between 2.95 - 6.57 g kg^{-1}. Chidozie et al. (2019) reported that surface soil samples collected in Nigeria had Ca contents varying between 0.4 and 2.6 cmol kg^{-1}, Mg contents varying between 0.1 and 1.6 cmol kg^{-1} and K contents varying between 0.01 and 0.22 cmol kg^{-1}. Mehdi et al. (2002) reported that surface horizons of alluvial soil series in Pakistan had $Ca^{++}Mg^{++}$ values ranging from 3.0 to 7.0 me L^{-1}, K^{+} values ranging from 0.13 to 0.30 me L^{-1} and subsurface horizons had $Ca^{++}Mg^{++}$ values ranging from 1.5 to 44.1 me L^{-1}, K^{+} values ranging from 0.10 to 0.31 me L^{-1}. Atmaca and Nalbant (2018) reported that all soil samples collected from around the western parts of the Avutmuş Creek contained large amounts of calcium and sufficient magnesium.

Findings from an analysis of the micro element contents of the study soils are reported in *Table 7*. Iron contents varied between 1.01 ppm and 85.50 ppm for surface soils, and between 2.22 ppm and 69.76 ppm for subsurface soils. Using the values provided by Lindsay and Norvell (1978), surface and subsurface soils from the sampling points 14, 18, 19, and 23 (Soğulcuk and Çatalkaya Stream beds) were classified as containing a moderate amount of iron. Sampling point 12 (Kepçeli Stream bed) had a moderate amount of iron in its surface soil, and little iron in its subsurface soil. Sampling points 15 (Acı Stream bed) and 21 (Yedikardeş Stream bed) had little iron in their surface soils, and a moderate amount in their subsurface soils. Surface soil from the sampling point 20 (Derin Stream intermittent bed), and subsurface soil from the sampling point 22 (Avutmuş Creek bed) contained a moderate amount of iron. All surface and subsurface soils samples apart from these were found to contain a large amount of iron.

Copper contents varied between 1.33 ppm and 6.48 ppm for surface soils, and between 1.30 ppm and 8.41 ppm for subsurface soils. All soil samples in the study were classified as having sufficient copper because they contained more than the threshold value of 0.2 ppm reported by Follett (1969).

Zn and Mn contents were classified on the basis of the threshold values provided by Anonymous (1990). Zinc contents varied between 0.06 ppm and 7.29 ppm for surface soils, and between 0.04 ppm and 4.70 ppm for subsurface soils. 17 of the surface soils contained little or very little zinc, and the rest contained sufficient or a large amount of zinc. Of the subsurface soils, on the other hand, 17 contained very little zinc, 4 contained little zinc, 1 contained sufficient zinc, and 2 contained a large amount of zinc. Sampling points 5 and 6 (Asarcık Stream bed) were found to have a large amount of zinc in their surface and subsurface soils. Surface and subsurface soils from sampling point 4 on the bed of Avutmuş Creek had a large amount and sufficient zinc. Sampling points 7 and 8 (Asarcık Stream bed), and 13 and 24 (Derin Stream active bed) had sufficient or a large amount zinc in their surface soils. All surface and subsurface soils samples apart from these were found to contain very little and little of zinc.

Manganese contents varied between 2.74 ppm and 58.82 ppm for surface soils, and between 1.75 ppm and 41.32 ppm for subsurface soils. Sampling points 1, 2, 3 (Avutmuş Creek bed), 7 (Asarcık Stream bed), 11 (Bayhasan Stream bed), 18 and 19 (Çatalkaya Stream bed), 20 (Derin Stream intermittent bed), 21 (Yedikardeş

Stream bed), 22 (Avutmuş Creek bed) had little manganese, sampling point 15 (Acı Stream bed) had very little manganese, and sampling points 6 and 8 (Asarcık Stream bed), 16 and 17 (Püsküllü Stream bed), and 24 (Derin Stream active bed) had sufficient manganese in their surface and subsurface soils. Sampling points 9, 12, 14, and 23 (Bayhasan, Kepçeli and Soğulcuk Stream beds) on the other hand, had little manganese in their surface soils, and very little in their subsurface soils. Sampling points 4 (Avutmuş Creek bed) and 10 (Bayhasan Stream bed) had sufficient manganese in their surface soils, and little manganese in their subsurface soils. Sampling points 5 (Asarcık Stream bed) and 13 (Derin Stream active bed), on the other hand, had a large amount of manganese in their surface soils, and sufficient manganese in their subsurface soils.

Atmaca and Nalbant (2018) reported that soils around the western parts of the Avutmuş Creek contained sufficient copper. They found that a majority of the soils contained a large amount of iron, but some of the soils were deficient in zinc and manganese. Their findings regarding iron, copper, zinc, and manganese contents are consistent with those of the present study. Atmaca and Boyraz Erdem (2016) found that surface soils in stream beds in the Central district of Tekirdağ in Türkiye had sufficient iron, copper, and manganese. On the other hand, they found that some of the soils were deficient in zinc. Atmaca and Boyraz Erdem's (2016) findings regarding iron, copper and zinc are consistent with those of the present study. But their findings regarding manganese are different with those of the present study. Soils samples collected from a stream terrace in Kahta, Adıyaman were found to contain low and a moderate amount of available iron, sufficient copper and manganese, and low available zinc (Çelik and Akça, 2017). In surface horizons of soils samples from the Buskerud region of Southern Norway, total Fe was found to vary between 11.35 and 27.12 g kg^{-1} and total Mn was found to vary between 0.25 and 0.37 g kg^{-1}. In subsurface horizons, on the other hand, total Fe ranged from 12.36 to 25.13 g kg^{-1} and total Mn ranged from 0.22 to 0.37 g kg^{-1} (Sowiński et al., 2020).

Table 6. Contents of macro elements of the research

Sample no.	Depth (cm)	Total N (%)	ppm				Sample no.	Depth (cm)	Total N (%)	ppm			
			P	K	Ca	Mg				P	K	Ca	Mg
1	0-30	0.05	4.96	192.27	7616.83	383.58	13	0-30	0.08	10.06	230.03	5394.24	821.66
	30-60	0.03	3.88	200.64	7937.54	405.77		30-60	0.04	4.33	203.18	5510.11	730.58
2	0-30	0.06	8.01	270.36	8165.15	607.91	14	0-30	0.05	7.53	220.58	6408.90	253.23
	30-61	0.02	5.08	199.73	7917.88	630.00		30-60	0.02	0.59	98.09	6452.18	296.07
3	0-30	0.03	5.57	123.98	5812.61	313.51	15	0-30	0.02	2.90	143.23	7139.20	285.98
	30-60	0.05	5.23	146.69	7203.12	592.64		30-60	0.01	1.26	81.81	6438.30	341.44
4	0-30	0.07	18.89	199.20	6552.37	353.73	16	0-30	0.03	7.75	67.47	6241.91	646.65
	30-60	0.04	10.27	168.65	4681.85	334.15		30-60	0.02	6.94	60.01	6099.97	633.47
5	0-30	0.21	80.80	579.63	3137.76	332.94	17	0-30	0.10	10.50	85.87	4533.57	459.74
	30-61	0.09	48.10	368.48	4266.27	357.01		30-61	0.05	7.25	57.61	4569.44	563.80
6	0-30	0.19	27.08	152.62	1601.04	191.23	18	0-30	0.06	2.76	176.10	8060.13	223.83
	30-61	0.11	21.33	154.29	1695.91	226.63		30-60	0.04	0.91	168.52	7592.28	255.27
7	0-30	0.02	5.78	244.31	7865.14	200.55	19	0-30	0.03	0.08	136.43	7763.85	197.26
	30-61	0.02	1.31	113.33	6715.42	154.29		30-60	0.02	0.86	90.43	7608.33	260.29
8	0-30	0.07	22.53	219.37	2952.76	188.61	20	0-30	0.03	2.99	153.90	6628.55	168.01
	30-62	0.02	12.76	114.53	1587.79	188.80		30-61	0.01	1.47	177.50	6485.80	209.83
9	0-30	0.03	2.37	233.32	7561.52	411.34	21	0-30	0.04	5.50	350.31	6287.77	185.45
	30-61	0.02	0.82	164.09	8606.08	736.33		30-61	0.03	1.18	204.39	6250.37	219.15
10	0-30	0.11	4.83	306.87	8549.18	228.73	22	0-30	0.11	10.53	626.92	6387.76	386.66
	30-62	0.03	2.58	197.96	7894.05	247.13		30-65	0.02	0.39	156.77	6355.61	327.30
11	0-30	0.05	3.18	323.88	9648.03	636.86	23	0-30	0.06	5.06	276.20	6468.89	263.46
	30-60	0.02	0.10	251.09	8794.55	829.02		30-60	0.02	0.65	114.28	5898.07	289.02
12	0-30	0.02	1.22	194.49	8627.93	246.95	24	0-30	0.09	16.48	222.90	6258.51	468.36
	30-60	0.02	0.01	126.02	8689.45	286.26		30-61	0.05	3.42	131.79	6555.82	440.10

Table 7. Contents of micro elements of the research soils

Sample no.	Depth (cm)	ppm				Sample no.	Depth (cm)	ppm			
		Fe	Cu	Zn	Mn			Fe	Cu	Zn	Mn
1	0-30	7.61	4.45	0.49	12.67	13	0-30	30.32	3.84	0.73	51.75
	30-60	11.40	5.33	0.13	9.19		30-60	37.01	3.65	0.20	41.32
2	0-30	6.91	1.89	0.26	8.00	14	0-30	3.24	1.33	0.19	4.99
	30-61	7.98	2.19	0.13	6.43		30-60	3.57	1.30	0.06	2.94
3	0-30	11.88	2.26	0.25	5.31	15	0-30	1.01	1.91	0.45	2.74
	30-60	21.30	4.11	0.26	4.24		30-60	4.24	1.47	0.07	2.75
4	0-30	18.33	5.57	4.00	16.46	16	0-30	14.61	1.88	0.13	39.32
	30-60	14.16	4.09	0.92	13.35		30-60	13.49	1.38	0.12	32.62
5	0-30	54.48	6.48	7.29	58.82	17	0-30	33.25	3.45	0.45	47.66
	30-61	33.62	8.41	4.70	38.50		30-61	21.37	2.69	0.10	30.13
6	0-30	85.50	3.88	6.53	44.55	18	0-30	3.69	2.10	0.40	5.72
	30-61	69.76	5.40	3.69	37.17		30-60	4.30	2.56	0.11	6.22
7	0-30	10.72	4.52	1.23	12.26	19	0-30	2.85	1.48	0.07	7.60
	30-61	8.22	3.19	0.19	7.07		30-60	2.82	1.34	0.05	4.76
8	0-30	45.77	3.55	2.44	34.53	20	0-30	3.43	1.64	0.11	10.81
	30-62	24.34	3.12	0.43	28.79		30-61	4.55	1.35	0.10	6.12
9	0-30	16.10	3.98	0.27	12.26	21	0-30	1.41	1.60	0.62	4.63
	30-61	5.86	2.39	0.09	2.51		30-61	3.95	2.18	0.13	4.53
10	0-30	7.87	3.43	0.32	14.80	22	0-30	6.07	4.17	0.56	8.46
	30-62	7.40	3.97	0.08	10.87		30-65	3.07	1.94	0.07	4.67
11	0-30	6.17	2.65	0.11	9.25	23	0-30	3.29	1.58	0.18	6.48
	30-60	7.17	2.57	0.07	5.79		30-60	2.97	1.66	0.04	2.95
12	0-30	2.91	1.87	0.06	5.54	24	0-30	17.40	2.65	0.84	35.04
	30-60	2.22	1.93	0.04	1.75		30-61	10.75	2.05	0.23	25.71

Table 8 reports descriptive statistics for the soil parameters in the study, and *Table 9* reports correlation coefficients and results of the correlation analysis carried out for surface and subsurface soil samples. For all surface and subsurface soils: Results of the correlation analysis showed that here was a positive (+) relationship between pH-lime, pH-Ca, total salt-Ca, total salt-Mg, OM-total nitrogen, OM-P, OM-K, OM-Fe, OM-Cu, OM-Zn, OM-Mn, total nitrogen-P, total nitrogen-Fe, total nitrogen-Cu, total nitrogen-Zn, total nitrogen-Mn, P-K, P-Fe, P-Cu, P-Zn, P-Mn, K-Cu, Fe-Cu, Fe-Zn, Fe-Mn, Cu-Zn, Cu-Mn, and Zn-Mn. On the other hand, there was a negative (-) relationship between pH-OM, pH-total nitrogen, pH-P, pH-Fe, pH-Cu, pH-Zn, pH-Mn, lime-OM, lime-total nitrogen, lime-P, lime-Fe, lime-Cu, lime-Mn, OM-Ca, total nitrogen-Ca, P-Ca, Ca-Fe, Ca-Zn, and Ca-Mn. In addition, a positive (+) relationship was found between total nitrogen-K in surface soils, and between pH-total salt and K-Zn in subsurface soils. A negative (-) relationship was found between lime-Zn in surface soils, and between total salt-Mn in subsurface soils. In a similar study, Atmaca and Nalbant (2018) performed correlation analysis of the surface soil (0-30 cm) characteristics of the orchards around the western parts of the Avutmuş Creek in Şebinkarahisar district. They determined that there was a positive (+) relationship between salt-lime, OM-total N, OM-P, OM-Fe, OM-Cu, OM-Zn, total N-P, total N-Fe, total N-Cu, total N-Zn, P-K and Fe-Cu. On the other hand, they found that there was a negative (-) relationship between pH-OM, pH-total N, pH-Fe, pH-Cu, salt-Mn and lime-Mn.

Table 8. Descriptive statistics for the soil parameters in the study

Parameter	Surface Soils (0-30 cm) (N:24)			
	Minimum	**Maximum**	**Mean**	**Std. Deviation**
pH	5.84	7.98	7.35	0.57
Tot.Salt (%)	0.01	0.05	0.02	0.01
Lime (%)	0.00	38.30	12.35	11.78
OM (%)	0.32	4.16	1.34	1.00
Tot.N (%)	0.02	0.21	0.07	0.05
P (ppm)	0.08	80.80	11.14	16.33
K (ppm)	67.47	626.92	238.76	132.60
Ca (ppm)	1601.04	9648.03	6485.98	1908.15
Mg (ppm)	168.01	821.66	352.34	175.99
Fe (ppm)	1.01	85.50	16.45	20.41
Cu (ppm)	1.33	6.48	3.01	1.40
Zn (ppm)	0.06	7.29	1.17	1.98
Mn (ppm)	2.74	58.82	19.15	17.56
Parameter	**Subsurface Soils (30-60/61/62/65 cm) (N:24)**			
	Minimum	**Maximum**	**Mean**	**Std. Deviation**
pH	6.06	8.05	7.45	0.58
Tot.Salt (%)	0.01	0.04	0.02	0.01
Lime (%)	0.00	37.90	13.37	12.31
OM (%)	0.14	2.16	0.65	0.48
Tot.N (%)	0.01	0.11	0.03	0.02
P (ppm)	0.01	48.10	5.86	10.27
K (ppm)	57.61	368.48	156.25	67.30
Ca (ppm)	1587.79	8794.55	6325.26	1906.17
Mg (ppm)	154.29	829.02	398.10	197.60
Fe (ppm)	2.22	69.76	13.56	15.42
Cu (ppm)	1.30	8.41	2.93	1.68
Zn (ppm)	0.04	4.70	0.50	1.16
Mn (ppm)	1.75	41.32	13.77	13.49

Table 9. Correlation coefficients (r) and results of the correlation analysis for surface and subsurface soil samples

Surface Soils (0-30 cm)												**$^{*}p<0.05$, $^{**}p<0.01$**
	pH	**Tot.Salt**	**Lime**	**OM**	**Tot.N**	**P**	**K**	**Ca**	**Mg**	**Fe**	**Cu**	**Zn**
Tot.Salt	0.289											
Lime	0.720**	0.105										
OM	-0.698**	-0.230	-0.417*									
Tot.N	-0.696**	-0.217	-0.412*	0.999**								
P	-0.542**	-0.245	-0.412*	0.788**	0.798**							
K	0.027	0.334	0.145	0.472*	0.482*	0.497*						
Ca	0.747**	0.650**	0.353	-0.668**	-0.660**	-0.650**	-0.046					
Mg	-0.266	0.444*	-0.355	0.009	0.010	-0.015	0.013	0.122				
Fe	-0.882**	-0.365	-0.608**	0.781**	0.777**	0.679**	0.041	-0.860**	0.011			
Cu	-0.485*	0.073	-0.568**	0.602**	0.593**	0.652**	0.436*	-0.363	0.106	0.546**		
Zn	-0.605**	-0.313	-0.453*	0.802**	0.799**	0.865**	0.299	-0.712**	-0.176	0.831**	0.683**	
Mn	-0.899**	-0.308	-0.719**	0.679**	0.680**	0.673**	0.039	-0.723**	0.377	0.799**	0.522**	0.597**
Subsurface Soils (30-60/61/62/65 cm)												**$^{*}p<0.05$, $^{**}p<0.01$**
	pH	**Tot.Salt**	**Lime**	**OM**	**Tot.N**	**P**	**K**	**Ca**	**Mg**	**Fe**	**Cu**	**Zn**
Tot.Salt	0.445*											
Lime	0.736**	0.117										
OM	-0.586**	-0.266	-0.506*									
Tot.N	-0.607**	-0.330	-0.505*	0.992**								
P	-0.549**	-0.389	-0.478*	0.747**	0.752**							
K	0.058	0.278	-0.189	0.425*	0.379	0.553**						
Ca	0.798**	0.733**	0.392	-0.517**	-0.550**	-0.586**	0.066					
Mg	-0.082	0.572**	-0.243	0.011	-0.041	-0.079	0.144	0.325				
Fe	-0.798**	-0.336	-0.556**	0.838**	0.843**	0.647**	0.209	-0.723**	0.060			
Cu	-0.420*	-0.123	-0.530**	0.770**	0.755**	0.817**	0.681**	-0.369	-0.023	0.644**		
Zn	-0.497*	-0.381	-0.390	0.841**	0.853**	0.933**	0.541**	-0.571**	-0.163	0.736**	0.795**	
Mn	-0.860**	-0.422*	-0.771**	0.640**	0.649**	0.655**	0.153	-0.695**	0.167	0.786**	0.507*	0.576**

4. Conclusions

This study presents climate classification for Şebinkarahisar district for the 48 years (1965-2012), on the basis of Thornthwaite (1948), De Martonne-Gottman (De Martonne, 1942; Baltas, 2007), and Erinç (Erinç, 1965; Erinç, 1984) methods. Using the Thornthwaite (1948) method, the climate index was found to be C_2 B'_1 s_2 b'_3 (Moist subhumid), and its climate feature was found to be first mesothermal, large summer water deficiency, summer concentration 54.57%. Using the De Martonne-Gottman (De Martonne, 1942; Baltas, 2007) method, aridity index was found to be 17.74, and the climate type was classified as semi arid - humid. According to the Erinç (Erinç, 1965; Erinç, 1984) method, precipitation efficiency index was found to be 39.32, and the climate type was classified as semi humid.

Analysis results showed that soils in the study area had deficiencies in organic matter, total nitrogen, phosphorus, potassium, magnesium, iron, zinc, and manganese, and a fertilization planning that includes poultry manure, green manure, compost, vermicompost, and organic fertilizers containing various macro and micro elements, in addition to barnyard manure, is recommended for fertility. Moreover, analysis of the pH values and lime contents of the soils should also be taken into account for important agricultural work such as irrigation, growing plants, and tilling the land. Plant species and varieties to be grown in the study area should be selected from among those suitable for transitional climate conditions. Waters of the streams in the areas from which soil samples were collected should also be analyzed. For agricultural activities (tillage, irrigation, harvesting, fertilization, etc.), it will be useful to map the elevation, slope and aspect characteristics of the lands located in the stream beds in the study area.

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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Şarapçılık Atığı ile Yapısı Güçlendirilmiş Balık Jelatininin Reolojik Özellikleri Üzerine pH ve Gamma Işınlamanın Kombine Etkisi

The Combined Effect of pH and Gamma Irradiation on the Rheological Properties of Winery Waste-enhanced Fish Gelatin

Tuncay GÜMÜŞ[1], Deniz Damla ALTAN KAMER[2*], Gülce Bedis KAYNARCA[3], Tuğba GÜNAYDI[4]

Öz

Balık jelatinindeki zayıf jel kuvvetini yükseltmek ve kullanım alanlarını genişletmek için bir takım modifikasyon çalışmaları yapılmaktadır. Bu çalışmada balık jelatininin reolojik özellikleri üzerine çapraz bağ oluşturmak amacıyla %20 şarap tortusu (WL) ilave edilerek farklı pH değerlerinde (3, 5 ve 7) yüksek doz gamma ışınlamanın(10, 20 ve 30 kGy), etkisi incelenmiştir . WL ilavesi ile jelatinde en yüksek jel kuvveti 2380.68±34.45 Pa olarak tespit edilmiştir. WL ilavesi örneklerin jel mukavemetini %52 oranında arttırmıştır. 10, 20 ve 30 kGy ışınlama dozuna göre solüsyonların jel kuvvetleri sırasıyla 1351.74, 646.80 ve 599.87 Pa olarak tespit edilmiştir. Uygulanan ışınlama dozları içerisinde jelleşme kinetiği yönünden en iyi sonuç 10 kGy ile elde edilmiştir. WL ilavesiz kontrol grubunun jelleşme oranı k_{gel} değeri 286.03 Pa, WL ilaveli kontrol grubunun k_{gel} değeri ise 332.64 Pa olarak tespit edilmiştir. Işınlama grupları arasında en yüksek kgel değeri 10 kGy ışınlanan örnekte 184.43 Pa olarak belirlenmiştir. Tüm jelatin solüsyonlarının Power-law modeli ile uyumlu olduğu ve elastik özelliklerinin viskoz özelliklerden daha baskın olduğu tespit edilmiştir. Kıvam indeksi K′ değeri 2373. 25 Pa.s olarak en yüksek ışınlanmamış WL ilaveli örnekte bulunmuştur. 10, 20 ve 30 kGy gamma ışınlama jelatinin erime derecesini önemli düzeyde arttırmış ve erime dereceleri sırasıyla 45.36, 43.61 ve 35.41 °C olarak belirlenmiştir. pH değerleri jelatinin jel kuvveti, jelleşme ve erime derecelerini önemli düzeyde etkilemiştir. pH3'de jelatin solüsyonlarının daha düşük jel kuvveti ve erime derecesi değerlerine sahip olduğu pH7'nin yapıyı değiştirmediği pH5'in ise tüm reolojik özellikleri arttırdığı tespit edilmiştir. pH5 ile k_{gel} değerinde %30 oranında bir artış ve erime derecesinde de kontrol örneğe göre 2 kat artış tespit edilmiş olup en yüksek erime derecesi 48.72 °C'ye ulaşılmıştır.

Anahtar Kelimeler: Balık jelatini, Şarap tortusu, Reoloji, Gamma ışınlama, Jel kinetiği

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Abstract

The effects of high-dose gamma irradiation, different pH values, and the addition of wine residue (WL) on the rheological properties of fish gelatin were investigated. The gelation kinetics, gel strength, gelation, and melting temperatures of gelatin with 20% WL addition were studied by subjecting it to gamma irradiation at 10, 20, and 30 kGy doses and pH 3, 5, and 7. With the addition of WL, the highest gel strength was determined as 2380.68±34.45 Pa in gelatin. The samples' gel strength increased by 52% with the addition of WL. The solutions' gel strengths were determined to be 1351.74, 646.80, and 599.87 Pa for 10, 20, and 30 kGy irradiation dosages, respectively. As for gelation kinetics, 10 kGy was the most effective irradiation level. The control group without WL had a gelation rate kgel value of 286.03 Pa, and the control group with WL had a kgel value of 332.64 Pa. The irradiation group with the greatest kgel value was found to be the 10kGy group, with a value of 184.43 Pa. It was determined that all gelatin solutions were compatible with the Power-law model and elastic properties were more dominant than viscous properties. The consistency index K′ value was found to be 2373. 25 Pa.s in the highest non-irradiated WL added sample. Gelatin's melting point was dramatically raised by gamma irradiation at 10, 20, and 30 kGy; the resulting melting points were 45.36 °C, 43.61 °C, and 35.41 °C, respectively. The degrees of melting, gelation, and gel strength of the gelatin were all considerably impacted by the pH levels. The gel strength and melting point values were found to be decreased in pH:3 gelatin solutions, but at pH:7 the structure was unaffected and at pH:5 all rheological properties were enhanced. In comparison to the control sample, pH:5 produced a 30% rise in kgel value and a 2-fold increase in melting point. The highest melting point was reached at 48.72 ^{o}C.

Keywords: Fish gelatin, Wine residue, Rheology, Gamma irradiation, Gel kinetics

1. Giriş

Jelatin, benzersiz fonksiyonel ve teknolojik özellikleri ile modern endüstrileride özellikle gıda, ilaç, kozmetik ve fotoğraf ürünlerinin işlenmesinde yaygın olarak kullanılan bir biyopolimerdir (Karim ve Bhat, 2009). Jelatin, stabilize etme özelliği, tüketiciye verdiği "ağızda erime" hissi, elastikiyet ve kıvam verici özellikleri sayesinde gıda uygulamalarında çok yönlü ve en çok kullanılan jelleştirici maddelerden biridir (Ahmed, 2017; Nieto-Suárez ve ark., 2016; Van Nieuwenhove ve ark., 2016). Gıda endüstürisinde şekerlemelerde (çiğneme, doku ve köpük stabilizasyonu), az yağlı sürülebilir ürünlerde (kremsilik, yağ azaltma ve ağız hissi), süt ürünlerinde (stabilizasyon ve tekstür), unlu mamullerde (emülsifikasyon, jelleşme ve stabilizasyon) ve et ürünlerinde (su bağlama) farklı teknolojik amaçlar için kullanılmaktadır (Schreiber ve Gareis, 2007). Bir gıda bileşeni olarak yenilebilir jelatin protein bazlı bir ürün olup, Avrupa Birliği ve Türk Gıda Kodeksinde katkı maddesi olarak kabul edilmemektedir. Ticari jelatin neredeyse tamamen (%98.5); domuz derisi (%46), sığır derisi (%29.54), domuz ve sığır kemiği (%23.1) ve balık derisi (%1.5) dahil olmak üzere hayvan kaynaklarından elde edilmektedir (Duconseille ve ark., 2015; Gómez-Guillén ve ark., 2009; Karim ve Bhat, 2009). Son on yılda, küresel jelatin üretimi yaklaşık 200.000 ton artış göstermiş olup, 2024 yılında küresel jelatin pazar hacminin yaklaşık 650.000 tona ulaşması beklenmektedir. Jelatinin 2020 yılında 3.2 milyar dolar olan piyasa değerinin de 2027 yılı sonuna kadar 6.7 milyar dolara çıkması öngörülmektedir (Anonim, 2020).

Domuz, sığır gibi hayvanlardan elde edilen kollajen ve jelatinler, Müslümanların helal gıda, yahudilerin Kosher kuralları ve Hint kültürünün inek kültür hassasiyetlerine uymayan, bu inançlarda kullanılması ve yenilmesi sakıncalı olan ürünlerdir (Sha ve ark., 2014). Ayrıca, memeli hayvanlardan üretilen jelatinin kullanımı, Bovine Spongiform Encephalopathy (BSE; Deli Dana Hastalığı) ve Food and Mouth Desease (FMD, Şap Hastalığı) gibi hastalık riskleri taşıması nedeniyle endişeye sebebiyet vermektedir (Karim ve Bhat, 2009; Songchotikunpan ve ark., 2008). Sonuç olarak, insan sağlığı, dini hassasiyetler ve çevresel sürdürülebilirlik ile ilgili endişeler nedeniyle hayvan jelatin alternatiflerine olan talep artmıştır. Bu alternatiflerin başında balık jelatini gelmektedir (Huang ve ark., 2017a; Sow ve Yang, 2015). Jelatin kaynağı olarak atık ve çevresel kirlilik oluşturan balık endüstrisi yan ürünlerinin sürdürülebilirliğini göz önünde bulundurmak önemlidir. Balık filetosu üretiminde ortaya çıkan atık oranı balık ağırlığı üzerinden %75 seviyelerine ulaşmakta ve bu atıkların da yaklaşık %30'u jelatin üretiminde kullanılabilme potansiyeline sahip deri ve kemiklerden oluşmaktadır (Mariod ve Fadul, 2013). Jelatinin ekonomik olarak önemli üç temel özelliği; jel gücü, viskozite ve erime sıcaklığıdır (Huang ve ark., 2019). Balık jelatininin gıda sektöründe kısıtlı kullanım alanı bulmasının temel nedeni düşük erime derecesi ve zayıf jel kuvvetine sahip olmasıdır (Voigt ve Botta, 1990). Balık jelatinin teknolojik özelliklerini geliştirmek amacıyla transglutaminaz ilavesi (Norziah ve ark., 2009), fenolik bileşiklerin ilavesi (Poungchawanwong ve ark., 2020), yüksek basınç gibi çapraz bağlanma ajanlarının kullanımı (Gómez-Guillén ve ark., 2005), UV radyasyon uygulamaları (Otoni ve ark., 2012), balık jelatinin çeşitli karbonhidratlar (Bostar ve Hosseini, 2021; Kołodziejska ve Piotrowska, 2007), proteinler (Cai ve ark., 2016), ve tuzlar (Sow ve Yang, 2015) ile muamele edilmesi gibi pek çok modifikasyon çalışması yapılmıştır. Balık jelatini bol miktarda bulunması, biyoçözünür özellikte olması, mükemmel film oluşturma özelliklerine sahip olması, düşük su içeriğinde oksijen ve aromalara karşı iyi bir bariyer etkisine sahip olması ve nispeten düşük maliyetli olması gibi avantajlara sahiptir. Yüksek dozda ışın uygulaması gibi fiziksel bir yöntem ise, jelatinin fiziksel, kimyasal ve biyolojik özelliklerini değiştirmede kullanılabilecek düşük maliyetli ve çevresel bir alternatif olabilir (Benbettaïeb ve ark., 2016). Ayrıca, ışınlama uygulaması daha az numune ile, daha hızlı ve herhangi bir katalizör veya sıcaklık artışı gerektirmeyen bir yöntemdir (Woods ve Pikaev, 1993). Gama radyasyonunun jelatinin mekanik özelliklerine etkisi hakkında çeşitli çalışmalar bulunmakla birlikte ışınlanmış balık jelatinin reolojik karakterizasyonu ile jel mekanizmasında meydana gelen değişimlerin incelenmesi ile ilgili çalışmalar kısıtlıdır.

Bitkilerde yüksek oranlarda bulunan polifenoller, doğal çapraz bağlayıcılar olarak dikkat çekmekte ve etkileşimleri bazı gıda ürünlerinde kilit rol oynamaktadır (Balange ve Benjakul, 2010). Prolinin halka yapısı hidrojen bağlarının sarmal bir konfigürasyona geçmesini engellediğinden, balık jelatini gibi bu amino asit açısından zengin proteinler fenolik moleküller için daha yüksek bir afiniteye sahiptir (Karabulut ve Yemiş, 2019). Bitki kaynaklı fenolik asitler ve flavonoidlerin (kafeik asit, klorojenik asit, ferulik asit ve rutin gibi), oksitleyici koşullar altında kovalent çapraz bağlantılar üretmek için jelatin yan zincirleri ile reaksiyona girdiği bildirilmiştir (Strauss ve Gibson, 2004). Şarap üretimi, milyarlarca dolar değerinde küresel bir endüstridir ve yüksek fenolik içeriğe sahip bol miktarda yan ürün üretmektedir (Brostrom ve Brostrom, 2008). Şarap endüstrisi, büyük miktarda yan ürün üretmekte ve bu yan ürünler biyoürünler üretmek için muazzam potansiyele sahiptir. Şarap üretiminde

ortaya çıkan atıkların %60'ını üzüm posası oluştururken, %25'ini de şarap tortusu (Wine lees) oluşturmaktadır. Tarımsal atıkların balık jelatininin yapısal iyileştirilmesinde doğrudan kullanımı bilimsel literatürde nadiren incelenmektedir. Şarap tortusu ile zenginleştirilmiş balık jelatinin reolojik karakterizasyonun ortaya konulduğu, aynı zamanda yüksek doz gama ışını uygulaması ile çapraz bağlanma etkisinin reolojik analizler ile ortaya konduğu bir çalışma bulunmamaktadır.

Bu çalışmada, şarap tortusu ile yapısı güçlendirilmiş balık jelatinin farklı pH değerlerinde yüksek doz ışınlanması ile reolojik özelliklerinde meydana gelen değişikliklerin incelenmesi amaçlanmıştır. Özellikle yüksek dozda gamma ışınlamanın tercih edilme nedeni, balık jelatini ile şarap tortusu arasında pH etkisiyle çapraz bağların oluşturularak yapının güçlendirilmesi hedeflenmiştir. Bu kapsamda ışınlanmış ve ışınlanmamış örneklerde jelleşme kinetiği, jelleşme ve erime dereceleri ile jel kuvvetleri incelenmiştir.

2. Materyal ve Metot

2.1. Materyal

Balık jelatini Çipura (*Sparus aurata* L.) derisinden üretilmiştir. Balık atıkları Dardanel Önentaş Gıda San. A.Ş.'den derisiz fileto üretim atığı olarak temin edilmiştir. Şarap tortusu Tekirdağ, Şarköy ilçesinde bulunan yerel bir şarap üretim firmasından *Cabernet Sauvingnon* üzümlerinin şarapçılık atığı olarak temin edilmiştir. Balık atıkları, ve şarap tortusu kullanılana kadar -18°C' de muhafaza edilmiştir.

2.2. Metot

2.2.1. Balık Jelatini Ekstraksiyonu

Balık jelatini üretiminde kullanılan atık balık derileri ekstraksiyon öncesi donuk formdayken bir makas yardımıyla yaklaşık 3x3 cm boyutlarında kesilmiştir. Kesilen deriler musluk suyu ile yıkandıktan sonra 5°C' deki 0.5 M NaCl içerisine daldırılarak 5 dakika boyunca bir baget yardımı ile karıştırılmıştır (Işık, 2018). Ardından deriler NaOH (1:5 w/v) içerisinde çalkalamalı bir inkübatör cihazında (The Lab Companion, IS-971R) 20°C ve 180 rpm'de 40 dakika karıştırılmıştır. NaOH içerisinde şişen deriler distile su ile üç kez yıkanarak ekstraksiyonun ikinci aşamasına geçilmiştir. Deriler 18 saat boyunca 50°C ve 180 rpm'de 0.1 M asetik asit çözeltisi ile ekstrakte edilmiştir. Ekstraksiyonun ardından çözelti kaba filter kağıdından geçirilmiş ve filtrat 70°C sıcaklıkta etüvde kurutularak yaprak jelatinler elde edilmiştir. Yaprak jelatinler öğütülerek -18°C' de muhafaza edilmiştir (Garcia ve del Carmen Guillen, 2003).

2.2.2. Şarap Tortusunun Ekstraksiyonu

Şarap tortusu ekstraksiyonun daha etkili uygulanması amacıyla öğütülerek kullanılmıştır. Öğütülmüş tortu %70 etanol ile 1:5 (w/v) oranında karıştırılmış ve ardından 25°C'de 48 saat boyunca bir orbital çalkalayıcıda (INFORS HT Ecotron, İsviçre) ekstrakte edilmiştir. Elde edilen ekstrakt kaba filtre kağıdından süzülmüş ve rotary evaporatör (SCI LOGEX, RE 100-PRO) yardımıyla 50-55°C'de çözgenleri uçurulmuştur. Ekstraktların geri kazanımı distile su ile çözülerek yapılmıştır. Ekstraktlar analizlerde kullanılıncaya kadar -18 °C' de muhafaza edilmiştir.

2.2.3. Şarap Tortusu İçeren Jelatin Solüsyonlarının Hazırlanması

Şarap tortusu ektraktı, jelatin solüsyonlarına % kuru madde oranı üzerinden hesaplanarak jelatin ağırlığının yüzdece %20'sini oluşturacak şekilde ilave edilmiştir. %20 konsantrasyon önceki çalışmalarımızda gerçekleştirilen optimizasyona göre belirlenmiştir (Kaynarca ve ark., 2022). Kontrol grubu solüsyonlar ve şarap tortusu ilaveli solüsyonların hazırlanışına ilişkin detaylar ve örnek kodlamaları *Tablo 1*'de gösterilmektedir. Tüm örneklerde jelatin oranı %6.67 (g mL^{-1}) olarak sabit tutulmuştur. Öncelikle tüm örnekler için jelatin tartıldıktan sonra distile su içerisinde hidratize olması amacıyla 20 dakika 25°C'de bekletilmiştir. Ardından jelatin homojen olarak çözününceye dek 60°C'de 200 rpm'de manyetik karıştırıcı ile karıştırılmıştır. Jelatin tamamen çözündükten sonra şarap tortusu ekstraktları ilave edilmiş ve 0.1N NaOH ve 1M HCl kullanılarak örneklerin pH değerleri 3.5 ve 7'ye ayarlanmıştır. Son hacim distile su ile tamamlandıktan sonra solüsyonlar 15 dk 50°C'de 200 rpm'de karıştırılarak homojen olması sağlanmıştır. Hazırlanan solüsyonların 10, 20 ve 30kGy dozunda ışınlama işlemine tabi tutulmuş ve ardından 4°C'de 24 saat beklendikten sonra reolojik analizler gerçekleştirilmiştir. *Tablo 1*'de jelatin solüsyonlarının formülasyonu verilmiştir.

Tablo 1. Jelatin solüsyonlarının formülasyonu

Table 1. Formulation of gelatin solutions

Örnek kodu	Örnek detayı	Balık jelatini (g mL^{-1})	Şarap tortusu ekstraktı (WLE, mL)	Distile su (mL)	pH	Işınlama dozu (kGy)
FG	Kontrol balık jelatini	1.33	-	20	-	-
WFG	Kontrol, %20 WLE	1.33	2.30	20	-	-
1G	%20 WLE, 10 kGy	1.33	2.30	20	-	10
2G	%20 WLE, 20 kGy	1.33	2.30	20	-	20
3G	%20 WLE, 30 kGy	1.33	2.30	20	-	30
2G/3P	%20 WLE, pH 3,00	1.33	2.30	20	3.00	20
2G/5P	%20 WLE, pH 5,00	1.33	2.30	20	5.00	20
2G/7P	%20 WLE, pH 7,00	1.33	2.30	20	7.00	20

2.2.4. Solüsyonların ışınlanması

Jelatin solüsyonları polipropilen (PP) kaplar içerisinde paketlenmiş ve Gammapak Sterilizasyon San. ve Tic. A.Ş., Çerkezköy Tekirdağ'da Cobalt 60 (1.25 MeV) gamma ışını (mds, Nordion, Kanada) kaynağına maruz bırakılmıştır. Gamma ışınlama 1 kGy h^{-1} doz oranı ile üç hedef doz (10,20 ve 30 kGy) olacak şekilde gerçekleştirilmiştir. Absorbans dozları, Horwell Amber Perspex dosimeter kullanılarak kontrol edilmiştir. Absorbe edilen radyasyon enerjisi için birim, 1 J Kg^{-1} ve 100 rad'a eşdeğer olan greydir (Gy) (Bilgin ve ark., 2022).

2.2.5. Şarap tortusu ilaveli jelatin solüsyonlatının reolojik karakterizasyonu

Işınlanmış jelatin solüsyonlarının reolojik özellikleri, sıcaklık kontrollü (peltier sistem) Discovery Hybrid Rheometer-2 (TA Instruments New Castle, ABD) cihazı kullanılarak gerçekleştirilmiştir. Ölçümler 35 mm çapında paralel plakalı geometri kullanılarak 750 µm mesafe (gap) aralığında yapılmıştır. Reolojik analiz verileri uygun model analizleri kullanılarak TA reometre Veri Analiz yazılımı (V3.0) ile test edilmiştir.

Solüsyonların reolojik karakterizasyonu ortaya koymak amacıyla solüsyonlara öncelikle jel kinetiği analizleri yapılmıştır. Time sweep analizi için solüsyonlar reometreye 24 ºC'de yerleştirilmiş ve ardından solüsyonun dengeye gelmesi amacıyla 1°C/dakika hızda sıcaklık 4°C'ye düşürülmüştür. Solüsyonların lineer viskoelastik bölgeleri belirlendikten sonra time sweep analizi 4°C'de 4000 saniye boyunca 1 Hz sabit frekansta ve %1 strainde gerçekleştirilmiştir (Kuan, Nafchi, Huda, Ariffin, ve Karim, 2016). Birikim modülü (G′) elastikiyet, kayıp modülü (G″) ise viskoelastik karakterin ölçüsü olarak ifade edilmiştir.

Jelleşme oranlarının analiz edilmesi amacıyla time sweep analizinde bulunan G′ değerleri Eşitlik 1'de yerleştirilerek kgel değerleri hesaplanmıştır.

$$G_t = k_{gel} \ln(t_{gel}) + C \quad \text{(Eş. 1)}$$

Gt; t süredeki G′ değeri

tgel; jelasyon süresi,

C; logaritmik denklem sabiti

kgel; jelleşme hızı sabiti

daha sonra Eşitlik 2 kullanılarak jelleşme sisteminin G′ref' e ulaşması için gereken süre (tmodel) hesaplanmıştır.

$$t_{model} = e^{(G'ref-C)/k_{gel}} \quad \text{(Eş. 2)}$$

Solüsyonların depolama süresince olan jel stabilitesini belirlemek frequency sweep testi yapılmıştır. Açısal dönme hızı 0.1-10 Hz (Anvari ve Chung, 2016) ve %1 strainde ölçüm yapılmıştır. Depolama uyumluluğu J′ değeri Eşitlik 3 ve Eşitlik 4 kullanılarak hesaplanmıştır.

$$J' = \frac{G'}{G'^2+G''^2} \quad \text{(Eş. 3)}$$

$$G_N^0 = \frac{1}{J_N^0} \quad \text{(Eş. 4)}$$

Son olarak solüsyonların erime dereceleri ve jelleşme derecelerini belirlemek amacıyla temperature sweep testi yapılmıştır. Öncelikle son sıcaklıkları 4°C olan örneklerin sıcaklığı 10°C'ye yükseltilmiş ve bu noktada dengeye gelmeleri beklenmiştir. Ardından 1°C/dakika hız ile örneklerin sıcaklığı 40°C'ye yükseltilmiştir. Erime sıcaklığı G′ ve G″'ın kesiştiği nokta olarak belirlenmiştir. G″'ın önemli ölçüde yükseldiği nokta ise jelleşme sıcaklığı olarak kabul edilmiştir.

2.2.6. İstatistiksel analiz

Örneklerin istatistilsel değerlendirmesi, reoloji grafiklerinin çizimi ve verilerin hesaplanması Origin 8 programında yapılmıştır. Tüm örnekler üç tekerrürlü olarak çalışılmış ve sonuçlar, ortalama ± SD (n = 3) olarak sunulmuştur. Ortalamalar arasındaki önemli farklar ($p<.05$), SPSS 17.0 (SPSS Inc., Chicago, IL, ABD) kullanılarak tek yönlü ANOVA ile Duncan'ın çoklu karşılaştırma testi aracılığıyla analiz edilmiştir.

3. Araştırma Sonuçları ve Tartışma

3.1.WL ilaveli jelatin solüsyonlarının jelleşme kinetiği

Şarapçılık endüstrisinde fermentasyon sonu atığı olarak çıkan, fenolik bileşen, organik asit, antioksidan, maya hücreleri ve inorganik maddelerce zengin tortu ekstraktının (WL) balık jelatini ile interaksiyonunda gamma ışınlanma ve farklı pH değerlerinin etkisini belirlemek amacıyla hidrojellerin stabilite ve kararlılıklarının tespiti için ilk olarak time sweep analizi yapılmıştır. 10, 20 ve 30 kGy dozunda uygulanan gamma ışınlamanın etkisi *Şekil 1'*de verilmiştir. Elastik sertliğin ölçüsü olan birikim modülünün (G') örneklerin tümünde viskoz sertliğin ölçüsü olan kayıp modülü (G") parametrelerinden yaklaşık 2 log daha fazla olduğu görülmektedir. En yüksek G' değerlerinin ışınlanmamış WL ilaveli balık jelatin solüsyonlarında olduğu tespit edilmiştir. Fenolik bileşiklerin, jelatin ile kovalent ve non-kovalent etkileşimlere girerek jellerin yapısal ve fonksiyonel özelliklerini geliştirdiği bilinmektedir (Kaynarca ve ark., 2022). Buna göre ışınlama dozu arttıkça örneklerin G' ve G" değerleri düşmüş dolayısıyla kararlılıkları azalmıştır. Işınlama işlemi jelatin zincirlerinin kovelent bağlarını kırarak geri dönüşümsüz moleküler değişikliklere sebebiyet verebilmektedir (Bessalah ve ark., 2022). Bu sebeple çalışmamızda gıda sterilizasyonunda kullanılan gamma ışınlama doz aralığı (5-30 kGy) (Bessalah ve ark., 2022) baz alınarak seçilen ışınlama gruplarını kendi aralarında karşılaştırdığımızda en iyi sonuçların 10 kGy ışınlanan örnek gruplarında olduğunu 20 kGy ve 30 kGy ışınlanan örneklerin arasındaki farkın az olduğu ve yapıyı olumsuz yönde etkiledikleri belirlenmiştir. 20 ve 30 kGy ışınlama arasındaki farkın az olması jelatin-WL interaksiyon zincirlerinin büyük çoğunluğunun 20 kGy'lik ışınlanma sonucu tahrip olması ile açıklanabilir (Lin ve ark., 2017).

Işınlama ve pH'ın etkisini birlikte incelemek amacıyla 3, 5 ve 7 pH değerlerinde hazırlanmış WL ilaveli jelatin solüsyonları orta nokta olan 20 kGy dozunda gamma ışınlamaya maruz bırakılmıştır. Bu örneklerin birikim ve kayıp modülleri *Şekil 2*'de verilmiştir. Bunun sonucunda 20 kGy ışınlanmış pH ayarlanmamış örneğe (pH 4.73) göre pH3 olan örneklerin daha düşük G' ve G" değerlerine sahip olduğu pH7'nin yapıyı değiştirmediği pH5'in ise G' ve G" parametrelerini arttırdığı tespit edilmiştir. pH değerinin jelatinin jel kuvveti, jelleşme ve erime dereceleri, emülsiyon stabilitesi ve zeta-potansiyelini etkilediği bildirilmiştir (Wang ve ark., 2022). Jelatinin farklı pH değerlerindeki davranışını incelemek gıda matrisinde kullanımı hakkında fikir sahibi olmamız açısından oldukça önemlidir.

Jelatin solüsyonlarının jelleşme kinetiklerine ait parametreler ve t_{model} değerleri *Tablo 2*'de verilmiştir. Jelatinin jelleşmesi, kovalent olmayan etkileşimlerle çapraz bağların oluşumuna yol açan zincirler arası ilişkileri içerir. Jelleşme ile jelatin sarmaldan (coil) helikse yapısal bir dönüşüm gerçekleştirmektedir. Bu dönüşüm büyük ölçüde pH, sıcaklık ve konsantrasyondan etkilenmektedir (Kuan ve ark., 2016). Sade balık jelatini içeren ışınlanmamış solüsyonun (FG) jelleşme oranını gösteren k_{gel} değeri 286.03 Pa iken WL ilaveli ışınlanmamış örneğin k_{gel} değeri ise 332.64 Pa olarak tespit edilmiştir. Gamma ışınlamasına maruz bırakılan tüm örneklerin k_{gel} değeri kontrol gruplarında istatiksel olarak düşük çıkmıştır ($p<0.05$). Işınlama grupları arasında en yüksek değer 10kGy ışınlanan örnekte bulunurken 20 ile 30kGy ışınlama arasında istatiksel olarak önemli bir fark bulunamamıştır ($p>0.05$). pH uygulamasının etkisini incelediğimizde 20kGy ışınlanmış örneğe göre k_{gel} değeri yaklaşık %30 oranında bir artış göstermiştir. Jel kinetiği parametrelerinden biri olan denklem sabiti (C) tüm örneklerde negatif değerlerde bulunurken en düşük değer WFG ve 2G/5P örneğinde tespit edilmiş ve en yüksek değer 2G/7P örneğinde bulunmuştur. Jel kinetiği denklemine ait R^2 değerleri ise tüm örneklerde 1'e oldukça yakın bulunmuştur.

Jelatin solüsyonların jelleşme süreleri hakkında bilgi sahibi olmak amacıyla t_{model} değerleri hesaplanmıştır. Bu maksatla öncelikle G'_{ref} değeri Eşitlik 1'de t model yerine analiz süresi olan 4000 saniye yazılarak hesaplanmıştır.

Sade balık jelatini için 4000 saniye sonunda G'_{ref} değeri 2001 Pa olarak bulunmuştur. Ardından diğer tüm solüsyonlar için G'_{ref} değerine ulaşılması için geçecek süre t_{model} olarak hesaplanmıştır. Buna göre WFG örneğinde bu süre yarı yarıya kısalarken diğer örneklerin tümünde bu süre oldukça yüksek bulunmuştur. WFG'den son en iyi sonuçlar 1G ve 2G/5P örneğinde tespit edilmiştir.

Jelleşme kinetiği sonuçlarına göre balıkçılık endüstrisi atıklardan ekstrakte edilen balık jelatinin gıda güvenliği açısından gamma ışınlamaya maruz bırakılması bir takım yapısal gerilemelere sebebiyet verse de bu durumun daha düşük dozlarda ışınlama ve pH optimizasyonu ile giderilebileceği düşünülmektedir.

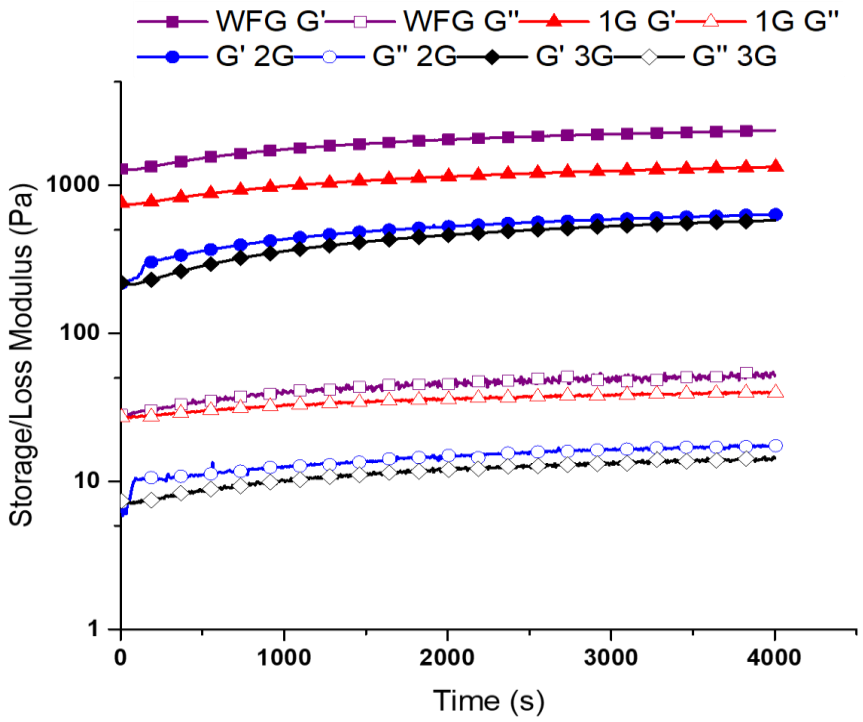

Figure 1. Rheograms of time-dependent storage and loss modulus of WL-irradiated gelatin solutions. G'; Storage Modulus, G''; Loss Modulus, WFG: non- irradiated WL-added fish gelatin, 1G, 2G, and 3G refer to 10, 20, and 30 kGy irradiated WL-added fish gelatins, respectively.

Şekil 1. WL içeren ışınlanmış jelatin solüsyonlarının birikim ve kayıp modüllerinin zamana bağlı değişim reogramları. G': birikim modülü; G'': kayıp modülü; WFG: ışınlanmamış WL ilaveli balık jelatini; 1G, 2G ve 3G sırasıyla 10, 20 ve 30 kGy dozunda ışınlanmış WL ilaveli jelatin solüsyonlarını ifade etmektedir.

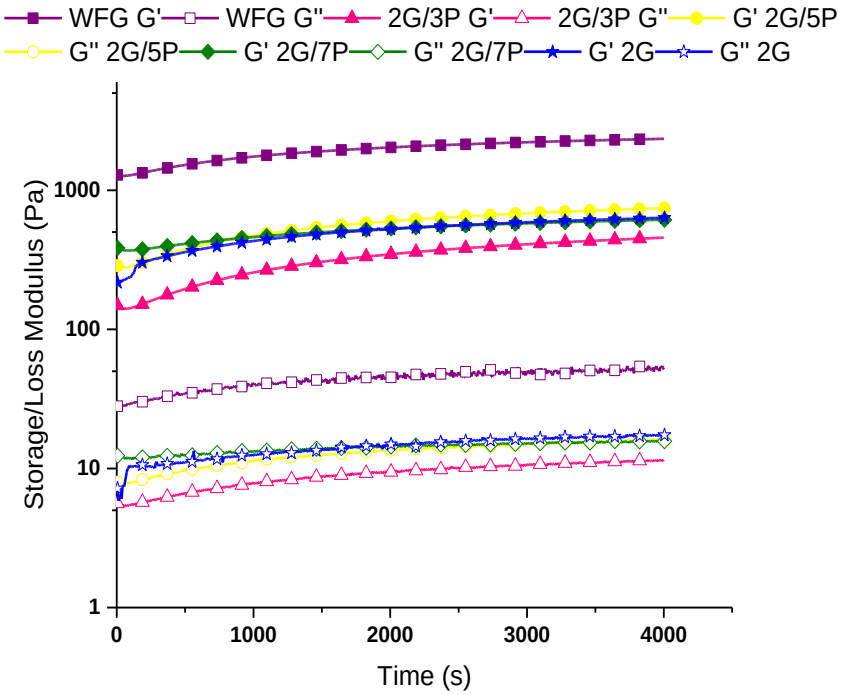

Figure 2. WL-added, 20 kGy-irradiated gelatin solutions showing time-dependent change rheograms of the storage and loss modulus at various pH levels. G'; Storage Modulus, G"; Loss Modulus, WFG: non-irradiated WL-added fish gelatin. 3P, 5P, and 7P refer to WL-added fish gelatin solutions with a pH of 3, 5, and 7, respectively.

Şekil 2. 20 kGy ışınlanmış WL ilaveli jelatin solüsyonlarının farklı pH değerlerinde zaman bağlı birikim ve kayıp modüllerinin reogramları G': birikim modülü; G": kayıp modülü; WFG: ışınlanmamış WL ilaveli balık jelatini; 3P, 5P ve 7P sırasıyla 3, 5 ve 7 pH değerlerindeki WL ilaveli jelatin solüsyonlarını göstermektedir.

3.2.WL ilaveli jelatin solüsyonlarının jel kuvveti

Frekans tarama testi (frequency sweep) solüsyonların mekanik kuvvetleri, reformasyonları ve deformasyonları hakkında bilgi vermektedir. Jelatin solüsyonları erime sıcaklarının altındaki derecelerde katı özellik göstermektedirler. G′ ve G″ değerleri arasındaki farkın yüksek olması jelatin ağlarının kuvvetini gösteren tanδ (G″/G′) değerinin düşük olmasına sebep olmaktadır. Tanδ değerinin 0.1'den düşük olması ise daha iyi jel ağı ve daha katı benzeri bir yapının sonucudur (Ahmed, 2017; Huang ve ark., 2017b). Farklı ışınlama dozlarına maruz bırakılmış ve ışınlanmamış örneklerin G' modüllerinin G" modüllerinde en az 2 log daha fazla olduğu görülmektedir (*Şekil 3*). Bu durum jelatin solüsyonlarının 10°C'de katı benzeri jel yapısına sahip olduğunu ayrıca bu sıcaklıkta moleküler arası etkileşimin arttığı ve yüksek jel kuvvetine sahip olduğunu göstermektedir. *Şekil 4*'te aynı ışınlama grubunda farklı pH değerlerine sahip örneklerin jel kuvvetlerini incelediğimiz ise bu örneklerinde katı benzeri davranış sergilediği ve jel mukavemetlerinin yüksek olduğunu sonucuna varılmıştır. Işınlama gruplarını kendi aralarında karşılaştırdığımızda en iyi sonucun 10kGy ışınlanan grupta olduğunu istatiksel olarak 20 ve 30 kGy ışınlanan örnekler grupları arasında ise fark olmadığı tespit edilmiştir ($p>0.05$). FG ve WFG örneğinde jel kuvveti 1556.50 ve 2380.68 Pa bulunmuştur. WL ilavesinin örneklerin jel mukavemetinin %52 oranında arttırdığı tespit edilmiştir. Işınlama dozu arttıkça 1G, 2G ve 3G solüsyonlarının jel kuvvetleri sırasıyla 1351.74, 646.80 ve 599.87 Pa değerine düştüğü gözlemlenmiştir (*Tablo 2*). Işınlama dozu arttıkça örneklerin mukavemetinin azalması gamma ışınlarının jelatinin daha fazla çapraz bağlanma bölgesini aktive etmesi ve jelatin hidrojellerin jel kuvvetinin azalması ile açıklanabilir (Lin ve ark., 2017). Jel kuvveti üzerine pH etkisini incelediğimizde ise k_{gel} ve tmodel değerleri ile benzer şekilde 2G örneğine göre pH3'ün jel kuvvetini düşürdüğü pH 7'nin yapıyı etkilemediği ve pH5'in ise yaklaşık %20 oranında arttırdığı sonucuna varılmıştır.

Farklı dozlarda gamma ışınlanmış jelatin solüsyonlarının açısal hıza bağlı kompleks viskozitelerindeki değişimler *Şekil 5*'te aynı ışınlama gruplarında farklı pH uygulanmış örneklerin kompleks viskozitelerindeki değişimler ise *Şekil 6*' da verilmiştir. Örneklerin tümünde kompleks viskozitenin açısal hız arttıkça azaldığı gözlemlenmiştir. Bu durum açısal hız ile moleküller arası bağların bozulması sonucu incelen psödoplastik davranış

ile açıklanabilir. Benzer bir kayma incelmesi farklı pH (3.6, 5 ve 9) değerlerinde gam arabik ile zenginleştirilen balık jelatinlerinde de görülmüştür (Anvari ve Joyner, 2017). Kompleks viskozite sonuçlarını incelediğimizde frequency sweep ve time sweep sonuçları benzer şekilde en yüksek viskozitenin WFG örneğinde olduğu ışınlama dozu arttıkça bu parametrenin düştüğü en iyi ışınlama sonucunun 10 kGy ışınlanan grupta olduğu tespit edilmiştir. Farklı pH uygulamalarında ise yine benzer şekilde pH3 yapıyı olumsuz etkilerken pH 5 olumlu etkilemiş ve pH7' de ise bir değişimin olmadığı belirlenmiştir

Jelatin solüsyonlarının jel kuvvetine bağlı olarak yapısal sıkılık ve kararlılıklarının değerlendirilmesi amacıyla açısal frekansın bir fonksiyonu olarak birikim ve kayıp modüllerinin Power-Law modeline uyumunu gösteren denklem parametreleri *Tablo 3*'de verilmiştir. Bu kısımda solüsyonların elastik ve viskoz modülerin kıvam katsayıları (K′-K″) ve akış davranış indeksleri (n′-n″) karşılaştırılmıştır. Örneklerin tümünde power law modeline uyumu gösteren R^2 değerleri (0.99-0.91) oldukça yüksek çıkmıştır. Jelatin solüsyonlarının tümünde elastik özelliklerinin viskoz özelliklerden daha baskın olduğunu gösterir nitelikte K′ değerleri K″ değerlerinden yüksek bulunmuştur. Akış davranış indekslerinin ise kıvam katsayısı değerleri ile ters orantılı olduğu tespit edilmiştir. K′ sonuçlarının kompleks viskozite sonuçları ile paralellik gösterdiği en yüksek sonucun 2373. 25 Pa.s ile WFG örneğinde olduğu onu sırasıyla FG, 1G ve 2G/5P örneğinin izlediği tespit edilmiştir. WL ilavesinin jelatin solüsyonlarının viskozitesini arttırdığı ışınlama işlemi ile bu değeri düştüğü fakat pH5 değerinde yapının olumlu yönde etkilendiği tespit edilmiştir. Akış davranış indeksi (n) değerlerinin düşük olması kayma incelmesi davranışının bir göstergesidir ve kompleks viskozite sonuçları ile paralellik göstermektedir.

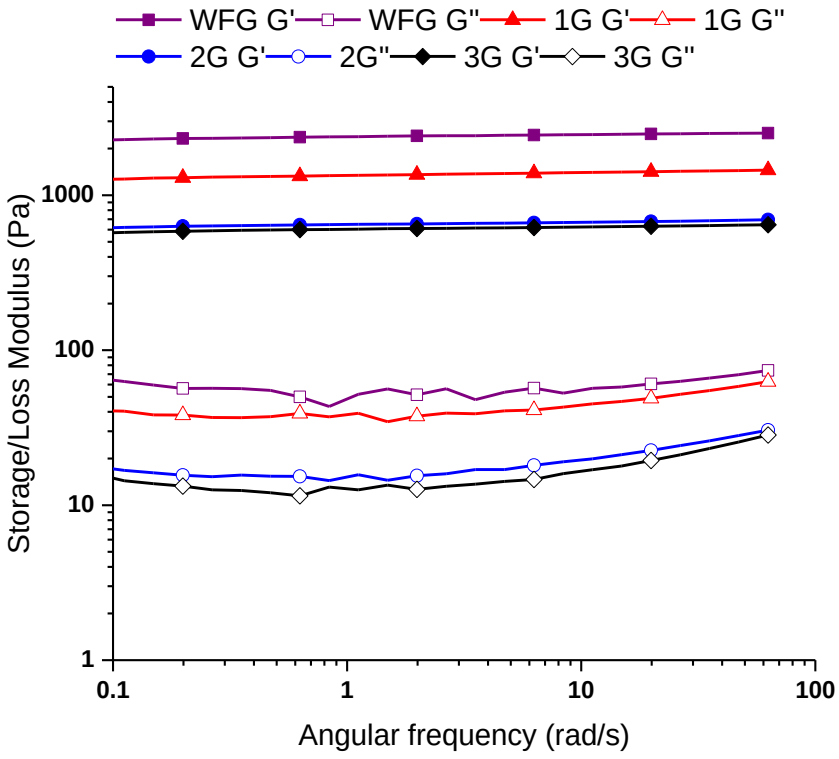

Figure 3. The change of the parameters G' and G" of the irradiated gelatin solutions depends on the angular frequency (rad/s). G'; Storage Modulus, G"; Loss Modulus, WFG: non-irradiated WL-added fish gelatin, 1G, 2G, and 3G refer to 10, 20, and 30 kGy irradiated WL-added fish gelatins, respectively.

Şekil 3. Işınlanmış jelatin solüsyonlarının açısal hıza bağlı G' ve G" parametrelerindeki değişim. G': birikim modülü; G": kayıp modülü; WFG: ışınlanmamış WL ilaveli balık jelatini; 1G, 2G ve 3G sırasıyla 10, 20 ve 30 kGy dozunda ışınlanmış WL ilaveli jelatin solüsyonlarını ifade etmektedir.

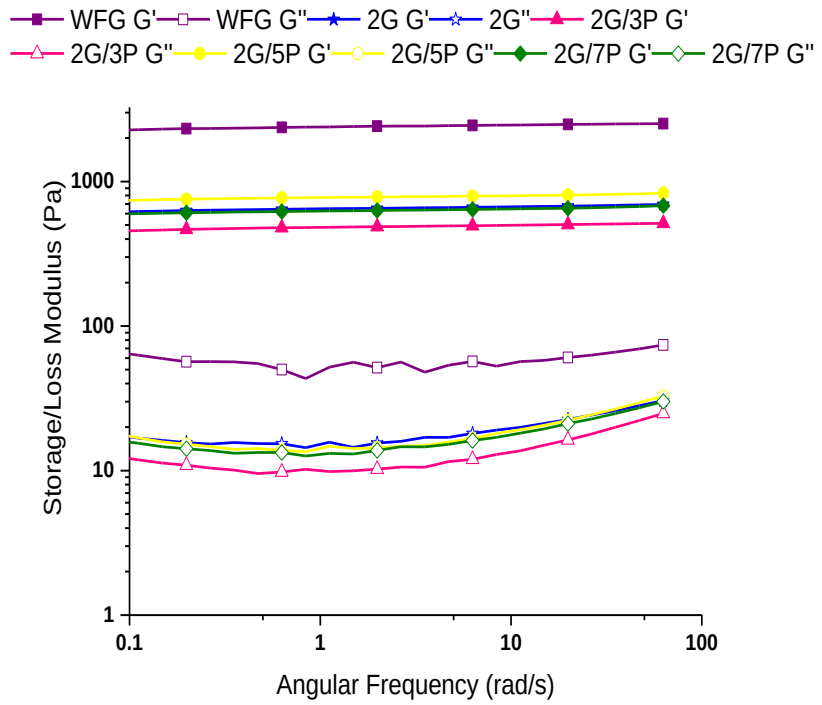

Figure 4. The change of G' and G" parameters at different pH values of 20 kGy irradiated gelatin solutions depending on angular frequency (rad/s). G'; Storage Modulus, G"; Loss Modulus, WFG: non-irradiated WL-added fish gelatin. 3P, 5P, and 7P refer to WL-added fish gelatin solutions with a pH of 3, 5, and 7, respectively.

Şekil 4. Farklı pH değerlerinde 20 kGy ışınlanmış jelatin solüsyonlarının açısal hıza bağlı G' ve G" parametrelerindeki değişim. G': birikim modülü; G": kayıp modülü; WFG: ışınlanmamış WL ilaveli balık jelatini; 3P, 5P ve 7P sırasıyla 3, 5 ve 7 pH değerlerindeki WL ilaveli jelatin solüsyonlarını göstermektedir.

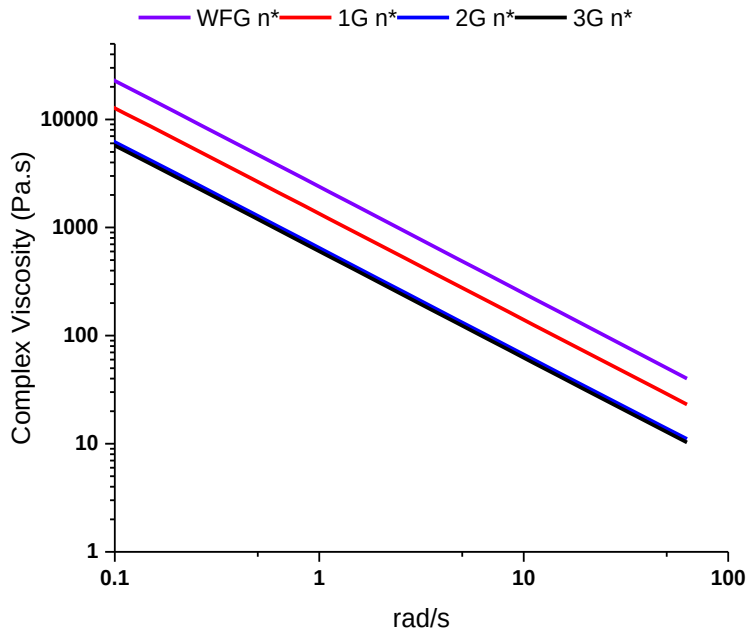

Figure 5. Complex viscosity of gelatin solutions exposed to gamma rays of 10, 20, and 30 kGy and not irradiated (WFG).

Şekil 5. 10, 20 ve 30 kGy dozunda ışınlanan ve ışınlanmamış (WFG) jelatin solüsyonlarının kompleks viskozite parametreleri.

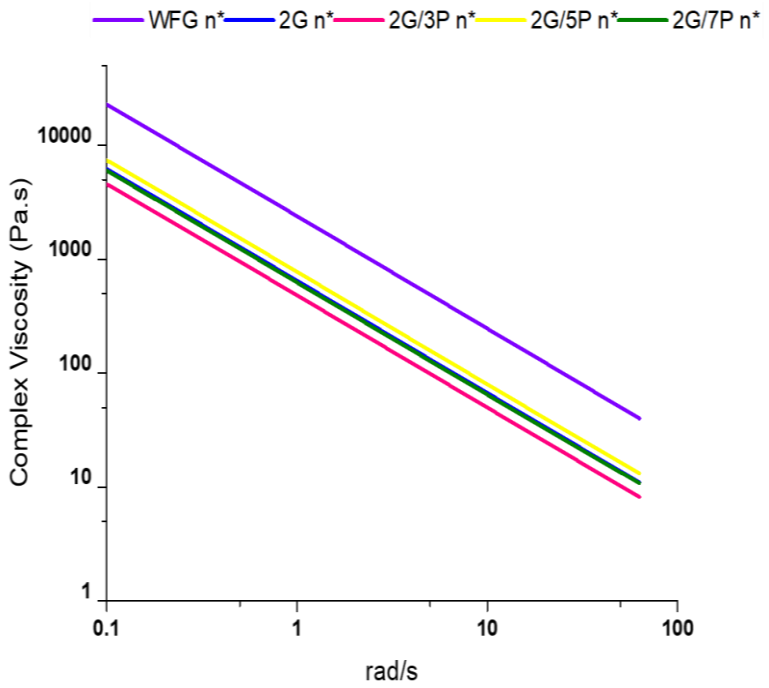

Figure 6. Complex viscosities of gelatin solutions irradiated at a dose of 20 kGy at different pH values.

Şekil 6. Farklı pH değerlerinde hazırlanmış ve 20kGy dozunda ışınlanmış jelatin solüsyonlarının kompleks viskozite parametreleri.

3.3.WL ilaveli jelatin solüsyonlarının erime davranışları

Jel kuvveti, visko-elastik özellikler ve jelleşme sıcaklığına ek olarak jelatinin erime sıcaklığı, en önemli fonksiyonel özelliklerinden biridir (Pranoto ve ark., 2007). Jelatin solüsyonlarının erime sıcaklıkları *Tablo 2*'de gösterilmektedir. Farklı dozlarda gamma ışınlanmış jelatin solüsyonlarının erime davranışları *Şekil 6*'da aynı ışınlama gruplarında farklı pH uygulanmış örneklerin erime davranışları ise *Şekil 7*' de verilmiştir. 10, 20 ve 30 kGy dozunda uygulanan gamma ışınlamanın erime sıcaklığı üzerinde önemli bir etkiye sahip olduğu tespit edilmiştir ($p<0.05$). Jelatin solüsyonları içerisinde en düşük erime derecesi sade balık jelatini içeren ışınlanmamış solüsyonda (FG) 24.03±0.18 °C olarak tespit edilmiştir. WL ilaveli ışınlanmamış örnek (WFG) erime derecesini kontrol örneğine göre 10.47 °C arttırmıştır. Balık jelatinlerinin (11-28 °C), memeli jelatinlerinden (28-31 °C) önemli ölçüde daha düşük erime sıcaklıkları, potansiyel ticari kullanımını ciddi şekilde sınırlandırmaktadır (Karim ve Bhat, 2009). Fenolik bileşikler balık jelatinlerinin erime sıcaklıklarını yükseltici etkiye sahiptir (Gilsenan ve Ross-Murphy, 2000; Kaynarca ve ark., 2022). WL ilavesi ile balık jelatinin erime sıcaklığı ticari olarak kullanılabilir noktaya taşınmıştır. Gamma ışınlamanın etkisi incelendiğinde uygulanan tüm ışınlama dozlarının erime derecesini ışınlanmamış örneklere göre önemli düzeyde arttırdığı görülmüştür ($p<0.05$). Işınlama dozları açısından incelendiğinde en yüksek erime sıcaklığı olan 45.36±1.12 °C'ye 10 kGy ışınlanmış örnek 1G'de ulaşılmıştır. 20 ve 30 kGy ışınlama dozunda erime sıcaklığı tekrar düşüşe geçmiş ve sırasıyla 43.61±1.06 °C ve 35.41±0.85 °C olarak tespit edilmiştir. Jelatin solüsyonlarının jelleşme kinetiği ve jel kuvveti sonuçları ile benzer şekilde 10 kGy ışınlama dozunda en iyi sonuçlar elde edilmiş, bununla beraber uygulanan tüm ışınlama dozlarının erime noktasını önemli düzeyde etkilediği tespit edilmiştir ($p<0.05$). 20 ve 30 kGy dozları ışınlanmamış örneklere göre jel kuvvetini olumsuz yönde etkilemekle birlikte, erime sıcaklığında ise ışınlama işleminin olumlu etki gösterdiği görülmektedir. Işınlama ile erime noktasındaki artışın nedeni, jelatin ağının hareketliliğini kısıtlayan yeni bağların oluşumu ile ilgili olabilir (Benbettaïeb ve ark., 2016).

Erime derecesi üzerine pH etkisini incelediğimizde uygulanan tüm pH değerlerinin erime derecesini önemli düzeyde değiştirdiği belirlenmiştir ($p<0.05$). Jelatin amfoterik bir protein olup molekül üzerinde hem pozitif hem de negatif yüklere sahiptir. Ham maddeye ve üretim yöntemine bağlı olarak izoelektrik noktası 5 ile 9 arasındadır (Osorio ve ark., 2007). Düşük pH değeri 3'te, erime sıcaklığının en düşük olduğu görülmektedir. pH 5'te ise en yüksek erime drecesi olan 48.72±0.90 °C'ye ulaşılmıştır. pH 5 değeri ile ışınlanmamış sade balık jelatinine göre erime sıcaklığında 2 kat artış sağlanmış, ışınlama uygulanmış tüm solüsyonlara göre de erime derecesinde artış

olmuştur. pH 3 ve pH 7'nin erime derecesini olumsuz yönde düşürdüğü, pH 5'in ise olumlu yönde artırdığı belirlenmiştir. Molekül içi elektrostatik etkileşimler izoelektrik noktada en yüksek seviyede olduğundan, protein çözünürlüğü bu noktada en düşüktür ve bu da dipolar molekülün en kompakt konformasyona sahip olmasına neden olur (Bae ve ark., 2009). pH 5'te en yüksek erime derecesinin gözlemlenmesi bu durum ile ilişkilendirilebilir. Elektrostatik etkileşimlerin artmasının protein ve şarap atığı ekstraktı arasındaki temasları azalttığı ve bu nedenle balık jelatininin pH 3'te reolojik özelliklerinin (jel kuvveti, jel kinetiği ve erime derecesi) azalmasına neden olduğu önerilebilir. Choi ve Regenstein (2000) domuz ve balık jelatini ile yapmış oldukları çalışmada benzer şekilde düşük pH 4'de düşük erime derecesi değerleri gözlemlemiş ve pH'taki düşüşün erime derecesini önemli ölçüde düşürdüğünü bildirmişlerdir.

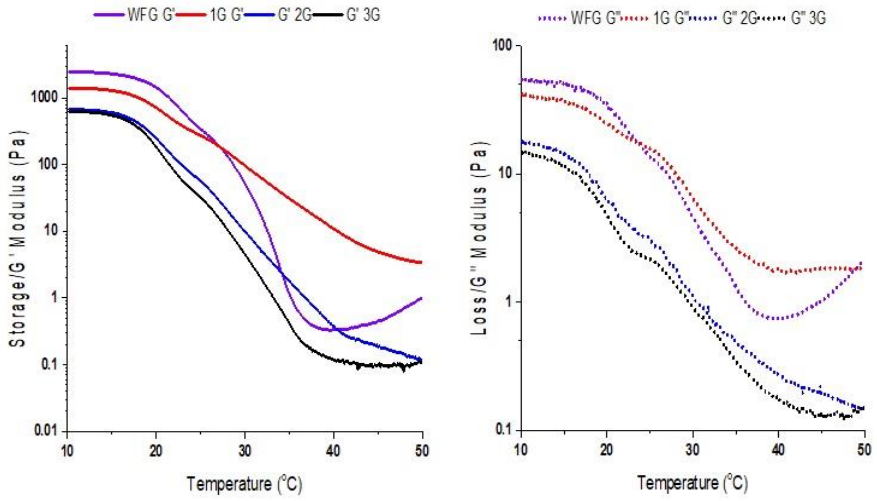

Figure 7. Melting behavior of gelatin solutions exposed to gamma rays of 10, 20, and 30 kGy and not irradiated (WFG).

Şekil 7. 10, 20 ve 30 kGy dozunda ışınlanan ve ışınlanmamış (WFG) jelatin solüsyonlarının erime davranışları.

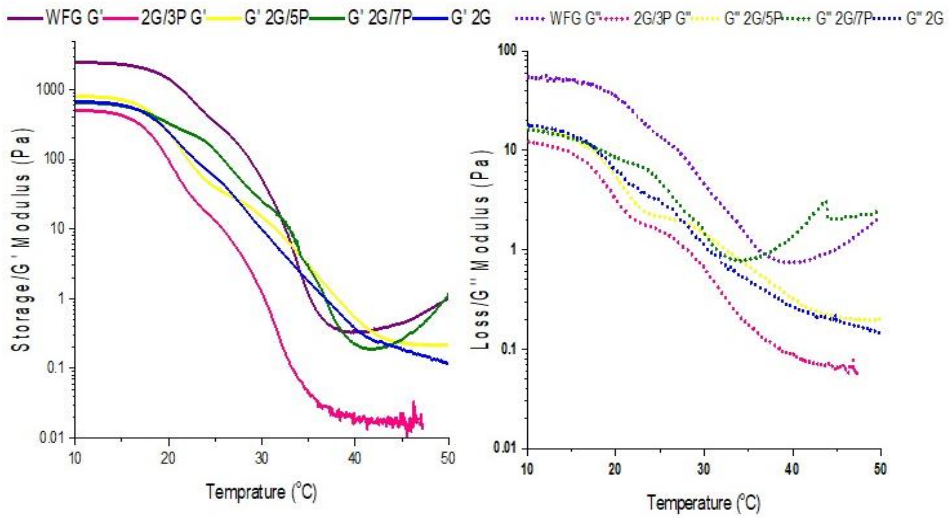

Figure 8. Melting behavior of gelatin solutions irradiated at a dose of 20 kGy at different pH values.

Şekil 8. Farklı pH değerlerinde hazırlanmış ve 20kGy dozunda ışınlanmış jelatin solüsyonlarının erime davranışı.

Tablo 2. Jelatin solüsyonlarının jelleşme profilinin logaritmik model parametreleri, jel kuvveti ve erime sıcaklıkları

Table 2. Gelation profile logarithmic model parameters, gel strength, and melting temperatures of gelatin solutions

Sample	G'_{ref}	Gelation Kinetics			t_{model} (h)	Gel strength (Pa)	Melting Temp. (°C)
		k_{gel} (Pa)	C	R^2			
FG	2001	286.03±10.37b	-370.00±14.07d	0.97±0.00	1.16	1556.50±25.22b	24.03±0.18g
WFG	2001	332.64±17.87a	-479.60±29.32f	0.95±0.00	0.53	2380.68±34.45a	34.50±0.87e
1G	2001	184.43±14.14c	-245.27±23.59b	0.93±0.00	81.70	1351.74±22.63c	45.36±1.12b
2G	2001	111.96±10.18e	-317.65±20.03c	0.96±0.00	705838.87	646.80±18.52e	43.61±1.06c
3G	2001	116.54±15.01e	-416.48±24.66e	0.94±0.00	2689046.40	599.87±20.27f	35.41±0.85e
2G/3P	2001	102.51±10.04ef	-423.17±24.90e	0.95±0.00	31328641.26	477.17±16.63g	31.35±0.63f
2G/5P	2001	145.59±15.13d	-498.02±35.20f	0.94±0.00	28343.10	773.05±15.13d	48.72±0.90a
2G/7P	2001	84.31±5.80f	-103.14±12.35a	0.95±0.00	54845060.74	623.62±20.64ef	36.94±0.33d

The logarithmic model equation is $G'_{ref}=k_{gel}\,ln(t)+C$. G'_{ref}: The target storage modulus (Pa) obtained from fish gelatin, k_{gel}: The gelling rate constant, C: the equation constant, and t: the gelling time.

Tablo 3. Jelatin solüsyonlarının kıvam katsayısı ve akış davranış indeksleri üzerine birikim ve kayıp modüllerinin etkisi

Table 3. The effect of storage and loss modulus of gelatin solutions on the consistency coefficient and flow behavior index values.

Sample	K'	n'	R^2	K"	n"	R^2
FG	1548.03±0.99b	0.017±0.000cd	0.98±0.00	42.13±0.97b	0.074±0.001d	0.94±0.00
WFG	2373.25±2.60a	0.016±0.001e	0.98±0.00	57.61±1.56a	0.012±0.001f	0.92±0.00
1G	1337.86±1.20c	0.020±0.001a	0.99±0.00	40.56±1.06b	0.065±0.001e	0.93±0.00
2G	644.7±0.76e	0.017±0.001bcd	0.98±0.00	17.10±0.62c	0.096±0.001c	0.92±0.00
3G	600.13±0.80g	0.018±0.001bc	0.97±0.00	14.31±0.66e	0.112±0.002b	0.94±0.00
2G/3P	478.83±0.79h	0.018±0.001bc	0.96±0.00	11.51±0.63f	0.129±0.002a	0.91±0.00
2G/5P	771.06±0.97d	0.016±0.001de	0.97±0.00	16.30±0.82cd	0.113±0.002b	0.93±0.00
2G/7P	623.23±0.78f	0.018±0.001b	0.98±0.00	15.23±0.69de	0.114±0.002b	0.93±0.00

4. Sonuç

Çalışmada balık jelatininin reolojik özellikleri üzerine farklı ışınlama dozlarının, pH'nın ve şarap tortusu (WL) ilavesinin etkisi ortaya konmuştur. Bu üç faktörün modifiye edilmesiyle farklı özelliklere sahip jelatin elde edilebileceği söylenebilir. WL ilavesi balık jelatininin viskoelastik özelliklerini arttırmıştır. Farklı gamma ışınlama dozajları jelatinin reolojik karakterizasyonunu önemli ölçüde etkilemiştir. Işınlama dozunun 20 Kgy'den sonra yapıda geri dönüşümsüz moleküler değişikliklere neden olabildiği ancak bununla birlikte WL ilaveli ışınlanmış jelatin jellerinin erime derecelerinin kontrol gruplarına göre önemli düzeyde yükseldiği ($p<0.05$) tespit edilmiştir. Işınlama dozları içerisinde en iyi reolojik sonuçlara 10 kGy ile ulaşılmıştır. Uygulanan pH değerleri jelatin jellerinin tüm reolojik karakterizasyonu üzerinde önemli düzeyde etki yaratmıştır. Jelatin jelleri pH 5'e göre hazırlandığında, k_{gel} değerinde yaklaşık %30 oranında bir artışa ulaşılırken, erime derecesi kontrol gruba göre 2 kat artış göstermiştir.

Sonuç olarak, jelleşme kinetiği parametrelerine göre balık jelatinin ışınlamaya maruz bırakılması durumunda reolojik özelliklerinde düşüşe neden olmakla birlikte, erime derecesini arttırıcı etki gösterdiği ve düşük dozlarda ışınlama ve özellikle pH optimizasyonu ile bu durumunun tamamen giderilerek istenilen teknolojik özelliklerde balık jelatini jelleri elde edilebileceği düşünülmektedir. Jelatinin farklı pH değerlerindeki reolojik davranışının belirlenmesi ile farklı gıda matrislerinde kullanım olanaklarını artıracağı düşünülmektedir.

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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Evaluation of the Impacts of Climate Change on Sunflower with Aquacrop Model*

İklim Değişikliğinin Ayçiçeği Üzerine Etkilerinin Aquacrop Modeli ile Değerlendirilmesi*

Hüdaverdi GÜRKAN[1*]

Abstract

Climate change has become one of the most significant risk factors in agricultural production. Plant productivity declines caused by climate change pose a serious threat to food supply and security. Crop simulation models have been widely used in recent years for the assessment of the impacts of climate change on agricultural production. In Konya, there have been limited studies on the potential effects of climate change on sunflower production. Sunflower, the main crop of the most imported agricultural product group, in which the production amount is currently insufficient to cover domestic consumption demand, is strategically important for the Turkish economy. The goal of this study was to examine the effects of climate change on sunflower yield in Türkiye by using the Aquacrop model. The data of the field experiment carried out on the Ekllor sunflower cultivar for two years in Konya conditions were used as material. The daily projection dataset of three Global Climate Models (HadGEM2-ES, MPI-ESM-MR, GFDL-ESM2M) and two scenarios (RCP4.5 and RCP8.5) were used to analyze climate change impacts. The 1971-2000 period was considered as the reference period and the 2022-2098 period was selected as the future period. The results confirmed that the Aquacrop model was able to satisfactorily simulate yield with NRMSE 2.10 % for the rainfed condition and 10.55 % for the irrigated condition, a d-index of 0.97, and a modeling efficiency of 0.91. Aqaucrop climate change impacts simulation which was based on 3 global climate models covering with 2022 -2098 period simulations projected that sunflower yield would be decreased in a range of 21% to 44% for RCP4.5 and 18% to 50% for RCP8.5 scenarios under rainfed conditions. In contrast, the yield would be increased in a range of 11% to 23% for RCP4.5 and 10% to 33% for RCP8.5 scenarios under irrigated conditions. The findings point to the use of appropriate water management measures for future sunflower production as a means of adapting to climate change.

Keywords: Aquacrop, Crop simulation model, Climate change, Sunflower, Crop yield changes

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Öz

İklim değişikliği tarımsal üretim için en önemli risk faktörlerinden biri haline gelmiştir. İklim değişikliğinin neden olduğu bitki verimliliği düşüşleri, gıda arzı ve güvenliği için ciddi bir tehdit oluşturmaktadır. Bitki simülasyon modelleri son yıllarda iklim değişikliğinin tarımsal üretim üzerine etkilerinin değerlendirilmesinde giderek yaygın olarak kullanılmaya başlamıştır. Türkiye'de iklim değişikliğinin tarımsal üretim üzerine olası etkileri ile ilgili çalışma sınırlı sayıdadır. Üretim miktarı henüz iç tüketim talebini karşılayamayan ve en fazla ithalatı yapılan tarımsal ürün gurubunun temel ürünü olan ayçiçeği Türkiye ekonomisi için stratejik öneme sahiptir. Bu çalışmanın amacı, Türkiye'de iklim değişikliğinin ayçiçeği verimi üzerine etkilerinin Aquacrop modeli kullanılarak analiz edilmesidir. Konya koşullarında iki yıl süreyle Ekllor ayçiçeği çeşidi üzerine yürütülen tarla denemesine ait veriler materyal olarak kullanılmıştır. İklim değişikliği etki analizi için ise 3 Küresel İklim Modeli (HadGEM2-ES, MPI-ESM-MR, GFDL-ESM2M) ve 2 senaryoya (RCP4.5 ve RCP8.5) ait günlük veriler kullanılmıştır. 1971 - 2000 dönemi referans, 2022 – 2098 dönemi ise iklim değişikliği etki analizi dönemi olarak ele alınmıştır. Çalışma sonuçları Aquacrop modelinin susuz koşullarda %2.10 ve sulu koşullarda %10.55 NRMSE değeri, 0.97 d-indeks ve 0.91 model etkinliği istatistiksel analizleri ile verimi başarılı bir şekilde simüle edebildiğini ortaya koymuştur. 3 küresel iklim modeli ve 2022-2098 yılları arası dönem özelinde oluşturulan Aquacrop iklim değişikliği projeksiyon sonuçlarına göre ayçiçeği veriminin susuz koşullarda RCP4.5 senaryosuna göre %21-44, RCP8.5 senaryosuna göre ise %18-50 aralığında azalması öngörülmektedir. Bunun aksine sulu koşullarda RCP4.5 senaryosuna göre %11-23, RCP8.5 senaryosuna göre %10-33 aralığında verim artışı sağlanabilecektir. Bulgular, iklim değişikliğine uyum sağlamanın bir yolu olarak gelecekteki ayçiçeği üretimi için uygun su yönetimi uygulamalarının kullanılmasına işaret etmektedir.

Anahtar Kelimeler: Aquacrop, Bitki simülasyon modeli, İklim değişikliği, Ayçiçeği, Bitki verim değişimleri

1. Introduction

As stated in the World Meteorological Organization (WMO) reports, in comparison to the pre-industrial period, the global mean surface temperature has increased by around 1.1°C (1850-1900). The WMO affirms that the last decade, 2015-2021, was the warmest on record. (WMO, 2022). According to the Intergovernmental Panel on Climate Change (IPCC), the global surface temperature rising is projected to hit 1.5°C by 2050 (IPCC, 2018). Different parts of the world are affected differently by changes in the climate. While some locations will experience greater warming and rainfall than others, others will experience more severe droughts. Türkiye has a semi-arid climate and is one of the regions most vulnerable to climate change. Temperatures are anticipated to rise, and precipitation will decrease in the future, based on the most recent IPCC projections for Türkiye (Demircan et al., 2017). Furthermore, according to World Food and Agriculture Organization (FAO) assessments, crop yields will decline in several countries, including Türkiye, due to climate change between 2030 and 2100 years (FAO, 2016).

Like many crops, sunflowers grow under rainfed conditions. Sunflower is the world's third crop of oilseeds, after soybean and rapeseed (USDA, 2021). Türkiye ranks sixth (FAO, 2022) in sunflower seed production. The main source of vegetable oil consumed in Türkiye is sunflower (TURKSTAT, 2022). There are significant decreases in productivity depending on drought years. Sunflower production dropped by 23.8 percent in 2007 compared to the prior year. (TURKSTAT, 2022).

Changes in temperature and precipitation factors, vital to plant production, directly affect the productivity, and vegetation period, suited to the growing region (Bulut, 2015). Temperature and precipitation threshold responses significantly affect crop yields (Easterling et. al., 2007). It is not possible to directly interfere with climate factors in open-field agricultural conditions. For this reason, the climate is the most important unknown factor in crop growth and agricultural production (Hoogenboom, 2000). Experiments or statistical methods are used to investigate the probable influence of climatic conditions on crop productivity. Crop simulation models (CSMs) with adequate modeling capabilities have grown in popularity in recent years (Boote et al., 2010). CSMs predict crop development by using meteorological and soil variables, cultivar features, management activities, and modeling mechanisms in the soil-plant-atmosphere system (Jones et al., 2003; Hoogenboom et al., 2004). CSMs can be used to model how climate change would affect crop productivity (White et al., 2011). In addition, the insights provided by the CSMs have become critical data for the agriculture assessment reports produced by the IPCC (White et al., 2011; Easterling et al., 2007; Gitay et al., 2001; Reilly et al., 1996). Crop models are classified into three main categories according to the basic components they consider in the calculations: carbon-driven, radiation-driven, and water-driven (Steduto, 2003; Todorovic et al., 2009). The Aquacrop model is one of the common models with its simulation capability in water-limited conditions, less input parameter requisition, and climate change studies.

This study aimed to inform decision-makers by simulating possible outcomes to quantify uncertainty in climate change impact assessment on sunflower production in Konya where one of Türkiye's top sunflower-growing regions by using the Aquacrop Model. The objectives were a) calibration and evaluation of the Aquacrop Model and b) estimation of changes in sunflower yields in the future periods using three different climate projections datasets.

2. Materials and Methods

2.1. Study Area

The study site was the Konya Soil, Water, and Deserting Control Research Institute's field in Türkiye (37°48'N, 32°30' E, 1031 m a.s.l.). Konya province, located in Türkiye's semi-arid climatic zone, is one of the primary sunflower production locations. According to the Turkish Statistical Institute (TURKSTAT) crop production reports of 2022, Konya positions third in sunflower production (tonnes) in Türkiye (TURKSTAT, 2022). (*Figure 1*).

Figure 1. Study region

The study area has clay soil characteristics, according to the results of the analysis applied to soil samples taken from the study area. Because of its high infiltration capacity, surface runoff is minimal. The organic matter content of the soil is low. (*Table 1*).

Table 1. Soil profile features

Depth (cm)	Clay (%)	Silt (%)	WP (cm cm^{-1})	FC (cm cm^{-1})	SP (cm cm^{-1})	BD (g cm^{-3})	OC (%)	pH in water
0-30	59.3	21.1	0.26	0.42	0.48	1.42	0.44	7.6
30-60	61.7	21.1	0.27	0.44	0.50	1.47	0.30	7.9
60-90	63.8	21.1	0.29	0.46	0.53	1.54	0.19	7.9
90-120	64.0	21.0	0.29	0.45	0.52	1.46	0.12	7.9
120-200	64.0	21.0	0.29	0.45	0.52	1.46	0.09	7.9

WP= wilting point, FC= field capacity, SP= saturation point, BD= bulk density, OC= organic carbon

2.2. Field experimental data

The field experiment was carried out for two years between 2015 and 2016 in Türkiye's Konya province (Gunduz et al., 2018). The Ekllor sunflower cultivar was chosen for the study, which took place in 2015 and 2016. The experimental layout was a Randomized Complete Block, with three replications. Plant and row spacing was determined as 70cm and 25cm, respectively. The sunflower vegetation period in the first year was 136 days, and the following phenological and growth stages were noted: May 5 emergence, July 21 starburst, August 4 seed formation, and September 18 maturity. The second year's sunflower vegetative period covered 133 days, and the development stages identified were emergence on May 12, starburst on July 22, seed formation August 4, and maturity on September 21.

There were two treatments in the field experiment: rainfed and irrigated. The drip irrigation method was used as the irrigation system. A total of 428 mm of water was used in 10 irrigation applications in 2015, and 465 mm of water was used in 12 irrigation applications in 2016.

In both years, a certain amount of fertilizer was used. The followings are the types and amounts of fertilization that were used: Before planting, 200 kg ha^{-1} of Di-ammonium Phosphate (DAP), 300 kg ha^{-1} of 20-20-20 compound fertilizer at planting, 50 kg ha^{-1} of Urea, and 50 kg ha^{-1} of Ammonium Nitrate (AN) at hoe.

2.3. Weather and climate projections dataset

The study area, Konya, is one of the aridest provinces of Türkiye. The long-term average total precipitation of the study area is 323.3 mm and the average temperature is 11.6°C. In comparison to 2015, the growth cycle in 2016 was hotter and drier. During the 2015 sunflower growing season (May-September), total precipitation was 163.8 mm, whereas it was 98.1 mm in the growing season of 2016 (*Figure 2*).

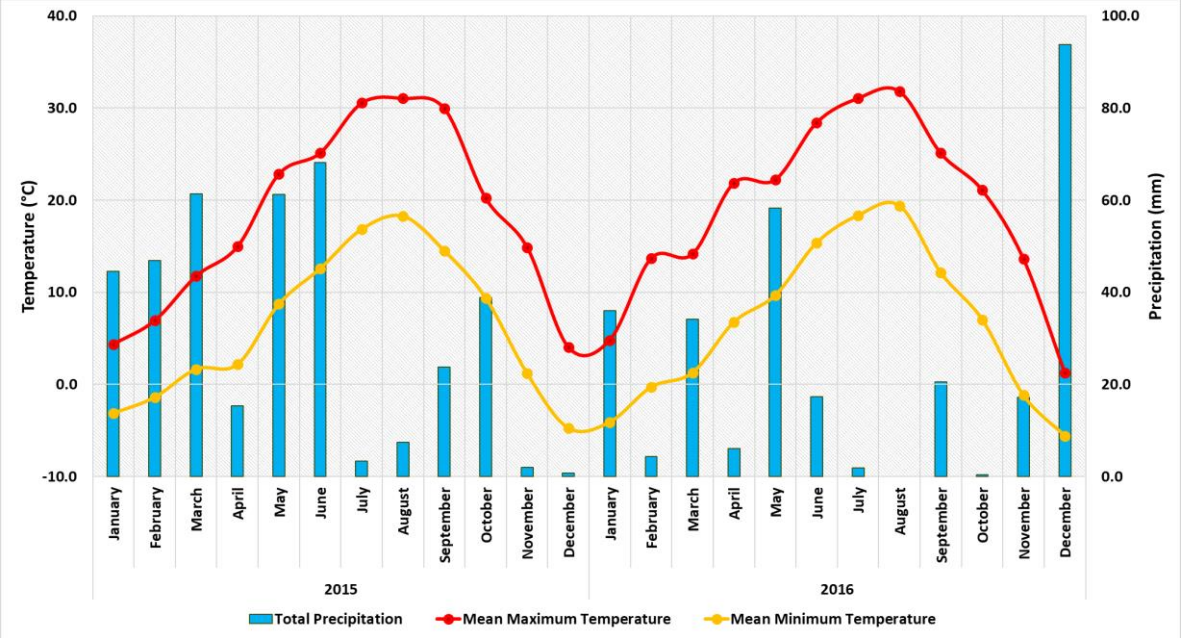

Figure 2. Observed monthly climate parameters at the research site in 2015 and 2016.

The observed daily weather dataset includes minimum and maximum temperature, total precipitation, average relative humidity, total radiation, and average wind speed parameters provided by The Turkish State Meteorological Service (TSMS). Due to the requirement for specific meteorological data for the Aquacrop model, observation data from a meteorology station 8 kilometers distant from the study area were used in the study.

The daily climate projections dataset downscaled by TSMS specifically for Türkiye was used for future climate change analyses. 3 GCMs (HadGEM2-ES, MPI-ESM-MR, GFDL-ESM2M) and two RCPs, i.e., 4.5 and 8.5 were selected for assessment of climate change impact. RCP4.5 reflects the most likely scenario, but RCP8.5 is referred to as the most catastrophic scenario due to the expected global temperature increase (Riahi et al., 2011; Thomson et al., 2011). The low-resolution GCMs dataset in the 130 - 220 km range was downscaled to 20 km resolution to conduct a detailed analysis specific to Türkiye conditions by TSMS. RegCM4.3.4 regional climate model and a nested dynamic downscaling approach were used to obtain a high-resolution climate projection dataset (Akcakaya et al., 2015).

For the 1971-2000 reference period, bias correction was performed between the model and observation data. Based on each parameter and each GCMs dataset, the bias adjustment was calculated for each day of the year. The bias correction results of each parameter was presented in *Table 2*.

For the climate change analysis study, the 1971-2000 period was considered as the reference period, and the 2022-2098 period was selected as the future period. The future term was divided into three segments: 2022–2040; 2041–2070; and 2071–2098.

Table 2. Daily average bias correction (observed-GCMs dataset) for each parameter

GCM	Tmin (°C)	Tmax (°C)	Precipitation (mm)	Wind (m/sec)	Rhum (%)	Radiation (w/m2)
HadGEM2-ES	0.1	1.0	-0.3	-1.1	-3.2	-2.5
MPI-ESM-MR	-0.1	1.1	0.3	-1.4	1.2	-1.1
GFDL-ESM2M	1.7	2.9	0.1	-1.2	-1.2	-1.2

Tmin: Minimum temperature, Tmax: Maximum temperature, Rhum: Relative humidity

The 2022-2098 climate projections of the study area were compared with the reference period of 1971-2000 specifically for the sunflower vegetation period. According to the climate projection results obtained for the sunflower vegetation period, based on the RCP4.5 and RCP8.5 scenarios, respectively, the total precipitation is projected to decrease by 18% and 21%, the minimum temperature is expected to increase in the range of 2.3°C and 3.6°C, and the maximum temperature is expected to rise by 2.5°C and 3.8°C.

2.4. The Aquacrop crop simulation model

The Aquacrop model produced by FAO aimed to increase water use efficiency in all irrigation conditions. Aquacrop mimics non-woody plant productivity reaction to water (Steduto et al., 2009; Raes et al., 2009; Hsiao et al., 2009). Since its creation in 2009, AquaCrop has been applied in a variety of regions all over the world. Aquacrop offers assistance with creating irrigation plans, identifying the best crop schedule, and estimating yield potential in various scenarios (including salinity and climate change). In the version of Aquacrop 7.0 released in 2022, simulations can be conducted for 17 different herbaceous crops. In this study, the previous version, Aquacrop v6.1, was used.

The model can visually present the estimation of yield and plant growth based on plant water consumption. The AquaCrop model calculates evapotranspiration separately as transpiration from the plant surface and evaporation from the soil surface. This distinction is of great importance on soil surfaces where there is insufficient vegetation and where soil water evaporation is too high. Another difference in the model compared to other models is that it uses the percentage of canopy cover instead of the leaf area index (LAI) in the calculations to better mimic the water deficit conditions. Aquacrop simulates the soil-water relationship in detail by considering capillary rise, deep infiltration, and groundwater level in the calculations.

Climate (daily, 10-day, or monthly), crop, soil, management (irrigation, tillage, etc.), and initial soil water content parameters are used as inputs in the model. Aquacrop stands out with its fewer input parameter requirements and modeling success in deficit water conditions compared to many other models. (Garcia-Vila et al., 2009; Todorovic et al., 2009; Stricevic, 2011; Kale et al., 2017; Elsheikh, 2015; Osman, 2018; Yigit and Candogan, 2019; Karimi, 2021).

In addition, the model is preferred in the studies of estimating the effects of climate change on crop yield productivity (Deveci et al., 2019; Gürkan, 2019; Konukcu et al., 2020; Raoufi et al., 2020; Yesilkoy, 2020; Stricevic et al., 2021). The Aquacrop model provides ease of use in climate change studies with the CO_2 projection data of IPCC's different climate scenarios (SRES and RCPs) presented in the database. Another superior feature of the Aquacrop model in climate change studies compared to other models is that it enables the automatic determination of parameters such as minimum temperature, average temperature, growing degree day, and precipitation, which are the determining factors of the planting window in line with the criteria determined by the user.

In this study, the future period simulations were carried out in the "successive years" mode. The start days of the next runs were linked to the crop maturity of previous years. Future sowing days were generated based on air temperature criteria. The minimum daily air temperature in 7 days of at least 5°C was determined as the threshold of the sowing date.

3. Results

3.1. Model calibration and evaluation

The data collected in 2015 were used to calibrate the Aquacrop Sunflower model, and the data obtained in 2016 were used to evaluate it. The calibration and evaluation procedures were conducted with the use of observed meteorological data. *Table 3* provided a list of the calibrated coefficients.

Table 3. The calibrated coefficients of the sunflower Ekllor cultivar

Parameter	Coefficient
Base temperature (°C)	5
Upper temperature (°C)	35
Initial Canopy Cover (CC_0)	0.29
Plant density (plants/ha)	57143
Canopy Growth Coefficient (CGC)	20.5
Maximum Canopy Cover (CC_x)	90
Canopy Decline Coefficient (CGC) (CDC)	0.401
Growing Degree Days (Sowing – Emergence)	147
Growing Degree Days (Sowing – Flowering)	1038
Growing Degree Days (duration of flowering)	268
Growing Degree Days (Sowing – Senescence)	1451
Growing Degree Days (Sowing – Maturity)	2233
Minimum effective rooting depth (Zr) - (m)	0.3
Maximum effective rooting depth (Zr) - (m)	2.0
Average root zone expansion (cm/day)	2.0
Crop transpiration coefficient (KcTr,x)	1.1
Crop water productivity (WP) g m^{-2}	20.5
Reference Harvest Index (HIo) (%)	37

Table 4 shows the findings of the phenological stage evaluation process. According to the vegetation period model simulation performance evaluation results, Aquacrop simulated the harvest period 6 days earlier.

Table 4. The model performance for evaluation of phenological stages

Phenological growth stages	Observed	Simulated	Model Error (day)
Emergence (DAP)	18	13	-5
Starburst (DAP)	71	66	-5
Maturity (DAP)	133	127	-6

DAP: days after planting

Before running the model for future periods, its performance under current conditions needs to be analyzed. Evaluation of the Aquacrop performance by comparing simulated data to field measurements was one of the primary goals of the research. This was accomplished using a statistical assessment approach that included relative error (RE), relative mean absolute error (RMAE), root mean square error (RMSE), normalized root mean square error (NRMSE), modeling efficiency (EF), index of agreement (d-index), and modified index of agreement (d1-Index). (Nash and Sutcliffe 1970; Willmott, 1982; Willmott et al., 1985). Inaccuracy is computed as the ratio in RE, RMAE, and NRMSE indexes. A lower number means a stronger connection, whereas zero symbolizes a great match. The RMSE methodology employs the unit used to compute the failure (in this case, kg/ha). A lower number means a closer link, while zero represents the perfect match. The d-index, d1-index, and EF indexes are dimensionless, with values ranging from 0 to 1. A perfect match is indicated by an index value of 1, and no match is indicated by a value of 0. A statistical examination of the simulations revealed that the model produced acceptable yield projected results. (*Table 5*).

Table 5. Evaluation of the Aquacrop for yield

Parameter		Yield								
Treatment	**Year**	**Observed (kg/ha)**	**Simulated (kg/ha)**	**RE (%)**	**RMAE (%)**	**RMSE (kg/ha)**	**NRMSE (%)**	**d-Index**	**d1-Index**	**EF**
Rainfed	**2015**	2388	2390	-2.3	-0.24	45.12	2.1	0.97	0.87	0.91
	2016	1913	2450	1.8						
Irrigated	**2015**	4361	4422	-12.8	-9.6	430.54	10.55			
	2016	3799	3759	-6.3						

3.2. Analysis of the impacts of climate change on sunflower vegetation duration

It is predicted that global warming and temperature increases will affect the sunflower vegetation duration. For this reason, the changes in the vegetation duration in the next periods were analyzed in the study. First, the reference period of 30 years (1971-2000) was analyzed for the assessment of the effects of climate change on the sunflower vegetation duration. 2022 and 2098 were chosen as prediction periods. Annual simulations of the Aquacrop model were performed for both the baseline and the future time frames.

The differences between the reference and the future periods were used to calculate the changes. The findings of the assessment showed that the sunflower crop will reach harvest maturity in less time due to climate change. Temperature increases caused by climate change would shorten plant growth durations, based on assessment results. The projection results of the RCP4.5 scenario revealed that the vegetation duration of the sunflower crop will be shortened by 8-14 days in the first period (2022-2040), 10-18 days in the second period (2041-2070), and 12-19 days in the last period (2071-2098) (*Figure 3*). For the overall 2022-2098 period, the average vegetation duration is expected to be shortened between 10-17 days. Sunflower vegetation duration will be shortened by 11-16 days in the first period, 16-20 days in the second period, and 23-25 days in the last period based on the RCP8.5 scenario. For the overall 2022-2098 period, the average vegetation duration is expected to be shortened between 17-20 days for the RCP8.5 scenario. (*Figure 3*).

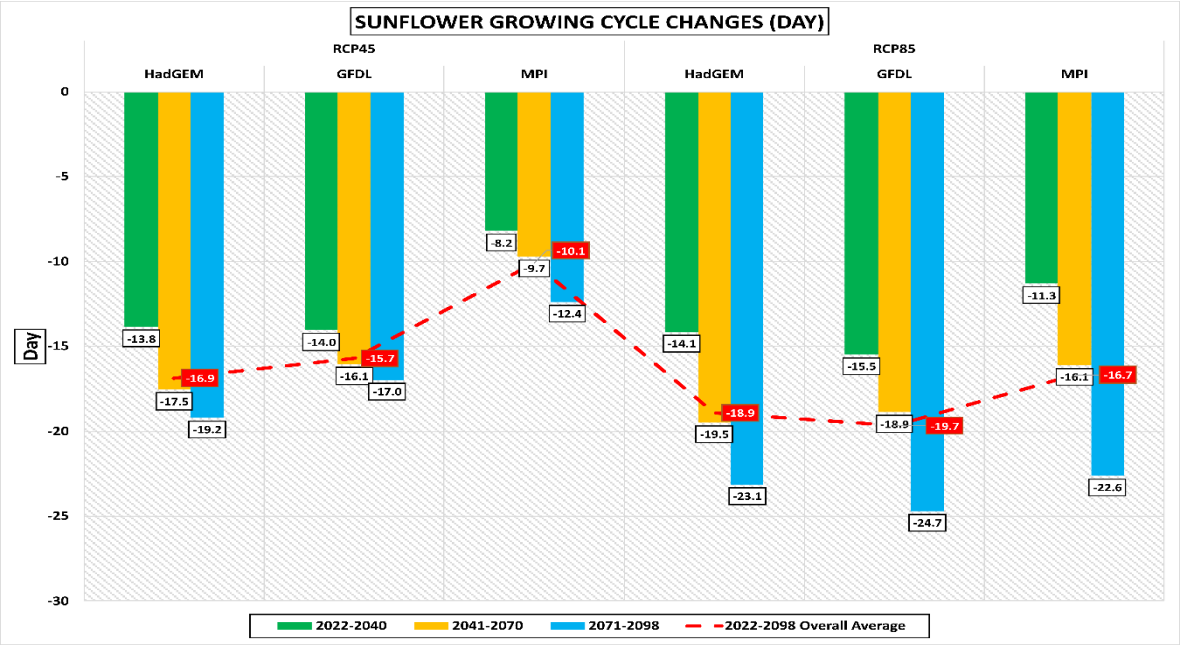

Figure 3. Changes in the length of the sunflower growth period (days)

3.3. Assessment of climate change impact on sunflower yield

Climate simulations for RCP4.5 were examined to determine how climate change may affect sunflower yield (*Figure 4*) and RCP8.5 scenarios (*Figure 5*). Rainfed and irrigated scenarios, as well as each GCM, were assessed independently for three periods (2022–2040, 2041–2070, and 2071–2098) and compared with the reference period (1971-2000) modeled yield results.

The 77-years (2022-2098) projections results based on the RCP4.5 scenario revealed the expectations of a decrease in yield under rainfed conditions and an increase in yield under irrigated conditions. For rainfed conditions, sunflower yield was simulated to decrease by 21% to 37% for the 2022-2040 period, decrease by 24% to 44% for the 2041-2070 period, and decrease by 23% to 42% for the 2071-2098 period. For irrigated conditions, it was proposed that sunflower yield will grow by 11% to 15% for the 2022-2040 period, increase by 14% to 23% for the 2041-2070 period, and increase by 18% to 22% for the 2071-2098 period (*Figure 4*).

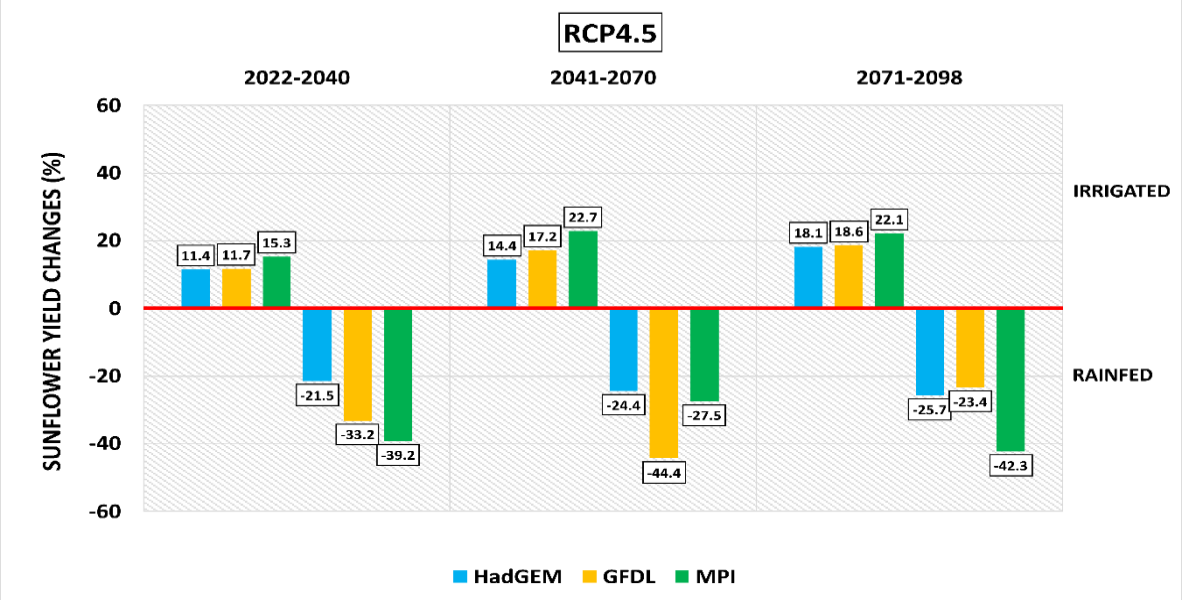

Figure 4. Sunflower yield changes based on RCP4.5 scenarios and three GCMs

Projection results of the RCP8.5 scenario revealed that the sunflower yield variation would be higher than the results of the RCP4.5 scenario in both rainfed and irrigated conditions. For rainfed conditions, sunflower yield was simulated to decrease by 18% to 44% for the 2022-2040 period, decrease by 23% to 40% for the 2041-2070 period, and decrease by 32% to 50% for the last period. For irrigated conditions, it was simulated that sunflower yield will rise by 10% to 14% for the 2022-2040 period, increase by 21% to 23% for the 2041-2070 period, and increase by 30% to 33% for the 2071-2098 period (*Figure 5*).

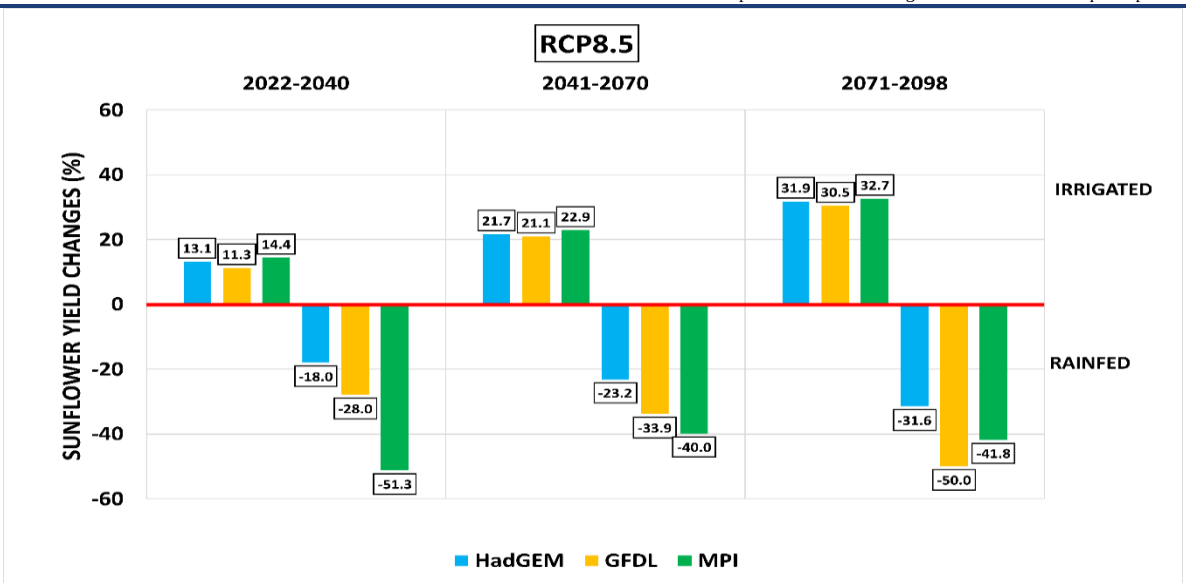

Figure 5. Sunflower yield changes based on RCP8.5 scenarios and three GCMs

3.4. Assessment of climate change impact on the total irrigation requirements of irrigated conditions

Due to climate change, it is predicted that temperatures will increase, and precipitation will decrease in the 2022-2098 period. According to the RCP8.5 scenario, precipitation decreases were expected to cause a greater negative impact in the 2071-2098 period. Precipitation is predicted to decrease by up to 25% in the RCP4.5 scenario and up to 41% in the RCP8.5 scenario (*Figure 6*).

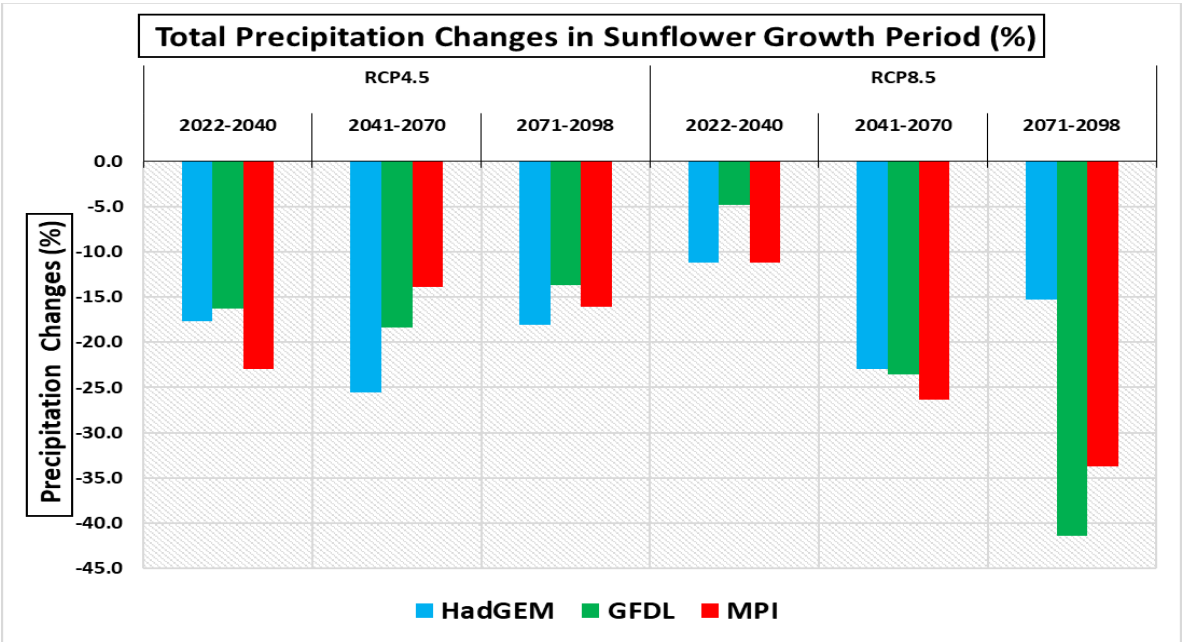

Figure 6. Konya sunflower growth period total precipitation projections

Temperature and precipitation changes will undoubtedly have an impact on sunflower growth and development. The projection results confirmed that and rising in temperature due to climate change would cause an increase in the water requirements for irrigated sunflower production conditions in future periods. The results of the research showed that the irrigation requirements will increase by 10% and 16% on average, respectively, in RCP4.5 and RCP8.5 scenarios in the 2022-2098 period under full irrigated conditions (*Figure 7*).

The results of the research indicated that if the rising irrigation requirements for the sunflower could be supplied, an enhancement in yield could be achieved. According to the RCP4.5 scenario, an average increase of 10% in irrigation water would contribute to an average 17% increase in the sunflower yield. The result for the RCP8.5 scenario projection indicated that an average increase of 16% in irrigation water would increase sunflower yield by 22% on average.

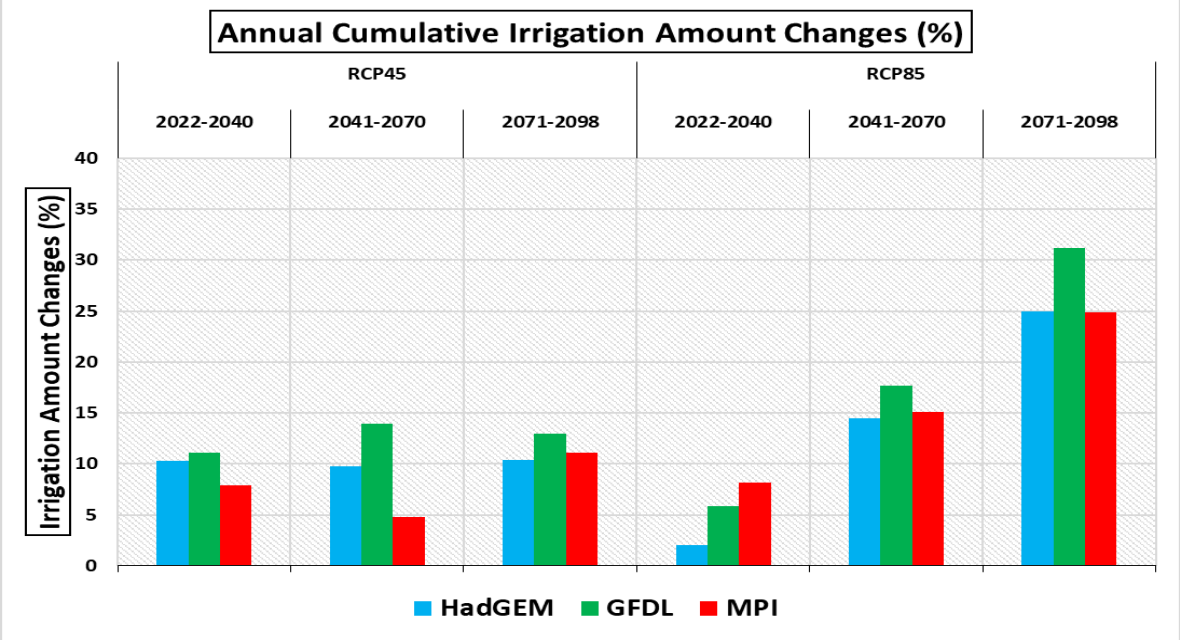

Figure 7. Konya cumulative irrigation requirement changes for irrigated conditions

4. Conclusion

The main objective of this study was the evaluation of the FAO Aquacrop model performance and the evaluation of how different climatic scenarios and GCMs simulations will affect sunflower yield. The yield and phenological stages were found to be faithfully predicted by the Aquacrop model. Under both rainfed and irrigated treatments, the model produced satisfactory simulation results. The Aquacrop model simulation ability was proven by statistical analysis.

The Aquacrop model achieved exceptional yield prediction ability under rainfed conditions with 2.10% nRMSE. Also, acceptable yield modeling findings in an irrigation environment with 10.55% nRMSE. The model's accuracy was comparable to earlier studies for conditions in Italy (Todorovic et al., 2009) with a 1.18% relative error and for conditions in Serbia (Stricevic et al., 2011) with a range of 0.3 to 5.0% relative error using the Aquacrop.

According to the findings of the climate change analyses, sunflower productivity in semi-arid regions will be severely affected under rainfed conditions. Obtained from two scenarios and three GCMs for rainfed situations, all yield estimates anticipated a yield loss ranging from 18 to 50 percent. Projection results revealed that there is a strong relationship between in-season total precipitation and yield. According to the simulation results, it is predicted that the maximum decrease in yield with 50% for the GFDL model on the RCP4.5 scenario will occur between 2071 and 2098 when the maximum decrease in precipitation with 48% is expected for the same model and scenario.

The results of the study match well with our earlier findings for the same location through the use of the DSSAT model (Gürkan et al., 2021). Obtaining results also outline similarities with other authors' research on climate change's effects on sunflower yield (Dellal, 2012; Demir, 2013; Deveci, 2015; Altürk et al., 2019; Gürkan, 2019; Gürkan et al., 2020; Yesilkoy, 2020) in Türkiye. Furthermore, the results are in line with previous studies conducted across Europe. In Southern and Eastern Europe, a decline in sunflower yield of between 10% and 30% was predicted for 2030. (Donatelli et al., 2015). Applying the ISAREG crop simulation model in Portugal

environments, sunflower yield declines to deviate from 6% to 10% for the 2011–2041 timeframe and deviating from 11% to 19% for the 2041–2070 timeframe was calculated (Valverde et al., 2015).

According to the modeling results, sunflower yield would increase by 10% to 33% in both RCP4.5 and RCP8.5 scenarios. The findings revealed that providing enough water through further irrigation in the agronomic period increases sunflower production. However, in the Konya region, which lacks adequate irrigation facilities even today, the future irrigation requirement should not be ignored. Decision-makers should consider this situation in future agricultural production strategies. The importance of irrigation as a climate change adaptation technique for sunflower production in semi-arid environments is highlighted in the assessment of climate change risks.

The findings show that a rise in CO_2 levels caused by climate change can boost sunflower efficiency under irrigated conditions. Several studies have indicated that higher CO_2 concentrations enhance the productivity of C3 plants including the sunflower by allowing them to photosynthesize at a faster rate (Long et al., 2006; Reddy, 2010; Debaeke et al., 2017). For future climate predictions, the IPCC scenarios considered in this study, RCP4.5 and RCP8.5, anticipate a rise in CO_2 levels.

Assessment results of the impacts of climate change prove that especially due to temperature increases, the sunflower life cycle will shorten in future periods. For the overall 2022-2098 period, the average vegetation duration is expected to be shortened between 10-17 days for the RCP4.5 scenario, and between 17-20 days for the RCP8.5 scenario.

The Aquacrop Crop Model is a valuable technological tool to simulate the potential effects of climate change on future crop productivity. The Aquacrop model provides ease of use in research where data collection difficulties are experienced with its fewer input requirements and high success capability. The assessment of the effects of climate change on other herbal crops in other regions of Türkiye using the Aquacrop model could be the main subject of future research.

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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Determination of Bioremediation Potentials and Plant Growth-Promoting Properties of *Bacillus* Species Isolated from The Rhizosphere of *Dactylorhiza urvilleana**

Dactylorhiza urvilleana Rizosferinden İzole Edilen *Bacillus* Türlerinin Biyoremediasyon Potansiyellerinin ve Bitki Büyümesini Destekleyici Özelliklerinin Belirlenmesi*

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Abstract

Industrial activities have been one of the biggest factors of environmental destruction by affecting natural resources for decades. Heavy metals, which are one of the greatest dangers especially for the biosphere, can be found in industrial waste. Heavy metals that enter agricultural areas through industrial wastewater cause heavy metals to accumulate in the soil after a certain period. These accumulated heavy metals become an important environmental problem, threatening the life of living beings due to their toxic properties. In soils contaminated with wastewater containing heavy metals, microorganism populations are severely damaged in terms of both number and diversity. This heavy metal accumulation in water and soil has become a global health threat. Alternative processes are needed in the fight against heavy metal pollution. Bioremediation activity, defined as the removal process of environmental pollutants through microorganisms and plants, has gained significant importance in recent years. In our study, the tolerance potentials of *Bacillus* species isolated from the rhizosphere of *Dactylorhiza urvilleana* (Steudel) Bauman in the Ovit plateau of Rize province to metals (such as copper, lead, zinc, iron and silver) were investigated. In addition, plant growth promoting Indole Acetic Acid (IAA) production, phosphate dissolution, and ACC (1-Aminocyclopropane-1-Carboxylic acid) deaminase production were determined. It was determined that the isolated *Bacillus* species had a wide pH growth range and some *Bacillus* species were salt tolerant. The results showed that *Bacillus* species have bioremediation potential and plant growth promoting properties. It is thought that the bacteria isolated from the study can be used to make areas with heavy metal pollution suitable for plant cultivation and act as plant growth promoters in these areas. These bacteria strains are planned to be used as cheaper and more effective methods in studies in agriculture or areas with heavy metal pollution.

Keywords: *Bacillus*, Bioremediation, Metal tolerance, Salt tolerance, Indole Acetic Acid (IAA)

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Öz

Sanayi faaliyetleri onlarca yıldır doğal kaynakları etkileyerek çevresel tahribatın en büyük etkenlerinin başında gelmektedir. Özellikle biyosfer için en büyük tehlikelerden biri olan ağır metaller sanayi atığının içeriğinde bulunabilmektedir. Endüstriyel atık sular yoluyla tarım alanlarına giren ağır metaller, belirli bir süre sonra ağır metallerin toprakta birikmesine neden olmaktadır. Biriken bu ağır metaller, toksik özellikleri nedeniyle canlıların yaşamını tehdit eden önemli bir çevre sorununa dönüşmektedir. Ağır metaller içeren atık sularla kirlenmiş topraklarda mikroorganizma popülasyonları hem sayı hem de çeşitlilik açısından ciddi zarar görmektedir. Suda ve toprakta meydana gelen bu ağır metal birikimi evrensel boyutlara ulaşan bir sağlık tehdidi haline gelmiştir. Ağır metal kirliliği ile mücadelede alternatif süreçlere ihtiyaç duyulmaktadır. Mikroorganizmalar ve bitkiler aracılığı ile çevresel kirleticilerin giderim süreci olarak tanımlanan biyoremidasyon faaliyeti son yıllarda büyük önem kazanmıştır. Çalışmamızda Rize ili Ovit yaylasındaki *Dactylorhiza urvilleana* (Steudel) Bauman rizosferinden izole edilen *Bacillus* türlerinin bakır, kurşun, çinko, demir, gümüş gibi metallere olan tolerans potansiyelleri araştırıldı. Ayrıca bitki gelişimini teşvik edici İndol Asetik Asit (IAA) üretimi, fosfat çözündürme, ACC (1-Aminosiklopropan-1-Karboksilat) deminaz üretimi gibi özellikleri belirlendi. İzole edilen tüm *Bacillus* türlerinin geniş bir pH üreme aralığına sahip olduğu ve bazı *Bacillus* türlerinin tuz toleransına sahip olduğu belirlendi. Sonuçlar, *Bacillus* türlerinin biyoremediasyon potansiyeline ve bitki büyümesini teşvik edici özelliklere sahip olduğunu göstermiştir. Çalışmadan izole edilen bakterilerin ağır metal kirliliği olan alanların bitki yetiştiriciliğine uygun hale getirilmesinde kullanılabileceği ve bu alanlarda bitki gelişimini destekleyici olarak görev yapabileceği düşünülmektedir. Bu bakteri suşlarının tarımda veya ağır metal kirliliği olan bölgelerde yapılacak çalışmalarda daha ucuz ve daha etkili yöntemler olarak kullanılması planlanmaktadır.

Anahtar Kelimeler: *Bacillus*, Biyoremediasyon, Metal toleransı, Tuz toleransı, İndol asetik asit (IAA)

1. Introduction

Efforts are being made to develop more environmentally friendly alternatives and to provide biological control in the fight against environmental pollutants and plant pathogens (Ongena and Jacques, 2008). Microorganisms in the soil have an important role in maintaining and protecting natural ecosystems. Microorganisms play a direct or indirect role in physical, chemical, and biological changes in plants and soil with specialized molecules and signals. In the soil ecosystem, there are microorganisms (bacteria and fungi) that have a positive effect on plant health, as well as microorganisms that are harmful to plant health. Beneficial bacteria in the plant root system promote plant growth by producing phytohormones, dissolving inorganic phosphorus, producing iron-chelate siderophores and increasing iron intake, producing plant hormones (such as auxin, cytokinin, and gibberellin), or facilitating the uptake of minerals in the environment. The plant growth-promoting properties of beneficial microorganisms are important for their use in agricultural production (Karapire and Özgönen, 2013). Some rhizosphere-resident bacteria, with their ability to produce aminocyclopropane carboxylate deaminase (ACC), reduce the amount of ethylene in plant roots and promote root elongation and development (Penrose and Glick, 2001). *Bacillus* species, which are frequently found in the soil and plant rhizosphere, attract attention with their stimulating properties on plant growth and with their inhibitory properties on pathogens (Güldoğan et al., 2022; Soylu et al., 2022). The characteristics that make *Bacillus* species biotechnologically attractive are their diverse secondary metabolisms and their ability to produce structurally different antagonistic substances (Stein, 2005). Therefore, *Bacillus* species are becoming a group of microorganisms with the potential for biotechnological applications.

Heavy metals that mix with and accumulate in soils affect soil fertility, microbial activity, and biological diversity (Yaldız and Şekeroğlu, 2013). Excessive amounts of heavy metals in the environment slow down the metabolic reactions of living organisms and may have toxic effects. In the world, the spread of heavy metals and environmental pollution with dyestuffs is increasing due to industrial development. Since industrial waste water contains a large amount of heavy metals, their uncontrolled discharge into the environment without treatment causes toxic and mutagenic effects on the living things in that environment. Although various metals are used in very little amounts to maintain life in some organisms, their high concentrations cause harmful effects on the cell. Although toxic metals such as Ag, Al, Au, Cd, Pb, and Hg have no biological significance, their presence in the cell, even in low concentrations, is dangerous (Bruins et al., 2000; Nies, 2004). Living organisms require certain heavy metals in trace amounts. However, negative results are observed due to high heavy metal intake due to toxic effects in plants, animals and humans. They can cause serious physiological and neurological damage, especially for the human body.

Our study includes the purification and biochemical characterization of *Bacillus* species isolated from the rhizosphere soil of *Dactylorhiza urvillenana* species grown on the Ovit plateau of Rize. In addition, it aimed to determine the salt tolerance, heavy metal tolerance, and plant growth-promoting properties of isolated bacteria.

2. Materials and Methods

2.1. Bacterial isolation from orchid specimens

Orchid samples were taken from the Ovit plateau in Rize province in 2012-2013. The tuber of the orchid plant and the rhizosphere soil at a depth of 20 cm were taken into sterile plastic bags. After the orchid samples were identified, bacteria were isolated from the root and rhizosphere soil. Orchid specimens were soaked in 70% ethanol for 3 minutes after the surfaces were washed in sterile tap water. The washing process was repeated three times with sterile distilled water to remove the alcohol. With the help of a sterile scalpel, 1 cm^2 pieces of orchid roots and tubers were cut and placed in Mueller Hinton agar petri dishes and incubated at 37 °C for 2 days. Soil samples were weighed equivalent to 10 g dry weight of wet soil and mixed in 90 mL sterile distilled water. Using the macrodilution method, 100 µL of dilution liquid was spread on MHA medium and incubated at 37 ºC for 2 days. Different colonies grown in the MHA media were examined microscopically by Gram staining. Gram-positive colonies grown in the medium were inoculated on MHA medium for pure culture with the single colony technique and incubated for 1-2 days at 37 ºC.

2.2. Biochemical identification of isolates

Bacteria purified as single colonies were incubated overnight at 36 °C in MHA medium. The bacteria's morphology was observed at 1000x magnification after Gram staining under a light microscope (BX63; Olympus, Tokyo, Japan). The isolated bacterial strains were identified by biochemical tests such as motility test, indole test,

methyl red test, gelatin test, citrate test, nitrate reduction test, lecithinase, catalase test, Voges-Proskauer (VP) test, urease test, starch hydrolysis test and sugar fermentation test(Cappuccino and Sherman, 1996; Kandler and Weiss, 1986; Holt et al., 1994).

2.3. Determination of the effect of temperature, pH and NaCl on bacterial growth

Mueller Hinton Broth (MHB) containing different pH (4.5, 5.5, and 8.5) and salt (10%, 15%) concentrations were used to determine pH and salt tolerance of bacterial isolates. A single colony was taken from the fresh cultures of the isolates and inoculated into MHB and incubated at 36 °C for 24 hours. Bacterial suspension McFarland 0.5 turbidity or $1x10^8$ CFU/mL was prepared from overnight culture. The Eliza plate wells were dispensed with 190 µL of MHB medium that had different salt and pH concentrations, and 10 µL of bacterial suspension was put into each well. The isolates were incubated for 48 h at different temperatures (10 °C and 45 °C), pH levels, and salt concentrations. The temperature, pH, and salt concentrations at which bacterial isolates could grow were determined. The experiments were carried out with 3 repetitions.

2.4. Detection of plant growth-promoting traits (PGPR) of the selected isolates

Phosphate solubilization activity was performed according to the methods of Fürnkranz et al. (2009) and Aydoğan et al. (2013). Bacterial isolates phosphate growth agar (NBRIP) (10 g glucose, 5 g $Ca_3(PO_4)_2$, 5 g $MgCl_2x6\ H_2O$, 0.25 g $MgSO_4x7H_2O$, 0.2 g KCl, 0.1 g $(NH4)_2SO_4$, and 15 g agar) was inoculated into the medium and incubated for 5 days. For testing of ACC(1-Aminocyclopropane-1-Carboxylate) deaminase activity, bacterial culture was seeded on ACC deaminase agar medium and incubated at 36±2°C for 2-7 days until colony formation. Colony growth was recorded as ACC-deaminase positive after incubation (Dworken and Foster, 1958; Brígido et al., 2015). All experiments were performed in triplicate. Chrome A qualitative test of siderophore production was performed on the agar medium of Chrome Azurole (CAS) (Schwyn and Neilands 1987). CAS agar plates were prepared and spot inoculated with bacterial isolates. Inoculated CAS agar plates were incubated at 36±2°C for 3-5 days. The development of a yellow-orange halo around the bacterial colony was considered a positive result (Schwyn and Neilands, 1987; Alexander and Zuberer, 1991). Indole acetic acid (IAA) production in bacterial isolates was tested by inoculating the bacterial suspension (10% v/v) in Mueller-Hinton broth containing 50 µg/mL L-tryptophan. Cultures were incubated at 36±2°C for 48 hours and then centrifuged at 10,000 g for 10 minutes. The concentration of IAA in the culture supernatant was tested using Salkowski's reagent (0.5 M $FeCl_3$ and 35% perchloric acid in a mixture 1:49). 1 mL of supernatant was placed on the spectrum plates, 4 mL of Salkowski reagent was added, and it was incubated at 37°C for 30 minutes after incubation in the dark, measurements were made at 535 nm in the spectrophotometer. Each sample was performed in triplicate and the mean value of the measured value was taken (Gordon and Weber, 1951; Fürnkranz et al., 2009).

2.5. Determination of Metal Tolerance of Isolates

It was aimed to determine the tolerance of the isolated bacteria to silver (Ag), copper (Cu^{+2}), iron (Fe^{+2}), zinc (Zn^{+2}) and lead (Pb^{+2}) metals. MHA medium containing metal salts ($AgNO_3$, $CuSO_4.5H_2O$, $FeCl_3.6H_2O$, $ZnCl_2$, and Pb $(NO_3)_2$) at different concentrations (1.0, 2.5, 5.0, and 10.0 mM/L) was used to determine metal tolerance (Velásquez and Dussan, 2009; Üreyen Esertas et al., 2020). The overnight cultures of bacterial isolates were incubated at 36±2°C for at least 5 days by spot seeding in MHA medium containing different concentrations of metal salts. Colony growth was recorded as positive by following the bacterial growth.

3. Results and Discussion

3.1. Identification of bacteria by traditional methods

It was observed that oxidase and catalase reactions were positive in the majority of bacteria, some showed weak positive reactions, most of them were R-type colonies and spore localization in the cell was mostly subterminal (*Table 1*).

Table 1. Biochemical characteristics of the Bacillus isolates.

NO	Species	Spore	O_2	Oxi	Cat.	Mot	Nit	Ami	Üre
5O7	*B. macerans*	Subterminal	FA	-	+	-	-	-	-
5O6	*B. mycoides*	Central	AE	+	+	-	+	2,8/3,4	-
1412	*B. insolitus*	Subterminal	FA	+	+	-	+	-	-
5O11	*Bacillus* sp.	Subterminal	AE	-/+	+	-	-	-	-
146	*Bacillus* sp.	Subterminal	A	-	+	-	-	-	±
5O5Y12	*Bacillus* sp.	Subterminal	A	+	+	-	-	-	-
112O3	*Bacillus* sp.	Subterminal	FA	+	+	-	+	-	++
141	*B. insolitus*	Subterminal	AE	+	+	-	+	-	-
5O5Y10	*Bacillus* sp.	Central	FA	-	+	-	+	-	-
142	*B. insolitus*	Central	AE	+	+	-	+	-	++
5O5Y2	*Bacillus* sp.	Subterminal	AE	+	+	-	-	-	±
144	*B. mycoides*	Subterminal	FA	+	+	-	+	1,8/2,8	-
5O3	*B. laterasporus*	Subterminal	FA	-	+	-	-	-	-
5O5Y13	*B. globisporus*	Central	FA	-/+	+	-	+	2,9/3,4	-

NO	Species	Les	Gel	İn	MR	Cit	D/Y	H_2S	G
5O7	*B. macerans*	4+	+	+	+	-	A/Al	-	-
5O6	*B. mycoides*	4+	+	-	+	-	Al/Al	-	-
1412	*B. insolitus*	nd	+	-	+	-	Al/Al	-	-
5O11	Bacillus sp.	-	-	-	-/-	-	Al/Al	-	-
146	*Bacillus* sp.	nd	+	-	-	±	Al/Al	-	-
5O5Y12	*Bacillus* sp.	nd	+	-	+	±	A/A	-	-
112O3	*Bacillus* sp.	nd	+	-	-	±	A/A	-	-
141	*B. insolitus*	4+	-	-	+	-	A/A	-	-
5O5Y10	*Bacillus* sp.	nd	+	-	-	++	A/A	-	-
142	*B. insolitus*	4+	-	-	+	-	A/A	-	-
5O5Y2	*Bacillus* sp.	nd	+	-	-	±	A/A	-	-
144	*B. mycoides*	4+	-	+	+	-	Al/Al	+	-
5O3	*B. laterasporus*	4+	+	-	+	-	A/A	-	-
5O5Y13	*B. globisporus*	4+	+	-	+	-	A/A	-	-

In; İndole, Mot; Motility, Oxi; Oxidase, Cat; Catalase, MR; Methyl-Red, Cit; Citrate, KIA: Kligler iron agar, D: Deep, Y; Surface, G; Gase, Nit; Nitrate, Ami: Amilase, Üre; Ürease Lest: Lesitinase, Gel; Gelatinase, nd; Not detected. -/-; Not Growth, -; Reproduction positive but negative test result, ±; weak positive, +; Positive, 4+; very good,

3.2. Determination of the effect of temperature, pH and NaCl on bacterial growth

Since temperature, pH and salt (NaCl) concentration have roles in enzymatic function as well as overall metabolic efficiency, these factors do have an effect on survivability. The temperature, pH profile and salt (NaCl) concentration of the strain indicate that the strain has the ability to survive in an adverse condition (Samanta et al., 2012). It was aimed to determine the growth potential of the isolated *Bacillus* strains at different temperatures and pHs. In our study, it was observed that *Bacillus* species can grow well in especially 10°C conditions with a wide temperature range (10-45°C). A similar result has been obtained in the study of two isolates *Bacillus sonorensis* and *Bacillus subtilis* that showed growth between 10-40°C (Jadhav et al., 2010).

It was observed that *Bacillus* species can grow well in both acidic and basic conditions with a wide pH range (4.5-8.5). In our study was consistent with previous studies, which reported a wide pH range (5.0–10.0) where Bacillus can grow. The study highest growth was observed at pH 7.0, 8.5, and 10.0, respectively. However, the results also indicated that isolates *Bacillus* could grow at an acidic pH of 5.0 (Wekesa et al., 2022).

In determining the salinity tolerance, it was observed that the growth changed depending on the species. It was determined that growth was better when the salinity rate was 10%, but some *Bacillus* species could not reproduce

when the salinity rate was 15%. Determining the growth of bacteria at different temperatures, pH and salinity rates is important because of adaptation to seasonally changing soil conditions. Because when the microorganism is applied to the plant, it should be able to adapt easily to the root rhizosphere and be able to benefit the plant by maintaining its vitality. It was observed that 85% of the isolates (except three) grew well in the presence of salt (10%), and the 5O5Y10 strain was very good in the presence of 15% salt. It was determined that only 5O5Y12 grew better in the presence of 10% salt (*Table 2*). In the study has been reported that the soil-based *Bacillus subtilis* strain AS-4 was able to tolerate high salt concentration in a growing medium that contained both 10% and 15% salt. Study according the growth was observed solely in the initial hours up to 25 hours of incubation and later on it decreased (Satapute et al., 2012). In general, enzymes/enzyme manufacturers that are resistant to harsh conditions such as high temperature, pH or salt are preferred in the industry (Duman et al., 2016). It is thought that the temperature, pH and salt tolerance abilities observed in the isolates of 5O7, 1412, 5O11, 112O3, 5O5Y10, 142 which have been identified as species, can also be evaluated industrially.

Table 2. Effect of Salinity, pH and temperature on bacterial growth.

Code	Species	10% NaCI	15% NaCI	10 °C	45 °C	pH 4.5	pH 5.5	pH 8.5
5O7	*B. macerans*	±	±	++	+	++	++	++
5O6	*B. mycoides*	-	-	+	+	+	++	++
1412	*B. insolitus*	±	+	+	+	++	++	++
5O11	*Bacillus* sp.	+	+	++	+	++	+	++
146	*B. macerans*	+	-	++	++	++	+	++
5O5Y12	*B. mycoides*	++	-	+	+	++	++	++
112O3	*B. insolitus*	+	+	+	+	+	+	+
141	*Bacillus* sp.	+	±	-	+	+	+	++
5O5Y10	*B. macerans*	+	++	++	+	++	+	++
142	*B. mycoides*	±	+	+	+	++	++	++
5O5Y2	*B. insolitus*	-	-	+	-	+	++	++
144	*Bacillus* sp.	+	-	+	-	-	+	+
5O3	*B. macerans*	+	-	±	+	++	++	±
5O5Y13	*B. mycoides*	-	-	+	-	++	++	+++

3.3. Detection of plant growth-promoting traits (PGPR) of the selected isolates

Plant-associated bacteria play an important role in the adaptation of the host to the changing environment by altering plant cell metabolism or promoting plant growth (Ma et al., 2011). The most well-known of features that plant growth promoting bacteria is promote plant growth under heavy metal stress conditions, including indole acetic acid (IAA) production, 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase, siderophore production, phosphate solubility, ammonia production and nitrogen fixation (Ullah et al., 2015).

Bacterial endophytes either directly by producing phytohormones, including indole-3-acetic acid (IAA), gibberellins, cytokinins, phosphate solubility, nitrogen fixation, or indirectly support the growth of host plants through the production of antibiotics, siderophores. Soil microorganisms can affect trace metal mobility and availability to plants (Idris et al., 2004). In addition, IAA, siderophores, ACC deaminase and phosphate solubilizing bacteria can stimulate plant growth (Rajkumar et al., 2012). In previous studies, metal-resistant bacterial isolates from different genera (such as *Pseudomonas* and *Bacillus*) were reported to produce IAA and enhance plant growth (Rajkumar et al., 2012). A number of plant growth promoting factors (Siderophore production, Phosphate solubility, ACC deaminase activity and Ammonium production) of the identified bacteria were tested in liquid or solid media (on agar plate). Strong siderophore production (≥20) ability was detected in 4 of the isolates, but moderate in most of the isolates. In a study to draw attention to the importance of siderophores, pyoverdine (Pvd), a bacterial siderophore produced by *Pseudomonas aeruginosa*, was applied. In metal-contaminated soil (Cd and Cu), the mobility, phytoavailability, and uptake of Cu by tomatoes and barley increased, while cadmium fate did not change (Cornu et al., 2014). In our study, low levels of siderophore were detected only in isolates 5O5Y2 and 144, while siderophore production was not detected in the strain 1412. Other *Bacillus* strains in the study showed a strong ability to produce siderophores by forming yellow zones on agar plates. Phosphate

solubility activity was detected in 6 of them, 112O3 and 144 better. It was observed that all isolates had the ability to produce ammonium, but some isolates were better than others (*Figure 1*, *Table 3*). In addition, ACC deaminase positivity was determined in 6 of the isolates, one of which was low. In this study, the ability of the strains to produce indole acetic acid was investigated by spectrophotometric method. Good level of IAA activity was detected in most of the samples, very good level of IAA activity in 146 and 5O5Y12 strains, while no activity was detected in 6 of them. The plant growth promoting properties of the strains in the study promise potential to be local PGPB strains due to concerns about the potential ecological risks of introducing non-native PGPR to the field, especially in recent years (Ambrosini et al., 2016).

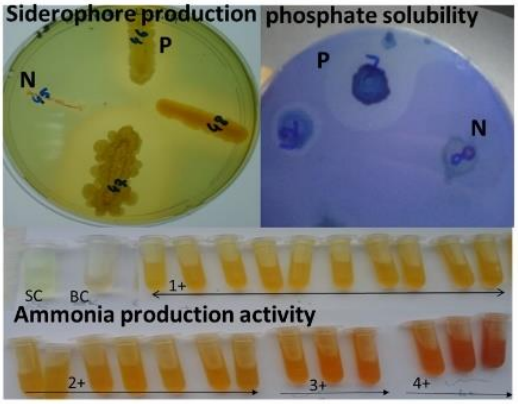

Figure 1. Siderophore production, phosphate solubility and ammonium production activity of bacterial isolates. P: Positive, N: Negative, SC: Nessler solution control, BK: Bacteri control. 1+;weak, 2+; moderate activity, 3+;good activity, 4+; strong activity

Table 3. Determination of plant growth-promoting some properties.

Code	Sid(mm)	Pho(mm)	ACC	Am	IAA activity
5O7	10/12	6/-	-	+++	7,54±0,26
5O6	18/48	5/-	-	++	-5,15±0,10
1412	-	-	-	+	-2,80±0,36
5O1	8/15	3/-	-	+++	10,92±2,45
146	17/20	6/-	+	+	15,29±0,26
5O5Y12	8/10	5/-	-	+++	11,10±0,12
112O3	8/10	6/14	±	++	18,14±0,63*
5O3	19/24	5/-	+	++	5,81±1,35
5O5Y10	9/14	7/9	-	++	3,76±0,65
5O5Y13	18/45	5/-	-	++	14,68±0,62
142	7/18	5/8	+	++	-5,56±0,17
5O5Y2	6/9	4/-	-	++	5,94±7,32
144	4/7	9/12	-	++	-4,18±0,95
141	6/10	7/9	-	++	17,00±0,51*

U; Growth, Z; Zone diameter(mm), -; negative activity, +; positive activity, ACC; ACC deaminase, Am; Ammonium production, Sid.; Siderophore production, Pho: phosphate solubility, 1-9 mm; positive activity, 10-19 mm; good activity, ≥20; very good activity

3.4. Determination of metal tolerances of bacteria

Studies show that a large number of plants are used to remove heavy metals from contaminated soils (Ullah et al., 2015). Accordingly, PGPB are widely used to inoculate plants as a biologically defined approach to increase the phytoremediation efficiency of toxic metals (Chen et al., 2017; Zornoza et al., 2017). The growth characteristics five different heavy metals salts (copper(II), lead(II), iron(II), silver and zinc(II)) of the Ovit strains tested in the study were examined on agar medium with 1, 2.5, 5 and 10 mM concentrations (*Table 3*, *Figure 2*). All strains in

the study were found to grow easily even in the presence of 10 mM iron. Five of the strains were able to grow in the presence of 10 mM silver, nine in the presence of 10 mM copper, and eleven in the presence of 10 mM lead. It was determined that all strains in the study did not grow in the medium in the presence of 10 mM zinc. In the presence of silver, obvious color change and transparent zone formation were observed in some strains. In the presence of lead, this event was detected as darkening of the colonies. In the presence of copper in the medium, no color change was observed in the petri dish, but a zone of transparency was observed around the bacterial colony. In the presence of zinc and iron metals, no shape changes were observed in the petri dishes. Colonies grown in the presence of copper showed a clear color change in the medium when kept at room temperature (*Figure 2*). It is thought that the metal salts will probably be reduced by accumulating on the surface of the bacteria or adsorbing the metals to the surface of the bacteria as they wait. Similar to our study, Njoku et al., (2020) reported in their study that the uptake of Pb, Cd and Ni from the media increased with the increase of the incubation period of *Bacillus megaterium* and *Rhizopus stolonifer*.

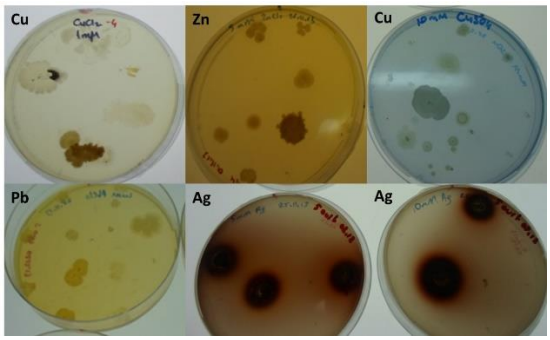

Figure 2. Petri image of bacterial isolates in an agar medium containing different metal salts

Table 4. Determination of the tolerance of bacteria to different metal concentrations

Code	Ag	Fe	Cu	Pb	Zn
5O7	nd	≤10 /+	nd	TE	TE
5O6	-	≤10 / +	≤10 / +	≤10 / +	≤5 / +
1412	nd	≤10 /+	nd	nd	Nd
5O1	1 / +	≤10 / +	≤10 / +	≤10 / +	≤2,5 / +
146	≤10 / +	≤10 / +	≤2,5 / +	≤5 / +	≤1 / +
5O5Y12	-	≤10 / +	≤10 / +	≤10 / +	≤5 / +
112O3	1 / +	≤10 / +	≤5 / +	≤10/ +	-/-
5O3	≤10 / +	≤10 / ++	≤5 / +++	≤1 / +	≤5 / +
5O5Y10	-	≤10 / ++	≤5 /+++	≤10 / ++	≤5 / +
5O5Y13	≤10 / +	≤10 / +	≤5 / +	1 (-/+)	≤5 / +
142	-	≤10 / +	≤10 / +	≤10 / +	≤2,5 / +
5O5Y2	≤2,5 / +	≤10 / +	≤10 / +	≤10 / +	≤5 / +
144	-	≤10 / ++	≤10 / ++	≤10 / +	≤2,5 / +
141	-	≤10 / +	≤10 / +++	≤10 / +	≤5 / +

≤0; negative activity, 0-9, positive activity, 10-15; good activity, ≥16; very good acitivity, Ability to grow in the presence of 1, 2.5, 5 and 10 mM metal, nd: not detected, (≤); 1-10 mM growth, (-); not growth, (+): growth, (2+); good growth, (3+); very growth.

Biomass that is used or turned into waste should be disposed of safely without harming the environment. Since the discharge or burning of spent adsorbents pollutes the soil, air and groundwater, suitable alternative techniques such as thermal desorption and maximum possible recycling of biosorbents are needed for sustainable use (Priyadarshanee and Das, 2021). Bacteria are characterized by their high adaptability to different environments, even under extreme conditions. Studies suggest many mechanisms to explain the rapid adaptation and tolerance

of bacteria to different environmental conditions (Casacuberta and González, 2013). Utilizing plant-beneficial rhizobacteria not only improves biocontrol and reduces the use of environmentally harmful pesticides, but also promotes plant growth and increases crop uptake. It is recognized that PGPB play a key role in agricultural practices and enable more sustainable farming opportunities (Zhang et al., 2022). PGPB can help growth by increasing the resistance of plants to different environmental stresses (Gururani et al., 2013). Studies show that these rhizobacteria, which are beneficial for plants, have high potential in applications for sustainable agriculture and this is of great importance for agricultural activities (Chen et al., 2022). As we stated in our study, it is seen that PGPB with high metal tolerance are of great importance for environmental and agricultural applications. Liu et al., (2014) stated in their study that the plant growth rate was more than 300% after the treatment of the soil with copper-tolerant bacteria. Bioremediation is the removal of waste with microorganisms and is one of the most effective strategies. For effective bioremediation, it is of great importance to identify metal-resistant bacteria that can promote plant growth and minimize the exposure of plants to metals in the soil. Different studies show that the application of heavy metal resistant PGPB in soils exposed to artificial heavy metal application prevents plants from being exposed to these heavy metals due to their microbial activities in the rhizosphere (Tak et al., 2013). It shows that plants should be treated with PGPR if heavy metal accumulation is increased. However, in parallel with the bacterial metal dissolving activity, other factors such as nutrient level, pH, metal species and plant variety in the soil highly affect the metal solubility in the soil, which in turn changes the metal uptake (Rajkumar et al., 2013). *Bacillus* genus can be grown in many culture media. In addition, it is mostly in the first place in studies carried out in heavy metal-contaminated areas, along with those based on enrichment techniques for the detection of metal-resistant microorganisms. In a field study, it was reported that 31.51% of the bacterial population belonged to the genus *Bacillus* in the microbiota examination of soil samples known to contain cadmium (0.5 to 1.6 mmol L^{-1}), zinc (138.2 to 900.7 mmol L^{-1}), and lead (6.1 to 442.1 mmol L^{-1}) (Lenart and Wolny-Koladka, 2013). Jing et al. (2012) reported that three of the four species isolated from heavy metal-contaminated soils belonged to the genus *Bacillus*. New approaches based on using contaminated biomass aim to produce catalysts for the synthesis of organic molecules (Grison, 2015). Among these studies, *Bacillus* genus is of great importance as it is the most isolated bacterium. Clavé et al. (2016) show that copper "eco-catalysts" prepared from the roots of *Eichhornia crassipes* are highly effective in the azide-alkyne cycloaddition reaction. Sevim and Sevim (2015) reported that *Bacillus* strains isolated from soil samples taken from different locations in Rize were resistant to copper, chromium, zinc, iron, and nickel. Another study revealed that inoculation with metal-resistant endophytic bacteria can effectively increase growth parameters in plants under Cd and Ni stress (Jan et al., 2019). Similar to these studies, the results of our study are considered to have potential for bioremediation and growth regulating properties for plants. *In vitro* and *ex vivo* studies on different conditions and plants should be continued in the future to fully demonstrate this potential.

4. Conclusions

Plant growth-promoting bacteria (PGPB) may be one of the most viable alternatives in the future to reduce negative stress threats with their natural, environmentally friendly and sustainable approach. In addition to improving plant health, using PGPB can optimize conditions for plants in the soil and the rhizosphere by maintaining nutrient availability, synthesizing phytohormones, and dissolving phosphate. Since the metal-bacteria interaction is initiated at the metal uptake level, the uptake mechanism is thought to be closely related to the metal resistance mechanism. Biodegradation abilities in bacteria may differ between species, even within species within the same genus. However, data on the bioremediation of heavy metals is limited, and further research is required to identify candidate species that can provide bio-recovery of heavy metal-contaminated soils.

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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Genetic Diversity of Kırklareli Honey Bee (*Apis mellifera* L.) Populations in Thrace Region of Turkey: Identification of Mitochondrial COI and ND5 Gene Regions*

Türkiye'nin Trakya Bölgesi Kırklareli Bal Arısı (*Apis mellifera* L.) Populasyonlarında Genetik Çeşitlilik: Mitokondriyel COI ve ND5 Gen Bölgelerinin İncelenmesi

İlknur GÖZE[1*], Fulya ÖZDİL[2]

Abstract

The main goal of genetic resource conservation is to keep as much genetic diversity as possible within each species. In this respect, some difficulties in the protection of honey bee gene resources make it necessary to reveal the genetic structures of the subspecies and the genetic relationships between the subspecies. In this study, Kırklareli honey bee populations which were officially registered as an ecotype of Turkey's honey bee (*Apis mellifera* L.) gene resources by the Republic of Turkey, Ministry of Agriculture and Forestry, were examined in the COI and ND5 genes of mitochondrial DNA. The restriction fragment length polymorphism (RFLP) together with the polymerase chain reaction (PCR) was used to define *Apis mellifera* populations. A total of 117 worker bee samples were used which were collected from mostly the Kırklareli province. A newly found single nucleotide polymorphism (SNP), G→A transition in the COI gene region formed a novel *Nco*I restriction site resulting in a new haplotype. This new haplotype has been abbreviated as haplotype C. As a result of the COI/*Ssp*I digestion, the previously reported C haplotype was determined. No restriction was found with the treatment of COI/*Sty*I enzyme. On the other hand, as a result of ND5/*Alu*I restriction, 2 restriction site and previously reported haplotype C was obtained in all of the studied samples. No restriction was screened with ND5/*Fok*I and ND5/*Hinc*II enzymes in the whole samples, only a reported uncut B haplotype was observed. Within this study, novel genetic information has been revealed for the Kırklareli honey bee ecotype registered as the Thrace honey bee of Turkey's honey bee gene resources. Moreover, detailed studies with larger sample sizes should be conducted to characterize the origin and the subspecies of Kırklareli honey bees in detail. It is thought that this study will be useful in the identification and registration of the Kırklareli honey bees to be carried out in the future, and also in the creation of a database.

Keywords: *Apis mellifera*, COI gene, ND5 gene, Kırklareli honey bee, RFLP technique

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*This study cited from Master thesis of İlknur GÖZE under titled as "Determination of genetic diversity in Kırklareli honey bee populations (*Apis mellifera* L.) by PCR-RFLP analysis in mtDNA COI and ND5 gene regions"

Öz

Genetik kaynakları korumanın temel amacı, her bir tür içinde mümkün olduğu kadar çok genetik çeşitliliği korumaktır. Bu açıdan bal arısı gen kaynaklarının korunmasındaki bazı güçlükler, alt türlerin genetik yapılarının ve alt türler arasındaki genetik ilişkilerin ortaya çıkarılmasını zorunlu kılmaktadır. Bu araştırmada, T.C. Tarım ve Orman Bakanlığı tarafından Trakya ekotipi olarak tescil edilen Kırklareli bal arısı populasyonlarını temsil eden bal arıları mitokondriyel genomda COI ve ND5 gen bölgelerinde farklı restriksiyon enzimleri ile incelenmiştir. *Apis mellifera* populasyonlarının tanımlanmasında restriksiyon parça uzunluk polimorfizmi (RFLP) yöntemi, polimeraz zincir reaksiyonu (PCR) ile birlikte çalışılmıştır. Ağırlıklı olarak Kırklareli ilinden örneklenen 117 adet işçi arı örneği materyal olarak kullanılmıştır. COI/*Nco*I kesiminde G→A transizyonu sonucu yeni bir kesim noktası ve ilk kez bu çalışmada bildirilen yeni bir haplotip elde edilmiştir. Bu haplotip daha önce bildirilen haplotipleri takip ederek C haplotipi olarak adlandırılmıştır. COI/*Ssp*I kesim sonucu olarak, birden fazla kesim noktası ve önceki çalışmalarda bildirilen C haplotipi elde edilmiş; COI/*Sty*I enzimi ile herhangi bir kesim noktası bulunamamıştır. Öte yandan, ND5/*Alu*I kesimi sonucunda, incelenen tüm örneklerde 2 kesim noktası ve daha önce bildirilen haplotip C elde edilmiştir. ND5/*Fok*I ve ND5/*Hinc*II enzimleri ile incelenen tüm populasyonda kesim tespit edilememiş ve tek bir bant profili sonucu B haplotipi görülmüştür. Bu çalışma ile Türkiye bal arısı gen kaynaklarından Trakya bal arısı olarak tescil edilen Kırklareli bal arısı ekotipi için yeni genetik bilgiler ortaya konmuş olup, Kırklareli bal arılarının orijin ve alttürlerinin detaylı olarak karakterize edilebilmesi için daha geniş örnek büyüklüğü ile detaylı çalışmalar yapılmalıdır. Bundan sonra yapılacak olan Kırklareli bal arısının tanımlanmasında ve tescil çalışmalarında ve ayrıca veri tabanı oluşturulmasında bu çalışmanın faydalı olacağı düşünülmektedir.

Anahtar sözcükler: *Apis mellifera*, COI geni, ND5 geni, Kırklareli bal arısı, RFLP tekniği

1. Introduction

Beekeeping has been one of the most effective production activities in Anatolia since ancient times. It has been reported that honey bees developed by spreading firstly in Europe, Africa, and the Near East until the 17th century, and after the 17th century, it was carried by immigrants, and beekeeping was carried out in all settlement areas (Fıratlı, 1988). While it was used to produce only honey to meet family needs, it has now become a commercial line of business. It is known that beekeeping is an activity that is more dependent on nature than other agricultural lines, and Türkiye is in an advantageous position for beekeeping with its climate pattern and rich flora.

It is known that the western honey bee (*Apis mellifera* L., 1758), (Hymenoptera: Apidae), has spread to all parts of the world due to its high adaptability and has adapted to the ecological conditions of the regions where it is found. A large number of geographical races have formed different ecotypes within these races. Adam (1983) reported that Turkey, due to its different climatic characteristics, is naturally a transition point between Africa, Europe, and Asia, and therefore it is a major gene pool that contains many bee races and ecotypes. Turkey has been one of the most important countries in beekeeping not only in terms of its natural riches but also because of its bee gene resources. Previous studies defined 27 subspecies of *Apis mellifera* based on mostly morphometric characters, and finally 29 *Apis mellifera* races (subspecies) identified so far with the help of molecular techniques. Considering this geography, it was concluded that Anatolian (*Apis mellifera anatoliaca* Maa, 1953), Caucasian (*Apis mellifera caucasica* Gorbachev, 1916), Iranian (*Apis mellifera meda* Skorikov, 1929) and Syrian bees (*Apis mellifera syriaca* Buttel-Reepen, 1906) were found in Turkey. The existence of the fifth honey bee subspecies in the Thrace region according to morphological (Güler et al., 2010) and genetic marker studies was also reported (Smith et al., 1997; Palmer et al., 2000; Kekeçoğlu et al., 2007, 2009; Özdil et al., 2009, 2022; Ünal and Özdil, 2018).

It is known that there has been an increase in the number of nomadic beekeepers in Turkey recently. For this reason, gene flow may increase between honey bee populations and uncontrollable genetic hybridizations may occur. Identification of the morphological and genetic variation in the honey bee subspecies and ecotypes, such as Thrace, Yığılca, Muğla, etc. ecotypes plays an important role in the formation of honey bee populations (Güler et al., 2010; Kekeçoğlu et al., 2007, 2009; Özdil et al., 2009, 2022; Güder et al., 2017; Gür et al., 2018; Ünal and Özdil, 2018).

In this study, in order to determine the genetic structure of honey bees in mostly Kırklareli and also in Tekirdağ provinces, possible mtDNA variations of COI and ND5 genes were analyzed and genetic markers were tried to be determined. *Nco*I, *Ssp*I, and *Sty*I restriction enzymes in the COI gene and *Alu*I, *Fok*I, and *Hinc*II restriction enzymes in the ND5 gene were studied. Thrace honey bee populations were considered as a different ecotype in Türkiye's honey bee gene resources and registered no need by the Republic of Türkiye, Ministry of Agriculture and Forestry (Anonymous, 2020). With this study, novel information has been revealed and updated for the ecotype registered as the Thrace bee.

2. Materials and Methods

2.1. Sample collection

In this study, a total of 117 DNA samples, representing the Thrace region, from mostly Kırklareli (107 samples) and Tekirdağ (10 samples) honey bee populations were examined.

2.2. Selection of genomic DNA samples

Genomic DNA isolation was previously carried out within the scope of the Tübitak 3001 Research Project. In this study, the honey bee DNA samples were selected according to the quantity and quality of the DNAs, samples were both checked on 1% agarose gels and controlled on a UV spectrophotometer.

Samples with good DNA quantity and quality were selected and used in this study. After checking the purity of DNA samples, PCR (Polymerase Chain Reaction) optimization was performed to amplify the targeted mitochondrial regions. All the analyses were performed in Molecular Genetics Laboratory in Tekirdağ Namık Kemal University in 2021.

2.3. PCR amplification of mitochondrial DNA COI and ND5 regions

PCRs were performed to amplify the 1028 bp of the COI (between the 2095-3123th mtDNA nucleotide sequence) and the 822 bp of the ND5 (between the 7395-8217th mtDNA nucleotide sequence) gene regions. COI (*Nco*I, *Ssp*I, *Sty*I) and ND5 (*Alu*I, *Fok*I, *Hinc*II) gene regions were amplified with the primers and digested with the restriction enzymes given in *Table 1*.

Table 1. Primers, restriction enzymes and the references used in this study

mtDNA Region	Primers (5'→3')	Enzymes	References
COI (1028 bp)		*Nco*I	Bouga et al., 2005
COI−*F	GATTACTTCCTCCCTCATTA	*Ssp*I	Stevanoviç et al., 2010
COI−*R	AATCTGGATAGTCTGAATAA	*Sty*I	Meixner et al., 2013
ND5 (822 bp)		*Alu*I	Bouga et al., 2005
ND5−*F	TCGAAATGAATAGGATACAG	*Fok*I-*Bts*CI	Ivanova et al., 2010
ND5−*R	GGTTGAGATGGTTTAGGATT	*Hinc*II	Meixner et al., 2013

Primers that were used to amplify the COI and ND5 gene regions, were designed based on the honey bee whole genome reference sequence (Access No: NC-001566) available in the NCBI GenBank database. PCR reactions for the amplification of COI and ND5 gene regions; prepared as 40 µl mixture, 20 ng genomic DNA, 0.5 µM of primer, 10X PCR buffer ($MgCl_2$), 2 mM dNTP, and 1U Taq DNA Polymerase.

The PCR cycling conditions were 94ºC for an initial denaturation for 5 min; 35 cycles of 94ºC for 1 min denaturation, 1,5 min at the primer annealing temperature, and 72ºC for 2 min extension; and a final 72ºC for 15 min. 1028 bp of the COI and 822 bp of the ND5 PCR products were digested with *Nco*I, *Ssp*I, *Sty*I, and *Alu*I, and *Fok*I and *Hinc*II (ER0571, ER0771, ER0411, ER0011, ER0871, ER0491, Thermo Fisher Scientific), respectively. The restricted products were controlled on 2% agarose gels.

3. Results and Discussion

In this study, mtDNA variations in COI and ND5 genes were determined and the genetic markers were tried to be presented. *Nco*I, *Ssp*I, and *Sty*I restriction enzymes were studied in the COI gene, and *Alu*I, *Fok*I, and *Hinc*II restriction enzymes were studied in the ND5 gene, and previously reported and newly found restriction sites were revealed.

3.1. Amplification of COI gene region and RFLP results

The COI region was amplified by PCR using the primers given in *Table 1* and 1028 bp PCR products were obtained. *Nco*I, *Ssp*I, and *Sty*I restriction enzymes were used to detect variation in this gene region.

After digesting the COI gene with the *Nco*I (ER0571 Thermo Fisher Scientific) restriction enzyme, a novel restriction site was formed as a result of a new SNP (G→A transition) at position 2345, and a new *Nco*I restriction site formed a profile of 246 and 782 bp on the gel (*Figure 1*). This newly found profile was abbreviated as the C haplotype in addition to the haplotypes reported previously (Bouga et al., 2005, Özdil et al., 2012). This haplotype was found in 2 out of 10 samples, belonging to the Tekirdağ region, and 12 samples of the Kırklareli population (10.26%). No restriction site was found in all the other studied samples and haplotype B (1028 bp) was found.

Cleavage of the COI gene region with the *Ssp*I (ER0771 Thermo Fisher Scientific) restriction enzyme revealed 4 restriction sites and 523, 213, 175, 85, and 32 bp bands on the gel (*Figure 2a*). This profile was previously reported as the C haplotype (Özdil et al., 2012). C haplotype was found in all of the studied samples.

As a result of the restriction of the COI gene with the *Sty*I (ER0411 Thermo Fisher Scientific) enzyme, two haplotypes were found in the studied populations. No digestion was seen in most of the samples resulting the haplotype A, which was reported previously, on the other hand, a point mutation at position 2150 (G→A transition) changed the restriction site to CCWWG**A** from CCWWG**G**, and 628 and 400 (*Figure 2b*) bp was obtained on the gel as a result of a single cut, and this profile was abbreviated as the B haplotype (Bouga et al., 2005).

B haplotype was determined in 52 (44.4%) of the Kırklareli samples studied. No restriction site was found in the remaining Kırklareli and Tekirdağ samples, only A haplotype (55.6%) was found.

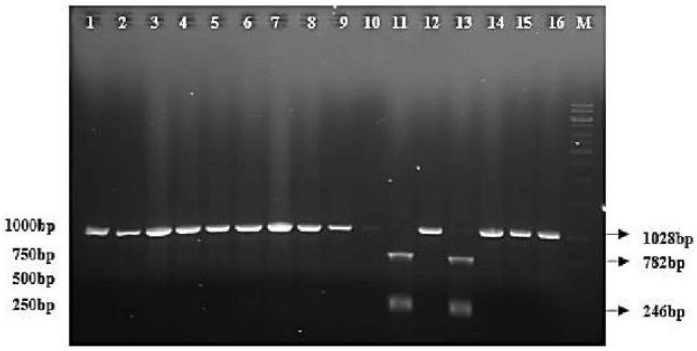

Figure 1. Cleavage of the COI gene region with the NcoI restriction enzyme on 2% agarose gel electrophoresis (1-10, 12, 14-16: B haplotype (1028 bp-PCR product); 11 and 13: C haplotype (782-246 bp); M: Fermentas GeneRuler^{TM} 1kb DNA Ladder.

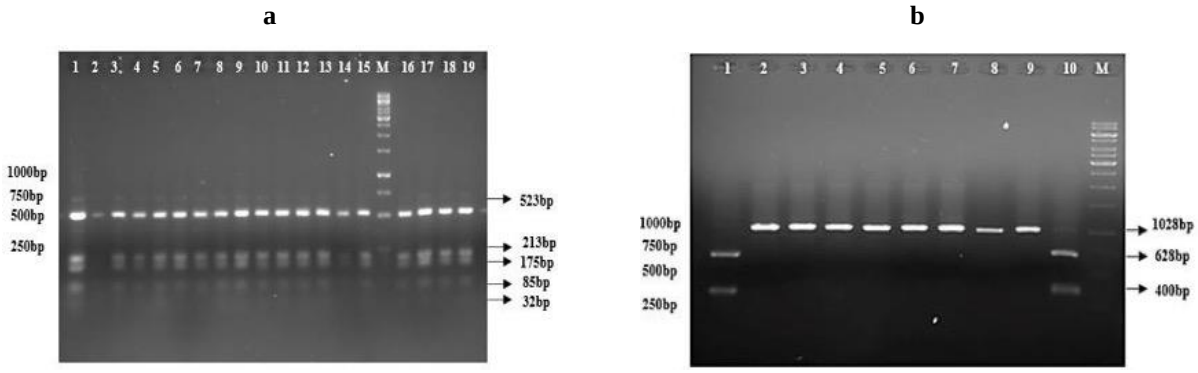

Figure 2. Cleavage of the COI gene region with the SspI (a) and StyI (b) restriction enzymes on 2% agarose gel electrophoresis.* (a) *1-19: C haplotype (523-213-175-85-35 bp); M: Fermentas GeneRuler^{TM} 1kb DNA Ladder.* (b) *2-9: A haplotype (1028 bp); 1 and 10: B haplotype (628-400 bp); M: Fermentas GeneRuler^{TM} 1kb DNA Ladder.

The cleavage of the COI gene region in different honey bee populations was performed in the previous studies. For example; in honey bee populations native to Greece (*Apis mellifera cecropia* Kieseweiter, 1860) and Cyprus (*Apis mellifera cypria* Pollman, 1879), Bouga et al. (2005) reported a different *Nco*I cleavage in this gene which is different from our study and abbreviated this haplotype as A (595 and 433 bp). In this study, A haplotype was not detected but a novel restriction was reported as the C haplotype. Kekeçoğlu et al. (2007) reported only the B haplotype (1028 bp-uncut) in the honey bee populations of Türkiye in the same gene region. And also, Ivanova et al. (2010) and Stevanovic et al. (2010), reported that Bulgaria, Serbia, Bosnia-Herzegovina (*Apis mellifera carnica* Pollman, 1879), and Macedonia (*Apis mellifera macedonica* Ruttner, 1988) honey bee populations, did not have the recognition site of the *Nco*I enzyme in the COI gene, only the B haplotype (1028 bp-uncut) was found in these populations.

In cleavage of the COI gene region with the *Ssp*I enzyme, different restriction patterns were reported as A haplotype (487-277-264 bp) (Bouga et al., 2005) and B haplotype (580, 439 bp) (Kekeçoğlu et al., 2007) but in our study, only C haplotype (523-213-175-85-32 bp) which were reported previously (Özdil et al., 2012) was found in all of the studied populations. Our results were found in accordance with Özdil et al. (2012). Ivanova et al., (2010) reported that the *Ssp*I and *Sty*I enzymes did not have a recognition site and a single PCR product (1028 bp) occurred in Bulgarian honey bee populations.

The digestion of the COI gene region with the *Sty*I enzyme revealed two different haplotypes, the A haplotype (1028 bp-uncut) (Ivanova et al., 2010; Stevanovic et al., 2010), and the haplotype B (626, 402) in honey bee populations.

It was emphasized that diagnostic patterns were revealed in the Macedonian populations after the digestion of the COI gene segment with the restriction enzymes *Nco*I (haplotype A) and S*ty*I (haplotype B) (Bouga et al., 2005). In this study, the B haplotype was determined in 52 of the Kırklareli samples out of the 117 samples which can be the result of the Macedonian origin.

3.1. Amplification of ND5 gene region and RFLP results

The 822 bp of the ND5 gene region was amplified by PCR using the primers in *Table 1*, and *AluI*, *Fok*I (BtsCI), and *Hinc*II restriction enzymes were used to detect variation in this gene region. Only *Alu*I digestion resulted in a restriction site, the remaining enzymes have no restriction sites in the ND5 gene region of the studied honey bee populations.

In this study, cleavage of the ND5 gene region with the *Alu*I (ER0011 Thermo Fisher Scientific) restriction enzyme revealed two restriction sites, and a profile consisting of 3 bands of 554, 211, and 57 bp in all of the studied samples (*Figure 3*) which were reported as the C haplotype (Özdil et al., 2012). The 57 bp long band formed a faint band in the gel, and cannot be seen properly. Our results were found similar to Özdil et al. (2012). Bouga et al. (2005) reported 2 haplotypes (A: 554, 268 and B: 554, 171 and 97 bp long) which had different cleavage sites compared to the haplotype found in our study.

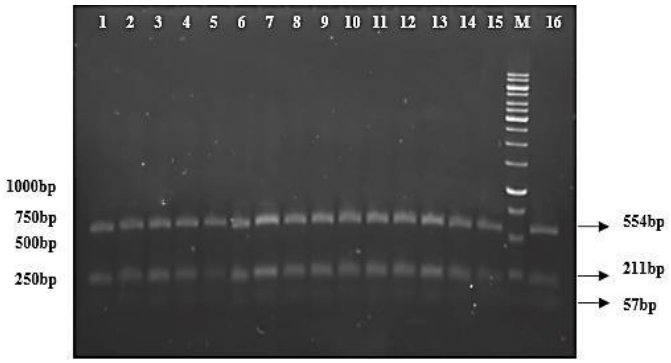

Figure 3. Cleavage of the ND5 gene region with AluI restriction enzyme on 2% agarose gel electrophoresis (1-16: C haplotype (554-211-57 bp), M: Fermentas GeneRuler^{TM} 1kb DNA Ladder.

No restriction sites were found with *Fok*I (*Bts*CI) (ER0871 Thermo Fisher Scientific) (*Figure 4a*) and *Hinc*II (ER0491 Thermo Fisher Scientific) (*Figure 4b*) restriction enzymes in the ND5 gene resulting in a single band profile of 822 bp in our study. This result was found in accordance with Ivanova et al. (2010). Bouga et al (2005) reported a single cleavage site in both of the restriction enzymes; the *Hinc*II enzyme, A haplotype (418-404 bp), and the *Fok*I enzyme, A haplotype (430-392 bp).

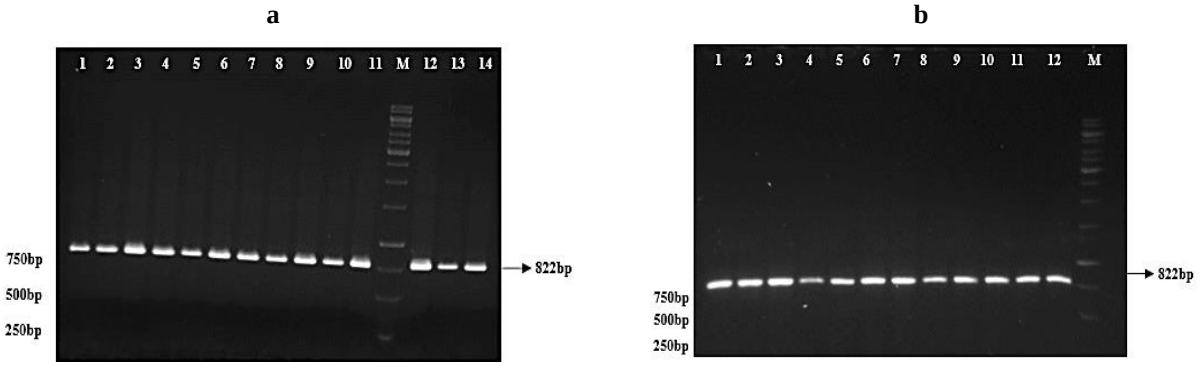

Figure 4. Cleavage of the COI gene region with the FokI (BtsCI)* (a) *and HincII restriction enzymes on 2% agarose gel electrophoresis.* (a) *1-14: B haplotype (822 bp); M: Fermentas GeneRuler^{TM} 1kb DNA Ladder.* (b) *1-12: B haplotype (822 bp); M: Fermentas GeneRuler^{TM} 1kb DNA Ladder.

In this study, COI and ND5 gene regions were restricted with the *Nco*I, *Ssp*I, *Sty*I and *Alu*I, *Fok*I, *Hinc*II restriction enzymes in order to determine possible new haplotypes in terms of mtDNA molecule in honey bees from Kırklareli and Tekirdağ representing Thrace honey bees.

The similarities and differences between races, ecotypes, and even populations can be investigated on the basis of nucleotide changes obtained as a result of cleavage with restriction endonuclease enzymes. In this way, genetic proximity and distance in all species can be revealed.

In this study, it is known that different mtDNA haplotype groups are formed with the restriction enzymes applied to the gene regions of the honey bees, and the following enzyme haplotype combinations were formed according to the enzyme recognition sites found in gene regions (*Table2*).

Table 2. The haplotype diversity of the populations in the mitochondrial genome of COI and ND5 genes

Gene Region	**COI**			**ND5**		
Enzyme/Haplotype	*Nco*I	*Ssp*I	*Sty*I	*Alu*I	*Fok*I	*Hinc*II
Type 1	B	C	A	C	B	B
Type 2	B	C	B	C	B	B
Type 3	C	C	A	C	B	B
Type 4	C	C	B	C	B	B

In this study, two different mtDNA regions (COI and ND5) were investigated and genetic similarities or differences in Kırklareli and Tekirdağ honey bee populations were revealed. As a result of the analysis, different variations were found as a result of cleaving with *Nco*I and *Sty*I restriction enzymes, which will be used to distinguish the populations as a result of treatment with different enzymes. As a result of the digestion of the COI region with the *Nco*I enzyme, variations were detected in 2 samples from Tekirdağ and 12 samples from Kırklareli populations observed and both populations were found to be polymorphic. Likewise, in the *Sty*I digestion of the COI gene region, 44.4% of Kırklareli populations were found as haplotype B, the rest as the haplotype A.

4. Conclusion

In this study, some of the haplotypes that were found in Kırklareli samples were found similar to the haplotypes detected in the Macedonian (*Apis mellifera macedonica* Ruttner, 1988) samples reported previously whereas some of them showed quite different haplotype profiles. The reason for this situation may be due to the low number of samples examined in this study, and also the intense use of Carniolan (*Apis mellifera carnica* Pollman, 1879) queen bees in the region. Intra-regional races and ecotypes should be revealed, these hypotheses should be confirmed or tested by conducting studies with different gene combinations in the Kırklareli population and throughout Thrace.

It is a necessity to define or protect the physiological, morphological, or behavioral characteristics of honey bee breeds in detail. In order to do this, genetic diversity must be determined. It is thought that this study will be useful in similar studies to be carried out in the future and also in the creation of a genetic database on Thrace honey bees in Turkey.

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