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THE UPTAKE OF ESSENTIAL MINERAL ELEMENTS BY ENDEMIC SALVIA ABSCONDITIFLORA (GREUTER & BURDET) GROWING IN NATURAL HABITATS

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Abstract: This study was conducted to determine the uptake of essential mineral elements by endemic *Salvia absconditiflora* species growing in the natural habitats of Kırşehir province and its dependence on some physical and chemical properties of soil. The plant and soil samples were collected from 10 different locations. It was found that in the collected plants, K concentration was inadequate (0.344%), Fe had an excessive amount (541.853 mg kg^{-1}), while Cu, Zn, Mn, Mg and Ca concentrations were adequate (6.154 mg kg^{-1}, 32.610 mg kg^{-1}, 43.395 mg kg^{-1}, 0.507% and 3.650%). The soils had sandy clay loam texture, slight alkaline reaction (pH=7.92), an average amount of organic matter (3.06%), high amount of calcareous (35.61%) and low amount of soluble salts. It was also determined that soils had adequate K, Ca and Mg concentrations and 80% of the soil samples had inadequate level of P. Since Cu concentration was adequate in terms of microelement stock, concentrations of Zn were inadequate (0.292 mg kg^{-1}). It was found that *Salvia absconditiflora* plant had the ability to grow in the soil with inadequate Zn and Mn.

Keywords: *Salvia absconditiflora*, soil properties, plant nutritions

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1. Introduction

Turkey is famous for growing many medicinal and aromatic plants and microclimate richness. *Salvia* species is one of the important medicinal aromatic plants which are cultivated widely throughout the world and belongs to Lamiaceae family. 89 species of *Salvia* genus have been identified in Turkey and 45 of these are endemic (Yılar and Altuntaş, 2017).

Salvia absconditiflora (Greuter & Burdet) is a perennial plant spread between 700 m and 2500 m altitude in Turkey and has been found to be endemic in lime soils (Saadia et al., 2010; Dogan et al., 2017). It prefers dry habitat like the other *Salvia* species and survives in the soil even in drought and at high temperature typically. It develops well in the places, having low groundwater, in rocky slopes and sunny places. Alkaline, sandy, loamy soil with high lime rate and well drainage that warms easily is ideal for *Salvia* farming (Yılar and Altuntaş, 2017). It is known that *Salvia sclarea* L. is a plant tolerant to heavy metals and can be cultivated in polluted soils. It is classified as the accumulator of zinc and cadmium and the hyperaccumulator of lead (Angelova et al., 2016).

Kırşehir, the study area, is located in the Central Anatolian region and in Irano-Turanian Floristic Region. This area includes the arid and sub-arid regions of the Anatolian Plateau. No study was found in the literature including the nutrition elements concentration of *Salvia absconditiflora* (Greuter & Burdet) plant growing in natural ecosystems, the physical and chemical properties of the soil where it grows and examining the heavy metal amounts of soil.

This study was conducted to determine the uptake of basic nutrition elements by endemic *Salvia absconditiflora* (Greuter & Burdet) species growing in the natural habitats of Kırşehir province and its dependence on soil physical and chemical properties. It will lead the studies on developing the non-agricultural rural industries in order to support the rural development.

2. Material and Methods

The plant and soil samples of *Salvia absconditiflora* (Greuter & Burdet) growing in the natural flora were collected from 10 locations in Kırşehir province and its districts between May and July 2018. Soil sample was taken from 0-30 cm depth. The collected plant and soil samples were brought to Kirsehir Ahi Evran University Central Research and Application Laboratory.

2.1. Analysis Methods of Soil Samples

The soil samples from the studied areas were collected in accordance with the principles reported by Jackson (1962), were dried in the laboratory and they were sifted out through a sieve of 2 mm. The soil reaction was performed according to Thomas (1996), electrical conductivity was performed according to Rhoades

(1996), texture and total calcerous were analyzed according to Klute et al. (1994), the organic matter was analyzed according to Nelson and Sommers (1996), exchangeable K, Ca, Mg, and Na concentrations determination was performed according to Helmke and Sparks (1996), available phosphorus was analyzed according to Olsen and Sommers (1982). The analyses of the plant available microelement (Fe, Cu, Zn, Mn) was performed based on DTPA (diethylenetriamine penta acetic acid)+TEA (triethanolamine) (pH:7.3) method stated by Lindsay and Norvell (1978) and the concentrations of the elements were determined in Atomic Absorption Spectrophotometer. The available Fe was evaluated according to the reference range reported by Lindsay and Norvell (1978), Mn, Zn and P were evaluated according to Silanpaa (1990), K, Ca and Mg were evaluated according to Sumner and Miller (1996), Cu was evaluated according to Follet (1969).

2.2. Analysis Methods of Leaf Samples

Salvia absconditiflora (Greuter & Burdet) plant samples were washed with tap water and then distilled water, placed into paper bags separately without using any metal tool and dried in circulating air drying oven at 65°C until reaching the fixed weight. The dried plant samples were pounded in porcelain mortar and were grounded with a porcelain pestle. They were kept in the locked plastic bags in a dark and cool environment. The plant samples were burnt using nitric acid and perchloric acid mixture following block digestion procedure, defined by Jones and Case (1990), and the homogeneous extractions of the samples were obtained. The elemental concentrations of the total Mg, Ca, Mn, Cu, Zn and Fe were determined in Atomic Absorption Spectrophotometer and the elemental concentrations of total K and Na were determined in flame spectrometer in the clear solutions.

2.3. Statistical Analysis

The results obtained were assessed by using analysis of variance and multiple comparison test (Duncan's test) (Genc and Soysal, 2018) of the SPSS software (version 21.0). Cluster analysis was also performed on data sets obtained through the study (Ali et al., 2019).

3. Results and Discussion

Figure 1 shows the results of the soil samples analyses obtained from each location of *Salvia absconditiflora* species. When the sand, clay and silt rates were examined, the texture of studied soils was classified as sandy clay loam (Ulgen and Yurtsever, 1995), in general. Accordingly, the habitat has suitable physical properties in terms of water holding capacity, porosity and ventilation for plant growing. Dengiz et al. (2007) reported that a great majority of the surface soil of Büyükçay basin such as 67.6% was included in sandy clay loam and clay loam texture classes.

The values of basic soil properties: pH and concentration of total salt, lime, organic matter, phosphorus and potassium were classified according to the limit values reported by Ulgen and Yurtsever (1995). The pH values of soils varied between 7.63 and 8.21, and 100% of them had slight alkaline properties. The soil organic matter concentration fell into the middle range and was found to be between 1.30% and 3.53%. The lime concentration in soils was quite changeable between 3.45% and 65.28% and the area was included in a high lime class according to the average value (Figure 1).

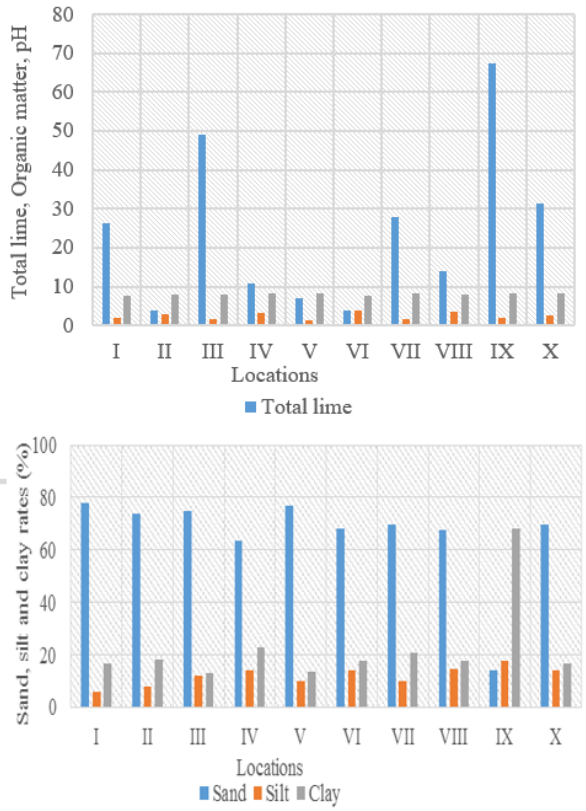

Figure 1. Analysis values of some chemical properties of soils.

The elemental concentration distribution of the soils obtained from each natural location of *S. absconditiflora* species was compared using Duncan's test by performing one-way analysis of variance (Table 1). It was found that the K concentration varied between 175.9 and 415.1 mg kg^{-1}, the available Mg between 165.410 and 761.600 mg kg^{-1}, Ca from 6525 to 10045 mg kg^{-1}, Na from 251.0 to 285.98 mg kg^{-1}, and available P varied between 2.701 and 21.215 mg kg^{-1}. It was determined that there was no problem related to K and Mg availability throughout the studied area and these nutrients were in an adequate level and the Ca had an excessive concentration.

In the studied area, the existence of the P inadequacy was determined as a result of the analyses. It was observed that *Salvia absconditiflora* plants could grow in soils with P deficit. It was observed that soils of VII, IX and X among the study locations had high pH and calcerous values and, on the contrary, the plant available P concentration was inadequate. It has also been reported in the studies by Zhou and Li (2001) that the available P concentration is low due to high amount of clay and inadequate humidity in addition to high pH and calcerous concentration.

Table 1. Duncan test results of plant nutrient concentrations of soils taken from each location belonging to *Salvia absconditiflora* species

Location number	Macro nutrient concentrations				Micro nutrient concentrations				
	K	Mg	Ca	P	Fe	Cu	Mn	Zn	Na
	mg kg^{-1}								
I	379.12±4.24^{b}	190.0±7.071^{i}	6525±63.639^{e}	3.914±0.067^{e}	0.100±0.007^{e}	0.373±0.032^{d}	3.499±0.019^{e}	0.345±0.028^{b}	251.0±0.414^{h}
II	301.10±1.41^{e}	281.4±0.099^{e}	9555±148.49^{b}	2.716±0.325^{e}	0.107±0.004^{e}	0.495±0.001^{c}	4.789±0.001^{d}	0.165±0.00^{g}	270.69±0.198^{fg}
III	238.52±0.71^{g}	223.7±0.099^{h}	7385±49.497^{d}	11.745±0.00^{b}	0.043±0.001^{f}	0.027±0.004^{g}	2.311±0.001^{g}	0.285±0.00^{de}	285.98±0.049^{a}
IV	331.50±0.71^{c}	261.3±0.198^{f}	9520±98.995^{b}	7.387±0.325^{c}	0.692±0.003^{c}	0.253±0.004^{e}	5.327±0.004^{b}	0.265±0.00^{ef}	277.27±0.098^{c}
V	290.09±1.41^{f}	165.4±0.099^{i}	6755±148.492^{e}	3.786±0.325^{e}	0.487±0.004^{d}	0.387±0.004^{d}	1.274±0.006^{i}	0.260±0.00^{f}	271.32±0.098^{f}
VI	182.51±3.53^{h}	534.3±0.149^{b}	6650±296.985^{e}	20.258±0.975^{a}	2.413±0.004^{a}	0.266±0.006^{e}	8.470±0.042^{a}	0.541±0.00^{a}	272.58±0.098^{e}
VII	242.02±1.41^{g}	410.6±0.099^{c}	9555±148.492^{b}	3.274±0.975^{e}	0.052±0.003^{f}	0.215±0.001^{f}	3.467±0.004^{ef}	0.269±0.00^{ef}	269.92±0.098^{g}
VIII	326.55±2.12^{d}	236.4±0.198^{g}	8225±49.497^{c}	6.956±0.325^{cd}	1.619±0.004^{b}	0.747±0.004^{a}	4.902±0.002^{c}	0.299±0.00^{cd}	244.09±1.198^{i}
IX	375.25±0.70^{b}	761.6±0.282^{a}	8500±141.421^{c}	5.784±0.651^{d}	0.108±0.003^{e}	0.246±0.006^{e}	1.811±0.001^{h}	0.317±0.00^{c}	278.50±1.141^{b}
X	415.11±0.00^{a}	388.8±0.099^{d}	10045±49.497^{a}	7.154±0.650^{c}	0.027±0.004^{g}	0.685±0.007^{b}	3.451±0.001^{f}	0.177±0.00^{g}	278.50±0.198^{d}

The average values indicated by the same symbol in the same column are not statistically different from each other at P≤ 0.05 level according to Duncan test.

Table 2. Duncan test results of macro and micro nutrient concentration of *Salvia absconditiflora* plant

Location number	K	Ca	Mg	Na	Fe	Cu	Zn	Mn
	%				mg kg^{-1}			
I	0.3905±0.0007^{a}	2.500±0.000^{e}	0.389±0.0035^{f}	0.017±0.00^{f}	639.500±0.000^{c}	6.405±0.007^{d}	24.240±1.400^{f}	28.250±0.35^{f}
II	0.3255±0.0007^{f}	5.750±0.353^{a}	0.865±0.0007^{s}	0.016±0.00^{i}	1357.525±0.035^{a}	5.860±0.0141^{f}	31.838±0.1768^{d}	93.250±0.35^{a}
III	0.3305±0.0007^{e}	3.750±0.353^{c}	0.511±0.0070^{d}	0.022±0.00^{a}	305.450±0.0707^{i}	6.160±0.0141^{e}	31.188±0.0035^{d}	7.250±0.35^{i}
IV	0.2955±0.0007^{h}	5.250±0.353^{a}	0.755±0.0028^{b}	0.017±0.00^{g}	345.725±0.0353^{h}	6.850±0.0707^{b}	28.468±0.0035^{e}	27.250±0.35^{g}
V	0.3750±0.0000^{c}	2.750±0.353^{de}	0.260±0.0014^{h}	0.017±0.00^{e}	518.450±0.0707^{e}	4.600±0.1414^{h}	39.640±0.0141^{b}	18.250±0.35^{h}
VI	0.2955±0.0007^{h}	3.000±0.000^{de}	0.494±0.0007^{d}	0.019±0.00^{c}	744.450±0.0707^{b}	5.260±0.0141^{g}	37.700±0.0141^{c}	18.250±0.35^{h}
VII	0.3155±0.0007^{g}	4.500±0.000^{b}	0.425±0.0353^{e}	0.016±0.00^{h}	250.050±0.0707^{i}	6.735±0.0212^{c}	20.760±0.0141^{g}	38.450±0.07^{e}
VIII	0.3905±0.0007^{a}	2.500±0.000^{e}	0.346±0.0028^{g}	0.019±0.00^{b}	369.075±0.0353^{f}	6.460±0.0141^{d}	31.773±0.0106^{d}	74.250±0.35^{b}
IX	0.3790±0.0014^{b}	3.250±0.353^{cd}	0.346±0.0035^{g}	0.012±0.00^{i}	364.750±0.0707^{g}	7.260±0.0141^{a}	37.338±0.0177^{c}	67.250±0.35^{c}
X	0.3455±0.0007^{d}	3.250±0.353^{cd}	0.681±0.0212^{c}	0.018±0.00^{d}	523.550±0.0707^{d}	5.945±0.0070^{f}	43.160±0.0141^{a}	61.500±0.00^{d}

Table 1 indicated that according to microelement levels of the soil samples, the plant available Fe concentration varied between 0.027 and 2.413 mg kg^{-1}, Mn between 1.274 and 8.470 mg kg^{-1}, Zn 0.165 and 0.541 mg kg^{-1}, and Cu varied between 0.027 and 0.747 mg kg^{-1}. Accordingly, it was found that in all of the soil samples, there was inadequacy of Mn, Fe adequacy limit, while Zn and Cu were inadequate in 10% of the soil samples and adequate in 90% of the soil samples.

The results of the analyses performed to determine some of the macro and micro nutrition elements of *Salvia absconditiflora* plant were assessed via Duncan's test by performing one-way analysis of variance and were presented in Table 2. The concentration of K in plants varied between 0.30% and 0.39%, Ca from 2.50% to 5.75%, Mg 0.26%-0.87% and Na 0.01%-0.02%. When the micro nutrition elements of the plant samples were examined, it was found that Fe was between 250.050 and 1357.525 mg kg^{-1}, Cu was between 4.600 and 7.260 mg kg^{-1}, Zn was between 20.760 and 43.160 mg kg^{-1} and Mn was between 7.250 and 93.250 mg kg^{-1}. It was observed that K was inadequate in all of the collected plant samples and these plants had high concentration of Ca and adequate level of Mg. In all of the study soil samples, K was at an adequate level and all the plants had inadequate K due to the antagonistic effect of Ca on K. The Mn inadequacy was determined mostly in the plants in III location, Zn inadequacy was determined in the plants in VII location, and Cu inadequacy was determined in the plants in V location. In general, the micro element levels of the plants were found to be adequate based on their average values.

Sardans et al. (2004) have stated that there is a low rate of extractable P concentration in the calcareous, alkaline soils with high pH. It has been reported in a study examining the growth of *Pinus halepensis*, *Rosmarinus officinalis* and *Cistus albidus* in calcareous and siliceous soils in PACA region of Southeastern France that there was higher yield from the calcerous-rich soils and based on the chemical analysis compound of calcerous soils, the available P was 44.67 mg kg^{-1}, Ca was 9.33 mg kg^{-1}, K was 590.20 mg kg^{-1}, Mg was 106.02 mg kg^{-1} and the Ca levels of the soils with high pH were high. When Ca is high in the soils of arid regions, it has an antagonistic effect on absorption of some nutrition macro and micro elements. Many studies have revealed that high concentration of Ca ion reduces the effectiveness of P in soil and K, Fe and other elements are converted into forms that plants cannot benefit. It has been reported that the presence of high concentration of K in soil does not make a harmful effect on plants contrary to excessive nitrogen and P or excessive amount of K that negatively affects the intake of Fe and Zn by plants (Bosgelmez et al., 2001). It has been revealed that the reasons of the inadequate plant available concentration of Zn in soil include high pH, clay percentage, calcerous concentration, low organic matter and moisture concentration and it was assessed as an expected result that the plant available Zn is low in the soils with high pH and calcerous concentration (Abu-Darwish et al., 2011). It was recorded that Zn was at least 95.81 mg kg^{-1} and Fe was 935.40 mg kg^{-1} in *Salvia officinalis* species in Ash-Shoubak University research farm and Cu concentration was between 7.02 and 13.07 mg kg^{-1} (Abu-Darwish et al., 2011).

The calcarous and alkaline soils of the arid and semi-arid regions with low organic matter lead the amount of plant available Fe to be inadequate by decreasing the solubility of Fe. The pH value of many soils containing calcium carbonate varies between 7.3-8.5 and the Fe solubility within these limits was at the lowest levels. In addition, the events such as soil compaction, flood, long-term precipitation or excessive irrigation cause iron inadequacy. In addition, high amount of Cu causes Fe inadequacy. Upon organic matter is bonding Cu very strongly, high amount of available Cu may be observed in the soils poor in organic matter. It has been reported that *Salvia* can grow in the soils polluted by heavy metals such as Cu and Zn in Southern Europe and it has tolerance to these metals (Bolat and Kara, 2017). It is reported by Yılar et al. (2020a) that *S. absconditiflora* species can grow in alkaline soils, high $CaCO_3$ levels, medium organic matter levels and clay loam soils. In another study, Yılar et al. (2020b) for *Salvia* species water saturation 58.3%, pH 8.09, total salinity 0.008%, total calcerous ratio 14.074%, organic matter 3.501%, K_2O 98.766 kg da^{-1} and P_2O_5 3.914 kg da^{-1} have obtained data.

Hierarchical clustering analysis was applied to categorize the physical and chemical properties included in the plant and soil data set examined in the study based on their similarities more easily. Figure 2 shows the dendrogram obtained as a result of the hierarchical clustering analysis performed in order to categorize the similarities of 10 different locations. When the dendrogram demonstrating the cluster analysis results was examined, it was observed that the soils were categorized in two main groups. According to the classifications in dendrogram, II, IV, VII, VIII, IX, and X locations were perceived to be similar with each other in the first group and I, III, V and VI locations were perceived to be similar in the second group.

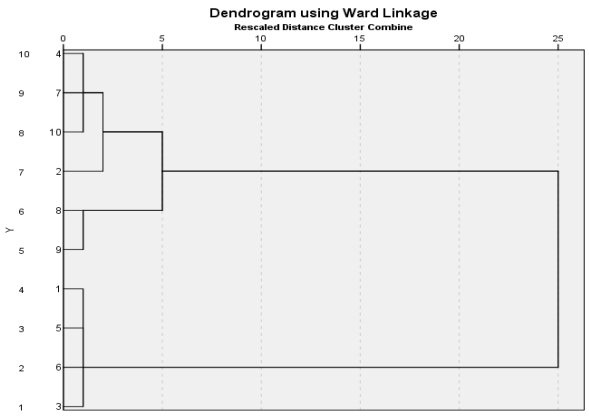

Figure 2. Dendrogram obtained by Cluster analysis of *Salvia absconditiflora* species.

4. Conclusion

In all of soil samples taken from Kırşehir province and its districts, potassium was adequate but all of the plants had inadequate K and this may be associated with the antagonistic effect of Ca on K. It was observed that K concentration in most of the plant sample is included in these limit values. According to the results, Ca concentrations in the soil and plant samples were parallel to each other and this was supported by the previous studies, as well. It was found that Zn, Mn and Cu had a high concentration in the plant samples of some areas included in the study.

Studied soils belongs to the class of alkaline soils with high pH and high calcerous concentration and these conditions negatively affected mainly Mn intake. It was determined that the studied soils with average amount of organic matter had inadequate P concentration for plant growth, but 70% of them were adequate in terms of K and 80% of them were adequate in terms of Mg and all of them were adequate in terms of Ca availability for plants.

According to the plant analysis results, 100% of the plant samples had inadequate K concentration, an excessive concentration of Fe and adequate levels of Ca, Mg, Mn, Cu and Zn. As a result of the analysis performed with *Salvia absconditiflora* plant and soil, it was determined that although Mn nutrition elements were inadequate in the soil, the plants absorb these nutrition elements adequately. It also could be conclude that soils in III, VII, IX and X locations had the most suitable properties for cultivation of *Salvia absconditiflora*.

Author Contributions

All tasks made by the single author of the manuscript; A.A.A.B. (100%). The author reviewed and approved final version of the manuscript.

Conflict of Interest

The author declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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STATISTICAL ANALYSIS OF LAMB SURVIVAL DURING THE REARING PERIOD WITH THE USE OF CLASSIFICATION TREES AND LOGISTIC REGRESSION

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Abstract: The presented research aimed to statistically analyse the survival of 44,133 Polish Merino and Polish Merino in Old-Type lambs between birth and the 100th day of their life, using classification trees and logistic regression. The study included lambs born between 2008 and 2017 and used in 43 flocks in Pomerania and Kujawy region (Poland). The results showed that 9.27% of all controlled lambs did not survive till the 100th day of life. The statistical analysis of the case of lambs' death during their first 100 days of life was carried out using multiple logistic regression as well as classification trees, using two algorithms CART and CHAID. The quality of multiple regression and decision tree models was compared considering the following criteria: percentage of misclassifications, average squared error and the area under the Receiver Operating Characteristic curve. The calculated quality criteria for tree models that were created during the research suggested that the classification trees formed based on CART algorithm were the most accurate in defining the variability of studied characteristics, i.e. survival of lambs up to the 100th day of age. For the best available classification model, the ranking of variable importance, developed based on the "Importance" measure, allowed to conclude that the type of lamb's birth, season, following by the year of birth, subsequent lambing, lamb's sex and its breed were the most significant differentiating factors. It was noted that the tree built with the use of CART algorithm was composed of 30 leaves. It was also shown that the highest frequency of lamb's death during the rearing period was to be expected among triplets born in winter or summer (37.14% of all deaths), while the highest chance (98.42%) of surviving till the 100th day of life showed singletons, born from their mother's 3rd to 6th litter, in the spring-winter season in the last year of the present research.

Keywords: Lamb mortality, Classification tree, CART, CHAID, Logistic regression

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1. Introduction

The beginnings of Polish Merino breeding in Poland date back to the 1870s. In the 1990s Polish Merino was improved by crossbreeding with prolific breeds, therefore, the number of sheep of the pure breed decreased considerably (Gut et al., 2008). In consequence, in 2008 the Polish Merino population (breed) was distinguished to represent the original breed pattern – Polish Merino in Old-Type (MPS) (maintains the breed purity) (PSBA, 2009). The Polish Merino is a breed that is normally used for two purposes: wool and meat. As measured in 2008, the ewes under assessment had an average prolificacy of 128.3%, while the average rearing for lambs until the age of approximately 100 days equaled 90.9%. In December 2020 the sheep population in Poland amounted to 277.9 thousand heads, including 148.7 thousand ewes (GUS, 2021). In the racial structure of ewes under the performance assessment, 1.71% were of Polish Merino (MP) breed and 10.39% of Old-Type Polish Merino (PSBA, 2021).

Lamb mortality resulting in high losses inevitably leads to a significant decrease in how profitable the production of sheep is. Hence, it is essential to discover the factors that are behind this. The common practice in Poland is to register lamb mortality but without specifying the time of death. This produces a binomial piece of information: 1 for the lamb that survived until the end of the rearing period, 0 for the deceased lamb. The statistical analysis of this type of trait is usually carried out with the use of logistic regression (Piwczyński, 2007, Schreurs et al., 2010; Piwczyński et al., 2012, Piwczyński et al., 2013a;). A possible alternative to logistic regression may be the classification tree technique (Piwczyński et al., 2012). It is an analytical technique that belongs to the field of data mining. The same field includes the cluster analysis and artificial neural networks (Grzesiak, 2003; Piwczyński et al., 2013a). The classification trees techniques have reportedly been used many times in animal farming and

breeding throughout the past several years (Piwczyński et al., 2012, Piwczyński et al., 2013a, Piwczyński et al., 2013b; Ghiasi et al., 2019; Kliś et al., 2021;). When graphical models of classification trees are created, this results in the occurrence of distinguishable subsets, having a "T" shape, within the repeated split of the set of observations (Piwczyński et al., 2013b). The reason for doing this is to obtain subsets that will have the highest possible homogeneity as regards a dependant variable's value. The same independent variables do not have to be used at various stages of the data set's multi-trait splitting. The variable that is selected is the one that warrants the best possible split of a node, which in turn results in the most homogeneous sets. The first thing one needs to do when forming a classification tree is to look at an entire data set, which is called the root node here. Subsequent nodes that occur as a result of splitting are known as child nodes. The name of a subset without any further division is a leaf. The measurement of each tree is made based on the number of its leaves. A tree's depth is determined by how many edges there are from its top to the leaves located furthest away.

The aim of the research was a statistical analysis of lamb mortality between their birth and the weaning time, using two algorithms of classification trees building (CART, CHAID), and comparison of obtained results with the results from logistic regression.

2. Material and Methods

The study included 44133 lambs born between 2008 and 2017 and used in 43 flocks in Pomerania and Kujawy region (Poland). The animals were maintained using indoor livestock farming. The data were obtained from breeding documentation from the Regional Association of Sheep and Goat Breeders, Bydgoszcz.

Table 1 presents statistical characteristics of the analyzed population in terms of lamb mortality in the period from birth to weaning (~100 days), depending on the examined factor: lambs' breed (MP, MPS), lambs' gender (ram, ewe) and type of birth (single, twine, triplet), dams' birth type (single, twin, triplet), subsequent lambing (1…8), year (2008-2017) and season of lambing (spring – III, IV, V; summer – VI, VII, VIII; autumn – IX, X, XI, winter – XII, I, II).

Table 1. Mortality of lambs classified by various factors

Factor	Level	Number of born lambs	Mortality (%)	Prob.
Breed of lamb	MP	15744	9.39	0.5328
	MPS	28789	9.21	
Gender of lambs	Ewe	22242	8.66	<0.0001
	Ram	22291	9.88	
Type of lamb birth	1	26259	6.03	<0.0001
	2	17831	13.58	
	3	443	28.22	
Type of dam birth	1	23045	9.13	0.5609
	2	20969	9.43	
	3	519	9.06	
Subsequent lambing	1	1509	12.99	<0.0001
	2	4021	9.65	
	3	4854	7.97	
	4	5736	8.47	
	5	6329	7.99	
	6	5110	9.43	
	7	4778	9.27	
	8	12196	10.18	
Year of lambing	2008	4896	8.17	<0.0001
	2009	4485	8.23	
	2010	3811	8.21	
	2011	4227	10.15	
	2012	4448	9.82	
	2013	4482	10.49	
	2014	4389	9.5	
	2015	4751	10.9	
	2016	4707	9.67	
	2017	4337	7.4	
Season of lambing	Spring	2122	11.26	<0.0001
	Summer	9542	9.00	
	Autumn	22470	8.22	
	Winter	10399	11.39	
	Total	44533	9.27	

The statistical analysis of the case of lambs' death during their first 100 days of life was analyzed using the chi-square test. Later multiple logistic regression as well as classification tree (using two algorithms CART and CHAID) were used. The classification tree technique was used to establish the factors that were to blame for the variability in lamb mortality. There were 44133 observations, of which 60% were used in the training set, and the other 40% formed the validation set. Stratified random sampling was applied to create the sets. The tree was formed using the former, whereas the latter was used for cutting. An assumption was made that the final node can have a size no lower than 100 observations, and that the maximum depth is 6. Such rules concerning leaf size and depth were selected so that overfitting of the tree to the training data was avoided – this could be a reflection of random links occurring inside the validation set. Each node that was formed and the resulting leaf were accompanied by the following details: ID (1); mortality percentage (2), and how many observations there were in a node or leaf (3) (Figure 1).

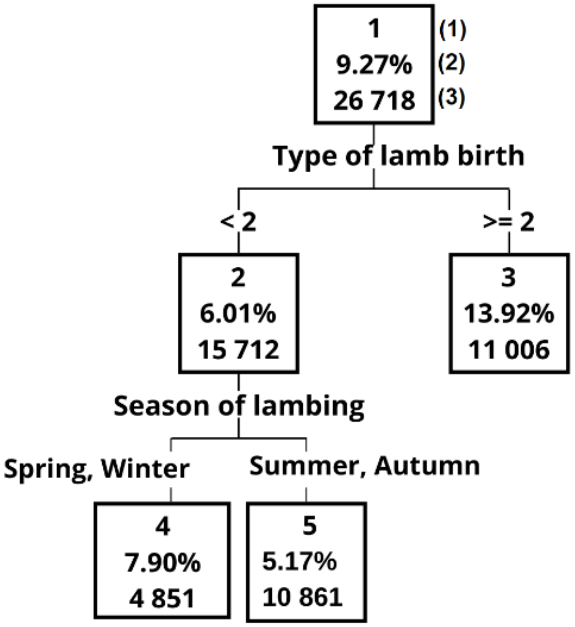

Figure 1. The graphical model of the classification tree - part 1.

Variables were ranked according to their importance for the creation of data set splits, with the use of the "Importance" measure (SAS Institute Inc., 2021). To calculate this measure (I(v,T) (Equation 1), a square root of the product of the Gini coefficient reduction (ΔGini) needs to be used. This, in turn, is calculated using the main and surrogate splits which in all nodes of the tree have the v variable (T), as well as the "agreement" measure (a(s_v, t)) connected with the rule that uses the v variable in the t node:

$$I(v,T)=\sqrt{\sum_{t=1}^{T}\Delta Gini(s(v,t))a(s_v,t)} \quad (1)$$

where: s(v, t) – the best surrogate split in the t node using the v variable (Equation 2)

$$a(s_v,t)=\begin{cases}1 & \text{if } s_v \text{ is the main splitting rule}\\ \text{agreement} & \text{if } s_v \text{ is the surrogate rule}\\ 0 & \text{Otherwise}\end{cases} \quad (2)$$

The values for the „agreement" measure are taken from the (0,1) range. This measure shows the share of agreeing on cases upon the comparison of the main and surrogate splits. The measures of "Importance" established for each variable were divided by the "importance" variable that had the highest importance.

This was followed by the verification of the effect of the factors under examination on lamb survival, conducted with the use of multiple logistic regression (SAS Institute Inc., 2021). The main factors were taken into consideration in the model, as were its interactions second degree. The forward method was used to determine statistical variables connected with lamb mortality. Wald statistics (SAS Institute Inc., 2021) were used to evaluate the importance of individual parameters within a model.

The quality of multiple regression and decision tree models was compared considering the following criteria: percentage of misclassifications, average squared error and the area under the Receiver Operating Characteristic curve. Statistical analysis was carried out using the Enterprise Miner 15.1 software included in the SAS package.

3. Results

The results showed that 9.27% of all controlled lambs did not survive till the 100th day of life (Table 1). The preliminary chi-square test showed that the mortality of lambs in the rearing period was highly significantly influenced by the influence of sex and birth type of lambs, dam's birth type, next lambing season, season and year of lambing. It was observed that the mortality of MPS lambs was 0.18 percentage points (pp) lower than MP, ewes by 1.22 pp. than rams. It was also observed that lambs from twin births were characterized by a higher risk of death during the rearing period and that the most deaths of lambs were recorded in the first and last of the studied reproductive periods. It was also shown that lamb mortality varied from one year to the next (from 7.4 to 10.90% and during the lambing season (from 8.22% to 11.39%) (Table 1).

The improvement in the quality of a given logistic and classification tree models can be proved based on a decrease in the values of the average squared error; misclassification rate; an increase in the values of the lift cumulative Kolmogorov-Smirnov statistics; and the area below the ROC curve (ROC index). Therefore, one may conclude that the classification trees formed based on CART algorithm were the most accurate in defining the variability of studied characteristics, i.e. survival of lambs

up to the 100th day of age (Table 2). For his model (CART), the ranking of variable importance, developed based on the "Importance" measure, allowed to conclude that the type of lamb's birth, season, following by the year of birth, subsequent lambing, lamb's gender and its breed were the most significant differentiating factors (Table 3).

Table 2. Model comparisons

Measure	CART	CHAID	Logistic regression
Average squared error	0.0819	0.0822	0.0823
Cumulative lift	2.1071	1.9743	2.0563
Kolmogorov-Smirnov statistic	0.2280	0.2260	0.2240
Misclasification rate	0.0928	0.0928	0.0931
ROC index	0.6540	0.6400	0.6500

Table 3. Variable importance and number of splits

Variable	Number of splits	Importance
Type of lamb's birth	2	1.000
Season of lambing	5	0.411
Year of lambing	10	0.334
Subsequent lambing	8	0.299
Gender	3	0.171
Breed	1	0.083
Type of dam's birth	1	0.052

It was noted that the tree built with the use of CART algorithm was composed of 30 leaves (Figures 1-4). In creating the tree, the largest number of splits was based on the following variables: year (10 splits) and subsequent of lambing (8), the season of lambing (5 splits), gender of lambs (3 splits) and type of lamb's birth (2 splits). In turn, variables breed of lamb and type of dam's birth were used once by the tree forming algorithm.

It should be emphasized that the results are shown in the nodes constituting the decision tree come only from the training set. Due to the complex nature of the tree, only the major splits were described in the study. The first and most important split of the lamb mortality dataset was based on the type of birth (Figure 1). As a result, subsets of lambs were created: from a single (Node 2) and multiple (Node 3) births, which were characterized by mortality of 6.01 and 13.29%, respectively. A further split of the set of lambs from multiple pregnancies (Node 3) was again based on the lamb's type of birth. It led to the separation of a subset of lambs from twin pregnancies (Node 6) with mortality of 13.53% and triplet pregnancies (Node 7) with mortality equal to 29.20% (Figure 4). Further divisions were also presented in provided Figures.

It was shown that in the construction of the classification tree the variable called calving year was used the greatest number of times (participated in 10 splits) by the CART algorithm, but the resulting subsets do not allow for the identification of unambiguous trends concerning the influence of this factor on lamb mortality. Multiple divisions of data subsets based on the mother's next litter allow concluding that lambs born to the youngest and oldest ewes (Node 30, 52, 56, 27, 37, 83) were characterized by higher mortality in the rearing period than those born from 2 to 7. at once (Node 31, 52, 53, 26, 36, 82). It was also observed that the rams (Node 10, 29, 5) had higher mortality than the ewes (Node 11, 28, 51) during the rearing period, and the differences ranged from 1.05 to 4.27 pp. It was also found that lambs of the MPS breed (Node 23) were characterized by 1.65 pp. lower mortality than MP (Node 22). Moreover, it was shown that lambs born to ewes from single litters (Node 38) were characterized by 1.85 pp. greater mortality than in multiple litters.

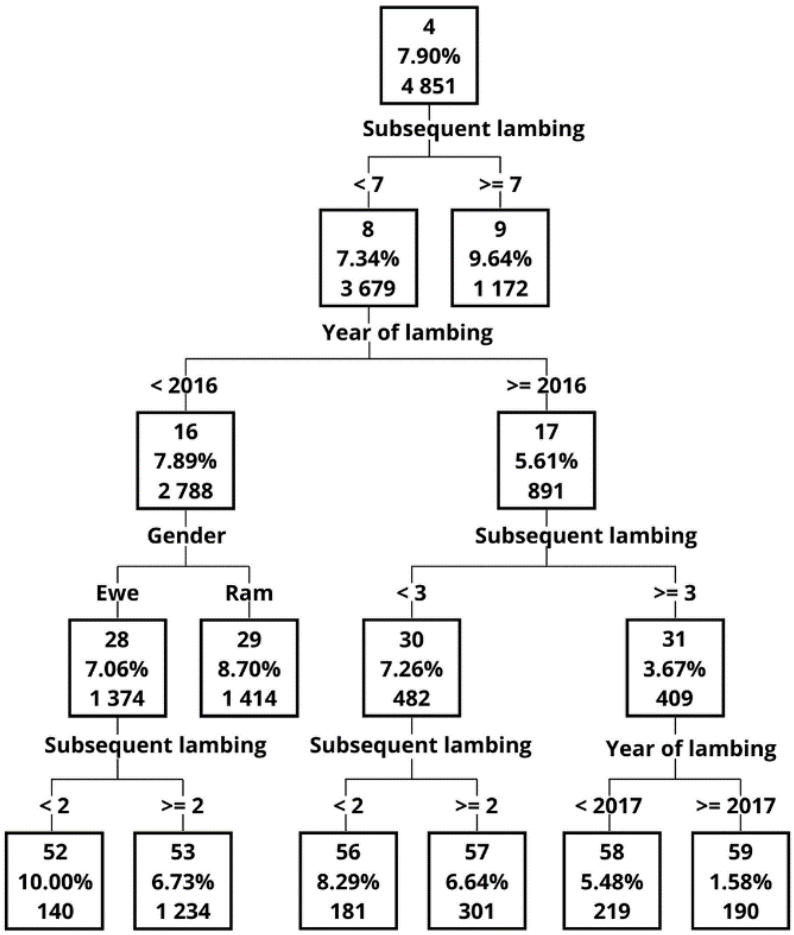

Figure 2. The graphical model of the classification tree - part 2.

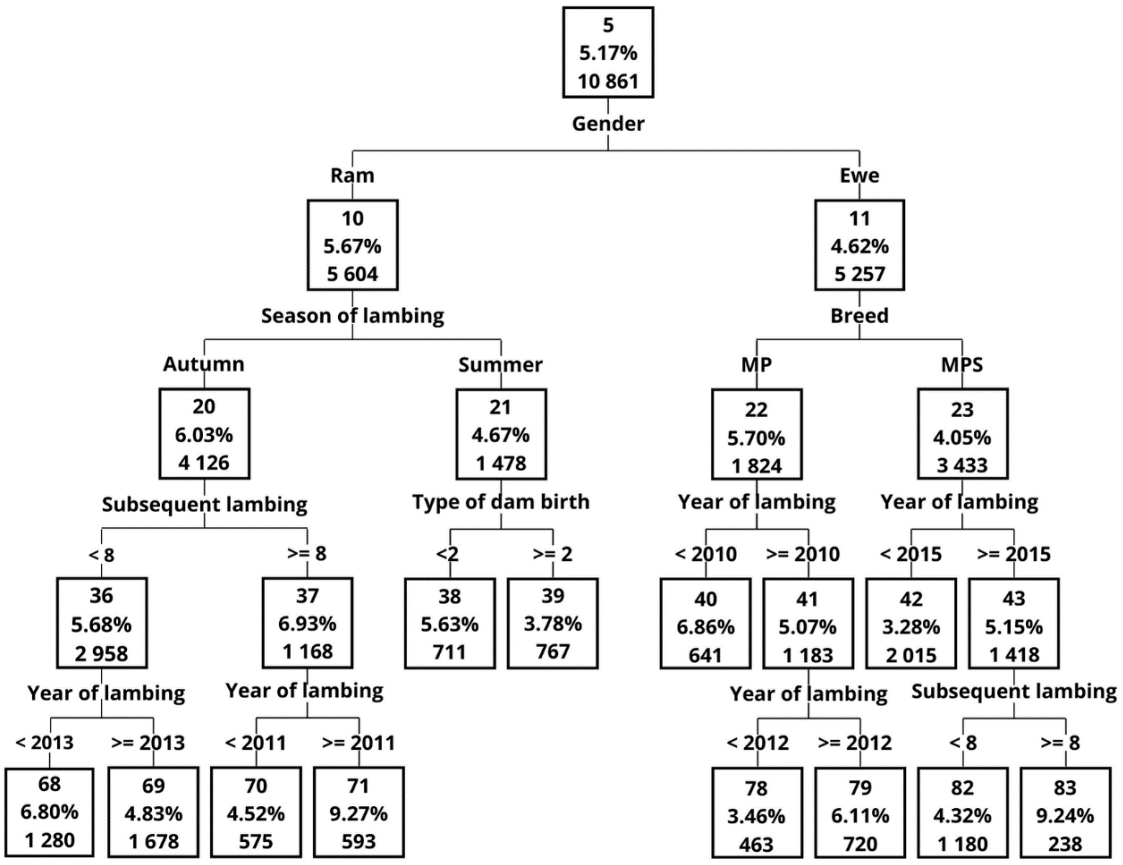

Figure 3. The graphical model of the classification tree - part 3.

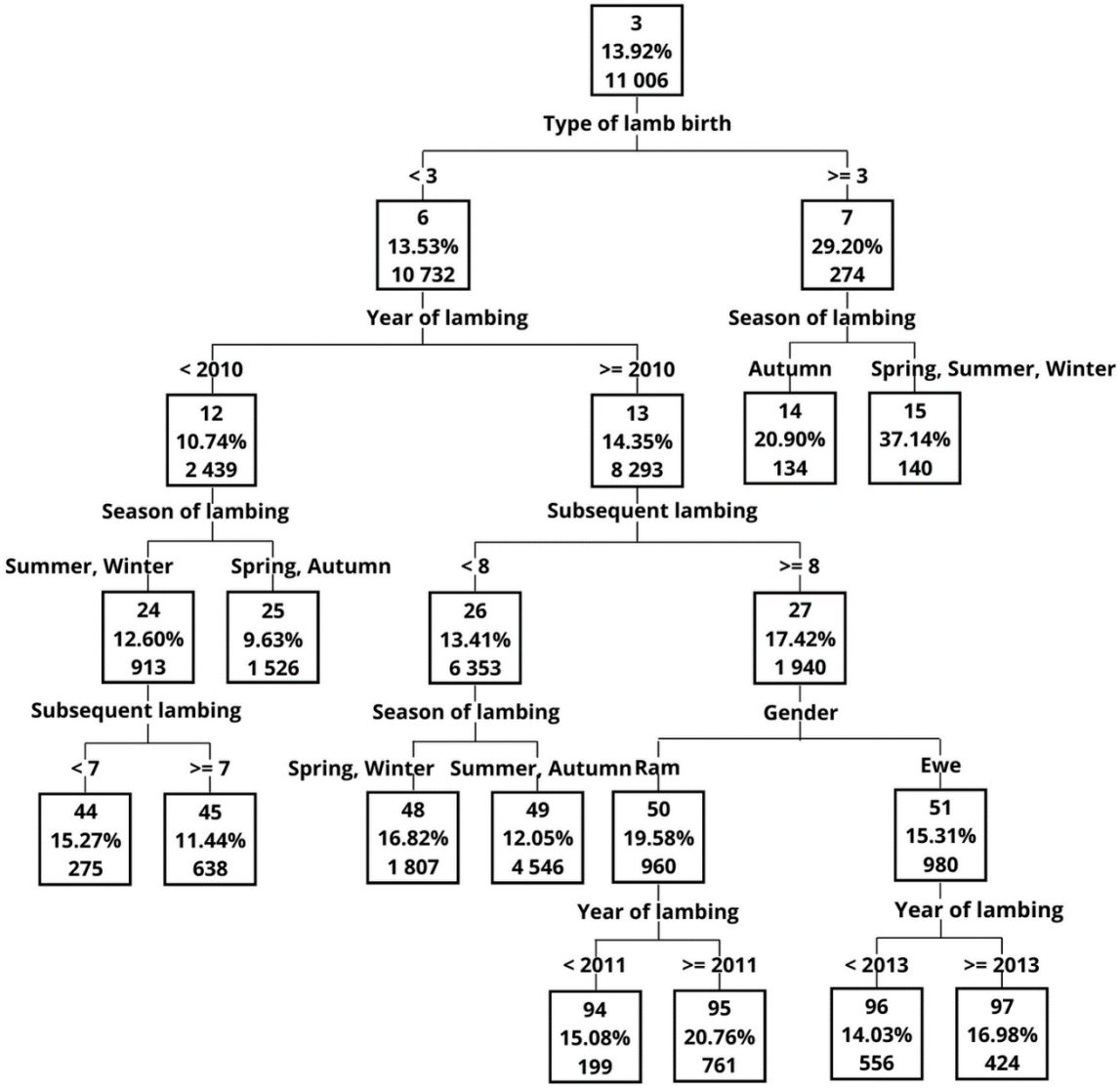

Figure 4. The graphical model of the classification tree — part 4.

4. Discussion

The percentage of lambs mortality kept in the Pomerania and Kuyawy region, in our research (9.27%) can be considered as slightly higher than for MP and MPS populations in all country. The Polish Sheep and Goat Breeders Association (PSBA, 2009) cites a corresponding index for the population of the Polish Merino in the year 2008 at 9.10%. In turn, the corresponding indicator for MP in 2020 was 6.5%, and MPS - 6.4% (PSBA, 2021). In earlier research (Piwczyński, 2007; Piwczyński, 2012), the observed proportion of Polish Merino lamb deaths in the period from birth to 100 days of age varied in the range of 6.74-9.19%.

Lamb mortality in the present study compared to other studies is proven to be relatively low. Hatcher et al. (2009) and Campbell et al. (2009) found mortality of the Australian Merino lambs in the weaning period (110 days) to be as high as an average of 14.3-27.6%. On the other hand, Cetin and Akcapnar (2005) found mortality in the range of 10.10% to 19.97% for Merino lambs. The climate in which the population of the assessed Polish Merino sheep were kept was a mild one. The fact that lamb mortality was low might suggest that the breeding conditions were favorable for the animals. Furthermore, it could be understood as proof that the applied breeding processes are effective as far as lamb survival is concerned. Mortality results obtained by some authors (Milerski, 2006; Hatcher et al., 2009) for different breeds of lamb, kept in different climatic conditions, signal possible considerable variations in terms of this trait between individual populations.

ROC index was one of the quality criteria chosen in respect of the classification tree and logistic regression models. Based on the values obtained for this index (0.650 to 0.654), it can be said that the prognostic capability in the case of all the models is moderate. A similar value of this index was established in studies on lamb mortality by Piwczyński et al. (2012) (0.6090-0.6476); on stillbirths Polish Holstein-Freisan calves by Piwczyński et al. (2013a) (0.6110) as well as in the work by Ghiasi et al. (2019) on modelling conception to first service (0.629-0.6323).

The research showed that the type of birth was the most important factor differentiating the mortality of lambs. The death rate for twins was twice as high as for singletons and for triplets four times as high as for the group of singleons. This is also confirmed by research by Piwczyński (2007), Aksakal et al. (2009), Hatcher et al. (2009), Piwczyński et al. (2012) have shown that the risk of death rises for twins. This tendency is supported by the results of our research. The reason for this is the lower weight of twins at birth, and also their more difficult access to udders, and contact with the dam. The type of birth is typically associated with body weight – it is lower in twins. The impact of body weight at birth on lamb mortality was shown in research conducted by Casellas et al. (2007), and Schreurs et al. (2010) – it was observed that both low as well as too high body weight at birth increase the risk of death.

According to Petersson and Danell (1985), the lambing season is a significant factor influencing lamb mortality. In the present research, this factor was indicated by the algorithm creating the classification tree as the second most important factor differentiating lambs in terms of their survival rate. The studies showed that lower mortality of lambs was observed among those born in the autumn and summer months than in the winter and spring months, which can be justified by a better feed base and more favorable climatic conditions at that time.

It was established by the authors that the year of birth was a key factor in variations in terms of the mortality of lambs. This may reflect different maintenance conditions, ways of feeding, and breeding work intensity. Evidence provided in studies by Petersson and Danell (1985), Mandal et al. (2007), Piwczyński et al. (2012), Everest-Hincks et al. (2014), supports the assumption that the year of birth has a statistical effect on lamb mortality. Moreover, the findings of our research as regards the impact of the lambing dam's age on the mortality among its offspring have been confirmed by what was established in the studies conducted by Petersson and Danell (1985), Aksakal et al. (2009), Hatcher et al. (2009), Salem et al. (2009). The results in the offspring of young female lambs were found to have been the least favourable in this respect. At the same time, in our research, we observed increasing mortality of lambs born to mothers in their 8th litter compared to previous, which can be justified by the deterioration of milk yield among these mothers.

It is not completely clear whether the lamb's sex has any effect on mortality. In our research, the classification treeS technique has revealed varied mortality among ewes and rams during the rearing period. Petersson and Danell (1985) had found comparable lamb mortality for both sexes; and the same had been established by Piwczyński et al. (2012). On the other hand, studies conducted by Hatcher et al. (2009), Morel et al. (2009), Everest-Hincks et al. (2014) indicate that mortality in ram lambs is considerably higher, which has also been confirmed in current studies; while Aksakal et al. (2009), who examined the Awassi breed, as well as Piwczyński (2007), in an earlier study on MP, found the mortality to be higher among ewe lambs.

The literature on the subject indicates many factors are responsible for lamb mortality in the period between birth and weaning. Results obtained by Freking and Leymaster (2004), Ekiz and Altnel (2006), Milerski (2006) prove that the mortality of lambs may be breed related. In our research, we found that MPS lambs had lower mortality in the rearing period than MP, which can be explained by the good maternal instinct of this breed. It should be emphasized, however, that the chi-square test conducted in the preliminary statistical analysis did not show a statistical relationship between breed and lamb mortality, and the value of the "Importance" measure of the "breed" variable was only 0.083. The dam's birth type played an even smaller role (Importance 0.052; $P_{chi\text{-}square} > 0.05$) than the breed of lamb in the structure of the classification tree, which is consistent with the results obtained in previous studies (Piwczyński et al. 2012). It should be noted, however, that in the current studies, lower mortality of offspring was observed among mothers from multiple births than in single births.

The undeniable advantage of the classification tree technique is the ability to study complex relationships between the variables responsible for the variability of the dependent variable (Piwczyński et al. 2013b). The result of our research showed that the highest frequency of lamb's death during the rearing period was to be expected among triplets born in winter or summer (37.14% of all deaths) (Node 15), while the highest chance (98.42%) of surviving till the 100th day of life showed singletons, born from their mother's 3rd to 6th litter, in the spring-winter season in the last year of the present research (Node 59).

5. Conclusion

The research showed that among the compared models of data analysis, the classification tree model based on the CART algorithm best predicted the mortality of lambs during the rearing period. In turn, the variables most strongly associated with the mortality of lambs were, in the order of decreasing importance: the type of lamb's birth. Season of lambing, year of lambing and subsequent lambing.

It was also shown that the highest frequency of lamb's death during the rearing period was to be expected among triplets born in winter or summer (37.14% of all deaths), while the highest chance (98.42%) of surviving till the 100th day of life showed singletons, born from their mother's 3rd to 6th litter, in the spring-winter season in the last year of the present research.

The advantage of using classification trees is that they present information in a clear graphic way, which makes it possible to understand splits which at times may be very complex. A graphical model of a tree created based on the Gini contamination indices is a proof of the

presence of several interactions between different factors.

Author Contributions

D.P. (100%) initiated the research idea, developed (100%), organized (100%), analyzed (100%) and interpreted the data (50%) and wrote the manuscript (30%); J.P. interpreted the data (50%) and wrote the manuscript (30%); M.K. wrote the manuscript (40%). All authors reviewed and approved final version of the manuscript.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans. The analyzed data on lamb mortality were taken from breeding documentation from the Regional Association of Sheep and Goat Breeders, Bydgoszcz.

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DEVELOPING SUSTAINABLE AGRICULTURE STRATEGIES: TURKISH FLORICULTURE CASE

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Abstract: While Floriculture maintains its importance for many countries and cultures with its commercial possibilities, global players had to differentiate their approach to the industry because of the shift of the production towards developing countries and the change in competitive dynamics. Türkiye's slow progress in Floriculture and the inefficacy to use its potential presents a unique opportunity to develop a sustainability-oriented strategy to differentiate Türkiye from its competitors. Hence, this work focuses on Turkish floriculture industry dynamics and aims to propose sustainable strategies using a Multiple Criteria Decision Making (MCDM)-based model. A comprehensive Strength Weakness Opportunities Threats (SWOT) analysis highlighting Turkish Floriculture's current state is used for that purpose. The economic, environmental, and socio-political dimensions of sustainability in the floriculture industry are also considered via an Analytical Network Process (ANP) model. The analysis results are used to define a sustainable floriculture strategy with its benefits-opportunities and costs-risks (BOCR) merits. Based on the findings, the economic dimension of sustainability takes precedence over the other two dimensions, and an efficient floriculture strategy needs to focus on logistics and marketing in a developing country like Türkiye.

Keywords: Floriculture industry, Sustainable agriculture, ANP-BOCR, SWOT analysis

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1. Introduction

Agricultural growth triggers other sectors and contributes to rural development and community well-being. Hence, classical views see agriculture as the main gear of development and industrialization (de Janvry, 2010). Despite this significant role, agriculture has lost its importance in the literature, although theories about long-term economic development continued to evolve. More recently, the interest in agriculture within the development framework has reappeared mainly due to climate change and food availability issues experienced in the economic, socio-political, and environmental context (Clapp et al., 2018; Loizou et al., 2019; Pawlak and Kołodziejczak, 2020; Norton, 2020). This increasing interest and renewed approaches to agriculture seem to differ from classical views by focusing on sustainability.

The concept of sustainable development provides a balance between economy, society, and environment and enables a guiding conceptual framework for global, national, regional, and institutional practices (Zilberman et al., 2018). Besides, sustainable agriculture has been essential for achieving various targets for rural areas and policymakers in developing countries (Lee et al., 2006; Fan, 2020). With the renewed approach to agriculture, increasing agricultural productivity has become critical for developing countries. However, due to the lack of advanced agricultural technologies, they could not reach the level of developed countries (Aker, 2011). Although similar concepts characterize the agricultural properties of developing countries, significant differences between these countries are ignored in global and national strategy formulation processes (Mekonnen et al., 2015). Therefore, the factors affecting the differences in agricultural productivity should be understood more thoroughly. This work uses Türkiye's case to demonstrate that a country-based analysis is necessary to develop a suitable strategy for sustainable agriculture development.

Even though historically agriculture has had an important place in Türkiye's development, its role has dramatically changed with the economic growth and new economic structure. While the composition of the outputs of the agricultural sector tends towards high-value products, such as horticultural products, the sector does not significantly affect the trade as in the past (Larson et al., 2016). In this work, horticultural products, specifically floricultural products, will be examined under the sustainable development framework.

So far, for the sustainable agriculture concept, there has not been agreed on a common analytical framework that can meet the needs of policymakers and researchers in

this field (Reig-Martínez et al., 2011). In order to cope with the versatile structure of sustainability, Pannell and Schilizzi (1999) proposed the use of MCDM methods in sustainable agriculture studies. Several works (Mulder and Brent, 2006; Dantsis et al., 2010; Veisi et al., 2016; Talukder et al., 2018) have extensively addressed criteria for various aspects of sustainable agriculture, using different MCDM approaches. For Turkish agriculture, Demirel et al. (2012) proposed a model for strategy selection based on two different MCDM methods. Although there are several works on sustainable agriculture, there is no work that focuses on sustainable Floriculture and related strategies. As evaluated in the comprehensive study of Wani et al. (2018), developing countries have many restrictions and criteria in internalizing sustainable floriculture applications. We believe that this work fills this gap while introducing the floriculture industry in Türkiye.

The paper's objective is to propose sustainable development strategies for Turkish Floriculture using a comprehensive MCDM model. In line with the sustainable floriculture definition by the Floriculture Sustainability Research Coalition (Hall et al., 2009; Burnett et al., 2011; Wani et al., 2018), our proposed model also aims to reduce any change or disturbance to the environment while continuing efficiency, productivity, and economic feasibility, protecting energy and resources and therefore improving life quality and providing reliable communities.

The remainder of the paper is organized as follows: Section 2 summarizes Floriculture Industry dynamics in Türkiye. Section 3 presents the methods used in the paper and their application in Türkiye's case. In the next section, results are discussed, and a sensitivity analysis is performed. The paper is concluded in Section 5 by presenting a strategic direction for the Floriculture industry in Türkiye.

1.1. Floriculture Industry in Türkiye

Floriculture is a sub-agricultural industry that plays a vital role in the economy of many developing countries. In the global arena, countries active in the floriculture industry show an alteration in the capital, entrepreneurship, labor, and labor productivity. Climate, flower diversity, labor costs, and technological superiorities are the most critical factors that cause this differentiation. Despite the increase in distance to the primary markets and logistics costs, it is observed that the floriculture industry has switched its production stage to developing countries with high labor force and lower capital requirements (Wijnands, 2005; Muhammad et al., 2010; Gebreeyesus, 2015).

Turkish Floriculture is a young and dynamic industry compared to competing countries. According to the recent industry report by Ornamental Plants Growers Union (SÜSBİR, 2020); despite having significant advantages such as its ecology and natural resources, suitable climatic and geographical conditions, proximity to market countries, and low-cost labor, Turkish Floriculture cannot get a sufficient share from the global floriculture market.

As shown in Table 1, while the Netherlands has almost 50% of the market share, the cumulative market share of the other four countries with the highest export ranks is almost 20%. Türkiye has the 23rd rank, with a 0.5% market share, which indicates that Türkiye cannot sufficiently use the advantage of being close to the market. In addition to these data, a significant decrease in imports and increase in exports since 2017 has been observed for Turkish Floriculture, shows that the trade deficit has turned into a trade surplus for the industry (ITC, 2021).

The production areas of the industry mainly consist of fragmented small lands, and the most important domestic market is still public procurement. In addition, due to marketing and logistics problems, it is preferred to establish businesses close to settlements. This makes it difficult to acquire land and results in high prices (SÜSBİR, 2020). Table 2 shows the floricultural production areas and yield figures that have been increasing each year since 2015. However, according to SÜSBİR (2020), this increase is quite limited, and the industry needs more production areas to reach its target.

Table 1. Top exporter countries list in floricultural products (ITC, 2021)

Rank 2020	Exporter countries	Value exported in 2020 (USD thousand)	Trade balance in 2020 (USD thousand)	Annual growth in value between 2016-2020	Share in world exports	Average distance of importing countries (km)
1	Netherlands	10963628	8371042	3%	49.33%	1239
2	Colombia	1431333	1393120	2%	6.44%	5175
3	Italy	1034440	497213	5%	4.65%	1240
4	Germany	1004208	-1947128	1%	4.52%	619
5	Ecuador	845741	824235	1%	3.81%	8383
…	…	…	…	…	…	…
23	Türkiye	106768	65269	8%	0.48%	2285
-	World	22225999	2572789	3%	100%	2314

Table 2. Sowed area and production numbers in Turkish floriculture (TUIK, 2021)

Ornamental plants (2015-2020)	Year	Flowers bulbs	Indoor ornamental plants	Outdoor ornamental plants	Cut flowers	Total
Area sown (m^2)	2015	612585	1465383	32293087	11826160	46197215
	2016	597305	1312793	34877416	12014172	48801686
	2017	426885	1650710	36263071	11748365	50089031
	2018	493930	2081527	37306970	11920217	51802644
	2019	412145	1992021	37699087	12374109	52477362
	2020	498830	1706388	39739347	12183481	54128046
Production (Number)	2015	27200330	40810719	451142538	1036147373	1555300960
	2016	25337330	38150927	409239917	1041173195	1513901369
	2017	21833825	56049665	490559391	1050584960	1619027841
	2018	88657000	60149981	507183040	1055783642	1711773663
	2019	62537229	51669029	510558039	1093333943	1718098240
	2020	71415654	48458815	529109699	1012465237	1661449405

The industry is foreign-dependent on production inputs. Relatedly, the fact that the increase in product prices does not meet the cost increase in production reduces the industry's profitability each year. The number of research and researchers on floricultural products in Türkiye is insufficient. Almost all of the R&D studies that are the foremost tool to reduce foreign dependency are carried out in research institutes affiliated with public institutions and organizations. In addition, informality continues to be one of the industry's most critical problems. With all its pros and cons, the existence of problems in terms of sustainability has been revealed by using the most up-to-date reports and data for the industry. Therefore, there is a need for studies involving different perspectives and approaches to support the industry at the national and international levels.

2. Materials and Methods

Ensuring sustainable development requires a deep understanding of the characteristics of the Turkish floriculture industry. For this, we performed a SWOT analysis. The results are consolidated from local studies related to Turkish Floriculture (Zencirkiran and Gürbüz, 2009; Baris and Uslu, 2009), policy documents (GTHB 2017; TÜSSİDE 2017), and expert opinions. Four different strategy processes are used in the Threats, Opportunities, Weaknesses, and Strengths (TOWS) matrix: WT: Minimize both weaknesses and threats, WO: Minimize the weaknesses and maximize the opportunities, ST: Maximize strengths to deal with threats, and SO: Maximize both strengths and opportunities (Weihrich, 1982). Table 3 and Table 4 represent SWOT analysis and TOWS matrix, respectively.

As SWOT and, similarly, TOWS analysis may not tell the expected consequences of future adverse developments, we applied BOCR analysis to consider all the potential aspects of factors and their relationships. BOCR analysis can be defined as a decision-making tool derived from benefit-cost analysis and is very similar to SWOT in many respects (Wijnmalen, 2007). Usually, ANP is also used with BOCR to handle the merits of a decision and represent them as separate networks. This approach, shortened as ANP-BOCR, provides an in-depth analysis of a decision's positive (B-O) and negative (C-R) aspects and synthesizes the decision alternatives through the help of strategic criteria. Strategic criteria are the main criteria for evaluating the BOCR values of all decisions, reflecting the organization's objectives to be fulfilled (Saaty, 2004).

3.1 ANP-BOCR Model for Turkish Floriculture

The first layer, i.e., the upper-level network, includes the control hierarchy in which the goal node, the strategic criteria, and the BOCR merits are presented. For example, we identified the goal node of our decision problem as: 'Evaluate sustainable development strategies for the Turkish floriculture industry. Accordingly, we have created three strategic criteria based on the sustainable development concept:

- Ensuring economic sustainability in the Turkish floriculture industry.
- Ensuring environmental sustainability in the Turkish floriculture industry.
- Ensuring socio-political sustainability in the Turkish floriculture industry.

The second layer, i.e., the decision networks, includes clusters and alternative strategies. Determining the alternatives for our selection problem, we used the SWOT analysis and TOWS matrix results. The strategies are gathered under four main alternatives to make the model more applicable.

- ST1: Establish an auction system and an efficient logistics network peculiar to the floriculture industry.
- ST2: Make investments to meet world standards in production systems and product diversity.
- ST3: Implement internal regulations to increase the competitive power of the industry.
- ST4: Restructuring the industry with R&D and educational revolution.

Table 3. SWOT analysis of Turkish floriculture industry

Strengths	Weaknesses
S1. Existence of natural resources	W1. Inadequate product variety and import dependence in seeds
S2. Various climatic characteristics which enable product differentiation	W2. Inadequate production and logistics infrastructure
S3. Areas that can be allocated to floricultural production	W3. High technology and input costs due to external dependence
S4. High production quality in specific products	W4. Lack of an auction system for export
S5. Richness in endemic species	W5. Weak consumption in the domestic market
S6. A certain level of production and development	W6. As yet unpublished quality standards
S7. High added value in the unit/area ratio	W7. Misapplication of agricultural spraying and irrigation
S8. Low-cost agricultural labor	W8. Insufficiently qualified personnel and intermediate staff
S9. Existence of occupational organizations	W9. Unfair competition, high informal production, and middleman commission
S10. Existence of industrial laws and sub-legislations	W10. Capital inadequacy and financing problems
Opportunities	**Threats**
O1. Appropriate geographic location	T1. Patent rights
O2. A downward trend in floricultural production in Europe	T2. Uncertainty and fluctuation in demand
O3. Availability of various transportation types	T3. Time-consuming custom bureau procedures
O4. New logistic related initiatives	T4. New trade routes that bypass Türkiye
O5. Presence of unsaturated foreign markets	T5. High electricity and water tariffs with insufficient fertilizer and fuel support
O6. Increasing importance on landscaping in the domestic market	T6. Insufficiencies in inspection
O7. Youth bulge	T7. Türkiye's narrow point of view on R&D activities
O8. Sufficient amount of educational institutions	T8. Political instability followed by high tax and exchange rates
O9. Stability of family-owned businesses	T9. Global warming, seasonal differentiation
O10. Ongoing European Union (EU) negotiations	T10. Low willingness to accept sustainable practices

Table 4. TOWS matrix of Turkish floriculture industry

[ST]1: Advance the existing development in production with sustainability studies and spread it to the whole product portfolio (S6-S4-S2-T10-T9).
[ST]2: Increase the R&D activities with a particular interest in endemic species (S5-T1-T7).
[ST]3: Increase contracted production using areas that can be allocated to avoid uncertainty in demand (S3-S1-T2).
[ST]4: Strengthen the relationship between state and occupational organizations to increase industrial incentives (S9-S10-S8-T8-T5-T6).
[WO]1: Improve the logistics systems according to Türkiye's advantageous geographic location and new initiatives (W4-W2- O1-O3-O4-O5).
[WO]2: Canalize the youth to floricultural education to improve the business cycle (W8-W5-W7-O7-O8-O9).
[WO]3: Reduce middleman commissions and informal production to watch the domestic competition (W9-W10-O6-O9-O10).
[WO]4: Improve the quality standards with modern production approaches to replace the falling production in Europe (W6-O2-O10).
[WT]1: Minimize the foreign-source dependency and costs both with R&D activities and governmental support (W1-W3-W7-T1-T3-T5-T6-T7-T8).
[WT]2: Improve the marketing system and logistics infrastructure and facilitate customs transactions to compete on a global scale (W4-W2-W7-T4-T2-T8-T10).
[SO]1: Give prominence to Floriculture among other agriculture-based industries (S7-S6-S5-S4-S3-S8-O9-O6-O5-O2-O7-O8).
[SO]2: Adapt the existing floriculture legislation to EU legislation (S10-S9-O10-O4).
[SO]3: Use elemental advantages to produce in a natural environment, expand product portfolio and increase market share (S1-S2-S3-O1-O3).

Table 5. BOCR criteria for Turkish floriculture industry

Benefits Sub-Network	Opportunities Sub-Network
Production	Production
B1: Increased modern agricultural practices.	O1: Increased environment-based good agricultural practices.
B2: Increased product variety.	O2: Increased use of indigenous seed and endemic flower species.
B3: Establishment of organized production areas.	O3: Increased number of domestic patents (production systems, equipment, and seed).
Logistics	Logistics
B4: More efficient use of logistics infrastructure and logistical advantages.	O4: Logistics village installation specific to the industry.
B5: Increased use of modern storage and cold chain applications.	O5: Increased green transport and storage applications.
Marketing-Labor	Marketing-Labor
B6: New job creation and employment.	O6: Growth of enterprises operating in the industry.
B7: Increased industrial recognition and reliability.	O7: Increase in export rate.
Environmental-Political	Environmental-Political
B8: More efficient use of soil and water resources.	O8: Delimitation to greenhouse gas emissions.
B9: Establishment of public institutions that supervise the industry.	O9: To be involved in the decision-making processes of international organizations.
Costs Sub-Network	Risks Sub-Network
Production	Production
C1: Input costs (energy, fuel, land, fertilizer, pesticide, patent ...)	R1: Import dependence on items such as technology, raw materials, and energy.
C2: Costs of production technologies	R2: Lack of sufficient results from R&D activities.
C3: R&D costs	R3: Problems in production finance.
Logistics	Logistics
C4: Transportation, storage, packaging, and deteriorated product costs	R4: Logistics investments fail to comply with the industry's needs.
C5: Logistics system installation costs	R5: Exclusion from the global floriculture distribution network.
C6: Customs costs	
Marketing-Labor	Marketing-Labor
C7: Training costs	R6: Failure to adapt to the change in trend and demand.
C8: Marketing costs	R7: Inadequate market share.
Environmental-Political	Environmental-Political
C9: Insurance costs	R8: Natural disasters, seasonal differentiation, global warming.
C10: Environmental tax	R9: Financial markets, political conflicts, and international problems.

After determining strategic criteria and alternatives, we selected the sub-criteria for the decision networks of the BOCR merits that we will use to evaluate the selection problem. For that purpose, production, logistics, marketing-labor, and environmental politics are defined as sub-criteria in all decision networks (Since the number of sub-criteria in the clusters are not sufficient for pairwise comparison, the clusters of marketing-labor and environmental-political have been combined). Table 5 shows our criteria system for the decision networks of the BOCR merits.

The next step involves determining the interactions between these elements and the internal and external dependencies and feedbacks. The developed network model with all its components is visualized in Figure 1. A uni/bi-directional arrow depicts an interactive relation between any two nodes at different clusters. Also, a loop typed arrow is used to identify the inner-dependency between the nodes under the same cluster.

A survey consisting of 53 questions with 255 pairwise comparisons is used to calculate the weights of identified relations. As the comparisons are prone to consistency issues, the consistencies of the individual surveys of the 12 experts (1 assoc. prof., 1 asst. prof. and 3 PhD candidates from the department of horticulture; 1 assoc. prof. and 1 asst. prof. from the department of agricultural economics; 1 prof. from the department of accounting and finance; and 1 PhD candidate from the department of public law which has a special interest on environmental tax law; 2 floriculture company owner; 1 agricultural engineer.) are calculated using Super Decisions software version 2.6.0, and they are all found less than the suggested threshold of 10% (Saaty, 1990).

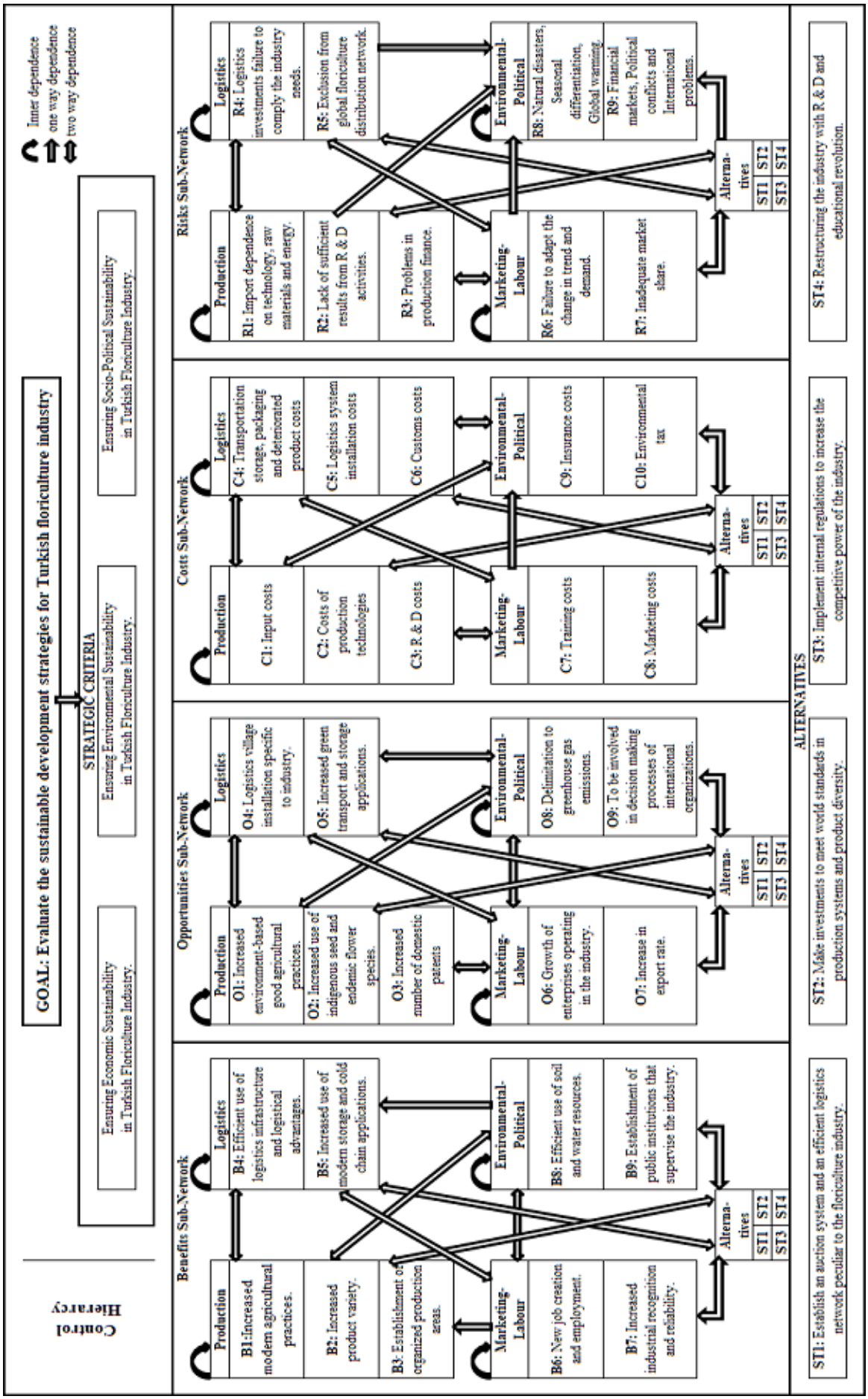

Figure 1. Proposed ANP-BOCR model for Turkish floriculture.

3. Results and Discussion

The separately applied surveys are gathered using the geometric mean of individual judgments for each pairwise comparison to obtain a group decision (Saaty, 2006). This approach, which has been mathematically demonstrated by Aczel and Saaty (1983), is a convenient procedure to unify the individual judgments, as it maintains the comparison matrices' reciprocal property. This procedure, including related priorities, is illustrated in Figure 2.

In Figure 2, *N* is the normalized value, prioritizing the criteria against the others in the cluster. *L* is the limiting values calculated in the limit super-matrices, which indicate the overall influence of the criteria in the network. The highest weighted normalized values at the related cluster and the highest weighted limiting values at the interconnected network are circled.

When cluster-based evaluations are performed, 'B3' and 'O3' are the sub-criteria with the most positive influence in production clusters, whereas 'C1' and 'R3' are the ones with the most negative influence. When other clusters are examined in a similar way, 'B5' and 'O4' are the sub-criteria with the most positive influence in logistics clusters, whereas 'C4' and 'R4' are the ones with the most negative influence. In marketing/labor clusters, 'B7' and 'O7' have the most positive influence, whereas 'C8' and 'R7' have the most negative influence. In politic/environmental clusters, 'B9' and 'O9' have the most positive influence, whereas 'C9' and 'R8' are the sub-criteria with the most negative influence. When the limiting values are observed, 'B3' in the benefits subnetwork and 'O7' in the opportunities subnetwork have the most positive influences overall, while in the cost subnetwork 'C1' and risk subnetwork 'R7' have the most negative influences.

Agricultural production in Türkiye has mostly been conducted in fragmented small lands (Atasoy, 2017). With the adoption of a sustainable floriculture approach, establishing organized production areas will eliminate inefficiencies and high rental costs. It is clear that an action plan should be formed in order to reduce the foreign dependency on important industrial inputs such as energy, fuel, fertilizer, and pesticide. Also in this context, the experts evaluate that increasing the number of domestic patents will significantly contribute to sustainable development by enhancing the competitive capacity and reducing costs in the long term.

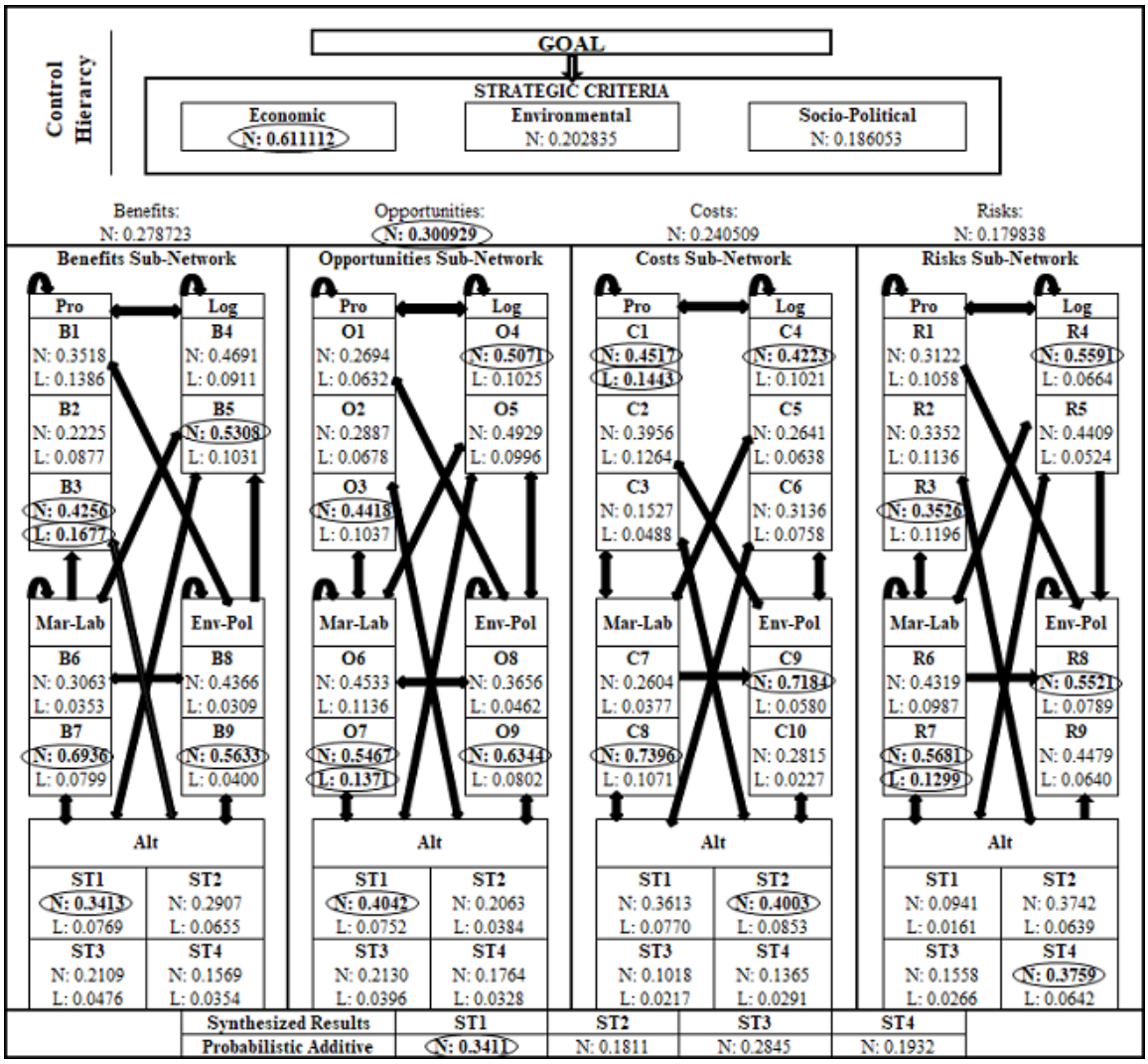

Figure 2. Consolidated group decision results

In Türkiye, the logistics of agricultural products mostly proceeds through commission merchants (Bignebat et al., 2009; Appel et al., 2014). Establishing individual logistics systems is not considered as a possible structure for small and medium-sized enterprises in the short term. The lack of opportunities for producers in terms of logistics and marketing at a later stage strengthens the hand of commission merchants. The competitive advantage arising from Türkiye's geopolitical position has not been supported with the right investments and could not establish a structure to meet today's needs. While inadequate market shares and marketing costs standouts as major concerns of the industry's representatives, it is clear that improvements in marketing channels will play an important role in the sustainability of Turkish floriculture.

According to the results 'ST1: Establish an auction system and an efficient logistics network peculiar to the floriculture industry' strategy has been computed as the best alternative. Meanwhile, the strategy of 'ST1' is the alternative with the most positive influence on the benefits and opportunities subnetworks. The strategy of 'ST3' has been evaluated as the alternative with the most negligible negative influence in terms of cost. Also, 'ST1' has been computed as the one with the most negligible negative influence in terms of risk. Therefore, the strategic criteria of 'Ensuring Economic Sustainability in Turkish Floriculture Industry' has been evaluated as more influential on the sustainable development of the Turkish floriculture industry.

Sustainable agriculture is bound up with the carbon footprints that evaluate the total volume of greenhouse gas emissions generated by a business activity or accumulated over the products' lives (Al-Mansour and Jejcic, 2017). If Türkiye wants to be involved in the decision-making processes of international organizations and to increase its share in foreign markets, awareness of these and similar concepts should be increased. It is not possible to talk about sustainability without considering its environmental dimension.

4.1 Sensitivity Analysis

As the ANP method carries out the comparisons based on individual perceptions, the results may differ depending on changes in the priorities of the BOCR merits. At this stage, we conducted a sensitivity analysis to examine whether the priority order of the alternatives would change. Super Decisions Software allows a "what-if" type sensitivity analysis. In Figure 3, the influences on the alternatives' weights are visualized with the selected independent variables: Benefits, Opportunities, and Costs, respectively. We did not include the graph for the risk merit since it did not cause a change in the order of the alternatives.

According to Figure 3, no matter how the weight of the Benefits independent variable changes, ST1 remains the best alternative. However, as the weight increases, ST2 surpasses other strategies in the ranking, indicating that ST2 will be a more positive strategy than ST3 and ST4 in a benefit-oriented evaluation. When we make the same assessment with the Opportunities independent variable, we can see that the ST3 strategy is superior in ranking compared to ST1 in a pretty small range. However, as the weight increases, ST1 has an absolute advantage as the best alternative. When the Costs merit is assigned as the independent variable, we encountered inconsistent results. As the weight of costs increases, ST1, which is observed as the best alternative, seems to have lost the lead with a dramatic decrease. In a cost-oriented evaluation, ST3 would be the best strategy.

The sensitivity analysis results reveal the importance of using MCDM methods to cope with the complicated structure of sustainability in agricultural practices. As can be seen, changing the weight of the concepts or a narrow-oriented approach in the decision-making stages would cause alteration in the rank of the strategies.

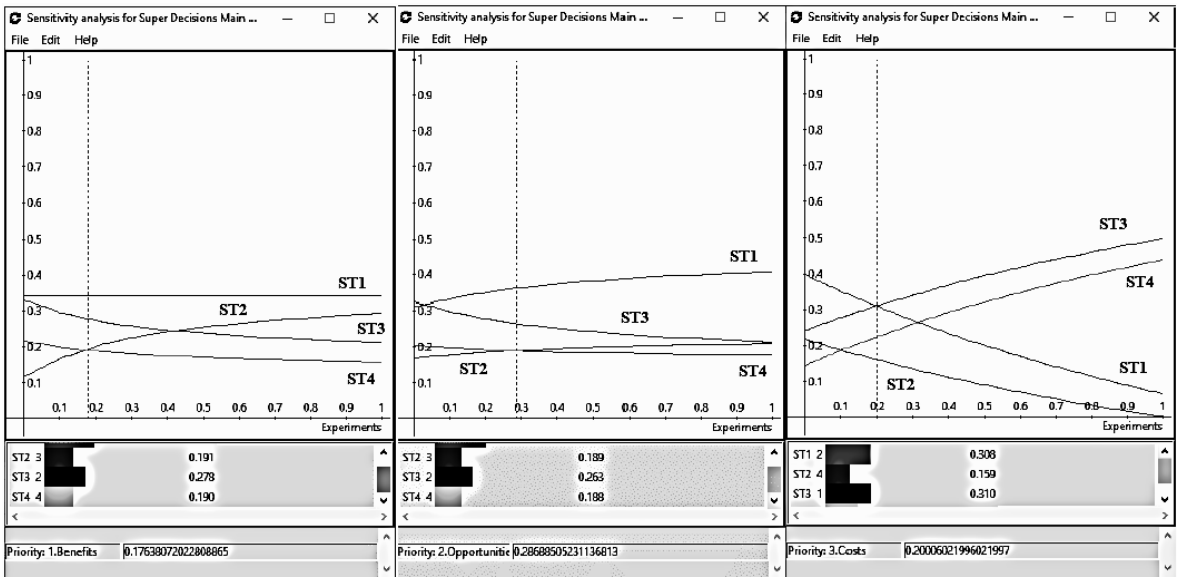

Figure 3. Sensitivity analysis: independent variables: benefits, opportunities, costs.

4. Conclusion

Based on the analysis performed and sensitivity results considered, the best strategy for the sustainable development of Turkish Floriculture has been evaluated as 'Establishing an auction system and an efficient logistics network peculiar to the floriculture industry. Underlying principles of this strategy is: Developing a sustainable, export-oriented logistics system in conformity with Türkiye's advantageous geographic location where effective intermodal transport networks are used, cold chain applications that catch the world standards are adopted, and the deterioration rate of floriculture products is minimized; without any break in the chain. With the proper location selection, supported with such studies on infrastructure, storage, packaging, product standardization, and quality standards, it is an investment that will improve the Turkish floriculture industry's position in the global arena and ensure its sustainable development.

The participated experts have evaluated that economic sustainability has the highest significance level between the three main components of the sustainability concept. Such a low evaluation of environmental and socio-political sustainability concepts in terms of significance level partially reveals the industrial sustainability perspective of developing countries through the Turkish floriculture case.

We believe that this study and the criterion system we have identified draws an applicable road map to Turkish Floriculture and similar countries and related industries.

Author Contributions

Concept: A.Ü.Ç. (100%), Design: A.Ü.Ç. (50%) and S.E.A. (50%), Supervision: S.E.A. (100%), Data collection and/or processing: A.Ü.Ç. (100%), Data analysis and/or interpretation: A.Ü.Ç. (100%), Literature search: A.Ü.Ç. (100%), Writing: A.Ü.Ç. (50%) and S.E.A. (50%), Critical review: A.Ü.Ç. (50%) and S.E.A. (50%). Submission and revision. All authors reviewed and approved final version of the manuscript.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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Research Article
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INCREASING THE PLANT PRODUCTIVITY USING THE AUTOMATIC CONTROLLED IRRIGATION SYSTEM: A COMPARATIVE EXPERIMENTAL STUDY

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Abstract: With the development of technology, today, the use of technology in the field of agriculture has become widespread. In order to meet the increasing demand for agricultural products, automation techniques should be used in agricultural areas in order to make the production of agricultural products simpler and more efficient. In this study, an automation system is designed by making use of technology against problems such as irrigation problem and water shortage, which have become an important problem in agricultural areas. The data coming from the humidity sensor placed in the soil is processed to the controller. According to these processed data, when the soil is dry and the plant needs water, the water-pumping set automatically activates and meets the water needs of the plant. Optimum use of irrigation water to be used in agriculture is prevented unnecessary agricultural irrigation, reducing excessive water waste and providing a very high level of energy savings. At the same time, the negativities caused by excessive irrigation have been prevented. It is observed that the automatic controlled irrigation system used in this study saves a lot of water compared to the conventional irrigation system and increases the productivity of the plants to a great extent.

Keywords: Drip irrigation, Automatic control, Productivity, Water savings

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1. Introduction

The developments in industry and technology, the increase in people's living standards and the living population have led to the emergence of water problems in the world and in our country since the last half of the twentieth century. Disruption of the ecosystem balance, continuous climatic changes and misuse of lands, as well as events such as floods and landslides, and climate changes can be seen as the main causes of water crisis. It is foreseen that this crisis and the demand for water are gradually increasing. As a result of the researches, it is shown that the increasing water demand on the Earth and the decreasing clean water supply curves will intersect in 2029. According to this result, it is concluded that there will be a serious water problem and a water crisis and even a situation leading to drought will occur throughout the world (Alparslan et al., 2008).

Most of the water around the world is used for irrigation of agricultural lands. Technological developments and irrigation in agriculture have a significant impact on Turkey's economic volume gain, the increase in the yield rate in agricultural areas and the gradual decrease in migration to cities. Irrigation method in agricultural lands is called as a concept covering systems such as the quality of the water network to be irrigated and the ways in which the water will reach the user (Erzurumlu, 2017; Wondatir and Belay, 2020). In order for the irrigation projects to be prepared realistically, the daily, monthly or periodic water costs of the plants that are planned to be produced should be measured. For the measurements of the water need in the area to be irrigated, the amount of water consumed by the plant must be determined (Özgüler, 1997; Kartal et al., 2019).

In Çakır and Calis (2007), a remote-controlled irrigation system was designed using PIC 16F877 integration for an automatic control system plant irrigation project. The system is irrigating automatically or manually over the PSTN telephone line. A system design that works with soil wetting and humidity sensor detection was used in this study. If the soil is moist, the system does not work, if the soil is dry, the system activates and performs the irrigation process. Since this system works automatically, it saves time and manpower. In Fidan and Karasekreter (2011), an SMS-controlled irrigation automation control unit (SKB) was developed. In the related study, the user can control via SMS and at the same time, it automatically adjusts the irrigation time and sends an information message to the user in case of rain. In the literature, there

are many studies on agricultural irrigation organization and water management problems (Acatay, 1996; Sayın, 1993).

In Milla and Kish (2006), infrared sensor and microcontroller are used for erosion prevention and a healthier irrigation system. With this designed system, information such as how long and how often the irrigated area needs to be irrigated is transferred to the computer and recorded in the memory. Al-Ali et al. (2001) designed an automatic irrigation system with solar rays and PLC. There is no control system for remote intervention in this system, the system consists only of a control unit. Kırnak (2006) developed a system that automatically performs drip irrigation that measures soil moisture via computer. With this system, with the right amount of irrigation at the right time, a great saving was achieved in water by eliminating the unnecessary irrigation process. Salivahanan et al. (2001) designed an intelligent irrigation system using fuzzy logic algorithm. There is no remote control unit in this system. In Jin et al. (2007), a system was designed to receive data from greenhouses by utilizing GSM/SMS technology. With this designed system, the success of measuring soil rate, soil temperature and greenhouse temperature has been achieved.

In Kırda et al (2007), the yield of mandarin is investigated under traditional restrained and newly introduced semi-wet irrigation applications and it is concluded that the deficit irrigation, both through partial root drying and conventional deficit irrigation must consider and balance savings of water and depreciation of marketable fruit quality. In Yıldırım et al. (2018), a smart automated drip irrigation system running with solar-powered energy is designed for a greenhouse system and it is concluded that it is possible to save water and fertilizer and increase the amount of energy by increasing the number of solar panels. In Zürey et al. (2020), an automatic nozzle control system is developed for the orchard sprayers to avoid the pesticide residues to the soil and it is observed that this system detects objects within 5 m distance with high stability. In Kesler et al. (2022), the normal and the fuzzy controlled irrigations ara compared with each other by using inputs e temperature, humidity, and soil moisture and an efficiency of 53.77% is obtained in irrigation water in seedling cultivation.

In this study, the water savings and the productivity of eggplant, tomato and pepper plants irrigated with conventional irrigation and micro-controller aided automatic controlled irrigation system are experimentally compared with each other.

2. Materials and Methods

An 8-bit, 8 MHz Atmega328 using Arduino Uno micro-controller is chosen for this study. ESP8266 Wi-Fi module is used to provide the remote control of irrigation process. In order to control the water requirement of plants, it can be provided by measuring the humidity of the soil. The used plant humidity sensor has 2 legs that measure humidity and these legs should be buried in the ground by the root of the plant without damaging the plant. When the plant humidity sensor legs are placed in the soil where the plant is located, a resistance occurs and this resistance creates a voltage difference between the two legs of the humidity sensor. It means that the higher the humidity of the plant, the higher the conductivity of the sensor. In Figure 1, the block diagram of soil humidity controlled plant irrigation system is given. First, humidity sensors are placed in the soil where the plant is planted. The operation of the water-pumping set is controlled by processing the information received from these sensors by the micro-controller. Yet, the water-pumping set also supplies the water to the drip irrigation system.

In this study, the plants planted in the first row are irrigated from the first water tank with the conventional method. The plants planted in the second row are irrigated with the system given in Figure 1 from the second water tank.

The plant water requirement is determined by the plant humidity sensor in the system and the system automatically gives the plant the water needed by the plant. Also, a Wi-Fi module is used for controlling and monitoring the irrigation system in order to check the irrigation system operation and manually control.

2.1. Plant Drip Irrigation System

Irrigation is the most important input in both increasing and improving the yield in plant production. The introduction of water, which is necessary for plant growth but cannot be met naturally, to the soil without creating environmental problems is called “irrigation”. Irrigation method refers to the way of the irrigation water is delivered to the plant root zone. Since the characteristics of agricultural areas (soil structure, topography and climate) are different, the way water is applied to the plant root zone is also different. Generally, plants are irrigated by one of the methods of surface, sprinkler and infiltration irrigation (Taş and Kırnak, 2015).

In order to obtain the highest yield and quality product from the unit area, it is necessary to know the amount of irrigation water and the irrigation time to be applied, along with other regional measures. Due to the limited water resources, drip irrigation is gaining importance all over the world. At the same time, this allows cultivation without stress on the plant. Ankara, which is the application area of this study, has a continental climate, limited water resources, the annual precipitation is not sufficient and drip irrigation is needed. Drip irrigation also provides energy savings in cases where water is forced. Drip irrigation prevents water and nutrient losses without creating surface runoff and infiltration. It allows irrigation with water with high salt content. At the same time, fertilization and spraying can be done with irrigation. Quality and standard products can be obtained. It allows irrigation in all kinds of areas and

early harvest. Importantly, it prevents erosion and soil loss. Irrigation can be done at low pressures. In drip irrigation, the labor cost is very low compared to conventional irrigation methods (URL). With the automatic control drip irrigation system designed in this study, it is aimed to save labor, time and water and to increase the product yield.

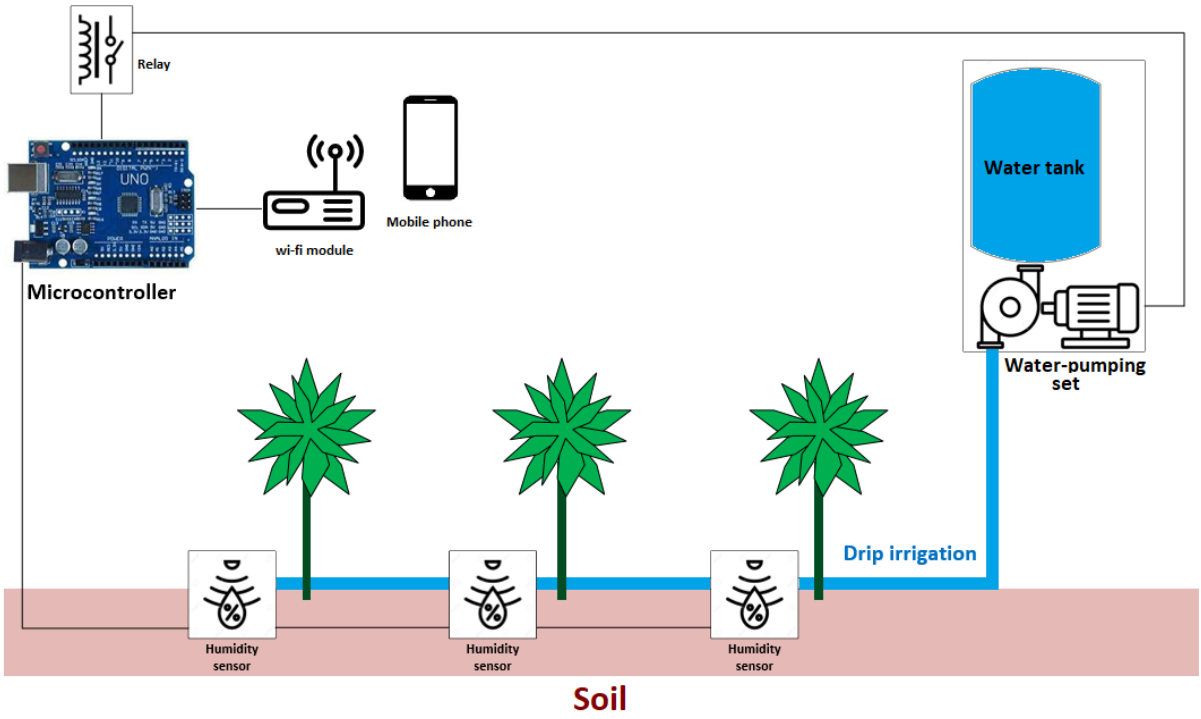

Figure 1. Soil humidity controlled plant irrigation system designed in the study.

3. Results and Discussion

In this study, an experimental comparison of crop yield and water savings is presented over plants irrigated with conventional methods and with automatic controlled drip irrigation. In the study, two 300-liter water tanks are used one for automatic controlled irrigation and for conventional irrigation. The amount of water consumed from the water tanks used is compared in quarterly periods as from May to June (first period), from June to July (second period) and from July to August (third period) and the monthly water savings are compared by the measurements. In Figure 2, the amounts of water consumed by conventional means (red color) and automatic controlled irrigation (green color) are given in the first, the second and the third months and in total. Figure 2 shows that for three months and total irrigation, the water consumed by automatic controlled irrigation is less than by conventional irrigation. The water savings are 13%, 19% and 22% in the first, second and third months, respectively. Therefore, the total water savings is 19% with automatic controlled drip irrigation.

In this study, three different plants, namely pepper, tomato and eggplant, are irrigated with two different methods as conventional and automatic controlled irrigation. Kızılcahamam district of Ankara is chosen as the application area of the study. The product yield and water savings in these two ways are also compared with each other. In Figure 3, the photos of these three plants irrigated in two different ways is given for the end of the 3rd month. In Figure 3, it is observed that the plants irrigated with the automatic controlled yield more products than the plants irrigated with the conventional way.

All cells at the boundaries of the membrane system of plant tissues play an important role in maintaining the normal course of the physiological process. Many proteins and enzymes are located in the membranes. For this reason, changes in membranes cause losses and normal physiological changes. Electrolyte leakage is defined as loss of cytoplasm in cells due to membrane damage (Fan et al., 2003). Membrane permeability can change due to environmental stress. Electrolyte leakage is used to describe the change in membrane permeability (Whitlow et al., 1992). In this study, ADWA AD3000 desk type EC meter is used to determine electrolyte leakage values. In Figure 4, electrolyte leakage values of three different plants irrigated with conventional and automatic controlled irrigation are given for three months. In Figure 4, Conv and Auto represent the conventional and automatic controlled irrigations, respectively. Pep (green colors), Egg (purple colors) and Tom (red colors) represent the pepper, eggplant and tomato, respectively. It is observed that the electrolyte leakage of plants irrigated by automatic controlled is less than the leakage of electrolyte from plants irrigated by In Figures 5a, 5b and 5c, the comparison of harvested products is given in terms of number. In Figures 5d, 5e and 5f, the comparison of harvested products is given in

terms of weight. In Figure 5, I and II represent the first and second harvest period, respectively. The first harvest period is from June to July and the second harvest period is from July to August. In Figure 5, orange and blue colors indicate conventional and automatic controlled irrigation, respectively. When the Figures 5a, 5b and 5c are compared within themselves, it is observed that the number of the crops irrigated with automatic controlled is more than those irrigated with conventional means for all three plants and two harvest periods. Similarly, when the Figures 5d, 5e and 5f are compared within themselves, it is observed that the weight of the crops irrigated with automatic controlled is greater than the weight of those irrigated with conventional means for three plants and two harvest periods. The yields with automatic controlled irrigation for pepper, tomato and eggplant are 53%, 69% and 13% for the first harvest period, respectively. The yields for the second harvest period are 42%, 30% and 15% for pepper, tomato and eggplant, respectively. The highest yield for the first harvest period is obtained in tomato and the highest yield for the second harvest period is obtained in pepper. While the yield of pepper and tomato decreases in the second harvest period, the yield of eggplant increases in the second harvest period.

As discussed in Section Introduction, in the literature, there are many studies that provide water saving and product efficiency with different methods. While in Kale et al. (2017), the maximum yield for wheat is 31%, in this study, the maximum yield is 69% for tomato. In Zhang et al. (2018), winter wheat production efficiency increases with water saving management in the North China Plain. In this study, it is concluded that the automatic controlled irrigation method has a positive effect on the development and productivity of the plants compared to the conventional irrigation method.conventional means for all three plants.

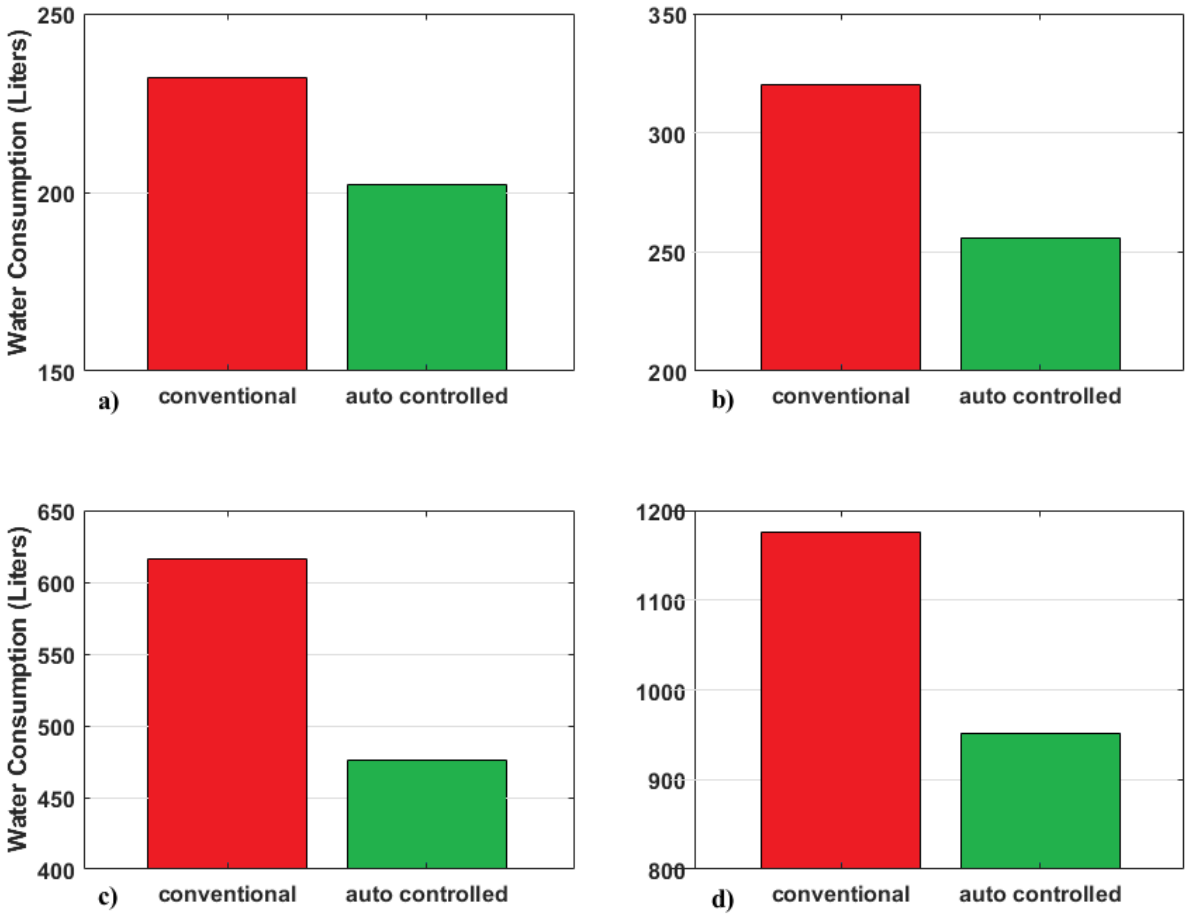

Figure 2. Consumption of water for: a) the first month, b) the second month, c) the third month and d) in total.

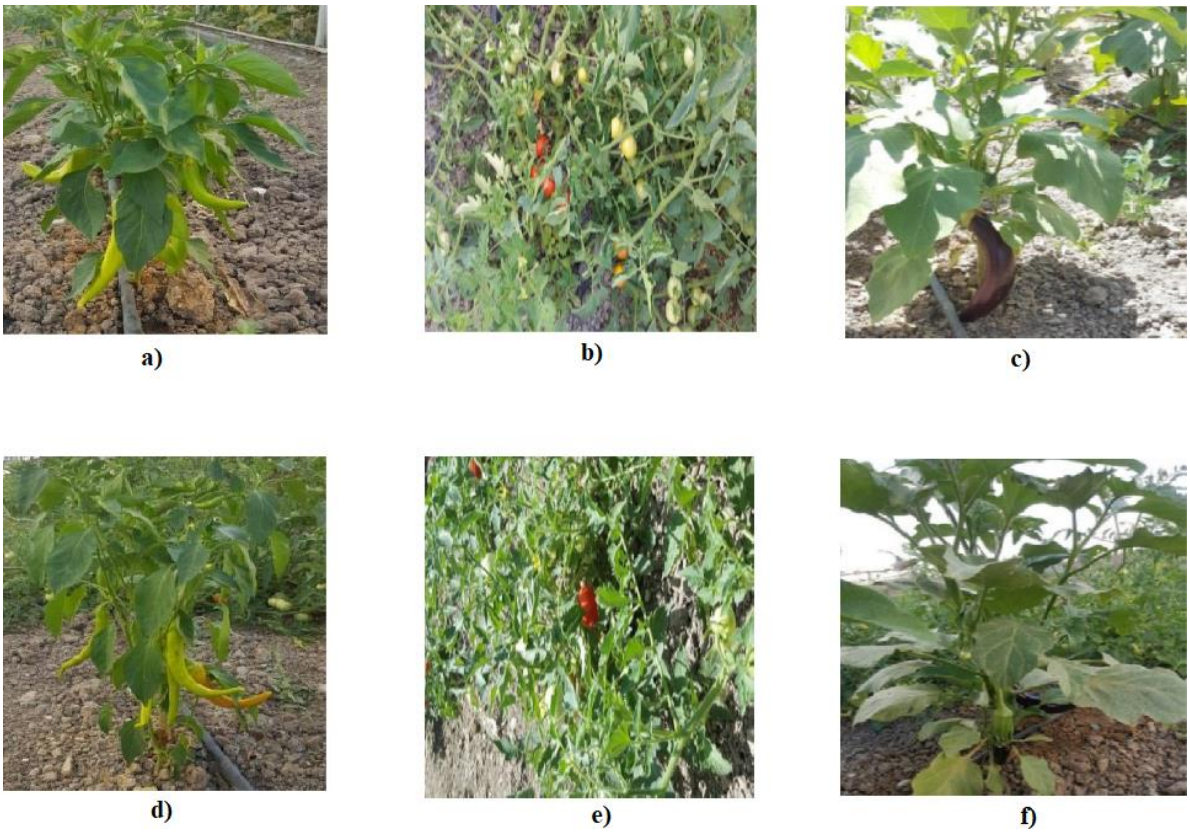

Figure 3. The photos of pepper, tomato and eggplant planted within the scope of the study. For automatic controlled irrigated a), b), c) and conventional irrigated d), e), f).

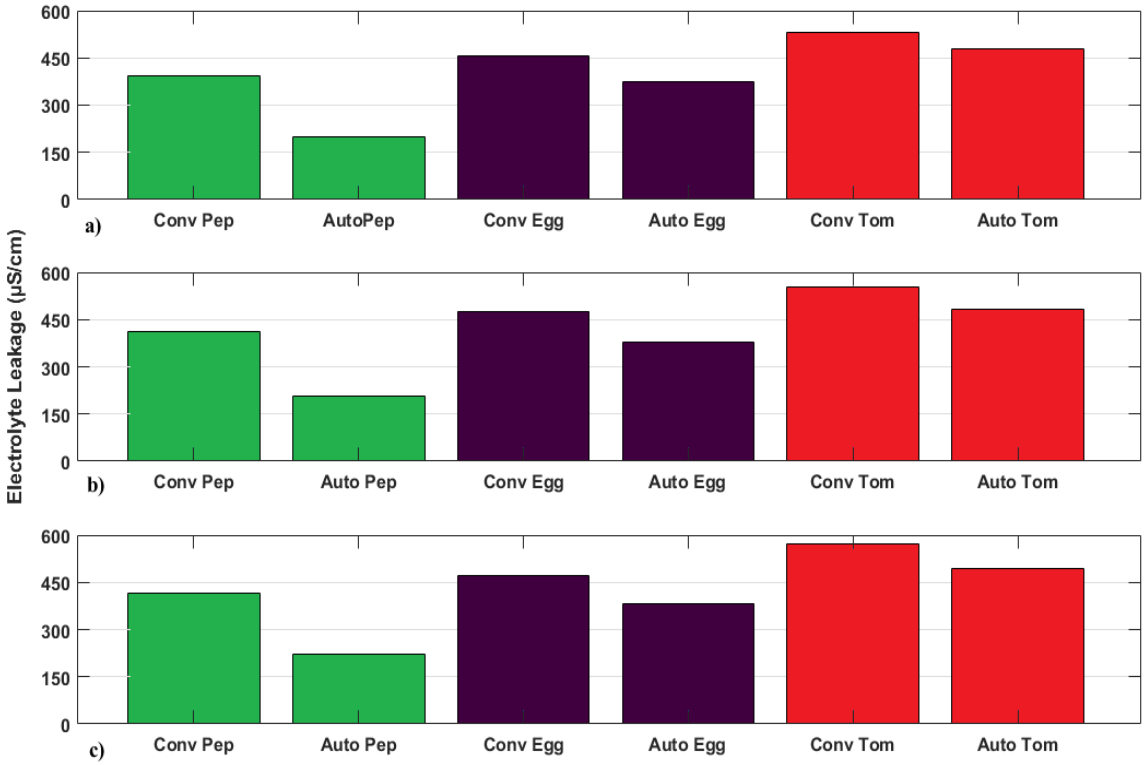

Figure 4. The monthly values of the electrolyte leakage of the three plants irrigate with the conventional means (Conv) and automatic controlled (Auto) for: a) first period, b) second period and c) third period.

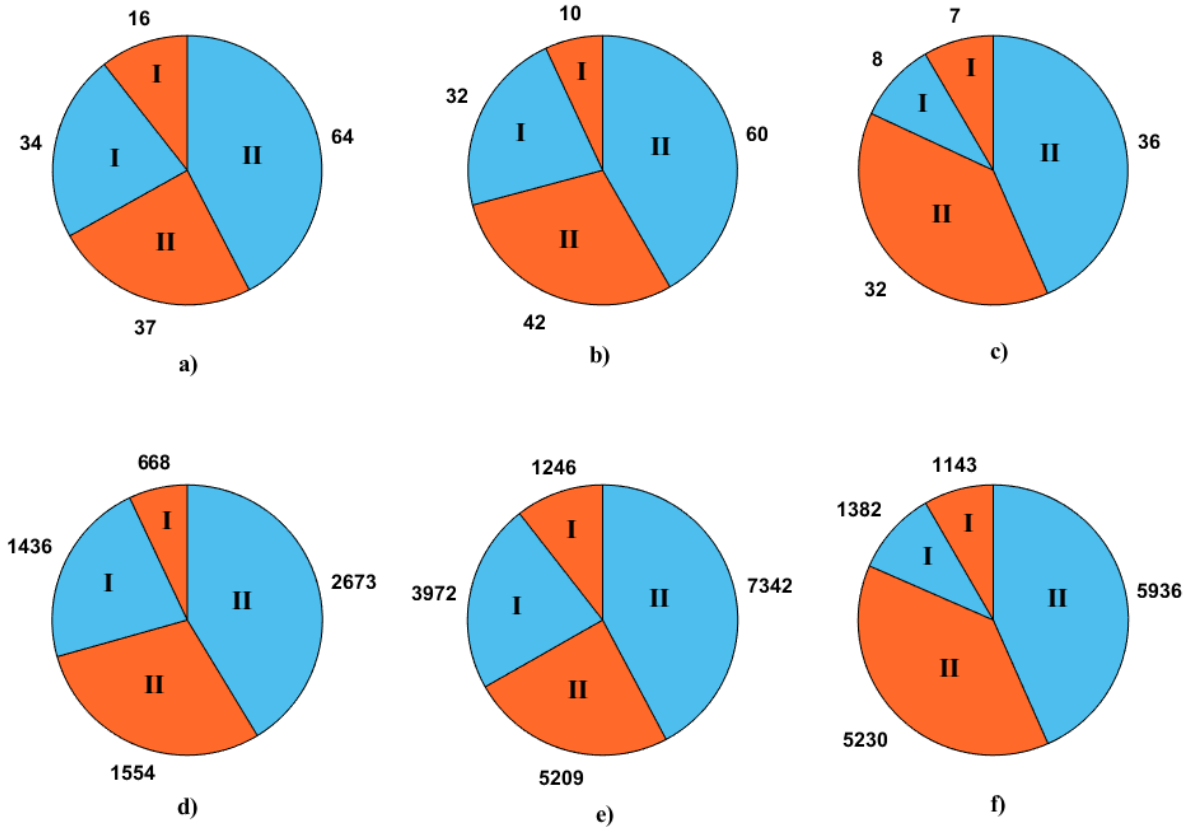

Figure 5. The numbers of: a) pepper, b) tomato and c) eggplant and the weights of: a) pepper, b) tomato and c) eggplant irrigated with conventional means (orange) and automatic controlled (blue) for the first (I) and the second (II) harvest periods.

4. Conclusion

In this study, the productivity of three crops, namely pepper, tomato and eggplant planted in Kızılcahamam, Ankara is experimentally compared using conventional and automatic controlled irrigation methods for two harvest periods. The crops are irrigated from May to June, June to July and July to August and harvested during July and August. Two 300-liter water tanks are used for the conventional and automatic controlled irrigation. The water requirement of the plants is determined using a remotely controlled plant humidty sensor. Only the root zone of the plant is irrigated using the drip irrigation system. As the plant humidity value decreases, the system automatically performs the irrigation process and when the humidity value determined for the plant is reached, the system automatically stops the irrigation process. In addition, the system can be controlled at the desired place and time with a mobile phone. With this control, up to 22% water savings are yielded with automatic controlled irrigation. The electrolyte leakage of plants irrigated by automatic controlled is observed to be less than those from plants irrigated by conventional means for all three plants. Finally, the numbers and the weights of the crops harvested in two periods are compared with each other and it is observed that up to 69% crops yield is observed from the first harvest period. As a result, it is observed that there is an increase in number and weight in the products irrigated with automatic controlled compared to conventional irrigation.

This system, which is applied to a small area, can also be applied to large agricultural areas without the need to use many humidity sensors. It can be improved by adding environmental elements such as humidity sensor and temperature sensor that can measure the ambient humidity value on the control unit of the irrigation system. The applied system is low-cost, increases productivity, reduces labor costs and provides water savings.

Author Contributions

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Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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Research Article
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BIOCIDAL EFFECT OF DELTAMETHRINE AGAINST *TRIBOLIUM CONFUSUM* DUV. (COLEOPTERA: TENEBRIONIDAE) AND *SITOPHILUS ORYZAE* L. (COLEOPTERA: CURCULIONIDAE) ADULTS IN LABORATORY CONDITIONS

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Abstract: *Tribolium confusum* and *Sitophilus oryzae* species are seen in human habitats, mainly in kitchens. They damage the storage products by feeding on crops and disturb people like urban pests in kitchens and homes. Until now, there were no biocidal that could be used to control these species. We carried out to determine the effect of deltamethrin, which is used against some urban pests, on *T. confusum* and *S. oryzae* under laboratory conditions. The recommended dose of the biocidal against urban pests (60 ml/10 liter of water), half dose (30 ml/10 liter of water), and quarter dose (15 ml/10 liter of water) were used in the study. The results showed that the highest amount of the biocidal (60 ml/10 liter of water) resulted in 87.5% mortality in *T. confusum* and 97.5% in *S. oryzae*. The LD_{50} value for *T. confusum* is 32.8 and LD_{90} is 61.3 ml, and for *S. oryzae*, these values were found to be 18.8 and 38.1 ml, respectively.

Keywords: Urban pests, Stored grain pests, Rice weevil, Confused flour beetle

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1. Introduction

Various insects and arthropods colonize and reproduce in urban environment. The urban entomology generally deals with the investigation of creepy crawly bugs that plague human-made structures. Some arthropods such as ants, bed bugs, cockroaches, and termites can grow in or around the indoor climate and invade a broad range of substrates, and food sources of humans. Depending on the insect species, these pests can be a reason for aggravation. They can prompt auxiliary harm, their nibbles can cause uneasiness, and presentation to this creepy crawlies can trigger hypersensitive responses. There are many urban pests in human habitats. In addition, some storage pests are seen at home in our kitchens and are considered urban pests from time to time. They often feed on and cause damage to products such as rice, wheat, and flour purchased from shops and used to prepare meals. (Shadia and El-Aziz, 2011; Kocak, 2016; Tyagi et al., 2019).

Tribolium confusum Duv. (Coleoptera: Tenebrionidae) and *Sitophilus oryzae* L. (Coleoptera: Curculionidae) are among the most critical species seen in kitchens. These pests significantly reduce grains' nutritional value and seeding characteristics and cause weight losses by feeding on the products. As a result of increasing populations of pests, mold, mildew, and rotting are observed in the product due to elevated humidity and temperature. Consumption of these products causes essential diseases in humans and animals. In addition, they cause certain diseases such as allergic asthma in humans with their secretions, body scraps, and dirt they produce during feeding. *Tribolium* spp. (Coleoptera: Tenebrionidae) can cause the formation of carcinogenic substances in the product (Stejskal and Hubert, 2006). These two species are widespread in our homes due to the foods found in kitchens. For this reason, these pests are considered urban pests when seen in living areas. These pests are mainly seen in agricultural areas and crop storage (Olejarski et al., 2013; Stejskal et al., 2015; Togantimur and Ozder, 2019). Insecticides and fumigants used against agricultural pests combat these pests in crop storage. In recent years, there have been studies on alternative methods of chemical control against these pests (Athanassiou et al., 2016; Karakas, 2016; Ayyıldız and Karaca, 2018; Cetinpolat et al., 2019; Göktürk et al., 2020). However, these insecticides do not apply to human habitats, especially in kitchens. In fact, it is even prohibited. Only licensed biocidal products can be used against urban pests in kitchens. Today, there is no licensed biocidal for controlling *T. confusum* and *S. oryzae*

species. Biocidal, whose active ingredient is deltamethrin used today in the control of urban pests such as mosquitoes, houseflies, cockroaches, and ticks (Cao et al., 2006; Barile et al., 2009; Romero et al., 2009; Jankov et al., 2012; Jahan and Shahid, 2013).

This study analyses the effects of this biocidal (Deltamethrin commercial name is Exdel 5 SC) on *T. confusum* and *S. oryzae* species in laboratory conditions.

2. Material and Methods

2.1. Insect Culture and Application of Biocidal

The insect species (*T. confusum* and *S. oryzae*) were used in the study and biocidal Exdel 5 SC (5% deltamethrin) were obtained from the entomology laboratory of Ondokuz Mayıs University's Faculty of Agriculture. Anonymous (2016) and Kocak (2016) methods were revised in the study. The recommended dose of the biocidal against urban pests (60 ml / 10 liter of water), half dose (30 ml / 10 lite of water), and quarter dose (15 ml / 10 liters of water) were used in the study. Four repetitions were made for each dose. The tests were conducted in the laboratory at 25±1ºC and 60±5% R. H. Plastic containers were used in the study. Different doses of the biocidal were sprayed in these containers (20 cm length, 10 cm width, and 6 cm depth) with a suitable device, 0.5 ml per container. After waiting for 24 hours, 10 adults were placed in the container and brought into contact with the surface. After 30 minutes of contact time, adults on the application surfaces were transferred into clean containers. The mortality was noted over 1st, 3rd and 5th days following application. Dead individuals were counted, and percent mortality was calculated.

2.2. Statistical Analysis

The mortality data were corrected by Abbott's formula (Abbott, 1925). Fifty percent lethal dose (LD50) and ninety percent lethal dose (LD90) were determined using the probit analysis by SPSS (Ver. 21) program. The effects of mortality of the *T. confusum* and *S. oryzae* were analyzed using a one-way analysis of variance (ANOVA) (P=0.05), followed by a comparison of means using Duncan's multiple range test (SPSS) (Genc and Soysal, 2018).

3. Results

Data obtained on the effect of different doses of deltamethrin biocidal on *T. confusum* and *S. oryzae* adults are given in Table 1.

There was a substantial increase in mortality in both species as the dose increased. At the highest dose of the biocidal, on day 1 (60 ml / 10 liter of water), 67.5% mortality was detected in *T. confusum* and 82.5% in *S. oryzae*. Day three showed 77.5% and 97.5% and day five showed 87.5% and 97.5% mortality in the same species, respectively. On day 5 of the study, the LD_{50} value for *T. confusum* was 32.8 and LD_{90} 61.3, and for *S. oryzae*, these values were 18.8 and 38.1 ml, respectively.

4. Discussion

Many typical insecticides such as flumethrin, lamda-cyhalothrin, permethrin, fenvalerate, deltamethrin, cyfluthrin, and some others had been successfully tested on their activity against a broad spectrum of insects, their activity against ticks was only occasionally a topic of investigations (Liebisch and Liebisch, 2008; Mehlhorn et al., 2008a, Mehlhorn et al., 2008b, Mehlhorn et al., 2010; Schmahl et al., 2008a, Schmahl et al., 2008b, Schmahl et al., 2009a). Deltamethrine, as biocidal, is used against urban pests such as mosquitoes, houseflies, cockroaches, and ticks (Cao et al., 2006; Barile et al., 2008; Romero et al., 2009; Jankov et al., 2012; Jahan and Shahid, 2013). Kemabonta et al. (2014) investigated the toxic and residual effects of Deltamethrin and Chlorpyrifos on *Blatella germanica* L. (Blattodea: Blatellidae). These insecticides were diluted in both aqueous and oil-based solvents and applied to adult insects in the study. In the acute toxicity study, it was concluded that Deltamethrin in an oil-based solution (100%-100%) was more effective than Deltamethrin in the form of a water-based solution (53.30%-43.30%) in both doses applied. In the acute toxicity study of Chlorpyrifos, all treatments on *B. germanica* caused a 100% mortality. As a result of the study, they reported that oil-based solutions of these insecticides were more effective than water-based solutions on *B. germanica* adults.

Table 1. Effects of different doses of deltamethrin on *Tribolium confusum* and *Sitophilus oryzae* adults

Pests	Dose (ml/10 lt water)	(Average % death±standard error)			LD_{50} -LD_{90} (ml) (for 5. day)
		1. day	3. day	5. day	
T. confusum	0	2.50± 2.50 C*e**	2.50± 2.50 De	2.50± 2.50 De	32.86 – 61.30
	15	20.00±5.77 Bc	20.00±5.77 Ce	22.50±4.78 Cd	
	30	27.50± 2.50 Bc	35.00± 2.88 Bc	47.50± 4.78 Bc	
	60	67.50± 4.78 Ab	77.50± 4.78 Ab	87.50± 4.78 Aab	
S. oryzae	0	2.50± 2.50 De	5.00± 2.88 Ce	5.00± 2.88 De	18.83 – 38.12
	15	27.50± 6.29 Cc	35.00± 8.66 Bc	45.00± 2.88 Cc	
	30	62.50± 4.78 Bb	82.50± 4.78 Ab	82.50± 4.78 Bb	
	60	82.50± 4.78 Aa	97.50± 2.50 Aa	97.50± 2.50 Aa	

*= upper case letters in the same column indicate intra-type dose comparison, **= lower case letters in the same column indicate all doses comparisons (P<0.05).

Shahi et al. (2008) investigated the biological activity of various active substances, including Deltmethrine, against *B. germanica* adults collected from the south of Iran. They reported that the recommended doses of Cypermethrin, deltamethrin, Lambda-cyhalothrin, diazinon, and permethrin+propoxur used in the study were found to have a maximum mortality rate of 20, 35, 90, 100, and 100%, respectively, in *B. germanica* adults.

Tribolium confusum is considered stored product. However, it can be found in the kitchen, dining rooms or shelves where the grains are stored in some areas. In such cases, people want to spray against this insect in their kitchens. Today, deltamethrin has products licensed as both biocidal and insecticide. Especially in recent years, various studies have been carried out to determine the biocidal effect of Deltamethrin against urban pests. Barile et al. (2008) conducted the effect of deltamethrin and piperonyl butoxide against *Cimex lactularus* L. (Hemiptera: Cimicidae), one of the most critical urban pests, was investigated. Their study reported that the application of deltamethrin alone was more effective than the application of piperonyl butoxide. Bergh and Quinn (2018) investigated whether deltamethrin-treated curtains could be effective against *Halyomorpha halys* (Stål, 1855) (Hemiptera: Pentatomidae). They stated that *H. halys* moved away from deltamethrin-treated nets in less than 5 minutes and later died, but they did not die completely. Researchers are reported that deltamethrin-treated nets can be an excellent inhibitor to keep *H. halys* away from humans. Papanikalou et al. (2021) studied the effects of 4 different insecticides (Thiamethoxam, Pirimiphos-Methyl, Alpha-Cypermethrin, and Deltamethrin) against *Ephestia kuehniella* (Zeller, 1879) (Lepidoptera: Pyralidae) and *Tribolium confusum* (Duv., 1863) (Coleoptera: Tenebrionidae). As a result these chemicals increased the mortality rates of *E. kuehniella,* and *T. confusum* compared to the control. Deltamethrine is authorized and can be utilized against urban pests in kitchens. Deltamethrine is not licensed as a biocidal against *T. confusum* and *S. oryzae* species.

5. Conclusion

As seen in our study, firstly, Exdel 5 SC biocidal was applied to a particular surface, after 24 hours, insects were brought into contact for 30 minutes, then they were transferred into clean containers, and mortality count was done in clean containers. In other words, this study investigated the residual effect of Exdel 5 SC biocidal on *T. confusum* and *S. oryzae* species. As a result of the study, it has been determined that this biocidal (Exdel 5 SC) has the potential to be used in human habitats against *T. confusum* and *S. oryzae* species.

Author Contributions

Concept: Ş.Y. (25%), A.K.A. (25%), İ.A. (25%) and İ.S. (25%), Design: Ş.Y. (25%), A.K.A. (25%), İ.A. (25%) and İ.S. (25%), Supervision: Ş.Y. (25%), A.K.A. (25%), İ.A. (25%) and İ.S. (25%), Data collection and/or processing: Ş.Y. (25%), A.K.A. (25%), İ.A. (25%) and İ.S. (25%), Data analysis and/or interpretation: Ş.Y. (25%), A.K.A. (25%), İ.A. (25%) and İ.S. (25%), Literature search: Ş.Y. (25%), A.K.A. (25%), İ.A. (25%) and İ.S. (25%), Writing: Ş.Y. (25%), A.K.A. (25%), İ.A. (25%) and İ.S. (25%), Critical review: Ş.Y. (25%), A.K.A. (25%), İ.A. (25%) and İ.S. (25%). Submission and revision. All authors reviewed and approved final version of the manuscript.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study due to the use of research material not included in the definition of experimental animals in the study (Animal experiment ethics committee regulation on working procedures and principles, Article 4-d).

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POTENTIAL NUTRITIVE VALUE AND ANTI-METHANOGENIC POTENTIAL OF POMEGRANATE PEEL FOR SHEEP

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Abstract: The aim of the current experiment was to determine potential nutritive value and anti-methanogenic potential of pomegranate peels obtained from 12 different pomegranate fruits in Turkiye. There are significant variations among pomegranate peel (PP) samples in terms of chemical composition, gas production, methane production, metabolisable energy (ME) and organic matter digestibility (OMD). The crude ash (CA), ether extract (EE), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF) and condensed tannin (CT) contents of PP samples ranged from 2.99 to 4.72%, 2.11 to 5.25 %, 2.53 and 6.36%, 20.79 to 27.29%, 11.71 and 17.96%, 0.79 to 3.39% respectively. Gas production and CH4 production of PP samples ranged from 41.07 to 57.22, 5.29 and 7.87 respectively whereas percentage of CH4 ranged from 12.51 and 14.03%. The ME and OMD of PP samples ranged from 7.92 to 10.84 MJ/kg and 54.43 to 68.95% respectively. Although PP samples studied in the current experiment have the low CH4 reduction potentials, the CP contents of PP samples are not sufficient to meet minimum level of CP requirement for sheep. Therefore protein supplementation is required for optimum rumen function and feed intake in ruminant animals when large amount of PP samples were included into ruminant diets. However before large implication, there is a need for *in vivo* experiment to test the mitigating effect of PP samples.

Keywords: Pomegranate peel, Chemical composition, Digestibility, Methane production

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1. Introduction

Pomegranate (*Punica gratum* L) is one of important edible fruit in most of parts of world (Seeram et al., 2006). After processing of pomegranate in juice industry, considerable amount of a by-product such as pulp or peel become available. Recently pomegranates by products have attracted great attention due to phenolic compounds (Jami et al., 2012). Although there are some researches about the use of pomegranate by products such as pulps or peels in ruminant diets to meet their nutrient requirements during the conventional feed shortage, otherwise being a wasted product (Shabtay et al., 2008, Canbolat et al., 2014; Kotsampasi et al., 2014, Omer et al., 2019; Moradi et al., 2020; Elmorsy et al., 2022) there is limited information about the nutritive value of pomegranate peel from different sources. Johnson and Johnson (1995) suggested that considerable amount of dietary energy lost occurs through enteric fermentation which is one of great contributors to greenhouse gasses. Recently some investigations showed that CT in feedstuffs had a mitigating effect on methane production (Bodas et al., 2012; Bhatta et al., 2013; Hixson et al., 2016). The pomegranate byproducts contain CT which may possible affect the enteric methane production when included into ruminant diets (Moradi et al., 2020). The chemical composition in company with in vitro gas production have been used to evaluate potential nutritive value and anti-methanogenic potential of previously uninvestigated feedstuffs (Kamalak and Canbolat, 2010; Jayanegara et al., 2011; Kaplan, 2011; Uslu, 2018). The aim of the current experiment was to determine potential nutritive value and anti-methanogenic potential of pomegranate peel obtained from 12 different pomegranate fruits in Turkiye.

2. Material and Methods

2.1. Pomegranate Peel Samples

In the current experiment pomegranate peel samples obtained from 12 different pomegranate fruits through removing the seed by hand from different parts of Turkiye and dried in shade until the constant weight (Table 1). The pomegranate peel samples were ground to pass 1 mm sieve for the subsequent analysis.

2.2. Chemical Analysis of Pomegranate Peel Samples

Pomegranate peel samples were analyzed separately in for DM, CA, EE contents of GP (AOAC, 1990). NDF and ADF contents of PP samples were analyzed with the method suggested by Van Soest (1991). The CT contents of PP samples were analysed with buthanol-HCl method (Makkar et al., 1995).

Table 1. Pomegranate peel samples obtained from 12 different pomegranate fruits

PPS	Site
PP1	Kahramanmaraş, Türkiye
PP2	Kahramanmaraş, Türkiye
PP3	Van, Türkiye
PP4	Kahramanmaraş, Türkiye
PP5	Kahramanmaraş, Türkiye
PP6	Kahramanmaraş, Türkiye
PP7	Kahramanmaraş, Türkiye
PP8	Şırnak, Türkiye
PP9	Şırnak, Türkiye
PP10	Şırnak, Türkiye
PP11	Konya, Türkiye
PP12	Antalya Türkiye

PPS= pomegranate peel samples.

2.3. Determination of Gas and Methane Production of Pomegranate Peel Samples

Approximately 200 mg PP samples were weighted into glass syringes in triplicate and subjected to fermentation with 40 ml of the buffered rumen fluid (1:2 V/V) in the bath set at 39 °C for 24 h incubation using in vitro gas production technique (Menke et al., 1979) to determine gas and methane production. The rumen fluid was obtained from slaughter house and filtered with four layered cheesecloth under flushing with CO_2 before use. The methane content (%) of gas was determined using infrared methane analyzer (Sensor Europe GmbH, Erkrath, Germany) (Goel et al., 2008). The methane production of pomegranate peel samples as mL was calculated as follows (equation 1);

CH_4 production (ml) = Total gas production (ml) × percentage of CH_4 (%) (1)

The ME and OMD of PP samples were calculated using the equations indicated by Menke and Steingass (1988) (equations 2 and 3);

ME (MJ/kg DM)= 0.72 + 0.15597GP + 0.068CP + 0.249EE (2)

OMD (%) = 15.38 + 0.8453GP + 0.595CP + 0.675CA (3)

GP: gas production of 200 mg sample at 24 h incubation (ml), CP: crude protein (%), EE: ether extract (%), CA: crude ash (%)

2.4. Statistical Analyses

Data obtained current study was subjected to one-way analysis of variance (ANOVA) to determine the effect of source on chemical composition, in vitro gas production, methane production, ME, OMD of PP samples. Differences ($P<0.05$) among the mean were determined with Tukey tests (Genc and Soysal, 2018).

3. Results and Discussion

The chemical compositions of PP samples are given in Table 2. There are significant variations among PP samples in terms of chemical composition. The crude ash contents of PP samples ranged from 2.99 in PP7 to 4.72% in PP3. The crude ash contents of PP samples are consistent of findings of Moradi et al. (2020) and Omer et al. (2019).

Ether extract contents of PP samples ranged from 2.11 to 5.25 % with highest being in PP12.

The crude ash contents of PP samples are in agreement with those reported by Moradi et al. (2020) and Omer et al. (2019).

Crude protein contents of PP samples ranged from 2.53 and 6.36 % with highest being in PP12 and lowest in PP2. Except for PP12, CP contents of PP samples are lower than those reported by Moradi et al. (2020) and Omer et al. (2019) but consistent with that by Mirzaei-Aghsaghali et al. (2011).

Van Soest (1994) suggested that crude protein contents of diets should be higher than the minimum level of 7-8% of DM for optimum rumen function and feed intake in ruminant animals. Therefore, the CP content of PP samples is not sufficient to meet minimum level of CP requirement.

Table 2. The chemical composition of pomegranate peel samples obtained from sources

Type	DM	CA	EE	CP	NDF	ADF	CT
PP1	89.90^{ab}	4.33^{b}	2.11^{c}	3.41^{cde}	23.52^{bcde}	15.25^{cd}	1.82^{bcd}
PP2	90.79^{a}	4.19^{b}	2.53^{c}	2.53^{e}	25.17^{a}	18.38^{a}	1.69^{bcd}
PP3	86.90^{cd}	4.72^{a}	2.90^{c}	4.54^{bc}	23.90^{bcd}	13.65^{f}	1.21^{cd}
PP4	88.67^{abc}	3.55^{de}	3.43^{abc}	3.84bcd	22.52^{cdefg}	11.71^{g}	3.39^{a}
PP5	88.80^{abc}	3.01^{f}	2.83^{c}	3.84^{bcd}	21.73^{efg}	11.92^{g}	2.50^{abc}
PP6	89.58^{ab}	3.40^{de}	3.56^{abc}	3.80^{bcd}	21.75^{defg}	13.65^{f}	1.68^{bcd}
PP7	85.82^{d}	2.99^{f}	4.81^{ab}	4.64^{b}	20.75^{g}	14.03^{ef}	1.05^{d}
PP8	90.04^{ab}	4.32^{b}	3.76^{abc}	3.45^{cde}	22.81^{cdefg}	15.03^{cde}	2.98^{ab}
PP9	87.91^{bcd}	3.99^{bc}	2.73^{c}	3.81^{bcd}	20.97^{fg}	14.30^{def}	2.54^{abc}
PP10	86.25^{d}	3.75^{cd}	3.16^{bc}	3.32^{de}	23.04^{bcdef}	15.46^{c}	0.79^{d}
PP11	88.86^{abc}	3.55^{de}	2.31^{c}	2.81^{de}	24.39^{bc}	16.84^{b}	1.98^{bcd}
PP12	85.95^{d}	3.38^{e}	5.25^{a}	6.36^{a}	27.29^{a}	17.96^{a}	1.73^{bcd}
SEM	0.600	0.100	0.508	0.328	0.601	0.305	0.395
Sig.	***	***	***	***	***	***	***

^{a,b}Column means with common superscripts do not differ at $P>0.05$. SEM= standard error mean.

Therefore protein supplementation is required for optimum rumen function and feed intake in ruminant animals when large amount of PP samples were included into ruminant diets.

The NDF contents of PP samples ranged from 20.79 to 27.29 with highest being in PP12 and lowest being in PP8 samples. The ADF contents of PP samples ranged from 11.71 and 17.96% with highest being in PP4 and 5 and lowest being in PP2 and 12 samples. NDF and ADF contents of PP samples were lower than those reported by Moradi et al. (2020) and Omer et al. (2019).

Condensed tannin contents of PP samples varied between 0.79 and 3.39 % with highest being in PP4 and lowest in PP10 samples. The effects of CT in diets depend on the amount and chemical structure (Makkar, 2003, Min et al., 2003, Mueller-Harvey, 2006, Goel et al., 2005, Tavendale et al., 2005; Galindo et al., 2008; McSweeney et al., 2011, Min et al., 2014). Low level of CT in diets may exert beneficial effect by preventing of protein from extensive degradation in the rumen but high CT level (6 and 10% of DM) reduces intake and growth of animal (Barry et al 1984). It is likely that all PP samples studied in the current experiment may have beneficial effect since their CT content lower than detrimental level. As can be seen from Table 2 there is a large variation among PP samples in terms of chemical composition. These variations among PP samples are likely related to type of pomegranate and growing site.

Gas production, CH_4 production, ME and OMD of PP samples are given in Table 3. There are significant variation among PP samples in terms of Gas production, CH_4 production, ME and OMD. Gas production and CH_4 production of PP samples ranged from 41.07 in PP2 to 57.22 in PP4, 5.29 in PP2 and 7.87 in PP4 respectively whereas percentage of CH_4 ranged from 12.51 in PP7 and 14.03% in PP5. The ME and OMD of PP samples ranged from 7.92 in PP2 to 10.84 MJ/kg in PP4 and 54.43 in PP2 to 68.95 % in PP4 ml respectively. Gas production and ME values of PP samples obtained in the current experiment were considerably higher than those of PP silage samples reported by Hatami et al. (2015) whereas OMD values was lower than those of PP silage samples reported by Hatami et al. (2015). On the other hand gas production and ME values of PP samples obtained in the current experiment was consistent with that reported by Mirzaei-Aghsaghali et al. (2011).

Lopez et al. (2010) the percentage of CH_4 of gas produced after 24 h incubation can be used to determine the CH_4 mitigation potential of feedstuffs. As can be seen from Table 3, PP samples studied in the current experiment have the low CH_4 reduction potential since the % of CH_4 in gas fell into range between 11 to 14% which is low potential group suggested by Lopez et al. (2010).

Table 3. The gas production, methane production, metabolisable energy and organic matter digestibility of pomegranate peel samples obtained from sources

Type	Gas	CH_4(ml)	CH_4 (%)	ME(MJ/kg/DM)	OMD (%)
PP1	47.36^{d}	6.55^{d}	13.85^{ab}	8.86^{bcd}	60.37^{bcde}
PP2	41.07^{d}	5.29^{a}	12.93^{ab}	7.92^{d}	54.43^{e}
PP3	50.10^{bcd}	6.73^{abcd}	13.44^{ab}	9.56^{abc}	63.62^{abcd}
PP4	57.22^{a}	7.87^{a}	13.78^{ab}	10.84^{a}	68.95^{a}
PP5	52.84^{ab}	7.41^{ab}	14.03^{a}	9.92^{abc}	64.36^{abc}
PP6	42.71^{cd}	5.95^{cd}	13.90^{ab}	8.52^{cd}	56.04^{de}
PP7	50.38^{abcd}	6.31^{bcd}	12.51^{b}	10.08^{ab}	62.75^{abcd}
PP8	51.74^{abc}	6.86^{abc}	13.27^{ab}	9.96^{abc}	64.09^{abcd}
PP9	52.84^{ab}	6.89^{abc}	13.01^{ab}	9.89^{abc}	65.01^{abc}
PP10	50.65^{abcd}	6.67^{abcd}	13.13^{ab}	9.63^{abc}	62.67^{abcde}
PP11	44.63^{bcd}	5.61^{cd}	12.60^{ab}	8.44^{cd}	57.17^{cde}
PP12	52.02^{abc}	6.72^{abcd}	12.92^{ab}	10.57^{a}	65.74^{ab}
SEM	2,800	0.415	0.420	0.436	2.371
Sig.	***	***	***	***	***

^{a,b}Column means with common superscripts do not differ at P>0.05. SEM= standard error mean.

4. Conclusion

There is considerable amount variation among PP samples in terms of chemical compositions, in vitro gas production, CH_4 production, ME and OMD. PP samples studied in the current experiment have the low CH_4 reduction potential whereas the CP content of PP samples is not sufficient to meet minimum level of CP requirement. Therefore protein supplementation is required for optimum rumen function and feed intake in ruminant animals when large amount of PP samples were included into ruminant diets. However before large implication, there is a need for *in vivo* experiment to test the mitigating effect of PP samples.

Author Contributions

Concept: İ.G (35%), A.K. (30%) AND Ç.Ö.Ö. (30%), Design: İ.G (35%), A.K. (30%) AND Ç.Ö.Ö. (30%), Supervision: İ.G (35%), A.K. (30%) AND Ç.Ö.Ö. (30%), Data collection and/or processing: İ.G (35%), A.K. (30%) AND Ç.Ö.Ö. (30%), Data analysis and/or interpretation:

İ.G (35%), A.K. (30%) AND Ç.Ö.Ö. (30%), Literature search: İ.G (35%), A.K. (30%) AND Ç.Ö.Ö. (30%), Writing: İ.G (35%), A.K. (30%) AND Ç.Ö.Ö. (30%), Critical review: İ.G (35%), A.K. (30%) AND Ç.Ö.Ö. (30%). Submission and revision. All authors reviewed and approved final version of the manuscript.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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DETERMINATION OF RHEOLOGICAL PROPERTIES OF ALTERNATIVE FLOUR SUBSTITUTED DOUGHS

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Abstract: All over the world, healthy foods, functional foods, diet foods and many similar terms are on the agenda. Consumers are offered different types of foods for a healthy diet. To this end, studies to improve the functional properties of bread have gained momentum. One of the ways to improve the functional properties of bread is to use flours with more functional properties than wheat flour. However, the effects of the added flours on the rheology of the dough are also different. The aim of this study is to determine the effects of flours (buckwheat (10-30%), carob (3, 6, 9, and 12), chickpea (10-50%), oat (10-50%), and barley (10-50%)) in different proportions added to bread flour on the rheological properties of the dough. The Mixolab® (Chopin) instrument was used to determine the rheological properties. A standard protocol for flour analysis was used for the analysis of bread flour and other flour mixtures. Various rheological and other dough properties were determined, such as water holding capacity, development time, stability, amylase activity, and degree of flour retrogradation. Using the obtained Mixolab® curve, C1 values for water retention and stability, C2 values for protein quality, C3 values for starch gelatinization, C4 values for amylase activity, and C5 values for degree of starch degradation were measured. C1 changed between 1.05 and 1.16 Nm, C2 between 0.33 and 0.58 Nm, C3 between 1.22 and 2.13 Nm, C4 between 0.96 and 1.98 Nm, and C5 between 0.95 and 2.81 Nm depending on the flour ratio and type used. As a result of the tests, it was determined that the most suitable flour for bread flour profile is 30% barley flour, 20% oat flour, 9% carob flour and 20% buckwheat flour, separately for each added flour.

Keywords: Buckwheat, Carob, Chickpea flour, Functional food, Rheology

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1. Introduction

Wheat is one of the cereals used for bread making. However, breads made from wheat flour dough are considered nutritionally inadequate (Sabanis and Tzia, 2009). Partial replacement of wheat flour with non-wheat flour increases the nutritional quality and flavour of baked goods. In recent years, consumers focus on a healthy diet and their demands for a variety of foods have necessitated the fortification of wheat flour with various components. To fortify wheat flour with important nutrients such as protein, fiber, calcium, iron, vitamin E, and polyphenols, fortification studies are being conducted with pseudocereals such as amaranth, quinoa, and buckwheat, (Rodriguez-Sandoval et al., 2012) and with legumes such as chickpeas, lentils, and soybeans (Sabanis and Tzia, 2009). In addition, in underdeveloped or developing countries where wheat cultivation is low, wheat flour is replaced by flour from other cereals such as maize and rice for economic reasons (Elkhalifa and El-Tinay, 2002).

For decades, oats were underutilized and usually grown for feed. However, oats are a good source of starch (55-59%), protein (15-26%), lipids (3-11%), and bioactive compounds such as β-glucan (3-8%) (Duque et al., 2020). Oats (*Avena nuda* L.) are considered a high-quality health food due to their cholesterol-lowering and antidiabetic properties (Ho et al., 2016; Martínez-Villaluenga and Peñas, 2017), and thus are widely used to make healthy snacks and even meals (Shukri et al., 2021). Barley flour is a good source of soluble fiber, arabinoxylans, and phenolic compounds (Moza and Gujral, 2017) and is nutritionally superior to wheat flour in terms of its bioactive composition (Moza and Gujral, 2018). Chickpea is the third most important legume in the world in terms of total production. Chickpea is a valuable source of protein, carbohydrates, fiber, and many essential vitamins and minerals (El-Sohaimy et al., 2020). Carob flour, obtained from the fruits of *Ceratonia siliqua* L. after removal of the seeds and subsequent roasting, has gained interest due to its remarkable composition, which has a preventive effect against various diseases. It is characterized by a high content of sugars, dietary fiber (~11%), minerals, and low protein (3-4%) and fat (0.2-0.8%) content, and a high content of phenolic compounds and vitamins (Papageorgiou et al., 2020). Buckwheat (*Fagopyrum esculentum*) is a pseudocereal that contains proteins with high biological value, as well as fiber, minerals, and flavonoids (Brites et al., 2019).

Replacing wheat flour with flour obtained from other raw materials changes both the rheological properties of the dough and the quality of the baked product. It is known that flours obtained from products other than wheat are not able to form the gluten network responsible for trapping the gas produced during fermentation (Arendt et al., 2002; Gallagher et al., 2003) Among different rheological techniques, Mixolab® has been likely used in many studies for probing dough behavior during processing conditions (Hadnadev et al., 2011). Mixolab® is a device developed by the technology company Chopin for measuring the rheological properties of doughs, which can be used to determine both the starch properties and the physical properties of doughs such as stability and strength. Mixolab® kneads the dough between two kneading arms and simultaneously subjects it to temperature changes. At the same time, the torque (Nm) achieved in the kneading arms is measured in real time (Anonymous, 2005).

There are a limited number of methods for determining the suitability of wheat flour for different end uses (Angioloni and Collar, 2011). The Mixolab® instrument is used to determine the rheological properties of gluten-free oat-based products (Duta and Culetu, 2015), to evaluate the quality of bread wheat (Şahin et al., 2014), and to determine the nutritional and rheological properties of grape seed flour (Mironeasa et al., 2012). Morover The Mixolab® instrument is used to determine the effects of additives on the kneading and baking properties of dough (Huang et al., 2010), the rheological properties of gluten-free flours from buckwheat and rice flour (Torbica et al., 2010), the rheological properties of the particle size of the dough in chestnut flour. In determining the effects on the behavior of flour (Moreira et al., 2010), determining the effects of hydrocolloids (Rosell et al., 2007), assessing the suitability of flour in terms of cake quality (Kahraman et al., 2008), determining the effects of enzymes on rheology (Bonet et al., 2006) was used.

An example of a Mixolab® diagram is shown in Figure 1. The diagram consists of five parts. In the first phase, the dough temperature is kept constant at 30°C and the dough's kneading properties, such as stability, elasticity and water retention, are measured. In this phase, the torque (Nm) exerted by the pallets increases until the deformation of the dough starts and reaches the maximum. The stability of the dough is expressed as the time (min) that the dough resistance remains above the torque of 1.1 ± 0.05 Nm (Anonymous, 2005). In terms of consistency and stability of the dough, it is desirable that the dough reaches a torque of 1.1 Nm and remains in this range for a long period of time. The longer this period, the stronger the protein structure (Rosell et al., 2007). In the second phase, the temperature is gradually increased from 30°C to 60°C. As the temperature increases, protein denaturation begins and the resistance of the dough to the pallets decreases. The α-angle determined in this phase indicates the slope of the curve drawn from the end of the C1 period at 30 °C to the end of the C2 period at 60°C and is used to evaluate the rate of protein attenuation by heat (Anonymous, 2005). In the third phase, when the temperature is increased to 90°C, there is an increase in consistency due to gelatinization of starch, which increases the torque acting on the pallets. At this stage, the starch molecules swell, absorb water, and displace the amylose molecules from the structure, causing an increase in viscosity (Kahraman et al., 2008). The β-angle determined at this stage indicates the slope of the curve between C2 and C3 and indicates the gelation rate (Anonymous, 2005). The steeper this angle is, the lower the dough viscosity, i.e., the harder the dough is, and in the opposite case, the dough is considered softer or liquid. In the fourth stage, where the temperature is kept constant, there is a decrease in consistency due to amylolytic activity. The γ-angle shows the slope of the curve between C3 and C4 and indicates the enzymatic degradation rate (Anonymous, 2005). Based on this value, an idea of the amylase activity of the product can be obtained. In the fifth stage, the temperature is gradually lowered from 90°C to 50°C. In this range, the gelling starch starts to liquefy and the retrogradation of the starch is detected here.

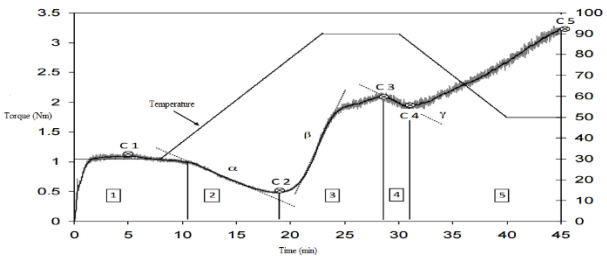

Figure 1. Typical Mixolab® diagram for dough (Anonymous, 2005).

There are studies in which the rheological properties of wheat flour, wholegrain wheat flour, buckwheat flour, amaranth flour, rice flour, corn flour, soybean flour (Hadnadev et al., 2011), sprouted whole wheat flour (Liu et al., 2017), oat dough (Huang et al., 2010), finger millet (Sharma et al., 2017), pearl millet-based composite flour (Awolu, 2017), millet flour (Maktouf et al., 2016), yellow pea flour (Dabija et al., 2017), *Cannabis sativa* L. skimmed flour (Apostol et al., 2015), grape epicarp flour (Oprea et al., 2018), cassava flour (Manano et al., 2021), milk thistle flour (Bojňanská et al., 2020), defatted mustard seeds (Mironeasa and Codină, 2017) triticale, rye, hulless barley, rice, maize (Sabovics et al., 2011), flaxseed flours (Codină et al., 2019), tomato seed flours (Mironeasa and Codină, 2019), legume flours (Bojňanská et al., 2021), quinoa and potato flours (Rodriguez-Sandoval et al., 2012), hulless barley flours (Moza and Gujral, 2018), grape seed flour (Mironeasa et al., 2012), hemp flour (Svec and Hruskova, 2015), chestnut flour (Moreira et al., 2012), *Agaricus bisporus* (Zhang et al., 2019) were determined using Mixolab®. However, there are no literature data on the determination of rheological

properties of chickpea flour and carob flour using Mixolab®. For barley, oat, and buckwheat flours, there are few data.

The aim of this study was to evaluate the effects of adding 5 flours (barley, oats, chickpea, buckwheat and carob) to wheat flour in different amounts on dough formation and rheological properties using the Mixolab® device (gluten, kneading index, amylase activity, retrogradation, water removal from the flour). In examining the literature data, studies on the determination of rheological properties using the Mixolab® are limited. In this study, alternative flours are added to wheat flour and their effects on the rheological behavior of the dough are investigated. In addition, some chemical compositions of the alternative flours are determined.

2. Material and Methods

2.1. Materials

Wheat flour (0.7 < % ash ≤ 0.8) was provided by Birsan Birlik Gıda San. A.Ş.in Tokat, Turkey. Alternative flours (carob, chickpea, oat, barley, and buckwheat) were purchased from a local company. All flours were stored in a cool and dry environment until use. All chemicals used in this study were from Sigma Chemical Company (MO, USA) or Merck KGaA (Germany) or Alfa Aesar GmbH and Co KG (Germany).

2.2. Methods

The dry matter of the flours were determined by the gravimetric method (AOAC, 2000). The total carbohydrate content of the samples was determined by the phenol-sulfuric acid method (Geater and Fehr, 2000). The micro-kjeldahl method was used to analyse the nitrogen content of the samples (AOAC, 2000). Crude protein content was estimated using a conversion factor of 5.75. Neutral detergent fiber were determined with the Ankom Fiber Analyser (Ankom Technology Corp., Macedon, NY, USA), following the Ankom Technology Method. Total fat were determined with Ankom Fat Analyser (Ankom Technology Corp., Macedon, NY, USA), following the Ankom Technology Method.

2.2.1. Determination of rheological behaviour using Mixolab®

Dough rheological investigations were performed by Mixolab® (Chopin, Tripette et Renaud, Paris, France), which simultaneously determinates dough characteristics during the process of mixing at a constant temperature, as well as during the period of constant heating and cooling. All the measurements were performed using the modified Mixolab® 'Chopin' protocol (ICC No. 173) which parameters are presented in Table 1.

The flour mixtures used in the study are as follows. 4 different ratios of barley flour, 10, 20, 30 and 50 percent, were added to wheat flour. 3 different ratios of buckwheat flour, 10, 20 and 30 percent, were added to wheat flour.

Table 1. Mixolab parameters used in modified Chopin + protocol

Settings	Values
Mixing speed	80 rpm
Target torque (For C1)	1100 Nm
Dough weight	75.0 g
Tank temperature	30°C
Temperature 1st step	30°C
Duration 1st step	8 min
1st temperature gradient	15 min 4°C / min
Temperature 2nd step	90°C
Duration 2nd step	7 min
2nd temperature gradient	10 min -4°C / min
Temperature 3rd step	50°C
Duration 3rd step	5 min
Total analysis time	45 min

4 different ratios of carob flour, 3, 6, 9 and 12 percent, were added to wheat flour. 4 different ratios of chickpea flour, 10, 20, 30 and 50 percent, were added to wheat flour. 4 different ratios of oat flour, 10, 20, 30 and 50 percent, were added to wheat flour. The amount of flour required for the analysis was calculated by the Mixolab® software based on the values entered for flour mixture moisture and water absorption.

2.2.2. Statistical analysis

SPSS statistical program (SPSS, Inc., Chicago, IL, USA) was used, variance analysis of the results (ANOVA) was performed and the differences between the groups were assessed statistically at a 95% confidence interval by the Duncan multiple comparison test.

3. Results and Discussion

Five different alternative flours were added to wheat flour. The composition of the added flours is shown in Table 2. Protein content varies between 4.6-22%, with chickpea flour having the highest protein content. The protein content of wheat and buckwheat is statistically similar (P=0.05). The fat content ranges from 0.5-7.5%. Oat flour has the highest fat content, while buckwheat flour has the lowest fat content. There is a statistically significant difference between the fat content of the flours (P<0.05). The fiber content is high in all added flours, with carob flour standing out as the flour with the highest fiber content at 40.1%. Chickpea and oat flour are characterized by their high protein and fat content, while chickpea and carob flour are characterized by their high fiber content. The high fiber content and composition are similar to those reported in the literature (Papaefstathiou et al., 2018; Papageorgiou et al., 2020).

The Mixolab® is an instrument that is used to determine the rheological quality of flour and to more accurately describe its behavior during bread making. The Mixolab® technique allows the complete characterization of the flours in terms of quality of proteins by determining their water absorption, stability, elasticity, and weakening properties; starch behavior

during gelatinization and retrogradation; consistency modification when adding additives and enzymatic activity of the proteases, amylases, etc. (Stoenescu et al., 2011). This device provides, a complex analysis of the rheological properties of wheat flour dough, considering dough behavior during mixing, protein coagulation, heating-up behavior at enzyme activity intensification, and starch gelatinization and retrogradation during the final cooling (Blandino et al., 2015). In this study, five alternative flours (barley, oats, carob, buckwheat, and chickpea) were mixed with wheat flour in different ratios, and the rheological properties of the obtained mixtures were determined separately using the Mixolab® instrument (Table 3).

Table 2. Chemical composition of flours

	Wheat Flour	Chickpea Flour	Buckwheat Flour	Carob Flour	Oat Flour	Barley Flour
Moisture (%)	13.20±0.24^{a}	9.60±0.30^{c}	8.50±0.40^{d}	6.90±0.56^{e}	10.20±0.60^{b}	10.55±1.02^{b}
Protein (%)	11.00±0.40^{b}	22.00±0.86^{a}	12.00±0.90^{b}	4.60±0.14^{e}	9.60±0.34^{c}	8.20±0.11^{d}
Fat (%)	0.50±0.10^{d}	4.80±0.62^{b}	0.50±0.10^{d}	0.70±0.15^{d}	7.50±0.20^{a}	1.50±0.05^{c}
T. Carbohydrate (%)	76.00±2.60^{b}	61.00±1.45^{c}	74.00±2.14^{b}	89.00±2.60^{a}	54.20±1.58^{d}	61.50±1.60^{c}
Fiber (g)	1.10±0.10^{e}	19.00±1.20^{b}	10.20±0.78^{d}	40.10±1.50^{a}	13.80±0.76^{c}	14.40±0.72^{c}

a,b= indicate statistical differences at the P<0.05 level of the samples in the same line.

Table 3. Mixolab analysis results of flours

		Development Time (min)	Stability Time (min)	C2 Torque (Nm)	C3 Torque (Nm)	C4 Torque (Nm)	C5 Torque (Nm)	α	β	γ	Water Absorption (%)
	0	4.70±0.1	8.97±0.1	0.49±0.1	1.78±0.1	1.80±0.1	2.66±0.1	-0.078	0.572	0.012	58.90±0.1
Barley Flour	10%	5.58±0.2	8.50±0.1	0.48±0.1	1.95±0.0	1.87±0.1	2.69±0.1	-0.098	0.398	-0.050	60.50±0.1
	20%	5.18±0.1	8.42±0.1	0.50±0.1	2.04±0.1	1.94±0.1	2.81±0.2	-0.090	0.414	-0.032	61.90±0.1
	30%	4.70±0.3	9.25±0.1	0.52±0.1	2.06±0.1	1.95±0.1	2.79±0.1	-0.080	0.574	-0.036	63.60±0.1
	50%	4.25±0.1	11.3±0.1	0.58±0.1	2.13±0.1	1.98±0.1	2.70±0.1	-0.066	0.704	-0.044	66.80±0.1
Buckwheat Flour	10%	5.48±0.2	6.42±0.1	0.41±0.1	1.45±0.1	1.27±0.1	1.72±0.1	-0.076	0.422	-0.028	67.50±0.1
	20%	5.32±0.1	5.48±0.1	0.39±0.1	1.23±0.1	0.98±0.1	1.24±0.0	-0.074	0.362	-0.026	76.50±0.1
	30%	6.78±0.1	5.65±0.1	0.40±0.1	1.05±0.1	0.76±0.1	0.95±0.1	-0.084	0.284	-0.040	85.40±0.1
Carob Flour	3%	4.18±0.0	6.22±0.1	0.34±0.1	1.79±0.1	1.72±0.1	2.28±0.1	-0.076	0.242	0.006	60.40±0.1
	6%	4.27±0.1	6.73±0.2	0.33±0.1	1.83±0.0	1.75±0.1	2.26±0.2	-0.080	0.356	-0.028	59.80±0.1
	9%	4.62±0.2	7.40±0.1	0.34±0.2	1.87±0.1	1.79±0.1	2.23±0.1	-0.076	0.402	-0.054	59.20±0.1
	12%	5.13±0.1	8.85±0.1	0.35±0.1	1.93±0.2	1.80±0.1	2.27±0.0	-0.082	0.532	-0.024	58.50±0.1
Chickpea Flour	10%	5.25±0.3	10.2±0.1	0.45±0.1	1.75±0.1	1.83±0.1	2.57±0.1	-0.096	0.562	0.014	60.50±0.1
	20%	5.12±0.1	8.90±0.1	0.39±0.1	1.70±0.1	1.77±0.0	2.35±0.1	-0.092	0.530	0.020	61.40±0.1
	30%	5.05±0.1	5.93±0.1	0.34±0.1	1.55±0.1	1.66±0.2	2.08±0.1	-0.088	0.348	-0.014	61.80±0.1
	50%	5.00±0.2	5.33±0.1	0.33±0.1	1.22±0.1	1.55±0.1	2.05±0.1	-0.076	0.170	-0.014	60.30±0.1
Oat Flour	10%	4.38±0.0	9.23±0.1	0.44±	1.81±0.1	1.74±0.1	2.43±0.0	-0.080	0.086	-0.018	60.70±0.1
	20%	5.03±0.2	9.10±0.1	0.42±	1.77±0.3	1.72±0.1	2.42±0.1	-0.084	0.078	0.012	60.70±0.1
	30%	5.27±0.1	9.33±0.1	0.40±	1.79±0.1	1.74±0.1	2.35±0.2	-0.092	0.076	-0.012	60.50±0.1
	50%	4.08±0.1	9.37±0.1	0.39±	1.70±0.1	1.63±0.1	2.19±0.1	-0.110	0.222	-0.006	61.80±0.1

Mixolab® torque curves of barley flour mixes are shown in Figure 2, Mixolab® torque curves of buckwheat flour mixes are shown in Figure 3, Mixolab® torque curves of carob flour are in Figure 4, Mixolab® torque curves of chickpea flour mixes are shown in Figure 5, and Mixolab® torque curves of oat flour mixes are shown in Figure 6. The first part of a Mixolab® curve describes dough development time, water absorption, stability and C2 value. Dough development time is the time required to achieve appropriate consistency at 1.1 Nm torque. The dough development times of the samples are shown in Table 3. Barley flour was added to wheat flour in four different amounts (10, 20, 30 and 50). The dough development time, which was 4.70 for the control flour, increased with the addition of barley flour. As the amount of barley flour added increased, the dough development time began to decrease. After the addition of 30% barley flour, the dough development time falls below that of the control flour. The addition of buckwheat and chickpea flour increased the development time at all rates. The addition of carob flour gradually increased the dough development time. The addition of oat flour up to 30% increased the dough development time, which tended to decrease at higher addition rates. It is believed that the decrease in dough development times is due to the free phenolic substances in the alternative flours. This can be explained by the ability of the phenolic compounds to react with the sulfhydryl groups of the gluten protein or to increase the rate of sulfhydryl-disulfide exchanges in the protein. For example, the addition of phenolic acids to dough reduces mixing time, tolerance, elasticity, and bread volume (Han and Koh, 2011).

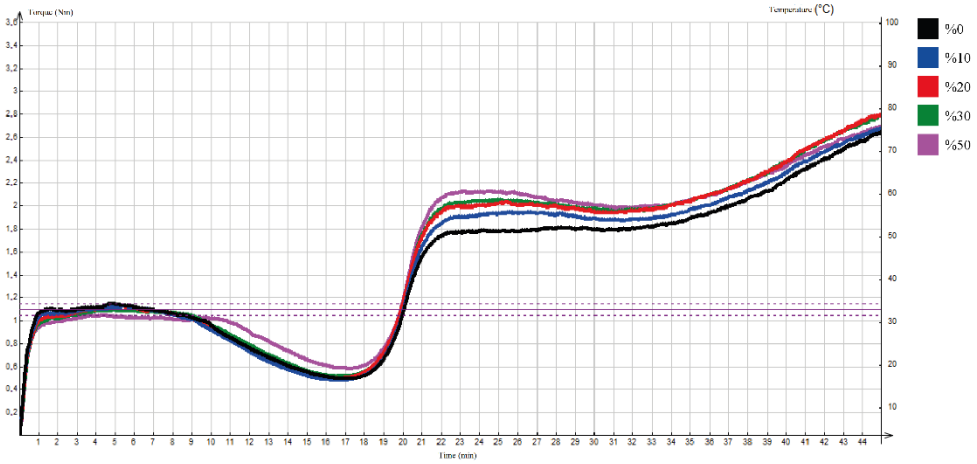

Figure 2. Mixolab torque curves of flours with barley flour (Nm).

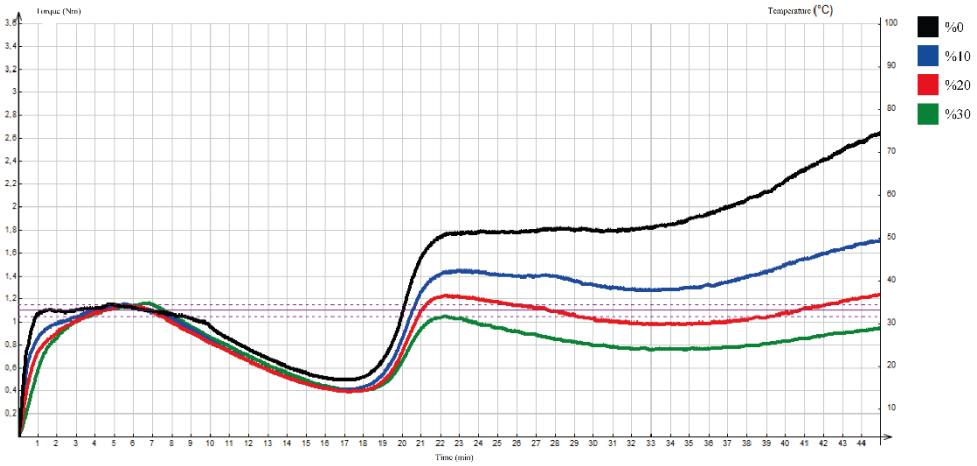

Figure 3. Mixolab torque curves of flours with buckwheat flour (Nm).

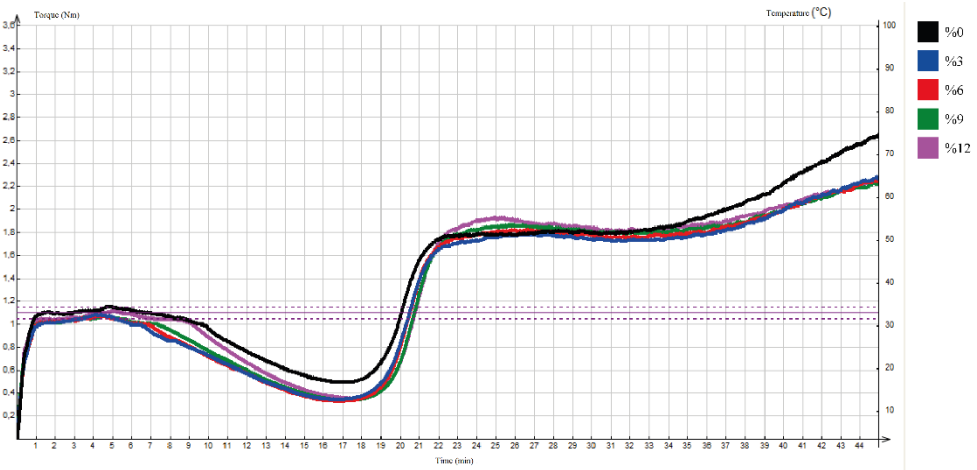

Figure 4. Mixolab torque curves of flours with carob flour (Nm).

The development time of buckwheat, barley and oat flour was higher than reported in the literature (Hadnadev et al., 2011; Huang et al., 2010; Sabovics et al., 2011).

The term expressed as "water holding capacity" in Table 3 is the amount of water taken by the samples until they reach a torque of 1.1 Nm during kneading (Şahin et al., 2014). In Mixolab® charts, C1 (initial maximum consistency (Nm)) is used to determine the water absorption; torque at the end of the holding time at 30 °C (Nm) capacity. The water-holding capacity of the control flour, which was 58.9%, increased for all alternative flour additives. The highest increase, 85.4%, was measured in the mix with 30% buckwheat. The development time refers to the time elapsed until the dough first begins to form, and the composition of the dough affects it (Rosell et al., 2007).

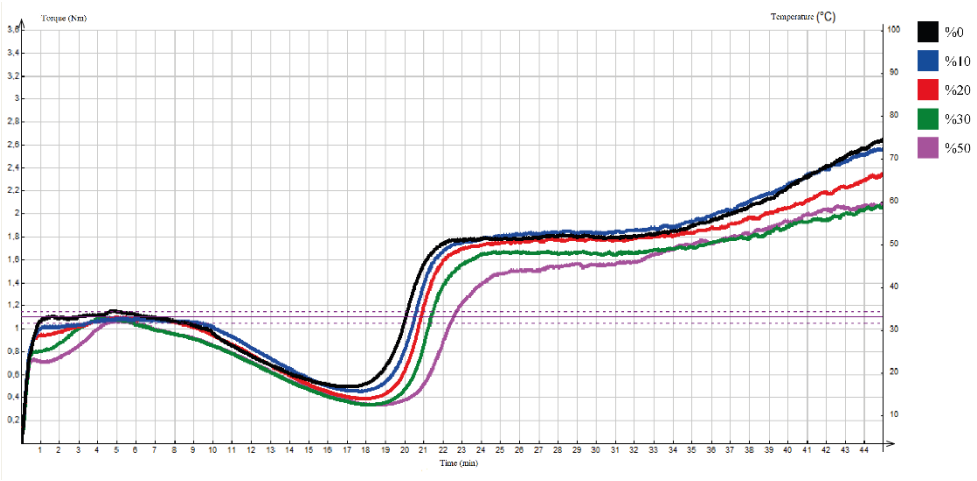

Figure 5. Mixolab torque curves of flours with chickpea flour (Nm).

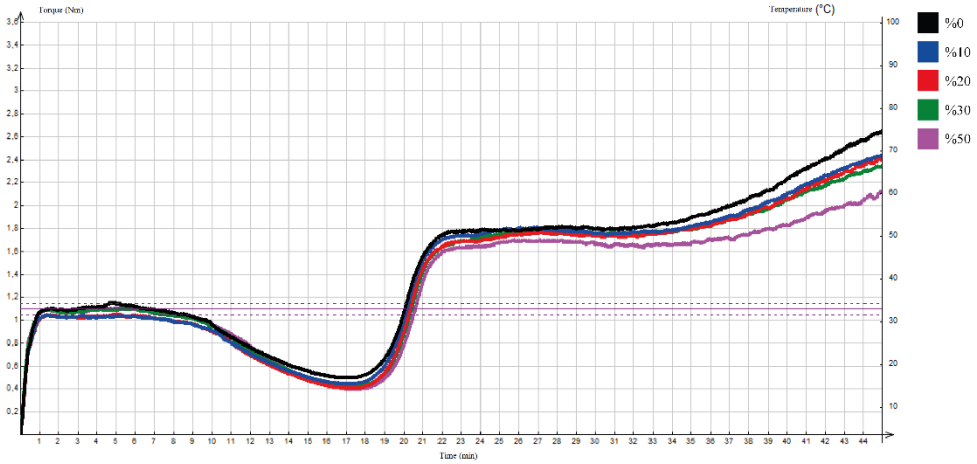

Figure 6. Mixolab torque curves of flours with oat flour (Nm).

The fact that the fiber content of all added flours is higher than that of wheat flour is thought to be the main reason for the increase in water-holding capacity.

Dough stability is defined as the time the dough that stands the applied force after reaching its maximum torque. Also, it refers to the resistance of dough to kneading (Rosell et al., 2007). Dough stability (min) or time until the loss of consistency is lower than 11% of the maximum consistency reached during the mixing. The dough stability time of the samples are shown in Table 3 and Figure 2-6. The stability value was found to be 8.97 min for the control flour. Barley and oat flours were found to increase dough stability time, while buckwheat, carob, and chickpea flours decreased stability time. This decrease is probably due to the gummy substances (locust bean gum, natural hydrocolloids, etc.) in buckwheat, carob and chickpea flours.

Protein destabilization occurs when the temperature rises from 30°C to 90°C during Mixolab® analysis. This part, which is expressed by the C2 value, is related to the protein content of the dough (Rosell et al., 2007). The C2 value is a data showing the degree of weakening of the proteins in the dough during kneading. In other words, C2 (minimum consistency (Nm) can be explained as the minimum value of torque produced by the dough passage while being subjected to mechanical and thermal constraints. In this region, the torque value decreases with kneading and heating. The value of C2 torque was determined to be 0.49 Nm for the control flour. Except for the addition of barley flour, all flours decreased the C2 torque value. Depending on the amount added, the C2 torque value increased up to 0.58 Nm when barley flour was added. The highest decrease was observed with carob flour, and it was found to decrease to 0.33 Nm on average. It is assumed that the gum substances in the structure of carob flour cause this situation. It is desirable that the proteins in the dough do not weaken during kneading and maintain the network structure (Cappelli et al., 2020).

The second part of the Mixolab® curve contains the C3, C4 and C5 values, α-, β- and γ-angles. The C3 value is defined as the resistance of the dough to the kneading arms together with the gelatinization of the starch. In other words, C3 (peak torque (Nm) can be explained as the maximum torque produced during the heating stage. The C3 torque value, which was 1.78 Nm for wheat flour, increased to 2.13 Nm with the addition of barley flour and to 1.93 Nm with the addition of carob flour. While the addition of buckwheat, chickpea and oat flours decreased the C3 torque value, the largest decrease was

observed with the addition of buckwheat flour. It is believed that the differences between the C3 torque values are due to the different carbohydrate composition of the flours and the gummy substances in their structure. Buckwheat, chickpea and oat flours are believed to reduce retrogradation of starch in dough (Figure 3, 5, 6), while barley and carob flours increase retrogradation (Figure 2, 4).

The C4 region, where the temperature is constant at 90°C, indicates the region where the formed starch gel remains stable. Another definition of C4 (minimum torque (Nm)) is the minimum torque reached during cooling to 50°C. In addition, the C4 region is also accepted as the amylolytic activity value. While the addition of barley flour increased the C4 torque value, there was a decrease in all other flours. As with the C3 torque value, the greatest decrease was observed with the addition of buckwheat flour (0.76 Nm). The difference between C3 and C4 is related to the stability of the starch gel during heating and its amylase activity (Şahin et al., 2014). C3 and C4 are also known as breakdown torque (Nm). The C3-C4 difference, which is 0.02 for control, The C3-C4 difference, which was 0.02 in the control, increased for all flours except chickpea flour with the different flour additives. This increase was highest in the blends with buckwheat, 0.30. The data with the closest C3-C4 difference from the control were obtained for the oat flour mixes.

The retrogradation of starch, which has an important place in the staling mechanism of bread, is associated with the C5 value (Şahin et al., 2014). C5 (final torque (Nm)) is the torque after cooling at 50°C. The viscosity of the dough increased due to the retrogradation of starch as the temperature decreased from 90°C to 50°C. The C5 torque value, which was 2.66 Nm for the control flour, increased to 2.81 Nm with the addition of barley flour. The fastest decrease was observed with buckwheat, and the C5 torque value decreased to 0.95 Nm with the addition of 30% buckwheat. A decrease in C5 torque was also observed with the addition of carob, oat and chickpea flours. Looking at the C5 torque values, the blend with buckwheat is the one where the viscosity value decreases the fastest, and the mixes with barley, chickpeas and oats are close in their values.

The angles between ascending and descending curves α, β and γ (Nm/min) are defined as protein network weakening, gelatinization and cooking stability rate, respectively (Hadnadev et al., 2011). Angle α gives the slope of the curve drawn from the end of period C1 to the end of period C2. This value is used to evaluate the rate of protein attenuation due to heat. The decrease in the angle is an indication that the dough has started to weaken and the structure will begin to deteriorate faster. The mixes closest to the control sample in terms of α-angle are those with 30% barley, 10% buckwheat, 3, 6, 9% carob, and 50% chickpea flour. There is an increase in α-angle with increasing addition of oatmeal to the mixtures and a decrease with the addition of barley flour. The addition of carob flour to the mixture did not result in a significant change in the α-angle. The β angle is determined, where the temperature is increased to 90°C. This angle value gives the slope of the curve between C2 and C3 and shows the gelation rate. The steeper this angle, the higher the dough viscosity. In other words, the closer the slope is to 1, the higher the dough viscosity resulting the formation of harder dough. The β-angle was determined to be 0.572 for the control flour. It was found that the addition of barley, carob, and oat flours to the mixture caused a gradual increase in the β-angle, while chickpea and buckwheat flours gradually decreased the β-angle. Angle γ shows the slope of the curve between C3 and C4 and gives the rate of enzymatic degradation and gives an idea about the amylase activity of the product. While the γ-angle in the control flour was measured to be 0.012, the values closest to the control flour were determined in the mixtures with 20% oat flour and 10% chickpea flour. Mixtures with the addition of barley and buckwheat are the flours that reduce the γ-angle the most.

4. Conclusion

The addition of alternative flours to wheat flour is becoming more popular by the day due to the nutritional benefits of various plant sources. However, these raw materials other than wheat flour often have negative effects on the technological properties of flour, dough and final product. The results of this study show that the addition of alternative flours to wheat flour at different rates and their effects on rheological properties. The analysis of all rheological data shows that the addition of alternative vegetable flour has an influence on the rheological properties of the dough. Considering all the data, the amounts of addition that would improve or not affect the rheological properties when added to wheat flour were determined to be 30% for barley flour, 20% for buckwheat flour, 6% for carob flour, 20% for chickpea flour, and 30% for oat flour. All added flours increase the water-holding capacity of the dough. While the addition of barley, oat, and chickpea flours increased the dough stability time, buckwheat and carob flours reduced the C2 torque value, which determines the degree of protein weakening, more than other flours. The C3 torque value, an indicator of the degree of gelatinization of starch, decreased more in buckwheat and carob flours with higher content of gummy substances and increased in barley flours. Considering the degree of retrogradation ((C5-C4)/C5*100), it was found that the addition of barley and oat flour reduced the degree of retrogradation of the control flour to a minimum, while the decrease was greatest for buckwheat, carob and chickpea flours. Due to the growing world population and the resulting insufficient raw material resources, the search for alternative products continues day by day. Wheat flour is mixed with various flours to improve its nutritional and technological properties. In this context, it is necessary to

study all the positive and negative effects of adding alternative herbal flours to wheat flour. This study represents an innovation in the literature by determining the effects of the addition of carob and chickpea to wheat flour on rheological properties. It is intended to support the literature in determining the effects of the addition of barley, oats, and buckwheat and to contribute to product manufacturing processes such as bread with the rheological data obtained.

Author Contributions

Concept: A.C. (50%) and T.Y. (50%), Design: A.C. (50%) and T.Y. (50%), Supervision: A.C. (50%) and T.Y. (50%), Data collection and/or processing: A.C. (50%) and T.Y. (50%), Data analysis and/or interpretation: A.C. (50%) and T.Y. (50%), Literature search: A.C. (50%) and T.Y. (50%), Writing: A.C. (50%) and T.Y. (50%), Critical review: A.C. (50%) and T.Y. (50%). Submission and revision. All authors reviewed and approved final version of the manuscript.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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THE RESISTANCE OF SOME TOMATO LINES AGAINST TOMATO SPOTTED WILD VIRUS, TOMATO YELLOW LEAF CURL VIRUS AND ROOT KNOT NEMATODES (*Meloidogyne* spp.) BY MOLECULAR MARKERS

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Abstract: In this study, it was attempted to determine the resistance of different tomato lines to tomato spotted wilt virus (TSWV), tomato yellow leaf curl virus (TYLCV), and root-origin nematodes (*Meloidogyne* spp.) using molecular DNA markers. For this purpose, out of 96 different tomato lines to be tested, Sw5-2 for resistance to tomato spotted wilt ripening virus (TSWV), Ty3P6-25 for resistance to tomato yellow leaf curl virus (TYLCV) and the DNA marker Mi23, which determines resistance to root-knot nematodes (*Meloidogyne* spp.), were used by PCR. In this study, Ty3P6-25, the marker that determines resistance to TYLCV, was found to be susceptible (rr) in 34 tomato lines, heterozygous resistant (Rr) in 56 tomato lines, and homozygous resistant (RR) in 4 tomato lines. In addition, no results were obtained in 2 tomato lines. Marker Sw5-2, which determines resistance to TSWV, was found to be homozygous susceptible (rr) in 57 tomato lines, heterozygous resistant (Rr) in 27 tomato lines, and homozygous resistant (RR) in 5 tomato lines. No results were obtained in 7 tomato lines. For the marker (Mi23) that determines resistance to root-knot nematodes (*Meloidogyne* spp.), 44 tomato lines were found to be homozygous susceptible (rr), 11 tomato lines were heterozygous resistant (Rr), and 35 tomato lines were homozygous resistant. No results were obtained for 6 tomato lines. It was concluded that the DNA molecular markers used are useful in determining resistance responses to TSWV, TYLCV and *Meloidogyne* spp. in tomato and can give reproducible and reliable results in a short time.

Keywords: Root-Knot nematode, Tomato yellow leaf curl virus, Tomato spotted wilt virus, PCR, Molecular marker

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1. Introduction

Tomato, which is one of the health-promoting foods with its various minerals and vitamins, is one of the most important vegetables grown and popularly consumed all over the world. It is an annual edible fruit (*Solanum lycopersicum* L.) belonging to the *Solanum* genus of the Solanaceae family. The homeland of the tomato is South American countries such as Peru and Ecuador. The tomato was first cultivated by the Mexicans and spread from the Americas to Europe and other parts of the world after the discovery of the New World. In our country, it was cultivated in Adana in the early 1900s (Oğuz, 2010). Nowadays, it is a crop of great economic importance because of its high productivity in a short growing season and its production and consumption quantities increase every year. Our country ranks fourth after China, India and the United States with a tomato production of 13.2 million tons. Tomato production in Turkey was 10.05 million tons in total on an area of 1.79 million decares in 2010, while the production has increased by about 35% resulted with 13.2 million tons (TUIK, 2020).

In the regions where tomatoes are grown, phytopathological and entomological problems occur due to cultivation errors and the difficulties resulting from the inability to adjust the humidity, temperature and ventilation of the greenhouse conditions. Fungal and viral diseases occupy an important place in phytopathological problems. One of the most important disease factors severely affecting tomato production in Turkey as well as worldwide is viruses (Yılmaz and Sipahioğlu, 2020). The most common viral diseases of tomato growing areas are Tomato Yellow Leaf Curl Virus (TYLCV), Potato Y Virus (PVY), Tomato Mosaic Virus (ToMV), Tomato Spotted Wilt Virus (TSWV), Tomato Ring Spot Virus (ToRSV) (Wani et al., 2010), Tomato Brown Rugose Fruit Virus (TBRFV) (Salem et al., 2016; Luria et al., 2017). Root knot nematodes as plant endoparasites of the *Meloidogyne* species and cause great harm on tomato worldwide. *Meloidogyne* species include *M. incognita, M. javanica M. hapla*, and *M. enterolobii* (syn. *M. mayaguensis*) (Kiewnick

et al., 2009), but *M. incognita* is the damaging oppression species (Agrios, 2005).

Systematic breeding studies in tomato have started in 1930s. New tomato cultivars were developed in the 1950s due to increased market demands for tomatoes (Crill et al., 1971; Gardner, 1982; Gardner, 2006). With the development of molecular markers and genetic mapping methods, the opportunity to develop higher quality tomato varieties has emerged. Genetic markers and maps were firstly developed and used in tomato crop (Tanksley, 1983; Tanksley et al., 1992). Marker assisted selection (MAS) technology using molecular markers have a powerful method to solve some of the challenges associated with phenotypic selection (PS). Molecular marker is a molecular technique that can be used successfully to screen for resistance gene loci related to disease resistance in an organism. Randomly amplified polymorphic DNA (RAPD), simple sequence repeat (SSR; microsatellite), amplified fragment length polymorphism (AFLP), sequence characterized amplified region (SCAR), cleaved amplified polymorphic sequence (CAPS), single nucleotide polymorphism (SNP), and insertion-deletion (InDel) were commonly used PCR-based methods for tomato breeding purposes (Floolad and Panthee, 2012).

Genetic markers are used in both public and private sector breeding programs to determine the resistance characteristics of tomato plants against plant diseases. The MAS method is faster than phenotypic selection. It is also a cheaper and more effective method for determining some disease resistance traits. However, all known molecular markers are not sufficient for tomato breeding programs. Since most commercial tomato cultivars are developed by the private sector, they often develop their own specific molecular markers adapted to their plant germplasm pools. Studies are needed to identify allele-specific molecular markers to improve the use of MAS method in tomato breeding programs (Floolad and Panthee, 2012).

The aim of this study is to screen some tomato lines developed by our breeding program in terms of virus and nematode-resistance using DNA molecular marker specific for *TYLCV*, *TSWV* and root-not nematodes.

2. Materials and Methods

Total genomic DNA from the young leaves of 96 tomato lines (F4) from our breeding program was extracted using 3% CTAB (cetyl-triethyl-ammonium-bromide)-extraction buffer (Doyle and Doyle (1990). The DNA samples were kept at 4˚C for further use. The concentration of total genomic DNA samples from 96 tomato lines were set by a spectrophotometer (Thermo ND-1000) at 100 ng/ml. The primers used for TSWV, TYLCV and root-not nematodes are detailed in Table 1.

PCR reactions for *TSWV* and for *TYLCV* were performed in a total volume of 25 µl; of which 2.2 µl DNA, 2.5 µl 10X Dream *Taq* Buffer (containing 20 mM $MgCl_2$), 4 µl dNTP (each dNTP 2.5 mM), 0.25 µl *Taq* (5U μl^{-1} *Taq* DNA polymerase), 1 µl forward and reverse primers and nucleotide-free de-H_2O for a total volume of 25 µl.

PCR cycle parameters for *TSWV* and *TYLCV* were examined as follows: initial denaturation at 94˚C for 3 minutes, denaturation for 35 cycles at 94˚C for 30 seconds, binding for 1 minute at 53˚C, elongation for 1 minute at 72˚C, and an additional 10 minutes final extension at 72˚C.

PCR reaction parameters of the PCR for *Mi*23: 3 minutes of initial denaturation at 94˚C, 35 cycles of denaturation at 94˚C for 30 seconds, binding for 1 minute at 57˚C, 1 minute of elongation at 72˚C, and an additional 10-minute final elongation at 72˚C.

The PCR products were separated by gel electrophoresis in 1.5% agarose gel with 0.5 TAE (Tris-acetate-EDTA) buffer. The agarose gel was stained in the ethidium bromide (0.5 mg/ml) for 10 minutes. The PCR results were visualized and recorded by using an ultraviolet (UV) light imaging system (Vilber Lourmat, France).

Table 1. Molecular marker and primer sequences used in study

Gene	Marker	Primer Sequences 5'……3'	References
Sw5	Sw5-2	F:AATTAGGTTCTTGAAGCCCATCT R:TTCCGCATCAGCCAATAGTGT	Dianese et al.,2010
Ty	Ty3 P6-25	F:GGTAGTGGAAATGATGCTGCTC R:GCTCTGCCTATTGTCCCATATATAACC	Jensen et al.,2007
Mi	Mi23	F:TGGAAAAATGTTGAATTTCTTTTG R:GCATACTATATGGCTTGTTTACCC	Seah et al., 2007

3. Results

The result of PCR studies with primers *Ty3*P6-25, *Sw5*-2 and *Mi*23: For *Ty3*P6-25, homozygous resistant (*RR*) genotypes produced a single band of 630 bp, while heterozygous (*Rr*) had two bands of 630 and 320 bp, and susceptible genotypes (*rr*) were found to have a single band of 320 bp (Table 2, Figure 1).

In *Sw*5-2, homozygous resistant (*RR*) genotypes showed a single band of 574 bp, while heterozygous (*Rr*) showed two bands of 574 and 470 bp and a single band of 470 bp was found in susceptible genotypes (*rr*) (Figure 2, Table 2).

On the other hand, in *Mi*23 gene, homozygous resistant (*RR*) genotypes formed a single band with a length of 380 bp, while in heterozygous genotypes (*Rr*) it was displayed as two bands of 380 and 430 bp, while in susceptible genotypes (*rr*) a single band of 430 bp was found (Figure 3, Table 2).

Table 2. Genotypic features of tomato lines analysed by PCR

Tomato Line #	*Ty3*P6	*Sw5*-2	*Mi*23	Tomato Line #	*Ty3*P6	*Sw5*-2	*Mi*23
1	*rr*	-	*rr*	49	*Rr*	*rr*	*rr*
2	-	-	*rr*	50	*Rr*	*Rr*	*rr*
3	*rr*	*rr*	*RR*	51	*Rr*	*rr*	*RR*
4	*rr*	*rr*	*RR*	52	*Rr*	*rr*	*RR*
5	*Rr*	*rr*	*rr*	53	*rr*	-	*rr*
6	*Rr*	*rr*	*rr*	54	*rr*	*rr*	*rr*
7	*Rr*	*rr*	*RR*	55	*Rr*	*rr*	*RR*
8	*Rr*	*rr*	*RR*	56	*Rr*	*rr*	*RR*
9	*Rr*	*Rr*	-	57	*Rr*	*rr*	*rr*
10	*Rr*	*Rr*	*rr*	58	*Rr*	*Rr*	*rr*
11	*Rr*	*Rr*	*rr*	59	*Rr*	*rr*	*RR*
12	*Rr*	*rr*	*rr*	60	*rr*	*Rr*	*RR*
13	*rr*	*rr*	*RR*	61	*rr*	*Rr*	*rr*
14	*rr*	*rr*	*RR*	62	*rr*	*Rr*	*rr*
15	*RR*	*Rr*	*Rr*	63	*Rr*	*Rr*	*rr*
16	*RR*	*rr*	-	64	*Rr*	*RR*	*rr*
17	*rr*	*rr*	*RR*	65	*Rr*	*Rr*	*rr*
18	*Rr*	*rr*	-	66	*Rr*	*rr*	*rr*
19	*rr*	*Rr*	*RR*	67	*rr*	*rr*	*RR*
20	*Rr*	*rr*	*Rr*	68	*rr*	*rr*	*RR*
21	*RR*	*Rr*	*rr*	69	*Rr*	*rr*	*RR*
22	*rr*	*Rr*	*rr*	70	*Rr*	*rr*	*RR*
23	*rr*	*Rr*	*RR*	71	*Rr*	*Rr*	*RR*
24	*rr*	*rr*	*RR*	72	*Rr*	*rr*	*Rr*
25	*Rr*	*Rr*	*rr*	73	*Rr*	*rr*	*RR*
26	*rr*	*Rr*	*rr*	74	*Rr*	*rr*	*RR*
27	*rr*	*rr*	*rr*	75	*Rr*	*rr*	*rr*
28	*Rr*	*rr*	*rr*	76	*rr*	*Rr*	*rr*
29	*Rr*	*rr*	*rr*	77	*rr*	*Rr*	*Rr*
30	*Rr*	*rr*	*rr*	78	*Rr*	*rr*	*Rr*
31	*Rr*	*rr*	*rr*	79	*Rr*	*Rr*	*rr*
32	*Rr*	*rr*	*rr*	80	*Rr*	*rr*	*rr*
33	*Rr*	*rr*	-	81	*rr*	*rr*	*Rr*
34	*Rr*	*rr*	*rr*	82	*Rr*	*rr*	*Rr*
35	*Rr*	*rr*	-	83	*rr*	*rr*	*Rr*
36	*Rr*	*rr*	*RR*	84	*rr*	*rr*	*Rr*
37	*rr*	*Rr*	*RR*	85	*rr*	*RR*	*rr*
38	*rr*	*rr*	*RR*	86	*Rr*	*Rr*	*rr*
39	*rr*	*rr*	*RR*	87	*rr*	*Rr*	*rr*
40	*rr*	*Rr*	*RR*	88	*Rr*	*Rr*	*rr*
41	*rr*	*rr*	-	89	*Rr*	*rr*	*RR*
42	*rr*	*rr*	*RR*	90	*Rr*	*RR*	*RR*
43	*rr*	*rr*	*RR*	91	*Rr*	*RR*	*Rr*
44	*Rr*	*rr*	*RR*	92	*Rr*	*rr*	*Rr*
45	*Rr*	*rr*	*rr*	93	*Rr*	-	*rr*
46	*RR*	*rr*	*rr*	94	*RR*	-	*rr*
47	*Rr*	*Rr*	*rr*	95	*Rr*	*Rr*	*RR*
48	*rr*	*rr*	*rr*	96	*rr*	*RR*	*RR*

RR= homozygote resistance, *Rr*= heterozygote, *rr*= sensitive genotypes, -= not detected.

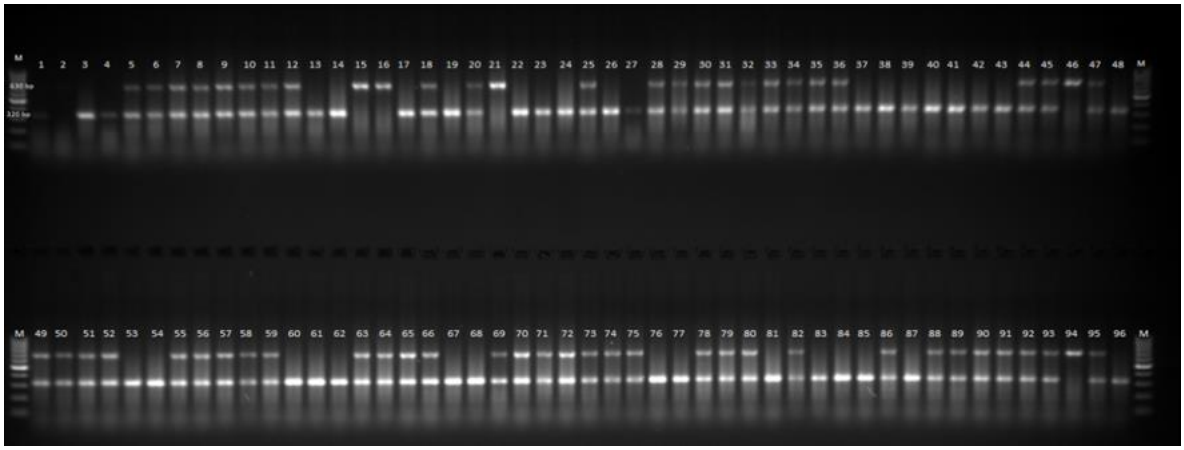

Figure 1. PCR reactions of different tomato lines for Ty3P6-25. M= 100 bp DNA marker, 1-96= tomato lines.

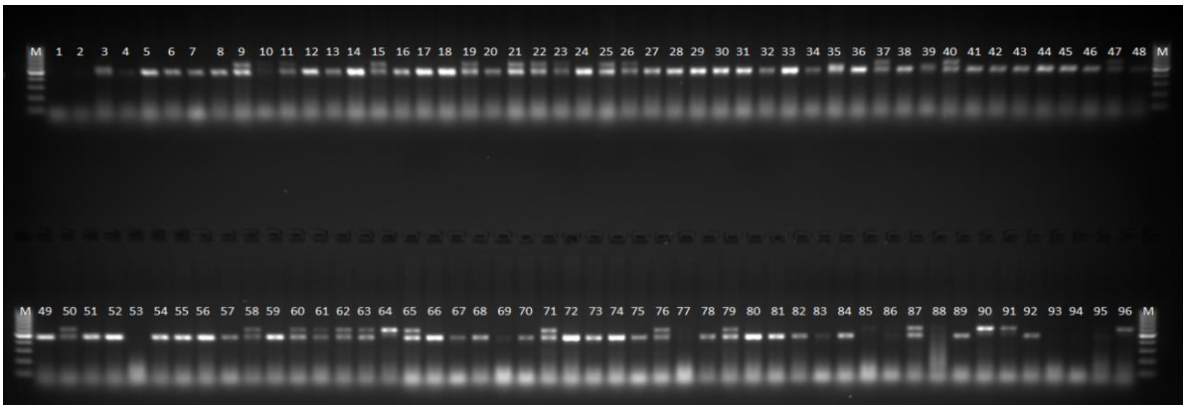

Figure 2. PCR reactions of different tomato lines for Sw5-2. M= 100 bp DNA marker, 1-96= tomato lines.

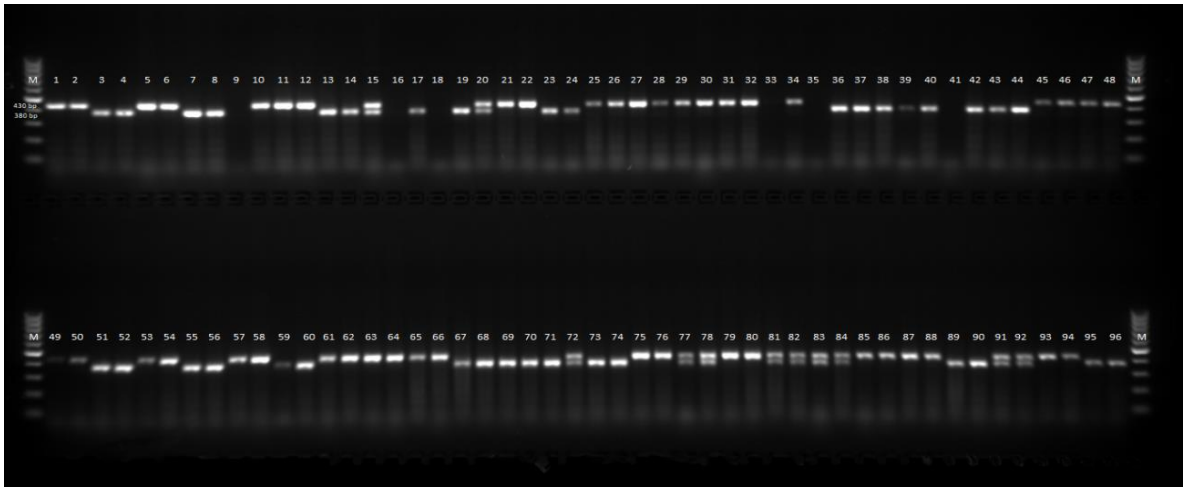

Figure 3. PCR reactions of different tomato lines for Mi23. M= 100 bp DNA marker, 1-96= tomato lines.

4. Discussion

Evaluation of the results of this study showed that molecular markers against Tomato Spotted Wilt virus (*TSWV*), Tomato Yellow Leaf Curl Virus (*TYLCV*) and root knot nematodes (*Meloidogyne* spp.) can be readily used for breeding existing tomato lines. Therefore, molecular markers (*Sw5*-2) determining resistance to Tomato Spotted Wilt Virus (*TSWV*), resistance to Tomato Yellow Leaf Curl Virus (*TYLCV*) (*Ty*3 P6-25) and root-knot nematodes (*Meloidogyne* spp.) (*Mi*23) should be used in various breeding programs to develop resistant cultivars to the above diseases. This will provide both the opportunity to test more material and increase the success rate by reducing the duration of breeding programs.

Although plant lines resistant to diseases have been determined in breeding programs with molecular markers, pathogenicity tests are essential for a safer resistance. Therefore, testing different genotypes of the pathogen against plants that have been identified as resistant may provide a more accurate and stable resistance against genotypes of the pathogen from

different geographic areas. For this purpose, in the next step of this study, tomato lines determined to be resistant by molecular markers will be tested *in vivo* against different genotypes of Tomato Spotted Wilt Virus (*TSWV*), Tomato Yellow Leaf Curl Virus (*TYLCV*) and root knot nematodes (*Meloidogyne* spp.).

The use of molecular markers and pathogenicity tests together in determining the resistance reactions against diseases is of great importance in terms of the reliability and sustainability of the resistance obtained. In such studies, it is essential to have different strains or genotypes of pathogens for pathogenicity tests.

5. Conclusion

In this study, determination of the resistance of tomato lines against Tomato Spotted Wilt Virus (*TSWV),* Tomato Yellow Leaf Curl Virus (*TYLCV*) and root knot nematodes (*Meloidogyne* spp.), and further studies confirming this resistance by pathogenicity tests, then will be carried out. Some of the tomato lines have resistance to all biotic factors used in this study. This will allow the development of new tomato varieties resistant to diseases. Based on the results of this study, it will also be possible to developed three biotic factor resistances in the same tomato variety.

Author Contributions

Concept: H.B. (100%), Design: H.B. (100%), Supervision: O.K. (100%), Data collection and/or processing: M.M. (50%) and R.İ. (50%), Data analysis and/or interpretation: H.B. (100%), Literature search: H.B. (25%), O.K. (25%), R.İ. (25%) and M.M. (25%), Writing: H.B. (100%), Critical review: H.B. (100%). Submission and revision H.B. (100%). All authors reviewed and approved final version of the manuscript.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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Research Article
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DETERMINATION OF FLIGHT ACTIVITIES AND POPULATIONS OF AMBROSIA BEETLES (COLEOPTERA: CURCULIONIDAE: SCOLYTINAE) IN HAZELNUT ORCHARDS IN SAMSUN, TÜRKİYE

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Abstract: Ambrosia beetles are highly invasive pests that cause thousands of hazelnut branches to dry out in hazelnut orchards each year. The manegement against these pests in hazelnut orchards, first of all, it is necessary to know the time of emergence of these pests during the year and the dates when their populations are concentrated. Between 2017-2019, studies were carried out in the Çarşamba and Terme districts of Samsun, which play an important role in hazelnut production in Türkiye. Populations of three invasive ambrosia beetle species (*Anisandrus dispar, Xylosandrus germanus and Xyleborinus saxesenii*) in hazelnut orchards were monitored using sticky traps for three years, from mid-March to mid-October. *A. dispar,* adults started to emerge in mid-March, their populations increased in April-May and started to decrease from mid-June. The emergence of *X. germanus* adults began in April, their populations peaked in late May and early June, and then the population began to decline. There was also a slight increase in their population in August. The emergence of *X. saxesenii* adults began in late March and a slight increase in their population was observed at the end of April. Populations of this species increased significantly in late June and early July, with a slight increase in populations in August. When these three ambrosia beetle species, which were caught in red sticky traps in hazelnut orchards for three years, were compared, the catch rates were determined as 56.28% for *A. dispar*, 24.20% for *X. germanus* and 19.52% for *X. saxesenii*, respectively. Thanks to this information obtained, it was determined when to start the combat against these pests in hazelnut orchards and when the management should be done most intensely.

Keywords: Hazelnut, Monitoring, *Anisandrus dispar*, *Xylosandrus germanus*, *Xyleborinus saxesenii*, Sticky traps

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1. Introduction

Ambrosia beetles (Coleoptera: Curculionidae: Scolytinae) are known worldwide as one of the most successful invasive species groups and are highly destructive pests for many tree species, especially fruit trees (Ranger et al., 2016; Rassati et al., 2016; Hulcr and Stelinski, 2017). Ambrosia beetles pierce the xylem of host trees and carefully infect the walls of these galleries they open with the symbiotic fungi they carry in their bodies. Ambrosia beetles cultivate these symbiotic fungi in the galleries, thus providing a food source for the adults and larvae in the gallery walls (Kirkendall et al., 2015; Hulcr and Stelinksi, 2017). Males cannot fly and they spend their entire life cycle in the host trees (Peer and Taborsky, 2007). Female ambrosia beetles, which have the ability to fly, spend the winter in the galleries of the host trees and begin to appear in the orchards when the first warm days of spring arrive (Miller and Rabaglia, 2009; Ranger et al., 2013; Reding et al., 2013).

Ambrosia beetles generally prefer weakened or dying trees, but they can be much more harmful when their populations are excessive (Oliver and Mannion, 2001; Ranger et al., 2016; Hulcr and Stelinski, 2017; Wang et al., 2021). Most ambrosia beetles follow olfactory cues from trees to distinguish old, stressed and dying trees from healthy trees, with a particular focus on ethanol (Oliver and Mannion, 2001; Ranger et al., 2015, 2021; Werle et al., 2019; Lehenberger et al., 2021), which is induced and spread by trees exposed to stress by biotic (Kelsey et al., 2013) or abiotic factors (Kelsey et al., 2014; Ranger et al., 2019). Ethanol is highly attractive to invasive ambrosia beetles and is therefore widely used in many different types of traps to monitor and control these pests in many orchards (Coyle et al., 2005; Miller and Rabaglia, 2009; Noseworthy et al., 2012; Kelsey et al., 2013; Ranger et al., 2014; Miller et al., 2018).

Samsun province realizes approximately 17% of Türkiye's hazelnut production and continues to increase its hazelnut production amounts every year (Türkstat, 2022). Ambrosia beetles are considered as one of the most destructive pests in hazelnut orchards in Türkiye. Three invasive species of ambrosia beetle (*Anisandrus dispar*, *Xylosandrus germanus* and *Xyleborinus saxesenii*) are common in hazelnut growing areas and cause serious damage to orchards (Ak et al., 2005; Tuncer et al., 2017; Aker, 2018). Traps prepared with ethanol were used in

the control and population monitoring of these invasive ambrosia beetles seen in hazelnut production areas in Türkiye (Ak et al., 2005; Saruhan and Akyol, 2012; Şahin and Özder, 2017).

Hazelnut branches infected by these pests die within a few years and new generations spread to the surrounding hazelnut orchards (Saruhan and Tuncer, 2000; Uygun et al., 2002). Hazelnut is a very valuable product and therefore it is very important to protect hazelnut orchards from these pests, so the right timing is as important as the right control technique against these pests. The aim of this study is to determine the first emergence times of these three invasive ambrosia beetle species, which are very harmful in hazelnut orchards, and the peak times of their populations during the year. In line with the information to be obtained, the most effective combat times against these pests will be determined better and the success of the combat will be increased.

2. Materials and Methods

2.1. Materials

The population monitoring study of these three species was carried out between 2017-2019 in Samsun province Çarşamba and Terme districts where hazelnut production is intense. Four hazelnut orchards (8 orchards in total) were selected from each of the two districts, and these selected orchards are a few meters above sea level, and there are mostly Tombul and Palaz cultivars in these orchards. Hazelnut trees were about 30-40 years old and were usually positioned in orchards with a distance of 3x4 meters between trees. Problems caused by ambrosia beetles in these selected hazelnut orchards had been experienced for years and growers were helpless. Coordinates of the 8 selected hazelnut orchards: 41°14'30.22"N-36°47'24.30"W, 41°14'39.45"N-36°47'37.78"W, 41°14'55.78"N-36°47'50.98"W, 41°15'9.20"N-36°48'8.97"W, 41°17'41.13"N-36°49'54.11"W, 41°17'45.86"N-36°50'5.18"W, 41°17'47.31"N-36°50'25.47"W, 41°18'0.09"N-36°50'39.47"W. Rebell®rosso sticky traps were used to catch ambrosia beetles (made by Andermatt Biocontrol AG-Switzerland). Plastic drums, which were located at the bottom of the sticky traps and will carry the attractive solution, were hung under these sticky traps with the help of a rope.

2.2. Preparation of the Attractive Solution

Dilute a mixture of 96% Ethanol (99.5% purity) (Merck-818760) + 1% Toluene (99% purity) (Merck-108323) (1:1) with distilled water at a ratio of 1:1 (Aker, 2018).

2.3. Method

Four sticky traps were hung in each of the four hazelnut orchards selected from each district. A total of 32 traps were hung on hazelnut branches 1.5 meters above the ground. Traps were placed in the same row in each orchard, 30 meters apart. Five hundred ml of attractive solution was placed in each of the plastic drums of the sticky traps. All traps were counted weekly, ambrosia beetle species in sticky traps were identified by the author and counted-recorded ambrosia beetles were removed from sticky traps with tweezers. After count-recorded processing, fresh attractive solutions were placed in the plastic drums of each sticky trap. Sticky traps were replaced with new traps every six weeks. All these studies were carried out for 30 weeks each year. The 2017 trials started on March 19 and ended on October 15, the 2018 trials started on March 18 and ended on October 14, and the 2019 trials started on March 17 and ended on October 13. Three years of climate data were prepared using the data provided by the Turkish State Meteorological Service (TSMS, 2020) (Figure 1).

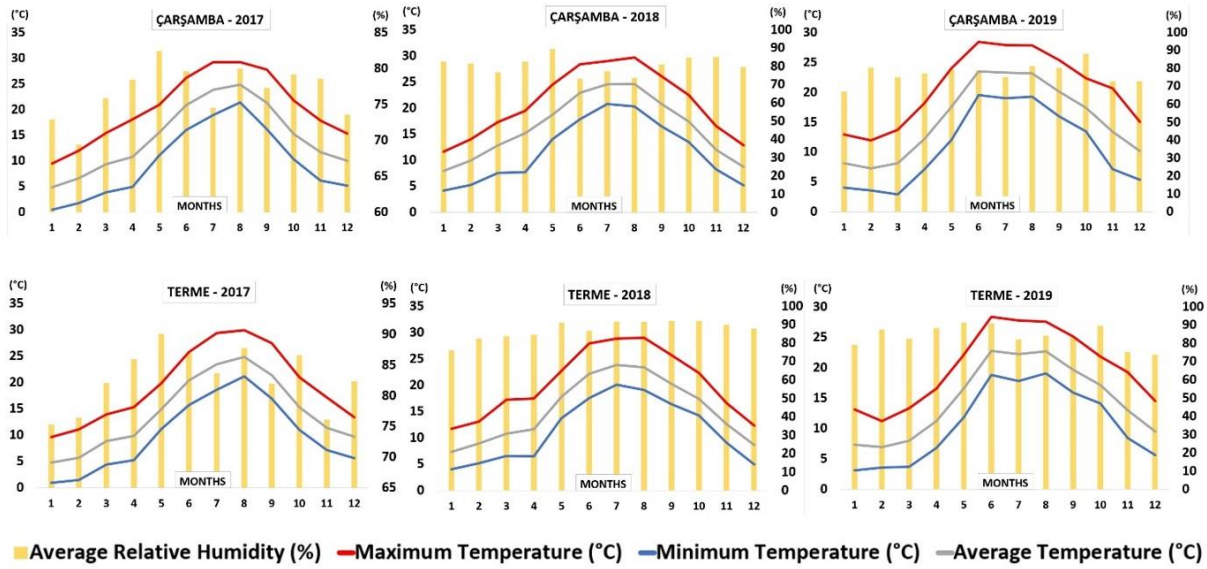

Figure 1. Monthly values of mean average relative humidity and temperature (maximum, average, minimum) in Çarşamba and Terme districts between 2017-2019.

2.4. Statistical Analysis

Shapiro Wilk-W Test was used to control the normal distribution of the obtained data and it was determined that the data did not show normal distribution. Results were expressed as mean ± SE. The significance of the differences in values was determined by the one-way ANOVA test and Duncan's multiple interval tests. P <0.05 was accepted as a significant difference. Graphs were prepared weekly based on numerical averages per sticky trap of different ambrosia beetle species caught in sticky traps each week for 30 weeks.

3. Results

3.1. Flight Activity and Population Monitoring of *Anisandrus dispar*

Female adults of this species were observed in hazelnut orchards for three years starting from mid-March, the first week of the trials, and many female adults were caught in sticky traps (125.31±3.02, 149.69±4.17, 17.88±1.02 for Çarşamba in 2017, 2018, 2019, respectively and 102.25±4.16, 117.94±3.67, 22.25±1.71 for Terme in 2017, 2018, and 2019, respectively). In the following weeks, the number of female adults caught in the sticky traps started to increase and the maximum number of catches in sticky traps was reached in late May and early June (857.5±19.74, 942.19±13.62, 951.69±13.46 for Çarşamba in 2017, 2018, 2019, respectively and 608.19±55.73, 593.63±36.29, 429.13±24.63 for Terme in 2017, 2018, 2019, respectively). After reaching the maximum number of adults caught in sticky traps, the number of adults caught in traps gradually decreased. While the number of adults caught in the trap decreases significantly in September and October, the number of adults caught in some sticky traps in the last week of trapping is negligible (2.31±0.18, 2.13±0.2, 1.81±0.2 for Çarşamba in 2017, 2018, 2019, respectively and 2.25±0.25, 0.75±0.21, 1.25±0.17 for Terme in 2017, 2018, 2019, respectively) (Figures 2 and 3).

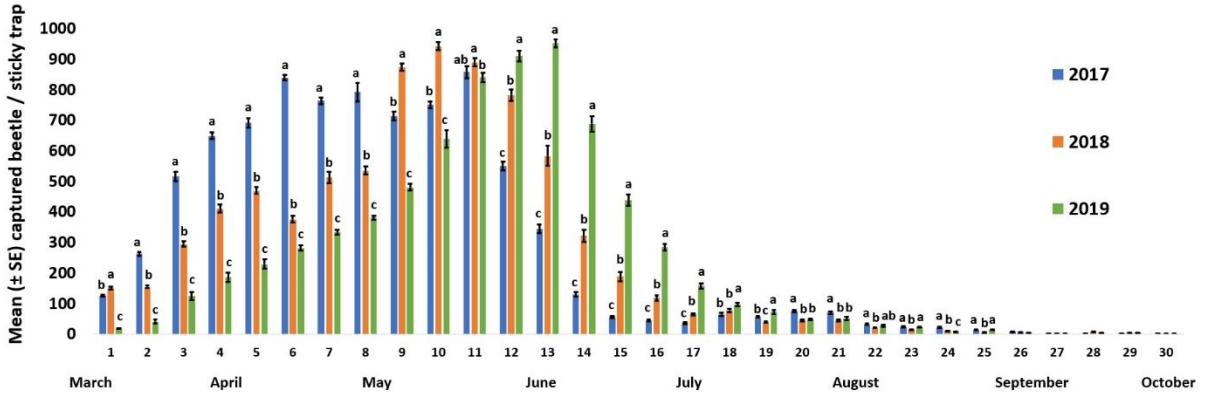

Figure 2. Weekly mean (± SE) number of new captures of *Anisandrus dispar* adults caught in sticky traps in Çarşamba district (P <0.05).

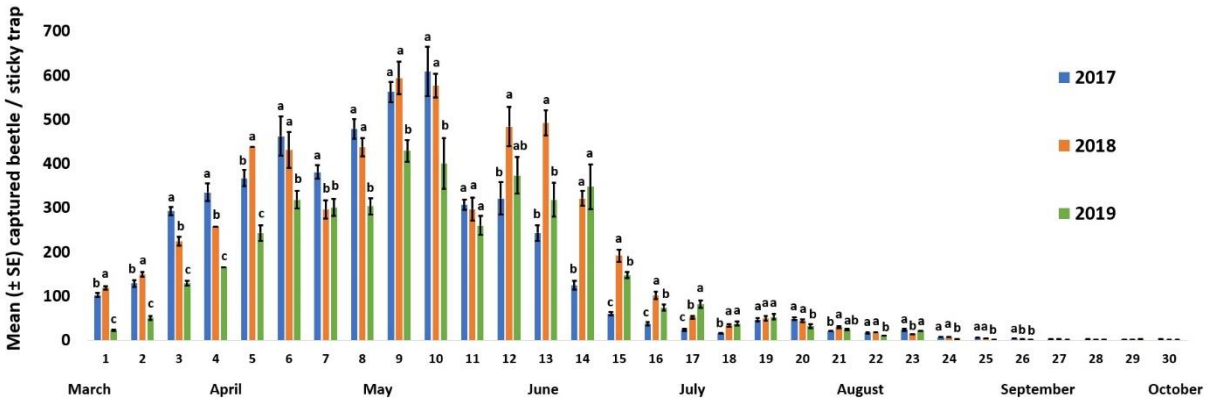

Figure 3. Weekly mean (± SE) number of new captures of *Anisandrus dispar* adults caught in sticky traps in Terme district (P <0.05).

3.2. Flight Activity and Population Monitoring of *Xylosandrus germanus*

This species, which was not observed when the traps were first set in hazelnut orchards, started to be counted in the sticky traps from the beginning of April, which corresponds to the third week in the traps (2.31±0.28, 0.25±0.08, 0±0 for Çarşamba in 2017, 2018, 2019, respectively and 1.38±0.2, 0.44±0.13, 0.31±0.12 for Terme in 2017, 2018, 2019, respectively). In the following weeks, the number of adults caught in sticky traps started to increase and the highest catch rate was reached in late May and early June (462.5±14.1, 687.94±6.48, 627.38±38.9 for Çarşamba in 2017, 2018, 2019, respectively and 400.69±26.1, 537.81±33.06,

407.38±21.56 for Terme in 2017, 2018, and 2019, respectively). While a decrease was observed in the number of adults caught in the traps in the following weeks, a slight increase was observed in the number of adults caught in the traps again in August. It was observed that the sticky trap catching decreased in the following weeks and almost no adults were caught in the sticky traps in the last week of trapping (0.69±0.12, 0.69±0.12, 0.69±0.12 for Çarşamba in 2017, 2018, 2019, respectively and 0.69±0.12, 0.69±0.12, 0.69±0.12 for Terme in 2017, 2018, 2019, respectively) (Figures 4 and 5).

3.3. Flight Activity and Population Monitoring of *Xyleborinus saxesenii*

Adults of *X. saxesenii* species, which have stuck to the traps since the first week when the sticky traps were hung in the hazelnut orchards, started to be seen more frequently in the hazelnut orchards as of the end of March (2.31±0.27, 1.94±0.21, 1.94±0.21 for Çarşamba in 2017, 2018, 2019, respectively and 1.0±0.27, 1.31±0.12, 0±0 for Terme in 2017, 2018, 2019, respectively). In the following weeks, the number of adults caught in sticky traps started to increase, and a significant increase was observed in the number of adults caught in the traps in late April and early May. Although the number of adults caught in the traps decreased in the following weeks, an increase curve was observed again from mid-June. The end of June and the beginning of July were recorded as the period when the adult females were caught in sticky traps the most (433.69±18.71, 442.25±18.05, 332.75±14.91 for Çarşamba in 2017, 2018, 2019, respectively and 451.75±20.62, 441.5±13.17, 333.5±18.93 for Terme in 2017, 2018, 2019, respectively). Gradual reductions followed in the following weeks. In the first weeks of August, a final increase in catches with sticky traps was observed, after which the catches of the traps started to decrease rapidly. Almost no adults were observed in the sticky traps during the last week of the trapping (0.63±0.13, 0.63±0.13, 1.06±0.21 for Çarşamba in 2017, 2018, 2019, respectively and 0.25±0.11, 0.75±0.11, 0.38±0.2 for Terme in 2017, 2018, and 2019, respectively) (Figures 6 and 7).

3.4. Comparison of population ratios of ambrosia beetle species in hazelnut orchards

The rates of catching of these three ambrosia beetle species in red sticky traps set in a total of eight hazelnut orchards were compared. In the studies carried out in hazelnut orchards for three years, it was determined that *A. dispar* was the most caught species in red sticky traps. *X. germanus* was identified as the second highest population species caught in sticky traps. *X. saxesenii* was determined as the species with the lowest rate of being caught in sticky traps set up in hazelnut orchards (Figures 8, 9, 10, 11, 12, 13 and 14). When the three-year general averages of these three species caught in red sticky traps are taken, it was determined as 56.28%, 24.20% and 19.52% for *A.dispar*, *X.germanus* and *X.saxesenii*, respectively.

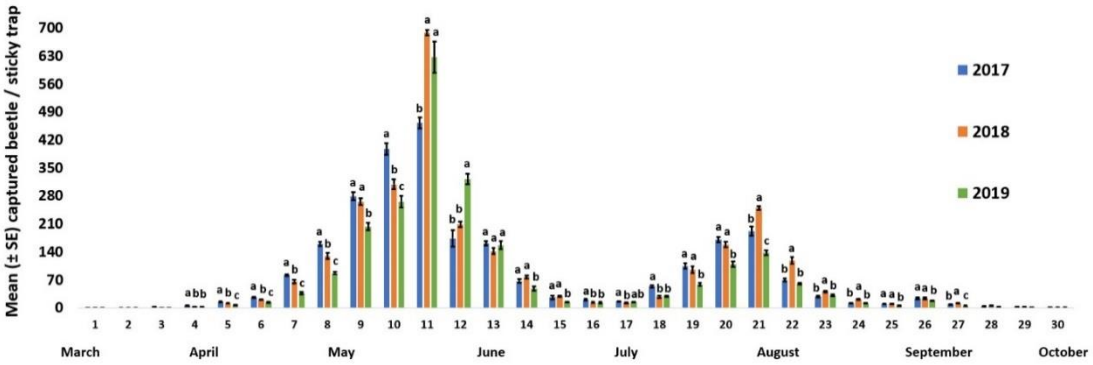

Figure 4. Weekly mean (± SE) number of new captures of *Xylosandrus germanus* adults caught in sticky traps in Çarşamba district (P <0.05).

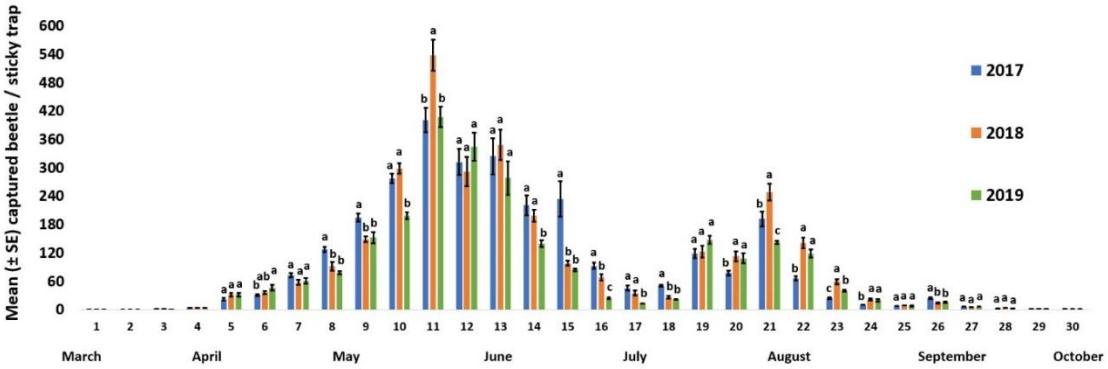

Figure 5. Weekly mean (± SE) number of new captures of *Xylosandrus germanus* adults caught in sticky traps in Terme district (P <0.05).

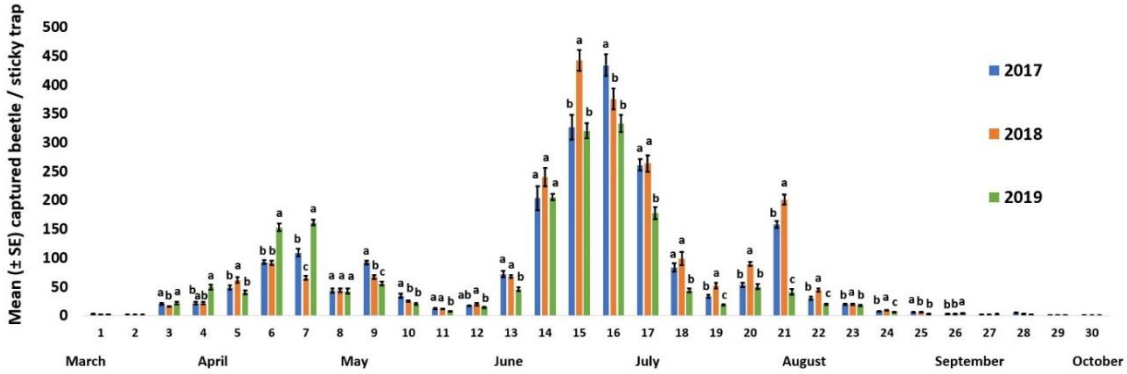

Figure 6. Weekly mean (± SE) number of new captures of *Xyleborinus saxesenii* adults caught in sticky traps in Çarşamba district (P <0.05).

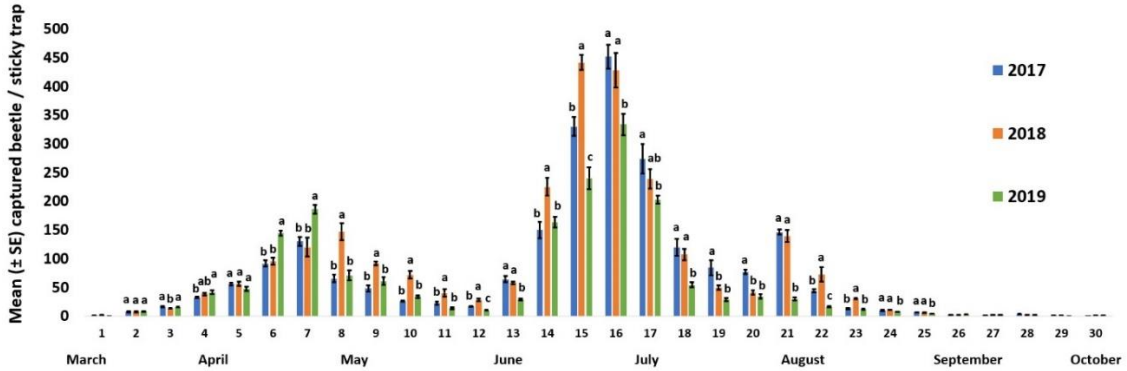

Figure 7. Weekly mean (± SE) number of new captures of *Xyleborinus saxesenii* adults caught in sticky traps in Terme district (P <0.05).

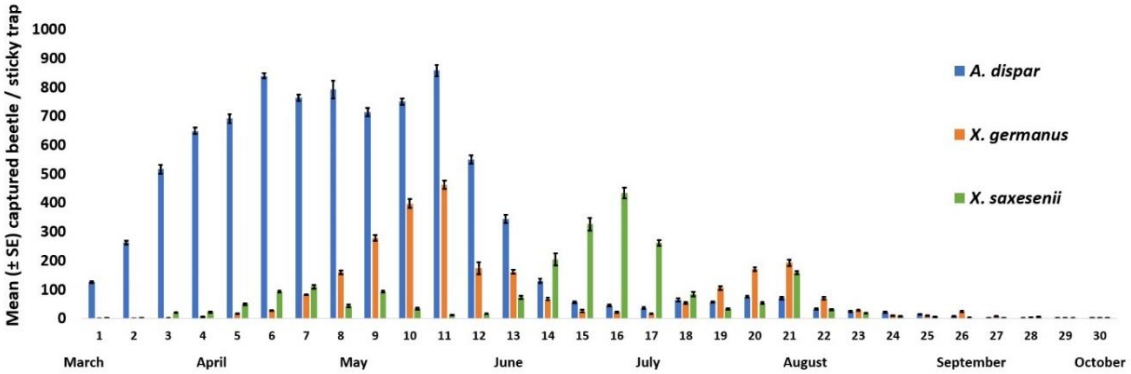

Figure 8. Weekly mean (± SE) number of new captures of ambrosia beetles caught in sticky traps in Çarşamba district in 2017 (P <0.05).

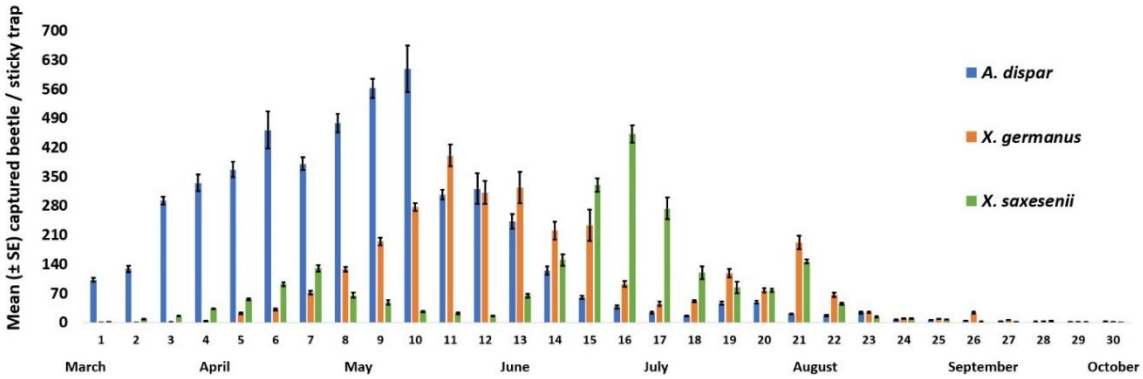

Figure 9. Weekly mean (± SE) number of new captures of ambrosia beetles caught in sticky traps in Terme district in 2017 (P <0.05).

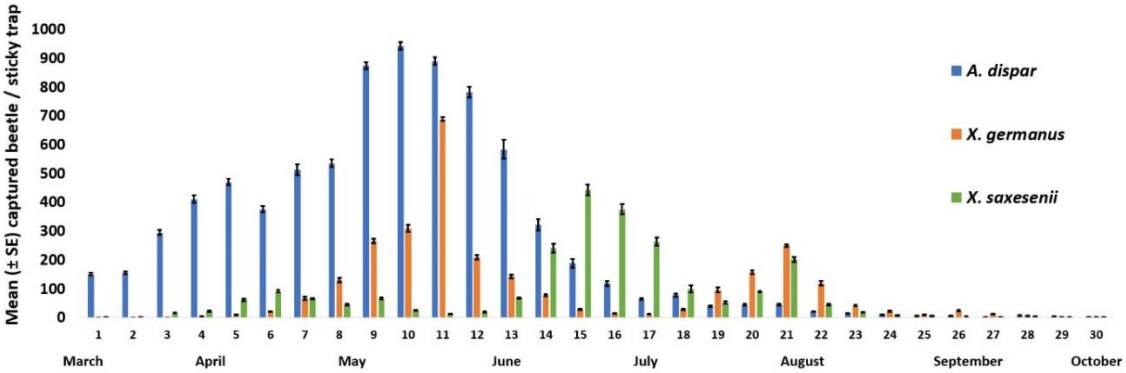

Figure 10. Weekly mean (± SE) number of new captures of ambrosia beetles caught in sticky traps in Çarşamba district in 2018 (P <0.05).

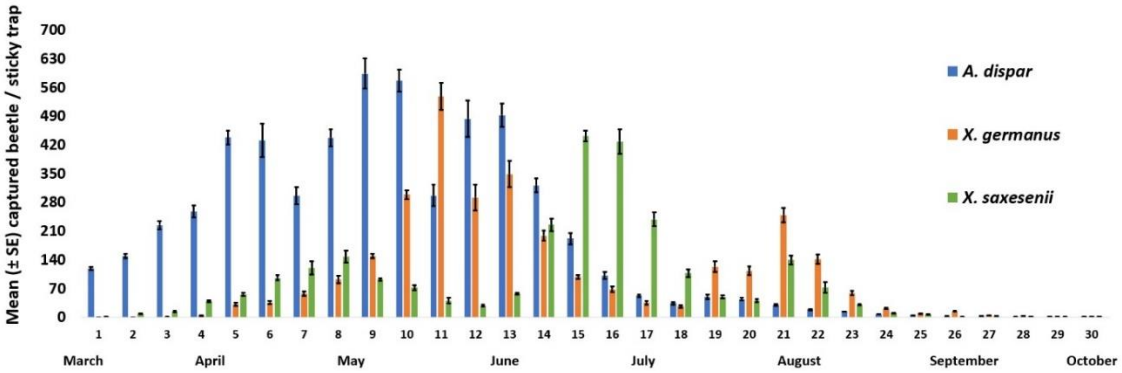

Figure 11. Weekly mean (± SE) number of new captures of ambrosia beetles caught in sticky traps in Terme district in 2018 (P <0.05).

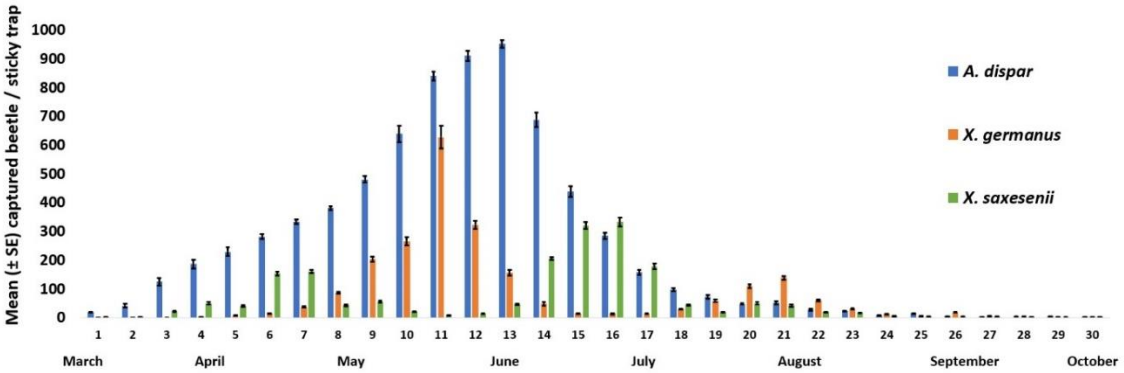

Figure 12. Weekly mean (± SE) number of new captures of ambrosia beetles caught in sticky traps in Çarşamba district in 2019 (P <0.05).

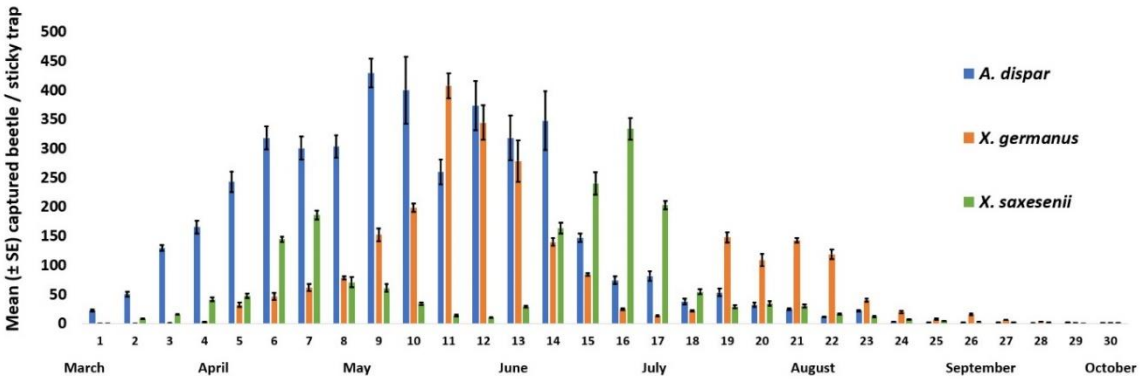

Figure 13. Weekly mean (± SE) number of new captures of ambrosia beetles caught in sticky traps in Terme district in 2019 (P <0.05).

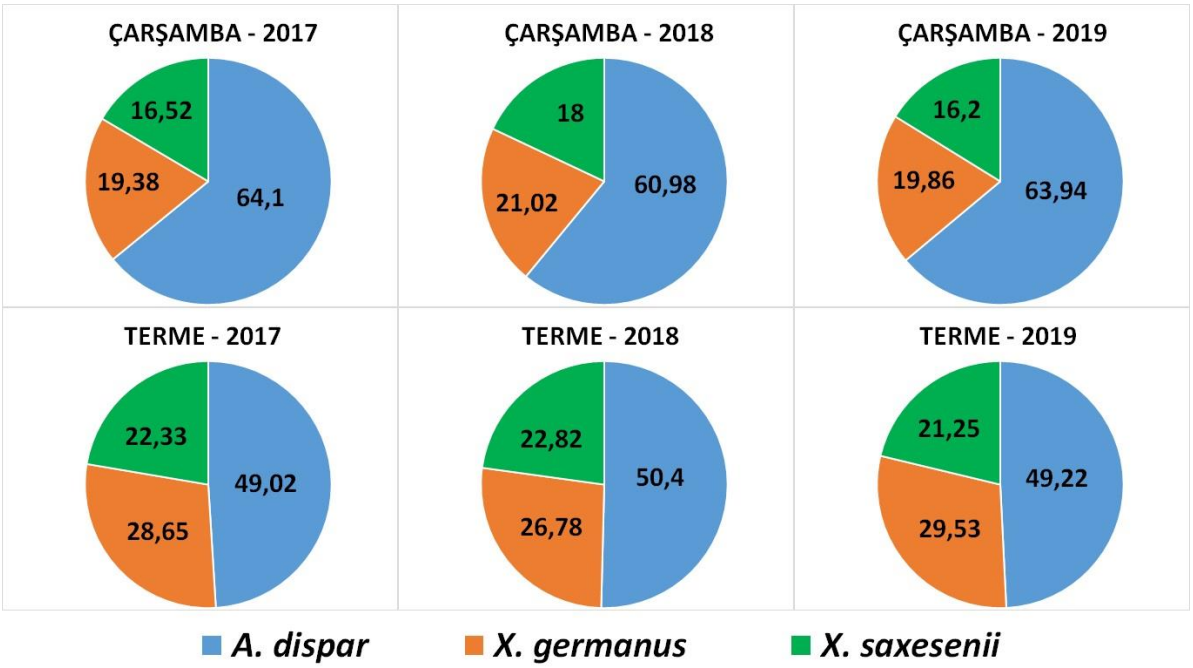

Figure 14. Capture rates (%) of ambrosia beetle species caught with red sticky traps in hazelnut orchards in Çarşamba and Terme districts between 2017-2019.

4. Discussion

In this study, the presence of ambrosia beetles was observed in hazelnut orchards from mid-March. In the 30-week studies that continued from mid-March to mid-October, it was determined that the presence of these three species in hazelnut orchards increased due to the increase in air temperatures from April (Figures 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 and 14). *A. dispar* has been observed in hazelnut orchards from mid-March. In similar studies, it was stated that *A. dispar* species started to appear in hazelnut orchards with the increase in air temperatures from mid-March, and an increase in adult emergence was observed from April when the daily average temperatures reached 18-20°C (Ak et al., 2005; Speranza et al., 2008; Saruhan and Akyol, 2012; Şahin and Özder, 2017; Aker, 2018). Saruhan and Tuncer (2000), stated that *A. dispar* was first seen at the beginning of April and the emergence continued until the beginning of August in hazelnut orchards between 1997-1998 in Samsun, Türkiye. Ak et al. (2005) stated that the first rises for *A. dispar* started in mid-March, peaked in May, and the second peak occurred in the July-August period in Giresun, Ordu, Samsun province between 2002-2003. Saruhan and Akyol (2012) stated that the first flights of *A. dispar* were seen in March-June and adult females were caught in the traps in hazelnut orchards between 2005-2007 in Samsun. In addition, the same researchers stated that the population densities of this species decreased in June-July, and the number of adults caught in the traps decreased significantly from the end of July. Sarıkaya and Sayin (2015) detected the first adults of *A. dispar* species in sticky traps as of the first half of April in their studies conducted in Kasnak Oak Forest Nature Reserve (Türkiye) in 2012-2013 and stated that overpopulation was observed in sticky traps in the second half of May. Şahin and Özder (2017) stated that the first emergence of *A. dispar* adults took place during the week of 13-18 March 2013, and the emergence of *A. dispar* adults took place on 12-19 March 2014 in hazelnut orchards.

X. saxesenii has been observed in hazelnut orchards from mid-March. In similar studies, it was stated that this species was observed in hazelnut orchards from mid-March and caught in traps and their population increased due to the increase in air temperatures (Saruhan and Akyol, 2012; Şahin and Özder, 2017; Aker, 2018). Saruhan and Akyol (2012) reported that *X. saxesenii* first appeared in March in the study they conducted in hazelnut orchards in 2005-2007, and there was a slight increase in their population in May. However, they stated that the peak population of this species was between July and August, and the number of adults caught in the traps decreased from September. Sarıkaya and Sayın (2015), in 2012-2013, detected the first adults of *X. saxesenii* in sticky traps as of the second week of April and stated that overpopulation was observed in sticky traps with the increase in air temperatures. They stated that the highest levels in the population were reached in the second half of August and the flight activities of this species continued until the end of September. Şahin and Özder (2017) stated that the first emergence of *X. saxesenii* adults took place during the week of 13-18 March in the studies in hazelnut orchards in 2013, and in the studies in 2014, the emergence of *X. saxesenii* adults took place on 20-27 March.

In this study, the first appearance of *X. germanus* started in the first days of April. In studies conducted by researchers doing similar studies on this species, it was

stated that this species first appeared in hazelnut orchards in April in parallel with the increase in air temperatures (Şahin and Özder, 2017; Aker, 2018). Şahin and Özder (2017) stated that the first emergence of *X. germanus* adults took place during the week of 20-27 March in the studies in hazelnut orchards in 2013, and in the studies in 2014, the emergence of *X. germanus* adults took place in April. In similar monitoring studies on ambrosia beetles, it is thought that the similarity or difference between the flight activities and population densities of these species and the data obtained in this study are related to the climate data. As a matter of fact, it has been stated by the researchers that the main factor affecting the emergence of these beetles from their winter quarters, the increase or decrease of their populations, and the flight times are the climate factors (Coyle et al., 2005; Sarikaya and Sayin, 2015; De Souza Covre et al., 2021; Hofstetter et al., 2022).

5. Conclusion

Although ambrosia beetles prefer old, weak, or stressed trees that will die in a short time, it is known that they turn to many healthy trees in years when their populations increase excessively. The success of the programs in the effective and sustainable control of these pests is possible by knowing the flight activities and population monitoring of these pests in hazelnut orchards. It is thought that the information obtained from this study will increase the success of integrated control efforts against these pests in hazelnut orchards in the future.

Author Contributions

All task made by O.A. (100%); Concept, Design, Supervision, Data collection and/or processing, Data analysis and/or interpretation, Literature search, Writing, Critical review, Submission and revision. The author reviewed and approved final version of the manuscript.

Conflict of Interest

The author declared that there is no conflict of interest.

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I would like to thank the valuable hazelnut producers in Çarşamba and Terme districts where the studies were carried out.

Ethical Consideration

Ethics committee approval was not required for this study due to the use of research materials that did not fall under the definition of experimental animals (The Scientific and Technological Research Council of Türkiye, Animal Experiments Local Ethics Committee Directive, 2018, Article 3-c).

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Research Article
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PROXIMATE COMPOSITION, PHYTOCHEMICAL PROFILE, ANTIOXIDANT, ANTIDIABETIC AND ANTI-INFLAMMATORY PROPERTIES OF JUSTICIA CARNEA LEAF POWDER

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Abstract: Determining the proximate composition, phytochemical profile, antioxidant, anti-diabetic and anti-inflammatory effects of *Justicia carnea* leaf powder (JLP) is the goal of this study. The results revealed that the crude fat (8.19%) had the lowest content in JLP and the nitrogen-free extract (37.85%) had the greatest. JLP included 9.98 percent crude protein, 17.54 % crude fibre, and 18.18 % ash, respectively. Phenol had the highest concentration (383.15 mg/g), whereas flavonoids (1.84 mg/g) had the lowest concentration. Alkaloids, tannins, saponins, and steroids all had concentrations of 24.03 mg/g, 84.81 mg/g, 188.13 mg/g, and 231.3 mg/g, respectively. JLP had a 62.21 % inhibition of lipid peroxidation and a 28.49 % scavenging of ABTS radicals, respectively. The percentages for the DPPH radical scavenging, Fe chelation, and hydroxyl radical inhibition were 54.05 %, 42.81 %, and 54.35 %, respectively. JLP's alpha-glucosidase and alpha-amylase inhibition activities were 65.96 and 65.82 %, respectively. JLP had a 35.51 % albumin denaturation inhibition and a 61.58 % antiprotease activity. Thus, these results suggested that JLP possesses antioxidant, antidiabetic and anti-inflammatory activities.

Keywords: Anti-oxidant, Anti-diabetic, Anti-inflammatory, *Justicia carnea*, Phytogens, Dietary supplements

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1. Introduction

The discovery of botanicals as significant sources of biologically functioning medicine has piqued researchers' curiosity, leading them to investigate the chemical make-up of medicinal plants and determine if they might be used as nutraceuticals (Dillard and German, 2000). Medicinal plants have been widely used as a source for the discovery of novel drug compounds because they are a rich source of secondary metabolites with interesting bioactivity. All portions of the plant are potential sources of bioactive substances because secondary metabolites are produced in all sections of the plant body, including the stem, bark, leaves, flowers, roots, fruits, etc. (Madhayan et al., 2022).

Varieties of phytochemicals are used in veterinary, human, and other scientific research studies, and recently, plants such as *Litsea floribunda* Gamble (Madhayan et al., 2022), *Momordica charantia*, *Ocimum gratissimum* (Oloruntola et al., 2021), *Ficus carica, F. exasperata, F. thonningii* (Osowe et al., 2021), *Anacardium occidentale* (Oloruntola, 2021), wild sunflower, goat weed (Adeyeye et al., 2020), mucuna (Oloruntola et al., 2022), *Tithonia diversifolia* (Hemsl.) A. Gray (Dada and Oloruntola, 2016) among others were reported for their neutraceutical values such as antioxidant, anti-inflammatory, antimicrobial, anti-plasmodial, and anti-diabetic properties among others.

Acanthaceae family, which encompasses over 600 species of shrubs, herbs, and delicate perennials, includes *Justicia carnea*, which is frequently found in tropical and subtropical regions (Correa, 2012). According to Uroko et al. (2017), the bioactive profile of *Justicia carnea* was linked to its medicinal properties, which included antioxidant, anti-cancerous, antimicrobial, and hypocholesterolemic effects. The plant species is also used in folk medicine to treat respiratory, gastrointestinal, inflammatory, and anaemic conditions

(Correa, 2021, Anthonia et al., 2019).

The evidence supporting the use of Justicia leaf to treat anaemia is sparse, however, there may be further health benefits from using *Justicia carnea* leaf as nutraceuticals to treat physiological issues and prevent the development of chronic illnesses (Wood et al., 2020). There is also a need for ongoing and further investigation or characterization of the bioactive composition profile of *Justicea cornea* leaf, as dietary supplements are considered nutraceuticals when they are used for health-related purposes rather than nutrition. Nutraceutical supplements are defined as products that have at least a vitamin, an amino acid, a mineral, a medicinal herb, a concentrate metabolite, extracts or a blend of these aforementioned ingredients (Nasri et al., 2014). Therefore, the goal of this study is to assess the *Justicia carnea* leaf powder's proximate composition, phytochemical profile, antioxidant, antidiabetic, and anti-inflammatory properties.

2. Materials and Methods

2.1. *Justicia carnea* Leaf Powder and Reagents

The *Justicia carnea* fresh leaves were gathered from a private garden in Akure, Nigeria. The plant was verified by a plant scientist from the Adekunle Ajasin University in Akungba Akoko, Nigeria's Department of Plant and Biotechnology. The samples were carefully cleaned with fresh water, drained, and shade dried for 14 days. They were then milled into *Justicia carnea* leaf powder (JLP) and kept at 4°C until used for analysis. Three duplicates of the parameters were analysed. The JLP samples underwent three iterations of analyses for each parameter. The analytical reagent grade chemicals utilised for chemical analysis were all acquired from Sigma-Aldrich.

2.2. *Justicia carnea* Leaf Proximate Analysis

JLP was analyzed for moisture, crude fat, crude fibre, crude protein, ash, and nitrogen-free extract using the AOAC method (AOAC, 2010).

2.3. Quantitative Phytochemical Analysis of JLP

Oloruntola (2021) described and reported the methods for determining phenols, saponins, flavonoids, and tannins; while the procedures for determination of steroids were reported by Madhu et al. (2016).

2.3.1. Phenols

A total of 2000 ml of 70% ethanol was added to 400 g of JLP, which was shaken for six hours, and then allowed to stand motionless for a further 48 hours before filtering through Whatman No 1 filter paper. The JLP ethanolic extract was vacuum condensed at 35–40 °C using a rotary evaporator. Whatman No. 1 filter paper was used to filter 200 g of JLP after it had been submerged in 1000 cc of 70% ethanol and vibrated continuously for six hours.

Using the Folin-Ciocalteau method described by Otles and Yalcin (2012), the phenolic content of JLP was measured. 250 mL of Folin-Ciocalteau reactive was added to 50 µL of JLP extract or standard solution. This mixture was left at room temperature in a dim setting for five minutes. At the conclusion of this time, a 750 microlitre solution of 7 percent Na_2CO_3 was added. The mixture was diluted to 5 mL using distilled water. The combination was then allowed to react for 120 minutes at room temperature in a dark area. At 760 nm, the absorbance of the standards and samples was measured. An 80 percent methanol solution (50µ l) was added to the blank solution in place of the 50 µl of extract. Using standards that are comparable to gallic acid, a calibration curve was used to determine the total phenolic content.

2.3.2. Alkaloids

To ascertain the alkaloid content of the leaf sample, the gravimetric method was employed (Adeniyi et al., 2009). 50 ml of acetic acid solution in ethanol (10% w/v) were mixed with 5 g of the JLP. Before being sieved, the mixture was vibrated and left alone for around 240 minutes. On a heated plate, the filtrate was reduced to one-fourth of its original volume. Then, by adding droplets of highly concentrated ammonium hydroxide, the alkaloids were precipitated. The precipitate was filtered through the filter paper and then washed with a 1 percent solution of ammonium hydroxide. The precipitate was then moved to desiccators and reweighed until it attained a constant weight after being oven-dried for 30 minutes at 60°C. Alkaloids' weight as a proportion of the sample weight was calculated.

2.3.3. Saponins

Saponin was measured using the vanillin and concentrated sulfuric acid colourimetric method (He et al., 2012). The 0.1 ml of JLP extract was combined with 0.5 ml of 50% ethanol, 4.0 ml of 77 percent sulfuric acid, and 0.5 ml of freshly made vanillin solution. The mixture was heated in a water bath to 60 °C for 15 minutes after being allowed to cool to room temperature. The absorbance at 545 nm was measured using a UV/Vis spectrophotometer. Utilizing a tea saponin calibration curve, the total amount of saponin in each sample was calculated and expressed as mg tea saponin equivalent per g (TSE/g DW).

2.3.4. Steroids

According to Madhu et al., (2016) reports, steroids concentration in JLP was identified. Steroids were determined as reported by Madhu et al. (2016). 10 ml volumetric flasks were filled with 1 ml of JLP steroid extract. Following the addition of potassium hexacyanoferrate (III) solution (0.5 percent w/v, 0.5 ml), sulphuric acid (4N, 2 ml) and iron (III) chloride (0.5 percent w/v, 2 ml) were added. The mixture was heated for 30 minutes at 70–20 °C in a water bath with periodic shaking before being diluted with distilled water to the proper concentration. At 780 nm, the absorbance was measured in comparison to a reagent blank.

2.3.5. Flavonoids

The concentration of flavonoids in JLP was measured according to Surana et al (2016) method. 0.1 ml of aluminium chloride solution, 1.50 ml of methanol, 0.1 ml of potassium acetate solution, and 2.8 ml of distilled

water were added to a test tube containing 0.50 ml of JLP extract. The same procedure was used to create sample blanks for extract and rutin standard dilutions (10-100 g/ml), but distilled water was used in place of aluminium chloride solution. The solutions were then filtered using Whatman filter paper after that (No. 1). At 510 nm, absorbance ratios were recorded in comparison to blanks. Following that, it was found that the overall flavonoid concentration was equal to 1 mg of rutin per gramme of the ethanolic JLP extract.

2.3.6. Tannins

Total tannins concentration was measured using the Folin-Ciocalteau method (Biswas et al., 2020). 1 ml of the JLP ethanolic extract was diluted with 49 ml of distilled water, 1.7 ml of 75% ethanol, 0.1 ml of metaphosphoric acid, 10 ml of 1.0 mol/ml Na_2CO_3, and 2.5 ml Folin-Ciocalteu in a volumetric flask (100 ml). After completely blending, the mixture was allowed to sit at room temperature for 15 minutes. The absorbance of the standard solution and JLP extract was then measured at 680 nm in a spectrophotometer in comparison to a blank. Tannic acid (TA) mg TA/g dry weight was utilised to express the sample's total tannin content as a reference against the standard curve (R^2 = 0.9972).

2.4. Antioxidant Activity

2.4.1. Lipid peroxidation inhibition

The lipid peroxidation inhibition of JLP extract was ascertained using a method previously described by Bajpai et al. (2015). In both the absence and addition of JLP extract (50-250 g/mL) or a control substance, the reaction mixture of 1 mM $FeCl_3$, 50 µl of bovine brain phospholipids (5 mg/L), and 1 mM ascorbic acid in 20 mM phosphate buffer was incubated at 37 °C for 60 minutes. Malondialdehyde (MDA), which was measured by the 2-thiobarbituric acid (TBA) reaction, was created as a byproduct of the process as hydroxyl radicals, which led to lipid peroxidation and lipid peroxidation. The proportion of inhibitory activity was calculated (Equation 1).

$$\% \text{ inhibition} = \frac{(\text{AC} - \text{AT})}{(\text{AC})} \text{x}100 \quad (1)$$

here, AC= absorbance of control, AT= absorbance of test.

2.4.2. 2,2'-Azino-Bis-3-Ethylbenzothiazoline-6-Sulfonic acid (ABTS)

The modified ABTS assay was conducted using a method outlined by Turkoglu et al. (2010) and Ozgen et al. (2006). ABTS was produced with potassium persulfate, dissolved in 20 mM sodium acetate buffer (pH 4.5), and then diluted in an acidic solution to achieve an absorbance of 0.700. (0.01 at 734 nm). Then, 3 ml of JLP extract in ethanol were combined with 1 ml of ABTS + solution at 100 g/ml concentrations. Thirty minutes after mixing, the absorbance was measured, and for each concentration, the radical scavenging percentage was determined in relation to a blank that had no scavenger. The percentage reduction of absorbance is used to determine the degree of decolourization. Different ABTS + concentrations were employed to create a standard curve. Using the following equation, the test compounds' ability to scavenge was determined (Equation 2):

$$\% \text{ inhibition} = \frac{(\text{AC} - \text{APJLP})}{(\text{AC})} \text{x}100 \quad (2)$$

here, AC= absorbance of control, APJLP= Absorbance in presence of JLP extract.

2.4.3. Ferrous chelating activity

Ebrahimzadeh et al. (2008) reported the procedures used for estimating the ferrous chelating activity of JLP. In a nutshell, 1 ml of various doses of the JLP extract (0.2, 0.4, 0.8, 1.6, and 3.2 mg/ml) were combined with 50 µl of 2 mM FeCl2. Thereafter, 0.2 ml of a 5 mM ferrozine solution was added to start the reaction. After giving the mixture a good shake, the mixture was given 10 minutes to stand at room temperature. The solution's absorbance was subsequently determined at 562 nm. . Na_2EDTA served as the positive control (Equation 3).

$$\% \text{ inhibition} = \frac{(\text{AC} - \text{AJLP})}{(\text{AJLP})} \text{x}100 \quad (3)$$

here, AC= absorbance of control, AJLP= Absorbance of JLP extract.

2.4.4. Hydroxyl radical inhibition

Outlines of Tijani et al. (2012) were followed to assess the hydroxyl radical inhibition activity of JLP extract. The reaction mixture included different quantities of the extract concentrations (50-350 g/ml) and 1.0 ml of reagent (3.0 mM deoxyribose, 0.1 mM EDTA, 2 mM H2O2, 0.1 mM L-Ascorbic acid, and 0.1 mM $FeCl_3$.6H2O in 10 mM phosphate buffer, pH 7.4). After the reaction mixtures had been incubated at 37 °C for 1 hour, 1.0 ml of 1 percent (w/v) TBA (in 0.25 N HCl) and 1.0 ml of 10 percent (w/v) TCA were added. The reaction mixtures were heated in a bain-marie of boiling water for 20 minutes at 100 °C, and the pink chromogen (malondialdehyde-(TBA) adduct) was extracted into 1.0 ml of butan-1-ol before the absorbance was measured at 532 nm against reagent blank (Equation 4).

$$\% \text{ inhibition} = \frac{\text{Abs (control)} - \text{Abs (sample)}}{\text{Abs (control)}} \text{x}100 \quad (4)$$

2.4.5. 2,2-diphenyl-1-picrylhydrazyl (DPPH)

The 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) radical degradation activity method (Otles and Yalcin, 2012) was employed to assess the antioxidant activity of the leaf sample. Pure methanol was used to develop the DPPH radical, and 2 µl of a methanolic DPPH solution was added to 100 µl of sample extract or standard solution. For 20 minutes, this combination was left in the dark. After then, the sample absorbance was ascertained at 515 nm. It was done with a 100% methanol blank

solution. In the control solution, 100 microliters of clean water were used in place of the 100 microliters of extract. Using a calibration curve created with different gallic acid solution concentrations (10-100 ppm), the antioxidant properties of sample extracts were assessed.

2.5. Antidiabetic Properties

2.5.1. Alpha-amylase inhibitory activity

The α-amylase inhibition study was carried out using the 3,5-dinitrosalicylic acid (DNSA) method (Wickramaratne et al., 2016). To create concentrations ranging from 10 to 1000 g/mL, the JLP extract was treated with at least 10 percent dimethylsulfoxide and then diluted in buffer ((NaCl (0.006 M, Na_2HPO_4/NaH_2PO_4 (0.02 M, at pH 6.9). 200 µL of extract and 200 µL of -amylase solution were mixed and incubated at 30 °C for 10 minutes. After that, each tube received 200 µL of the starch solution (1 percent in water (w/v)) and was incubated for 3 minutes. The reaction was stopped by adding 200 µL DNSA reagent (12 g sodium potassium tartrate tetrahydrate in 8.0 mL 2 M NaOH and 20 mL 96 mM 3,5-dinitrosalicylic acid solution) to a water bath at 85–90 °C and boiling for 10 minutes. The mix was cooled to room temperature and diluted with 5 mL distilled water before being analysed with a UV-Visible spectrophotometer at 540 nm. By substituting 200 µL of buffer for the plant extract, a blank with 100% enzyme activity was created. In the absence of the enzyme solution, a blank reaction was generated using the plant extract at each concentration. As a positive control sample, acarbose (100–200 µg/mL) was employed, and the reaction was conducted in the same manner as the plant extract reaction. Using the equation below, the inhibitory activity of -amylase was calculated and reported as a percentage of inhibition. By plotting the percentage of α-amylase inhibition versus the extract concentration, the IC50 values were determined (Equation 5).

$$\%\ \alpha - \text{amylase inhibition } = 100\ \text{X}\frac{\text{A\%C} - \text{AS}}{\text{A\%C}} \quad (5)$$

Here, A%C= absorbance 100% control, AS= absorbance sample.

2.5.2. Alpha-glucosidase inhibitory activity

An assay for assessing the glucosidase inhibitory activity of JLP was described by Dej-adisai and Pitakbut (2015). The glucosidase enzyme converts the substrate, p-nitrophenol-D-glucopyranoside (pNPG), into the yellow product, p-nitrophenol (pNP), which is used to analyse the glucosidase reaction. 50µL of a 10 mM phosphate buffer solution (pH 7) containing 0.2 mg/mL sodium azide and 2 mg/mL bovine serum albumin were added to a well plate.

One unit/mL of Saccharomyces cerevisiae α-glucosidase and 50 L of an 8 mg/mL sample solution were added to the phosphate buffer solution (Type I, lyophilized powder, Sigma, EC 3.2.1.20). The solvent control was a 5 percent DMSO solution, and the positive control was 8 mg/mL of acarbose in each well. The mixes were incubated at 37o C for 2 minutes. 50 microlitres of 4 mM pNPG were then put into the well. The mixture has to incubate for a further five minutes in the same circumstances. For 5 minutes, the pNP was carried out and timed using a microplate reader at 405 nm every 30 seconds. The following linear relationship equation between absorbance and time was used to calculate the velocity (V) (Equation 6).

$$\text{Velocity} = \frac{\Delta Absorbance\ at\ 405\ nm}{\Delta\ Time} \quad (6)$$

Each sample's initial reaction's highest velocity was gathered, and the equation below was used to calculate the percentage of inhibition (Equation 7).

$$\%\ \text{Inhibition} = \frac{\text{V control} - \text{V sample}}{V\ control} X\ 100 \quad (7)$$

2.6. Anti-inflammatory Activities

2.6.1. Albumin denaturation inhibition

The assay was carried out as outlined by Osman et al. (2016). Ibuprofen and diclofenac, two positive standards, were produced at a concentration of 0.1 percent each (1.0 mg/ml), along with the JLP extracts. Each mixture's reaction vessel was made up of 1000 µl of the test extract, 1400 µl of phosphate-buffered saline, and 200 µl of egg albumin. As a negative control, distilled water was utilised in place of the extracts. The mixtures were then heated for 5 minutes at 70 °C after 15 minutes of incubation at 37 °C. Their absorbances at 660 nm were measured after cooling. This formula was used to determine the protein denaturation inhibition percentage (Equation 8):

$$\%\ \text{DI} = \left(1 - \frac{\text{ARTS}}{\text{ARTS}\ (-\text{ve control})}\right) * 100\% \quad (8)$$

DI= denaturation inhibition, ARTS= absorbance reading of the test sample.

2.5.2. Antiproteinase activity

The test was performed as outlined by Rajesh et al., (2019). 1 ml of 20 mM Tris-HCl buffer (pH 7.4), 0.06 mg of trypsin, and 1 ml of the test sample with varying concentrations (100–500 g/ml) were all included in the reaction mixture (2 ml). For five minutes, the mixture was kept heated at 37°C. 1 ml of 0.8 percent (w/v) casein was then added to the mixture. A further 20 minutes were spent keeping the mixture heated. To stop the process, 2 ml of 70% perchloric acid was added to the mixture. The murky suspension was then centrifuged after that. The supernatant's absorbance was then measured at 210 nm using a buffer as a blank. Three times the experiment was conducted. The following formula was used to calculate the % inhibition of proteinase inhibitory activity (Equation 9):

% inhibition = (Abs control-Abs sample)*100/Abs control (9)

3. Results

Figure 1 shows the proximate composition of JLP. The crude fat (8.19%) had the lowest content in JLP and the nitrogen-free extract (37.85%) had the greatest. JLP included 9.98 percent crude protein, 17.54 percent crude fibre, and 18.18 percent ash, respectively.

Phenol, one of the phytochemicals examined, had the highest concentration (383.15 mg/g), whereas flavonoids (1.84 mg/g) had the lowest concentration. Alkaloids, tannins, saponins, and steroids all had concentrations of 24.03 mg/g, 84.81 mg/g, 188.13 mg/g, and 231.3 mg/g, respectively (Figure 2).

The antioxidant activity of JLP is displayed in Figure 3. JLP had a 62.21 percent inhibition of lipid peroxidation and a 28.49 percent scavenging of ABTS radicals, respectively. The percentages for the DPPH radical scavenging, Fe chelation, and hydroxyl radical inhibition were 54.05 percent, 42.81 percent, and 54.35 percent, respectively.

JLP's alpha-glucosidase and alpha-amylase inhibition activities were 65.96 and 65.82 percent, respectively (Figure 4). JLP had a 35.51 percent albumin denaturation inhibition and a 61.58 percent antiprotease activity, respectively (Figure 5).

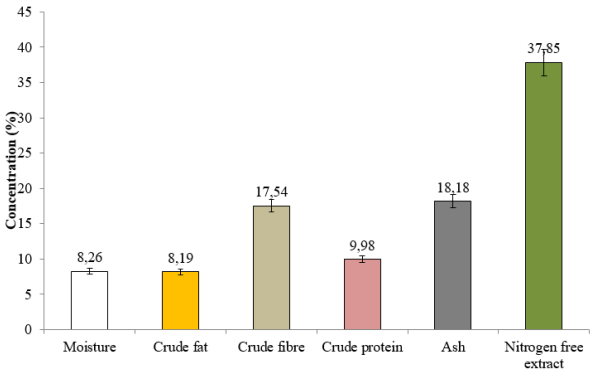

Figure 1. Proximate composition of *Justicia carnea* leaf powder.

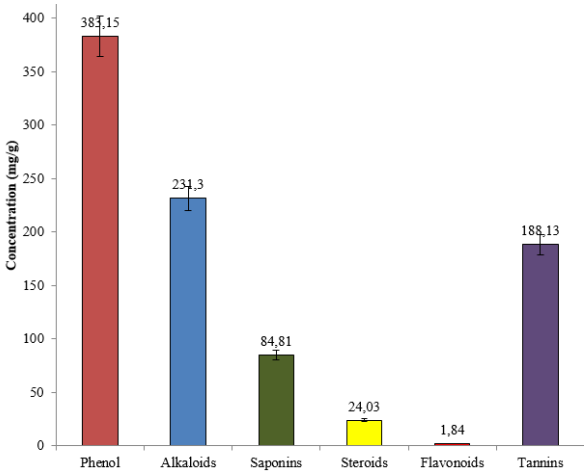

Figure 2. Phytochemical composition of *Justicia carnea* leaf powder.

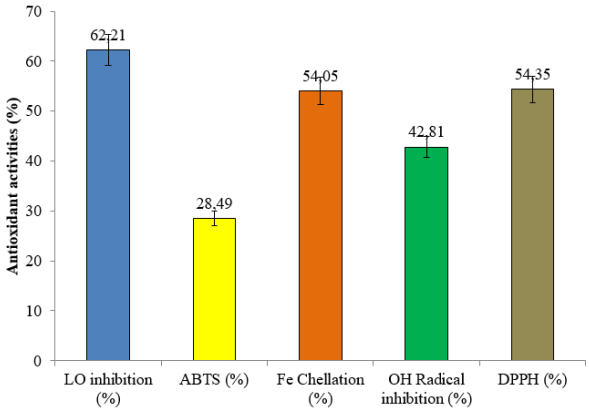

Figure 3. Antioxidant assays of *Justicia carnea* leaf powder (LO= Lipid peroxidation, ABTS= 2,2'-Azino-Bis-3-Ethylbenzothiazoline-6-Sulfonic Acid, OH= Hydroxyl, DPPH= 2,2-diphenyl-1-picrylhydrazyl).

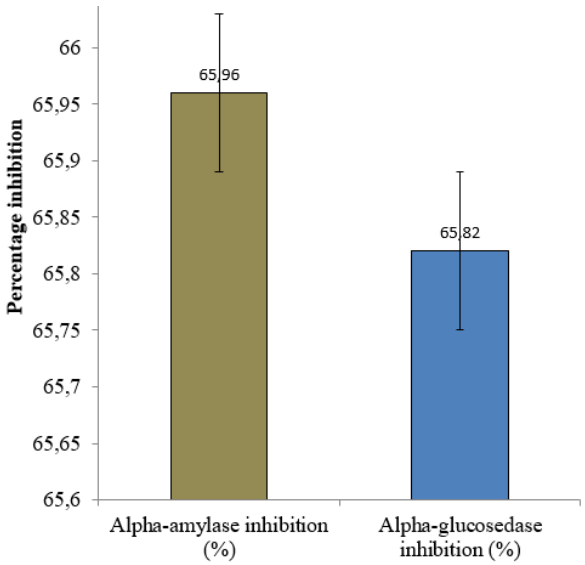

Figure 4. Antidiabetic properties of *Justicia carnea* leaf powder.

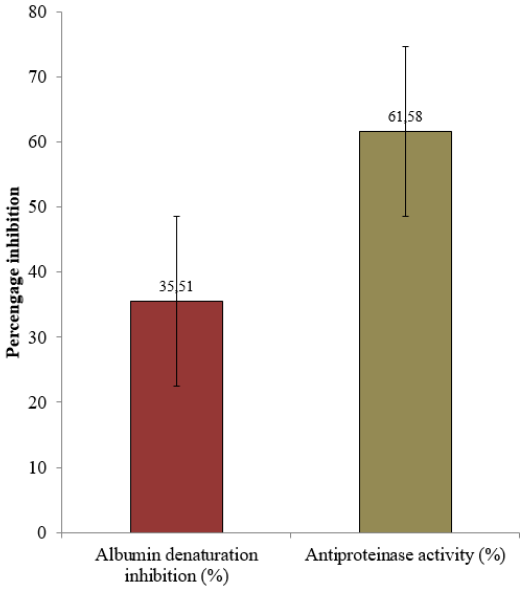

Figure 5. Anti-inflammatory properties of *Justicia carnea* leaf powder.

4. Discussion

Investigating the proximate composition can help determine whether certain foods, feeds, or supplements are appropriate to include in diets (Wu and Wu, 2017; Oloruntola, 2022). When taken as a dietary/food supplement, JLP's relatively high ash level suggests that it might augment the minerals present in foods or diets. Leaf meals were previously described by Somarsiri et al. (2010) as a dependable recipe for supplying dietary minerals. Additionally, the substantial amounts of crude fat and crude protein found in JLP reveal its nutritional value, indicating that when used as a feed ingredient or supplement, JLP, like several other phytogens or phytosupplements, may complement or contribute to the levels of fat and protein in diets (Sugiharto et al., 2019). When compared to other compositions, the nitrogen-free extract, which was noticeably more concentrated in JLP, also demonstrates its potential contribution to delivering dietary energy, especially when utilised at a macro level in feed or food formulation (Oloruntola, 2022). The 17.54 percent crude fibre content of JLP demonstrates that when added at a macro level in the diet, there may be a rise in feed bulkiness, which could affect protein intake, impair digestibility, and impair some monogastrics' performance (Buragohain 2016).

The use of phytochemicals or dietary supplements with secondary metabolites as feed supplements is presently in vogue due to the present global legal prohibition of the use of antibiotics as a growth promoter in diets of meat animals (Valenzuela-Grijalva et al., 2017; Kurek, 2019). The phenol and alkaloids, which are substantially more abundant in JLP than the other compounds identified in this study, have health benefits because it has been linked to a decreased risk of degenerative diseases like diabetes, Alzheimer's disease, cancer, and cardiovascular disease (Gutierrez-Grijalva et al., 2016; Kurek, 2019). Phenols are thought to have greater antioxidant potential, even than the widely consumed dietary vitamin E (Hollman, 2001). The hydroxyl groups connected to the phenyl ring of phenols are thought to be responsible for their antioxidative properties (Hollman, 2001). The alkaloids demonstrate antioxidant activities by inhibiting NADPH-oxidase activity by activation of the nuclear factor Nrf2 pathway (Macakova et al., 2019). According to reports, phenols and alkaloids are the two most significant compounds in phytogens, with alkaloids having a stronger link with antioxidant activity than phenols (Gan et al., 2017). The presence of saponins at a worthwhile proportion inJLP is of health importance because, along with their use in treating hypercalciuria, as an antidote for acute lead poisoning, and as an inhibitor of dental cavities, saponins are another substance with a reputation for strengthening immune function and so lowering cancer risks, lowering blood sugar and blood lipids (Shi et al., 2004). Tannin, a phenolic molecule with a high molecular weight that is also present in JLP, is well-known for its capacity to form complexes with proteins, alkaloids, carbohydrates, and gelatin as well as its antibacterial and antioxidant properties (Widsten et al., 2014). In addition, tannins and related compounds were reported to exhibit moderate cytotoxicity against tumour cell lines (Li et al., 2013). Steroids change the fluidity of membranes and serve as signalling chemicals. Because of the immune-modulating and anti-inflammatory effects of steroids, the presence of steroids in JLP in this study adds to its nutritional and health benefits. (Ericson-Neilsen and Kaye, 2014). The prevention of disease may be aided by flavonoids, secondary metabolites with a benzopyrone ring carrying phenolic or polyphenolic groups at various locations (Cavalcante et al., 2018). For instance, the total flavonoids or subclasses were said to have qualities that protect the cardiovascular system, are anti-inflammatory and antioxidant, and fight cancer, obesity, and diabetes (Ballard and Maróstica, 2019). Flavonoids, albeit having the least concentration, compared to other chemicals evaluated in this study, its presence in JLP also contributed to the nutraceutical quality of the phytogens.

The loss of free radical electrons from the lipid cell membranes during the process of lipid peroxidation resulted in a decrease in physiological performance, an increase in the permeability of the cell membranes, and a loss of membrane fluidity. Lipid peroxidation generally has negative effects on the nutritional value, texture, flavour, and appearance of food and food products (Balu et al., 2005). Since superoxide and hydroxyl radicals, which produce peroxy radicals that enhance the lipids' chain reaction, start the process of lipid oxidation, antioxidants with the ability to scavenge peroxi radicals could inhibit the process (Bajpai et al., 2015). Medicinal plants have various phytochemical components (e.g. flavonoids, tannins and phenyl propanoids and phenolic acids) that have antioxidant and free radical scavenging efficacy (Bajpai et al., 2015). In this study, the JLP showing 62.21% inhibitory effects on lipid peroxides may be a result of its hydroxyl radical scavenging abilities. Some other botanicals were also reported for having protection against lipid oxidation (Geetha and Vasudevan, 2004; Bajpai et al., 2015).

Since the production of free radical species is inhibited by the addition of antioxidants, methods being used to measure the free radical scavenging capacity are typically based on the inhibition of the accumulation of oxidised products, which gives rise to a reduction of the endpoint by scavenging free radicals, regardless of the individual compounds which contribute towards the total capacity of a phytogens or botanicals in scavenging free radicals. The 2,2-azino-bis (3-ethylbenzenthiazoline-6-sulphonic) acid radical (ABTS) assay is a technique for assessing a plant's ability to scavenge free radicals (Turkoglu, 2009). According to this study, the JLP has the potential to be a phytogenic antioxidant due to its 28.49 percent ABTS radical scavenging activity.

The myocardium, spleen, endocrine glands, and liver accumulate ferritin and hemosiderin due to the body's inability to eliminate iron released from the breakdown

of transfused red blood cells, which causes tissue damage and complications like hypothyroidism, liver failure, diabetes, heart failure, and early death (Ebrahimzadeh et al., 2008). According to the results of this study, JLP had a significant amount of iron chelating activity (50%) which suggests the phytogens might be used as dietary iron chelators to mobilise tissue iron by creating soluble, stable complexes that are then eliminated in urine and/or faeces. Chelation therapy is said to lessen issues associated with iron, according to earlier reports (Ebrahimzadeh et al., 2008).

Being the most reactive free radical, the hydroxyl radical can be created by combining hydrogen peroxide and superoxide anion in the presence of Fe2+ and Cu2+. The hydroxyl radicals are recognised for generating DNA strand breaks, lipid oxidation stimulation, and mutagenicity, carcinogenicity, and cytotoxicity as a result of connecting nucleotides in DNA (Valko et al., 2007; Bajpai et al., 2015). In this study, JLP demonstrated substantive radical scavenging activity with a radical inhibition capacity of 42.81%. The hydroxyl radical scavenging activity of *Terminalia chebula*, *Terminalia belerica* and *Emblica officinalis* was reported (Hazra et al., 2010). The DPPH radical scavenging of JLP being 54.35% in this study shows that the phytogens have antioxidant and free radical scavenging properties. This agreed with the previous report on DPPH radical scavenging activity in some selected medicinal plants (Amari et al., 2014; Kaur and Mondal, 2014).

By selectively blocking pancreatic α-amylase and intestinal α-glucosidase, hyperglycemia in type 2 diabetes cases caused by hydrolysis of starch by uptake of glucose by intestinal α-glucosidases and pancreatic α-amylase can be effectively managed. The inhibition of these enzymes causes a delay in the breakdown of carbohydrates and lengthens the time it takes for carbohydrates to be completely digested. As a result, the rate of glucose absorption is slowed and the postprandial rise in blood sugar is also slowed (Kwon et al., 2007). JLP demonstrated a 65.96 percent and 65.82 percent inhibition of α-amylase and α-glucosidases in this investigation, demonstrating the phytogens' ability to block hydrolyzing enzymes. This was in line with earlier research showing that white and ginger suppressed *in vitro* α-amylase activity (Oboh et al., 2010).

Inflammatory factors, such as heat, toxic chemical irritants, microbial infections, and physical injuries by inflammation, elicit responses in living tissues. The inflammatory response of the cells results in a variety of pathological reactions such as swelling, redness, discomfort, and heat as well as some diseases like cancer, arthritis, and stroke (Osman et al., 2016). Because there is a correlation between tissue damage and the denaturation of protein cells or intercellular components, the ability of phytogens or metabolites to prevent protein denaturation indicates apparent potential for anti-inflammatory effect (Osman et al., 2016). JLP's capacity to prevent albumin protein denaturation (35.51 percent inhibition) in this investigation so supports the claim that it has anti-inflammatory characteristics. Furthermore, arthritic responses have been linked to proteinases and leukocyte proteinase, in particular, is crucial for the occurrence of tissue damage during inflammatory responses (Rajesh et al., 2019). This study's observation of JLP's antiproteinase activity (61.58 percent) further demonstrates or supports the anti-inflammatory properties of JLP.

5. Conclusion

Thus, these results suggested that JLP could be a source of protein, energy and vitamins. In addition, JLP possesses antioxidant, anti-diabetic and anti-inflammatory activities. The JLP could be suitable as a dietary phytogenic supplement and is recommended for use as a dietary supplement in feeding trials with an animal model.

Author Contributions

Concept: O.D.O (35%), S.O.A. (30%) and C.O.O. (30%), Design: O.D.O (35%), O.S.F. (30%) and T.O.G. (30%), Supervision: O.D.O (100%), Data collection and/or processing: O.D.O (20%), S.O.A. (20%), S.A.A. (20%), C.O.O. (20%) and T.O.G. (20%), Data analysis and/or interpretation: O.D.O (20%), S.O.A. (20%), S.A.A. (20%), C.O.O. (20%) and T.O.G. (20%), Literature search: O.D.O (20%), S.O.A. (20%), S.A.A. (20%), C.O.O. (20%) and T.O.G. (20%), Writing: O.D.O (15%), S.O.A. (15%), S.A.A. (15%), C.O.O. (15%), O.S.F. (20%) and T.O.G. (20%), Critical review: O.D.O (15%), S.O.A. (15%), S.A.A. (15%), C.O.O. (15%), O.S.F. (20%) and T.O.G. (20%). Submission and revision: O.D.O (15%), S.O.A. (15%), S.A.A. (15%), C.O.O. (15%), O.S.F. (20%) and T.O.G. (20%). All authors reviewed and approved final version of the manuscript.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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CHEMICAL COMPOSITION, ANTIOXIDANT, ANTIFUNGAL AND HERBICIDAL ACTIVITIES OF ESSENTIAL OILS FROM THREE THYMUS SPECIES

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Abstract: The current study aimed to research the chemical composition, antioxidant, herbicidal and antifungal effect three essential oils, as obtained from *Thymus canoviridis* Jalas., *Thymus eriocalyx* (Ronni.) Jalas. and *Thymus fallax* Fisch. et C.A. Mey. Antioxidant capacities of essential oils were determined by 1.1dipheny1-2-picrylhydrazyl (DPPH) method. The antifungal potential was tested *in-vitro* against *Fusarium equiseti* (Corda) Sacc., *Fusarium graminearum* Schwab., *Fusarium moniliforme* J. Sheld and *Fusarium oxysporum* Schlec. Bioherbicidal effect was studied *in-vivo* and *in-vitro* against weed seeds of *Amaranthus retroflexus* L., *Convolvulus arvensis* L. and *Chenopodium album* L. Essential oils were assayed in laboratory three concentrations (5, 10 and 20 µL/petri) and one (20 µL/pot) concentration in the greenhouse. The chemical composition of essential oils was analyzed by (GC) and (GC/MS). The major constituents were *p*-cymene, 1,8-cineole and γ-terpinene. As a result of the antioxidant study, it was determined that *Thymus* essential oils had remarkable antioxidant activity. On the other hand, oils decreased mycelial growth of pathogens at different rates due to increasing doses and inhibited 9.33-100% *in-vitro* conditions. In the bioherbicidal study assays showed that tested essential oils had inhibitory effects on the seed germination and seedling growth of weeds. The study concludes that *Thymus* essential oils might has the potential use as bioherbicide and biofungicide can constitute an alternative process of weed control and fungus.

Keywords: Antioxidant effect, Antifungal effect, Bioherbicidal effect, Essential oil, *Thymus*

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1. Introduction

Turkey due to take place at the intersection of different climates, plant species and is a very rich country in terms of diversity. It is one of the leading countries in the world market in the export of tea plants and spices, and Lamiaceae (Labiatae) family takes the first place among the plant species traded (Kocabas and Karaman 2001; Özkan, 2007). In addition, the Lamiaceae family includes medicinal and aromatic herbs with powerful antimicrobial and antioxidant properties (Goudjil et al., 2020). Lamiaceae is a plant family represented by 236 genera and 7280 species and is distributed worldwide, especially in the temperate zone. In Turkey Flora Lamiaceae family of 45 genera, 565 species represented by and a total of 735 taxa (Davis, 1970). The biological and pharmacological plant species of this family have been known for many years. The phytotherapy feature of these plants is mostly due to the essential oils they contain (Bozin et al., 2006). The essential oil yield of the Lamiaceae family is very high, and the important known species are *Thymbra*, *Thymus*, *Origanum*, *Satureja*, *Mentha*, *Teucrium*, *Ballota*, *Stachys*, *Salvia*, *Ajuga*, *Prunella*, *Melissa*, *Lamium*, *Sideritis* and *Marrubium*. *Thymus* is one of the 8 largest genera of Lamiaceae family in terms of number of species. The species belonging to the genus *Thymus* are known as "thyme" or "stone thyme" in our country (Tümen et al., 1998; Bagci et al., 2005). The leaf and flowering parts of the *Thymus* plant with high essential oil content are mostly used as herbal tea, tonic and flavoring (Zargari, 1990; Amin, 2005). In addition, *Thymus* essential oils are also used in medicine and pharmacology because of their antiseptic, antibacterial, antifungal, antispasmodic, antitussive, expectorant, analgesic properties (Cosentino et al., 1999; Hedlili et al., 2002; Kabouche et al., 2005; Rasooli et al., 2006). *Thymus* have been found to be approximately 270 terpenes and one or more of them have been reported to be dominant. Especially thymol, carvacrol, linalool, p-cymene, geraniol, borneol are the most important terpenes. *Thymus* species is the most important source of monoterpenoid phenols in the plant kingdom (Stahl-Biskup, 2002). Therefore, considering that the main components of essential oils of vegetable origin are terpenes, it can be said that they have a potential to be used in weed control and fungal diseases (Kordali et al., 2009; Üstüner et al., 2018). Studies have shown that

essential oils obtained from plants in our country and in the world have the potential to prevent the growth of plant pathogenic fungi and bacteria. (Zambonelli et al., 1996; Bianchi et al., 1997; Wilson et al., 1997; Türküsay and Onoğur 1998; Ristic et al., 2000; Walter et al., 2001; Abou-Jawdah, 2002; Bouchra et al., 2003; Daferera et al., 2003; Bowers and Locke, 2004; Cakir et al., 2005; Soylu et al., 2005a; Soylu et al., 2005b; Soylu et al., 2006; Lee et al., 2007; Kotan et al., 2010). Some essential oils create an isolated area where the plants can grow easily by preventing the germination and growth of weed seeds (allelopathic effect). Effects of essential oils on germination and plant growth; They damage their intracellular structures, inhibit cell growth and development, slow down photosynthesis and respiration, and stop germination, seedling and plant growth by acting on oxygen uptake (Abrahim et al., 2000). The use of essential oils obtained from plants in the fight against plant diseases has started to attract the attention of researchers today. It is thought that essential oils penetrate through the cell wall of fungi and disrupt the structure of the cell wall, stop fungus growth and conidia production, and cause deformations in hyphae and create cytoplasmic currents (Chang et al., 2001; Ultee et al., 2002; Soylu et al., 2006).

In the present study, the purpose were to evaluate the antioxidant, herbicidal and fungicidal effect of the essential oil isolated from *Thymus canoviridis*, *T. eriocalyx* and *T. fallax* on some fungi and weeds. In addition, it is thought that this study conducted with different *Thymus* species will provide a source for antifungal and herbicidal studies and contribute to the literature.

2. Materials and Methods

2.1. Plant Material and Essential Oil Extraction

Weed seeds of *A. retroflexus*, *C. arvensis* and *C. album* were collected from Erzurum region of Turkey between June-September of 2015-2016. *Thymus canoviridis* Jalas. from Erzurum-Kırkdegirmenler (2122 m), *Thymus eriocalyx* (Ronniger) Jalas. from Igdir-Tuzluca (2028 m) and *Thymus fallax* Fisch. et C.A. Mey. from Igdır-Korhan (1899 m) were collected at flowering stage from the different localities of Turkey between June 2014 and August 2015. Plant herbariums have been deposited in the Department of Plant Protection, herbarium at Ataturk University in Erzurum, Turkey. The tested plants were identified by Prof. Dr. Vladimir I. DOROFEYEV Komarov Botanical Institute (Herbarium), Russian Academy of Sciences, St. Petersburg-Rusya; Prof. Dr. Ali KANDEMİR Erzincan Binali Yildirim University, Faculty of Arts and Sciences, Department of Biology Erzincan-Turkey; Prof. Dr. Tuncay DİRMENCİ Balıkesir University Necatibey, Faculty of Educatıon, Department of Biology Education, Balıkesir-Turkey and Prof. Dr. Meryem ŞENGÜL KÖSEOĞLU Atatürk University, Faculty of Science, Department of Biology, Erzurum-Turkey. Aerial parts of the plants were dried for 8 days in the shade and ground in a grinder (nearly 0.200-0.500 mm). The dried plant samples (500 g) were subjected to hydro distillation for 3-4 hours using a Clevenger-type apparatus. The oils were stored at 4 ^{o}C until used for antioxidant activity, herbicide and fungicide bioassays.

2.1.1. Antioxidant activity

DPPH free radical scavenging activity

The free radical scavenging activity of essential oils was determined using the method proposed by Doshi et al., (2015). Briefly, 50 µl of diluted (1:10 v/v) essential oil in methanol were added to 950 µl of the DPPH methanolic solution (60 µM, freshly prepared). The mixture was vortexed and maintained at room temperature for 30 min in the dark then the absorbance was measured at 517 nm against the corresponding blank. A mixture consisting of 50 µl methanol and 950 µl of DPPH solution was used as control. Each determination was carried out in triplicate, and results of the radical-scavenging activity were expressed as microgram Trolox equivalent per gram of essential oils (µg TE/g EO). The inhibition % values were calculated according to the Equation 1 shown below.

$$\text{Inhibition } \% = [(A_{DPPH}-A_{E.O})/A_{DPPH})*100] \quad (1)$$

2.2. Fungal Isolates and Antifungal Test

The plant pathogenic fungi; *Fusarium equiseti*, *F. graminearum*, *F. moniliforme* and *F. oxysporum* were obtained from the collection Mycology of Prof. Dr. Berna TUNALI (Ondokuz Mayıs University, Department of Plant Protection. Primarily, fungi were plated on potato dextrose agar (PDA, Oxoid, CM0139) mixed with P-aminobenzoic acid 10 mg/L (Sigma, A-9878). The cultures incubated at the darkness with 25 ± 2°C in the incubator for 7-10 days. The antifungal effects of essential oils evaluated by contact phase effects against mycelial growth of *Fusarium equiseti, F. graminearum, F. moniliforme* and *F. oxysporum*. From 7-10 days old cultures, 5 mm agar blocks containing hyphal tips from the colony margins cut with the fungal borer. And, the blocks transferred to PDA mixed with different concentrations of essential oils (5 µL (250 ppm), 10 µL (500 ppm) and 20 µL (1000 ppm), in each 20 mL PDA medium) from *Thymus canoviridis*, *T. eriocalyx* and *T. fallax*. To mix the essential oils in the medium 100 µL absolute ethanol (Sigma-Aldrich) in each 20 mL PDA was used. In controls, 100 µL absolute ethanol mixed with 20 mL PDA without essential oil. The 9x1.5 cm plastic Petri dishes selected for the experiment. For each concentration, three replicate plates used. After each 24 hours, the colony diameter of treatments and control measured. The measuring of colony diameter continued until the colony growth reaches to the sides of the petri dish in controls.

2.3. Fungal Inhibitory Test

The mean growth of the pathogen determined by measuring the colony diameter in two directions. The growth of fungi isolates in oil treated Petri dishes compared with the control plates. To indicate the fungal

hyphae growth, the initial fungal discs diameter (5 mm) subtracted from the final colony diameter of each treatment and control. Mycelia Growth Inhibitory (MGI) values were obtained using the equation 2:

$$MGI\ (\%) = [(C-T)/C] \times 100 \quad (2)$$

where C and T represent mycelia growth diameter in control and treated Petri plates respectively.

2.4. *In-Vitro* Herbicidal Activity Experiments

Weed seeds were sterilizied with sodium hypochloride (15 %) for 10 minutes and then they were washed 3-7 times with sterile distilled water. The sterilized seeds (n=50) of *A. retroflexus, C. arvensis* and *C. album* were placed into petri dishes (9 cm diameter) with 2 layers of filter paper (Whatman No.1) (Kordali et al., 2007; Kordali et al., 2008; Üstüner et al., 2018). To determine the contact herbicidal effects of oils, the oils were dissolved in ethanol–steril water solution (10%, v/v) and adjusted into 5, 10 and 20 µL/mL final concentrations. The prepared solutions were transferred into petri dishes (8 mL Petri^{-1} dishes). The petri dishes were immediately closed and covered tightly with parafilm and petri dishes were incubated kept at 25±2 ºC in a growth room providing with 12 h of fluorescent light and humidity of 80%. At the end of 7-10 days, the number of germinated seeds percentages (%) was determined and their root and shoot lengths (mm) were measured by using ruler and calculated with the following Equation 3 and 4. Petri dishes containing 8 mL Ethanol–water solution (10%, v/v) was used as the negative control. In addition, Beststok 330 EC (330 g/L Pendimethalin) (5, 10 and 20 µL/mL) was used as positive control. All experiments were prepared in a completely randomized design with three replications.

$$Germination\ \% = \frac{Number\ of\ Germinated\ Seeds}{Number\ of\ Total\ Seeds} \text{X } 100 \quad (3)$$

$$Inhibition\ \% = \frac{C-T}{C} \text{X } 100 \quad (4)$$

C: % germination and seedling (root and radicle) length in control

T: % germination and seedling (root and radicle) length in control in treatment with essential oil

2.5. *In-Vivo* Herbicidal Activity Experiments

Post-emergence experiment were applied study the effect of *T. canoviridis*, *T. eriocalyx* and *T. fallax* essential oils on 1-2-week-old *A. retroflexus*, *C. arvensis* and *C. album* plants of the weedy species under controlled greenhouse conditions. Firstly, pots (14 x 17 cm) were filled with 300 g soil (soil/potting soil 3:1, w/w) sterilized in the autoclave. Then, 50 seeds sterilized of the weeds were planted into the pots and kept under photoperiod conditions (20 ± 3 ºC, 12 h light and 12 h dark photoperiod) and relative humidity (60 ± 3%) in a growth room to allow germination and growth of the plant samples. The pots were irrigated with tap water when necessary. The number of germinated seeds of the respective weed samples in each pot was considered. Then, the oils was emulsified in 10 mL of Ethanol-water solution (10%, v/v) and was used for control treatment. The latest concentration of applications was 20 µL/mL. Prepared emulsions were sprayed equivalently with a glass spray bottle on the surface of all plants in each pot in the stage of 3-4 real leaves. The plants sprayed on Best Amin 500 SL (2,4-D Amin 500 g/L) (20 µL/mL for each pot) were used as a positive control. At the end of 24 and 48 hours the dead plants was recorded by counting. The experiments were performed in a completely randomized plan with four replications including controls. The phytotoxicity of the treatments was expressed as percent mean of dead plants (Kordali et al., 2008). The percentage of effect was calculated following the Equation 5:

$$Percentage\ of\ effect\ \% = \frac{\text{Dead Leaves}}{\text{Number of Total Leaves}} \text{X } 100 \quad (5)$$

2.6. GC-MS Analysis

The chemical constituents of the essential oils of *T. canoviridis*, *T. eriocalyx* and *T. fallax* were decided by Gas Chromatography-Mass Spectrometry (GC-MS). DB-1 fused silica non-polar capillary column (30 m × 0.25 mm I.D., film density 0.25 µm) was used for the analysis. Helium was used as carrier gas with 1.4 mL/min flux ratio. The ion source, injector and MS transfer route temperatures were 200, 220 and 290°C, respectively. The injection volume was 0.2 µL with a separate rate of 20:1. Ionization energy of EI–MS evaluations were taken at 70 eV. Mass area was from m/z 28 to 650 amu. Scan time was 0.5 s with 0.1 s interscan retardations. The oven heat was keep at 60°C for 5 min, then increased up to 240°C with 4°C/min rising and kept at this temperature for 10 min. Recognition of constituent of the essential oil was based on GC retention index and computer matching with the libraries of Wiley, NIST-2008 and TRLIB, as well as by comparison of the disintegration versions of the mass spectra. Quantitative data of the essential oils was acquired from the FID area rates. (Usanmaz Bozhüyük, 2020).

2.7. Statistical Analysis of Data

Study results was take estimating the statistical significance of differing treatments mean values against negative and positive control treatment using ANOVA and Duncan test at levels P<0.05 (Genç and Soysal, 2018). All assays were done SPSS program (version 17.0, SPPS Inc., Chicago, IL, USA) software package.

3. Results and Discussion

3.1. Yield and Chemical Composition of the *Thymus canoviridis*, *Thymus eriocalyx* and *Thymus fallax* Essential Oils

The essential oils obtained by hydrodistillation of dried *T. canoviridis*, *T. eriocalyx* and *T. fallax* were flowers and leaves yellow color and emitted a strong smell. The essential oil yields of *T. canoviridis*, *T. eriocalyx* and *T. fallax* were 0.70, 0.60 and 1.5% (w/v, dry weight basis), respectively. The essential oils compounds identified by GC–MS method are listed in Table 1. Based on GC-MS results, identified 100, 99.60 and 97.74% of the compounds present in the essential oils *T. canoviridis*, *T. eriocalyx* and *T. fallax*, respectively. The *Thymus* essential oils were characterized by the predominance of the monoterpene hydrocarbons class, among which *p*-cymene (23.06-32.97%), 1,8-cineole (11.34-25.34%) and γ-terpinene (14.40-24.79%) were the present. This class was followed by oxygenated monoterpenes and sesquiterpene hydrocarbons, while oxygenated sesquiterpenes were found in minor quantities.

Table 1. Chemical constituents (%) of the essential oils of *Thymus* species

No	Chemical constituent	*Thymus canoviridis*	*Thymus eriocalyx*	*Thymus fallax*
1	α-thujene	0.66	0.71	0.76
2	α-pinene	1.00	5.90	1.69
3	α-fenchene	0.49	0.67	0.59
4	Myrcene	1.05	1.93	0.80
5	α-terpinene	4.77	1.49	2.23
6	*p*-cymene	23.06	32.97	24.23
7	1,8-cineole	11.34	25.34	22.64
8	γ-terpinene	24.79	19.15	14.40
9	Camphor	-	1.07	1.36
10	Thymol	-	-	-
11	Thymol methyl ether	0.84	0.99	tr
12	Carvacrol methyl ether	1.97	0.80	1.55
13	Borneol acetate	0.58	0.52	1.55
14	α-terpineol acetate	17.00	2.62	3.27
15	β-bourbonene	-	-	1.03
16	β-caryophyllene	5.69	1.86	12.70
17	β-bisabolene	6.13	1.62	4.91
18	Methyl-α ionone	-	0.72	0.93
19	γ-cadinene	0.63	0.49	1.37
20	Caryophyllene oxide	-	0.75	1.73
Monoterpene hydrocarbons (%)		55.82	62.82	44.70
Oxygenated monoterpenes (%)		31.73	31.34	30.37
Sesquiterpene hydrocarbons (%)		12.45	4.69	20.94
Oxygenated sesquiterpenes (%)		-	0.75	1.73
Total (%)		100	99.60	97.74

Identification method: GC, identification based on the tR of authentic compounds on a SGE-BPX5 capillary column; MS, tentative identification based on computer matching of the mass spectra with those listed in the Wiley7N and TRLIB libraries and published data (Adams 2007). tr: Traces (< 0.10%).

Concerning the previously reported content of *Thymus canoviridis* (Azaz and Celen 2012), *Thymus eriocalyx* (Amiri, 2012) and *Thymus fallax* (Kucukbay et al., 2014) essential oils, it is point out that there were important quantitative differences suggesting that the environmental factors and genotypes strongly impact its chemical composition. For example, *p*-cymene (32.97%) was found to be the major constituent of *T. eriocalyx* essential oil in our research (Table 1). On the contrary, *p*-cymene component was found are very low concentration (4.1%), while the major component thymol was determined at 42.6% (Amiri 2012). *Thymus canoviridis* is very rich in thymol (Azaz and Celen 2012), *T. fallax* in thymol and *o*-cymene. Studies show that thymol is the main compound in almost all samples. It is accepted that the terpenes, thymol, p-cymene, 1.8-cineole and carvacrol are the major volatile components of *Thymus* species. Some studies have reported that thyme essential oil has high levels of phenolic substances, p-cymene, and γ-terpinene (Kabouche et al., 2005). The comparison between these results and the results of other reports showed differences, possibly due to plant types or areas, as well as harvest time, altitude, temperature.

3.2. Antioxidant Activity of *Thymus canoviridis, Thymus eriocalyx* and *Thymus fallax* Essential Oils

Antioxidant activity of *T. canoviridis*, *T. eriocalyx* and *T. fallax* essential oils was measured by DPPH scavenging assay, while BHA, BHT, Trolox and α-Tokoferol was used as controls. Compared to standard BHA, BHT, Trolox and α-Tokoferol; it was determined that as the doses of essential oils increased, the inhibition values (IC_{50}) also increased (Table 2).

Table 2. Antioxidant activities of the *Thymus* essential oils tested as compared to standard BHA, BHT, Trolox and α-Tokoferol

Essential Oils and Standards	% Inhibition Values (IC_{50}) Concentrations		
	0.5 µg/mL	1 µg/mL	1.5 µg/mL
BHA	56.437	61.468	78.719
BHT	61.552	78.899	79.661
Trolox	91.534	93.578	94.350
α-Tokoferol	76.014	73.211	77.589
T. canoviridis	57.672	76.880	86.629
T. eriocalyx	58.201	79.266	85.876
T. fallax	77.072	79.633	83.428

The scavenging effects on the DPPH radical expressed as IC_{50} value was the highest for concentration of 1.5 µg/mL in *T. canoviridis* essential oil (86.629%) followed by *T. eriocalyx* (85.876%) and *T. fallax* essential oils (83.428%), showing a radical scavenging activity highest important than that the standarts BHA (IC_{50} 78.719%), BHT (IC_{50} 79.661%) and α-Tokoferol (77.589%). But, *Thymus* essential oils determination a antioxidative activity clearly less important than that the standart Trolox (IC_{50} 94.350%) (Table 2).

In the study conducted with *Thymus capitatus* essential oil and its 2 major components; antioxidant properties, determined by 2,2-diphenylpicrylhydrazyl assay, revealed that IC_{50} values were 119.403 and 105 µg/mL for oil, thymol and carvacrol respectively and especially carvacrol active compounds with strong antioxidativity (Džamić et al. 2015). Besides, thymol and carvacrol, the main component of *Thymus* and other thyme essential oils, are potent antioxidants and their use could be beneficial in the antioxidative conservation (Faleiro *et al*. 2005; Hazzit et al. 2006). It is hard to property the antioxidant activity to one or few active compounds of total essential oils, since both minor and major constituents could perform significant addition to the essential oil activity (Wang et al., 2008). Because, in our study, the high proportion of *p*-cymene and 1,8-cineole compounds in *Thymus* essential oils may suggest that they increase antioxidant activity. In general, it can be said that plant essential oils and especially *Thymus* essential oils have antioxidant capacity.

Table 3. Antifungal activities of *Thymus* essential oils against *F. equiseti*, *F. graminearum*, *F. moniliforme* and *F.oxysporum* fungi

Essential oils	5 µL/petri Growth Inhibition (mm)(%)	10 µL/petri Growth Inhibition (mm) (%)	20 µL/petri Growth Inhibition (mm) (%)	P. Control (10 µL/petri) Growth Inhibition (mm) (%)	N. Control Growth (mm)
			Fusarium equiseti		
T. canoviridis	0.0 ± 0.0 a 100	0.0 ± 0.0 a 100	0.0 ± 0.0 a 100	33.7 ± 0.40 b 36	52.7 ± 0.92 c
T. eriocalyx	0.0 ± 0.0 a 100	0.0 ± 0.0 a 100	0.0 ± 0.0 a 100	33.7 ± 0.40 b 36	52.7 ± 0.92 c
T. fallax	0.0 ± 0.0 a 100	0.0 ± 0.0 a 100	0.0 ± 0.0 a 100	33.7 ± 0.40 b 36	52.7 ± 0.92 c
			Fusarium graminearum		
T. canoviridis	0.0 ± 0.0 a 100	0.0 ± 0.0 a 100	0.0 ± 0.0 a 100	1.71 ± 0.10 ab 96.5	49.2 ± 1.0 g
T. eriocalyx	9.33 ± 1.01 d 81	0.0 ± 0.0 a 100	0.0 ± 0.0 a 100	1.71 ± 0.10 ab 96.5	49.2 ± 1.0 g
T. fallax	0.0 ± 0.0 a 100	0.0 ± 0.0 a 100	0.0 ± 0.0 a 100	1.71 ± 0.10 ab 96.5	49.2 ± 1.0 g
			Fusarium moniliforme		
T. canoviridis	0.0 ± 0.0 a 100	0.0 ± 0.0 a 100	0.0 ± 0.0 a 100	17.7 ± 1.48 d 68.7	56.7 ± 1.50 f
T. eriocalyx	0.0 ± 0.0 a 100	0.0 ± 0.0 a 100	0.0 ± 0.0 a 100	17.7 ± 1.48 d 68.7	56.7 ± 1.50 f
T. fallax	0.0 ± 0.0 a 100	0.0 ± 0.0 a 100	0.0 ± 0.0 a 100	17.7 ± 1.48 d 68.7	56.7 ± 1.50 f
			Fusarium oxysporum		
T. canoviridis	0.0 ± 0.0 a 100	0.0 ± 0.0 a 100	0.0 ± 0.0 a 100	0.0 ± 0.0 a 100	41.5 ± 0.60 c
T. eriocalyx	0.0 ± 0.0 a 100	0.0 ± 0.0 a 100	0.0 ± 0.0 a 100	0.0 ± 0.0 a 100	41.5 ± 0.60 c
T. fallax	0.0 ± 0.0 a 100	0.0 ± 0.0 a 100	0.0 ± 0.0 a 100	0.0 ± 0.0 a 100	41.5 ± 0.60 c

*The differences between the averages containing different letters in each column are statistically significant. (P≤0.05).

3.3. Antifungal Activity of *Thymus canoviridis, Thymus eriocalyx and Thymus fallax* Essential Oils

The effectiveness of *T. canoviridis, T. eriocalyx* and *T. fallax* essential oils on plant pathogens *F. equiseti*, *F. graminearum*, *F. moniliforme* and *F. oxysporum* fungi was investigated *in-vitro.*

Antifungal activity in bioassay, our results showed that this essential oils strong fungicidal effect potential at different concentrations (5, 10 and 20 µL/petri). A rise in essential oils concentration increased inhibition of pathogens after 7 days of incubation. Compared to controls, 5, 10, and 20 µL/petri concentrations essential oils of *T. canoviridis, T. eriocalyx and T. fallax* from inhibited *F. equiseti* mycelium growth by 100%. It has been observed that positive control has 36% inhibition rate in the mycelium growth of the pathogen *F. equiseti*, but its effect rate is lower than *Thymus* essential oils. In contrast to the *F. equiseti* pathogen, 5 µL/petri concentration essential oil of *T. eriocalyx* showed lower than antifungal effects against the *F. graminearum* pathogen. But, *T. eriocalyx* (10 and 20 µL/petri), *T. canoviridis* and *T. fallax* (5, 10 and 20 µL/petri) of concentration inhibited the pathogen by 100%. Looking at the table, the fungicidal effects of the *Thymus* essential oils on *F. graminearum* pathogen mycelium growths are mostly higher than commercial fungicide, Captan 500 FL (10 µL/petri) (Table 3). *Fusarium moniliforme* and *Fusarium oxysporum* mycelium growth was completely inhibited at 5, 10 and 20 µL/petri concentration by 100%. Similarly, in the positive control the *F. moniliforme* drastically inhibited (68.7 %) the mycelium growth, while mycelium growth was completely (100%) inhibited at *F. oxysporum* (Table 3). The highest fungicidal effect was observed at a concentration of 20 µL/petri in all applied concentrations and *T. canoviridis*, *T. eriocalyx* and *T. fallax* essential oils pathogen by 100% inhibited. When the antifungal activities of essential oils were compared among themselves, the least micellar growth diameters was observed in *T. eriocalyx* essential oil at concentrations of 5 µL/petri. Biological activity of *Thymus* essential oils and extracts on different microorganism, pathogens, weed and test plants have also been reported in other studies (Rasooli and Mirmostafa 2002; Mossa, 2019). In a study with *Satureja* one of the important thyme species, the antifungal effects of essential oils from 10, 20 and 30 µL/petri concentration *Satureja* species (*Satureja cilicica* P. H. Davis, *Satureja cuneifolia* Ten., *Satureja hortensis* L., *Satureja montana* L., *Satureja spicigera* (C. Koch) Boiss. and *Satureja thymbra* L.) tested for their antifungal effect against eight *Fusarium* species (*Fusarium avenaceaum, F. culmorum, F. equiseti, F. graminearum, F. oxysporum, F. sambucinum, F. semitectum* and *F. solani*). The higher concentrations of oil (10, 20, and 30 µl/20 ml) prevents the colony growth of *Fusarium* in the medium and *Satureja* oils showed effective control of the plant pathogenic fungi growth in the medium with 100% inhibitory rates (Usanmaz bozhuyuk et al., 2019). In our study; it was determined that 5, 10 and 20 µL/petri doses of *T. canoviridis*, *T. eriocalyx* and *T. fallax* essential oils formed 100% inhibition zone in *F. equiseti*, *F. graminearum* and *F. oxysporum* fungi. The studies seem to be similar to each other. The results further reveal the toxicity of *Thymus* essential oils against fungicides.

3.4. Herbicidal activity of *Thymus canoviridis, Thymus eriocalyx and Thymus fallax Essential Oils*

The seed germination, root and shoot growth of *A. retroflexus*, *C. arvensis* and *C. album* plants were strong touched by *T. canoviridis, T. eriocalyx* and *T. fallax* essential oils. The effects differed depending on the concentration of essential oils and the test weed seed, and the herbicidal effect increased with the increased concentration. The increase in *T. canoviridis*, *T. eriocalyx* and *T. fallax* essential oils concentration decreased the seeds germination and seedlings root and shoot growth of *A. retroflexus*, *C. arvensis* and *C. album* than negative and positive control. Compared to negative and positive control, the application of essential oils at 5, 10 and 20 µL/petri completely (100%) inhibited seed germination and seedlings root and shoot growth of two weeds *A. retroflexus* and *C. album* (Table 4).

Thymus canoviridis at 5 µL/petri concentration decreased the germination of *C. arvensis* seeds by 80.2 than control respectively, while germination was completely (100 %) stopped at 10 and 20 µL/petri concentration (Table 4). At 5 µl/petri concentration, the root and shoot growth was inhibited strongly by 89.6 % and 85.3 in order of, whereas, the root growth was completely inhibited at 10 and 20 µL/petri concentration.

In the seeds *C. abum*; *T. canoviridis*, *T. eriocalyx* and *T. fallax* essential oils and positive control at 5, 10 and 20 µL/petri concentration inhibited the seed germination, root and shoot growth were completely (100%) inhibited (Table 4). In a study, the 5 and 10 µL/petri dose of *T. fallax* essential oil completely inhibited germination, root and shoot growth of *Avena sterilis*, *Cucumis sativus* and *Lactuca sativa* seeds (Yilar et al., 2013). The essential oils of *T. fallax*, *O. vulgare* and *M. dumetorum* completely inhibited the seed germination and seedling growth of *A. theophrasti* at a 15 µL dosage (Onaran et al. 2014). They reported that thymol and carvacrol, the main compounds of thyme, completely prevented the germination of

Amaranthus retroflexus, *Chenopodium album* and *Rumex crispus* seeds (Kordali et al., 2008). In this study, herbicidal effects of *T. canoviridis*, *T. eriocalyx* and *T. fallax* essential oils have been demonstrated. The *Thymus* essential oils decreased the germination, root and shoot growth of *A. retroflexus, C. arvensis* and *C. album* at higher doses than negative and positive control.

3.5. Herbicidal activity of *Thymus canoviridis, Thymus eriocalyx and Thymus fallax* Essential Oils Under Greenhouse Conditions

In the pot studies in which essential oils are practical in post-emergent conditions are limited. Therefore, *T. canoviridis*, *T. eriocalyx* and *T. fallax* essential oils was sprayed on 1-2-week-old weed plants.

Table 4. Herbicidal effects of *Thymus* essential oils on germination, root and shoot growth on *A. retroflexus*, *C. arvensis* and *C. album* seeds.

Essential Oils	C	Germination (%)	Germination Inhibation (%)	Root Growth (mm)	Root Growth Inhibation (%)	Shoot Growth (mm)	Shoot Growth Inhibation (%)
				Amaranthus retroflexus			
	5	0.00±0.00 a	100	0.00±0.00 a	100	0.00±0.00 a	100
T. canoviridis	10	0.00±0.00 a	100	0.00±0.00 a	100	0.00±0.00 a	100
	20	0.00±0.00 a	100	0.00±0.00 a	100	0.00±0.00 a	100
	5	0.00±0.00 a	100	0.00±0.00 a	100	0.00±0.00 a	100
T. eriocalyx	10	0.00±0.00 a	100	0.00±0.00 a	100	0.00±0.00 a	100
	20	0.00±0.00 a	100	0.00±0.00 a	100	0.00±0.00 a	100
	5	0.00±0.00 a	100	0.00±0.00 a	100	0.00±0.00 a	100
T. fallax	10	0.00±0.00 a	100	0.00±0.00 a	100	0.00±0.00 a	100
	20	0.00±0.00 a	100	0.00±0.00 a	100	0.00±0.00 a	100
	5	94.0±0.00 c	2.69	1.76±0.54 c	64.6	3.60±0.85 a	80.0
P. Control	10	90.0±0.00 b	6.83	1.38±0.43 b	72.2	2.74±0.76 a	84.8
	20	88.0±0.00 b	8.90	1.18±0.38 b	76.3	1.66±0.63 a	90.7
N. Control	-	96.6±1.44 d	-	4.98±2.65 d	-	18.04±6.82 b	-
				Convolvulus arvensis			
	5	15.3±0.54 b	80.20	2.20±3.26 a	89.6	6.25±8.74 b	85.3
T. canoviridis	10	0.00±0.00 a	100	0.00±0.00 a	100	0.00±0.00 a	100
	20	0.00±0.00 a	100	0.00±0.00 a	100	0.00±0.00 a	100
	5	0.00±0.00 a	100	0.00±0.00 a	100	0.00±0.00 a	100
T. eriocalyx	10	0.00±0.00 a	100	0.00±0.00 a	100	0.00±0.00 a	100
	20	0.00±0.00 a	100	0.00±0.00 a	100	0.00±0.00 a	100
	5	0.00±0.00 a	100	0.00±0.00 a	100	0.00±0.00 a	100
T. fallax	10	0.00±0.00 a	100	0.00±0.00 a	100	0.00±0.00 a	100
	20	0.00±0.00 a	100	0.00±0.00 a	100	0.00±0.00 a	100
	5	56.0±0.00 e	27.55	1.64±0.94 a	91.57	5.76±2.82 b	86.52
P. Control	10	54.0±0.00 d	30.14	1.50±0.99 a	92.29	4.38±2.42 ab	89.74
	20	52.0±0.00 c	32.72	1.22±0.82 a	93.73	3.82±2.22 ab	91.06
N. Control	-	77.3±2.88 f	-	19.46±9.44 b	-	42.73±16.9 c	-
				Chenopodium album			
	5	0.00±0.00 a	100	0.00±0.00 a	100	0.00±0.00 a	100
T. canoviridis	10	0.00±0.00 a	100	0.00±0.00 a	100	0.00±0.00 a	100
	20	0.00±0.00 a	100	0.00±0.00 a	100	0.00±0.00 a	100
	5	0.00±0.00 a	100	0.00±0.00 a	100	0.00±0.00 a	100
T. eriocalyx	10	0.00±0.00 a	100	0.00±0.00 a	100	0.00±0.00 a	100
	20	0.00±0.00 a	100	0.00±0.00 a	100	0.00±0.00 a	100
	5	0.00±0.00 a	100	0.00±0.00 a	100	0.00±0.00 a	100
T. fallax	10	0.00±0.00 a	100	0.00±0.00 a	100	0.00±0.00 a	100
	20	0.00±0.00 a	100	0.00±0.00 a	100	0.00±0.00 a	100
	5	0.00±0.00 a	100	0.00±0.00 a	100	0.00±0.00 a	100
P. Control	10	0.00±0.00 a	100	0.00±0.00 a	100	0.00±0.00 a	100
	20	0.00±0.00 a	100	0.00±0.00 a	100	0.00±0.00 a	100
N. Control	-	95.3±0.54 b	-	18.1±4.63 b	-	20.7±4.01 b	-

Differences between the averages containing different letters in each column are statistically significant (P≤0.05), C= concentration.

Table 5. Phytotoxic effects of *Thymus canoviridis*, *Thymus eriocalyx* and *Thymus fallax* essential oils against weed seedlings growth in greenhouse conditions

Applications	Phytotoxic effect (% Mean death)	
	24. hour	48. hour
	Amaranthus retroflexus L.	
Essential oils		
T. canoviridis	24.1 ± 2.20 c	70.0 ± 3.81 c
T. eriocalyx	35.8 ± 0.83 d	60.0 ± 1.44 b
T. fallax	34.1 ± 3.00 d	57.5 ± 1.44 b
Controls		
Positive Control	9.16 ± 0.83 b	90.8 ± 0.83 d
Negative Control	0.0±0.0 a	0.0±0.0 a
	Convolvulus arvensis L.	
	Essential oils	
T. canoviridis	5.00 ± 1.44 b	22.5 ± 1.44 b
T. eriocalyx	20.8 ± 0.83 d	29.1 ± 0.83 c
T. fallax	30.0 ± 1.44 e	46.6 ± 0.83 d
Controls		
Positive Control	12.5 ± 1.44 c	87.5 ± 1.44 e
Negative Control	0.0±0.0 a	0.0±0.0 a
Chenopodium album L.		
Essential oils		
T. canoviridis	25.8 ± 0.83 c	67.5 ± 3.81 cd
T. eriocalyx	25.0 ± 2.88 c	64.1 ± 3.0 c
T. fallax	30.8 ± 1.66 d	55.0 ± 1.44 b
Controls		
Positive Control	12.5 ± 1.44 b	87.5 ± 1.44 e
Negative Control	3.33 ± 1.66 a	6.66 ± 0.83 a

Differences between the averages containing different letters in each column are statistically significant. (P≤0.05).

Thymus essential oils and commercial herbicide, Best Amin 500 SL (500 g/L 2,4 Amin salt) also tested for their phytotoxic effects against 3-4 leaf stage seedlings of pots on the weeds at greenhouse condition and the results showed that the oils caused mortality rate of 5.0-35.8% at 24 h and 22.5-70.0% at 48 h after the treatment as compared with negative and positive controls (Table 3). In the laboratuvary parallel with petri experiments, *Thymus* essential oils exhibited similar her effects against *A. retroflexus*, *C. arvensis* and *C. album* in the pots greenhouse conditions. In the plants phytotoxic effects of essential oils increase depending on the exposure time. In particular, the phytotoxic effect of essential oils was higher than the commercial herbicide at 24 hours. Furthermore, between *Thymus* essential oils, the most phytotoxic effect with 35.8 % mean death in 24 hour wasfound to be *T. eriocalyx* oil against *A. retroflexus.* Again, the *A. retroflexus* the highest phytotoxic effect was determined in *T. canoviridis* essential oil with 70.0% seedlings death in 48 hour. In negative control, while there was no death in *A. retroflexus* and *C. arvensis* seedlings; in *C. album* seedlings, 3.33% death at 24th hour and 6.66% at 48th hour were determined. In addition; commercial herbicide Best Amin 500 SL (500 g/L 2,4 Amin salt) showed the highest phytotoxic effect at 24 h with 12.5% death rate in *C. arvensis* and *C. album* seedlings. The highest phytotoxic effect was detected at 48 th hour with 90.8% mortality in *A. retroflexus* seedlings. In a study conducted under greenhouse conditions; *T. capitata* essential oil was sprayed on *Portulaca oleracea*, *Avena fatua* and *Echinochloa crus-galli* seedlings and showed bioherbicidal effects at different rates depending on the hours (Verdeguer et al., 2020). In another study; *Nepeta meyeri* essential oil it showed a phytotoxic effect between 28.7-42.7% after 24 hour in *Amaranthus retroflexus*, *Chenopodium album*, *Cirsium arvense* and *Sinapsis arvensis* seedlings; between 53.3-64.0% at the end of 48 hour (Kordali et al., 2015). At the same time, it can be said that this may be related to the plant leaf structure and the cuticle layer. In the pot work done in the greenhouse; peppermint and caraway essential oils in *Chenopodium album* and *Avena fatua* seedlings with herbicidal effects were determined (Synowiec and Drozdek, 2016). In the study conducted with twenty-five different essential oils; the essential oils were applied to shoots of common lambsquarters, common ragweed, and johnsongrass in the greenhouse; shoot death occurred within 1 h to 1 d after application. Cinnamon essential oil was found to have high herbicide activity (Tworkoski, 2002). Generally, it can be said that the phytotoxic effect increases as the hour and day increase in seedling death.

4. Conclusion

Thymus canoviridis, *T. eriocalyx* and *T. fallax* essential oils showed antifungal effects on plant pathogens *F. equiseti*, *F. graminearum*, *F. moniliforme* and *F. oxysporum* pathogens and herbicidal effects on *A. retroflexus*, *C. arvensis* and *C. album* weeds in conditions in pre-emerge (*in-vitro*) and post-emergence (*in-vivo*). According to the results of the present study; *T. canoviridis*, *T. eriocalyx* and *T. fallax* essential oils have strong antifungal, antioxidant and bioherbicidal effects. Therefore, essential oils are a potential source for the development of new bioherbicides and biopesticides. More studies are needed with different doses and volumes of essential oils. However, studies should be carried out not only in petri dishes and greenhouses, but also in the field stage. However, with more trials compared to synthetic pesticides, further studies need to be addressed to show essential oils form of effect, cost-effectiveness, safety and phytotoxicity against the plants as potential pesticides and herbicides.

Author Contributions

Concept: A.U.B. (50%) and Ş.K. (50%), Design: A.U.B. (50%) and Ş.K. (50%), Supervision: A.U.B. (50%) and Ş.K. (50%), Data collection and/or processing: A.U.B. (50%) and Ş.K. (50%)„ Data analysis and/or interpretation: A.U.B. (50%) and Ş.K. (50%), Literature search: A.U.B. (50%) and Ş.K. (50%), Writing: A.U.B. (50%) and Ş.K. (50%), Critical review: A.U.B. (50%) and Ş.K. (50%). Submission and revision. All authors reviewed and approved final version of the manuscript.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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THE INVESTIGATION OF ARTIFICIAL WETLAND SYSTEMS FOR THE IMPROVEMENT OF AGRICULTURAL DRAINAGE WATER

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Abstract: Harmful substances in the wastewater negatively affect the ecological balance by reducing the dissolved oxygen concentration. High phosphorus and nitrogen compounds discharged into surface water resources may cause to eutrophication, due to increased algal growth. In this study, Elodea densa (Egeria), Lemna minor (Duckweed), Micranthemum micranthemoides (Micra), Pistia (Water Lettuce), Ceratophyllum demersum (Foxtail) plants were investigated for the removal of nitrate and ammonium from rice field drainage water. Synthetic drainage water was prepared to rely on the literature and real rice field drainage water composition from the Samsun Bafra region. Artificial wetland studies were carried out as individual systems in order to understand the treatment ability of each plant and as a combined system in batch and continuous flow mode. pH, electrical conductivity, dissolved oxygen, nitrate, and ammonium values were measured periodically. Results of the individual plant wetland system represented that the wetland plant Elodea densa revealed the highest nitrate removal efficiency (77%) at the end of 1 day, followed by Pistia (76%). It was observed that the treatment efficiency increased with increasing hydraulic retention time. In the combined system, the nitrate removal efficiency was 40%, while the ammonium removal efficiency was 51% at the end of the first day. It was concluded that the wetland plants successfully reduced the ammonium and nitrate content of the drainage water and showed resistance to the drainage water with high salinity. In the wetland experiments carried out under continuous flow mode, ammonium was reduced from 0.80 mg/L to 0.10 mg/L, while nitrate was reduced from 1.90 mg/L to 1.40 mg/L, yielding approximately 90% and 30% at the end of the 16th day. Studies have in general revealed the importance of plant type as well as residence time.

Keywords: Artificial wetland, Agricultural drainage water, Residence time, Ammonium and nitrate

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1. Introduction

Artificial wetlands have been used in two ways, as subsurface and free surface area flow, for the improvement and treatment of wastewater since the 1950s (Shutes, 2001). While wetlands directly contribute to the treatment processes, they also contribute to the collection of precipitation waters, balancing the water in the system, and supporting biodiversity. Wetlands used today are accepted as an efficient treatment method for the removal of organic compounds and suspended solids. The removal efficiencies of nitrate (NO_3-N), ammonium (NH_4-N), and phosphate (PO_4^{3-}) pollutants vary depending on the type of artificial wetland system (Vymazal, 2019).

In wetlands formed by different plant species; subterranean plants grow horizontally and vertically, creating a large surface area for the uptake of nutrients ions while also providing bottom oxygenation (Brix, 2003). Surface plants provide natural filtration under photosynthesis and aerobic conditions while also contributing to the removal of many pathogenic microorganisms (Brix, 2003).

In recent years, the effect of systemic design parameters on pollutant levels has been continuously investigated. Each parameter influencing the system has advantages and disadvantages. In recent years, high-efficiency systems have been more suitable for land integration. In addition, it offers many opportunities as systems that are relatively more advantageous in terms of cost compared to other systems (Borin et al., 2007). Influences of agricultural drainage waters are on the nitrogen and phosphate cycle and salinity of receiving waters. Drainage waters arising from agricultural activities are generally directly given to the receiving environment (Borin et al., 2007). Artificial wetland systems composed of plant species with high salinity tolerance decrease the levels of drainage water parameters such as NO_3-N, NH_4-N, PO_4^{3-}, and others that negatively affect living beings and nature (Kadlec and Wallace, 2009). The influence of nitrogen and phosphorus compounds especially on surface waters is well known (Tırınk, 2021).

In this study, the potential of Elodea densa, Lemna minor, Micranthemum micranthemoides, Pistia, and Ceratophyllum demersum, in individual and combined wetland systems, in removing NO_3-N and NH_4-N under batch and continuous flow mode was investigated.

2. Materials and Methods

2.1. Drainage Water

The composition of agricultural drainage waters varies depending on the type and amount of pesticides and fertilizers used (Tanji and Kielen, 2002). Based on the literature and the analyzed drainage water obtained from Samsun Bafra, the NO_3-N and NH_4-N level of the synthetically prepared drainage water was determined as 1.90 mg NO_3-N /L, 0.80 mg NH_4-N /L, and 1.00 g/L NaCl.

2.2. Artificial Wetland Plants and Wetland Structure

Shown in Figure 1, the plants selected for use in the study were Elodea densa, Lemna minor, Micranthemum, Ceratophyllum, Pistia, and Bafra seaweed. The Bafra seaweed obtained from the Kızılırmak Delta of the Samsun region was used only in the batch studies. All other plants were obtained from the WOOF Company that supplies aquarium plants. All of the plant pictures in Figure 1 were obtained from the internet (Leslie, 2007; Lovell, 2009; Vidéki, 2009; Shaun, 2018).

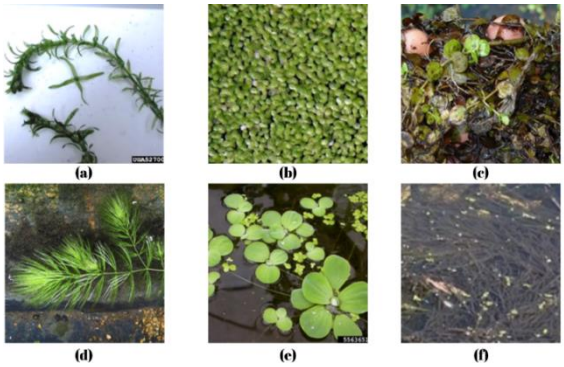

Figure 1. (a) Elodea densa, (b) Lemna minor, (c) Micranthemum, (d) Ceratophyllum, (e) Pistia, and (f) Bafra seaweed.

Submerged plants like Ceratophyllum demersum were planted into a 10-15 cm lava pea gravel layer which was initially placed into the individual or combined system tanks, while free-floating plants like Lemna minor were homogeneously distributed along the water surface of tanks.

2.3. Experimental Methods

In the individual wetland system study, each plant species was placed into a 10 L tank containing synthetic drainage water to understand their treatment potential under high salinity conditions (Figure 2). Parameters of daily samples were determined according to standard procedures.

In the combined wetland system study under batch mode conditions, the plants were taken into a common tank containing 5 L drainage water and exposed to the same hydraulic retention time. In both studies, the parameters of daily samples were determined according to standard procedures (Figure 3). In order to investigate the potential of a combined wetland system under continuous flow mode, wetland plants were placed into a 40 L tank operating with a flow rate of 12.5 ml/min and the NO_3-N and NH_4-N removal efficiencies were followed by taking samples with 2-day intervals.

After the measurement of dissolved oxygen (DO), pH, and electrical conductivity (EC), samples were passed through a 0.45µ MF-Millipore MCE membrane syringe filter and further analyzed for NO_3-N and NH_4-N.

Figure 2. Individual wetland systems.

Figure 3. Continuous flow combined wetland system.

2.4. Analytical Methods

Dissolved Oxygen: Measurements were carried out with a HACH-HQ430d flexi device containing a Luminescent/optical dissolved oxygen electrode.

pH and Electrical Conductivity (EC): Thermo Scientific-Orion Star A215 with a DuraProbe 4-cell conductivity sensor and a ROSS Ultra Triode epoxy-body pH/ATC electrode was used for pH and EC measurements.

Nitrate (NO_3-N): Spectrophotometric measurements were done by selecting 353 N, NO_3-N MR PP 400 nm from the recorded programs in the HACH Lange DR6000 device. HACH Method 8171 is based on the cadmium reduction method.

Nitrite (NO_2): Spectrophotometric measurements were carried out by selecting 373 M, NO_2 HR PP 585 nm from the registered programs in the HACH Lange DR6000 device. HACH Method 8153 is based on the ferrous sulfate method.

Ammonium (NH_4-N): Spectrophotometric measurements were done by selecting 380 N, NH_4-N Ness 425 nm from the recorded programs in the HACH Lange DR6000 device. HACH Method 8038 is based on the Nessler method.

3. Results

3.1. Batch System Operation

3.1.1. Individual wetland system

The variation in NO_3-N and NH_4-N concentrations with respect to different hydraulic retention times for each wetland plant is given in detail in Figure 4. Results generally showed that treatment efficiencies increased over time for all plants but at different levels. NO_3-N removal efficiencies for a residence time of one day were determined to be 72%, 38%, 22%, 38%, 77%, and 22% for Lemna minor, Micranthemum, Pistia, Bafra seaweed, Elodea densa, Ceratophyllum demersum plants respectively. When the residence time was increased to five days, these values increased to approximately 94%, 94%, 88%, 72%, 94%, and 78%, respectively.

NH_4-N removal efficiencies at the end of the first day were found to be 59%, 64%, 77%, 63%, 69%, and 64% for Lemna minor, Micranthemum, Pistia, Bafra seaweed, Elodea densa, Ceratophyllum demersum plants, respectively. These values increased on the fifth day and reached values of approximately 81%, 85%, 88%, 77%, 85%, and 85%, respectively. The highest NO_3-N removal efficiencies obtained for a retention time of one day were for Lemna minor and Elodea densa. Regarding NH_4-N removal efficiencies, all plants revealed efficiencies above 60% except for Lemna minor. The highest NO_3-N removal efficiencies obtained for a retention time of five days were for Lemna minor, Micranthemum, and Elodea densa, reaching values as high as 94%. NH_4-N removals by all plants were more than 80%.

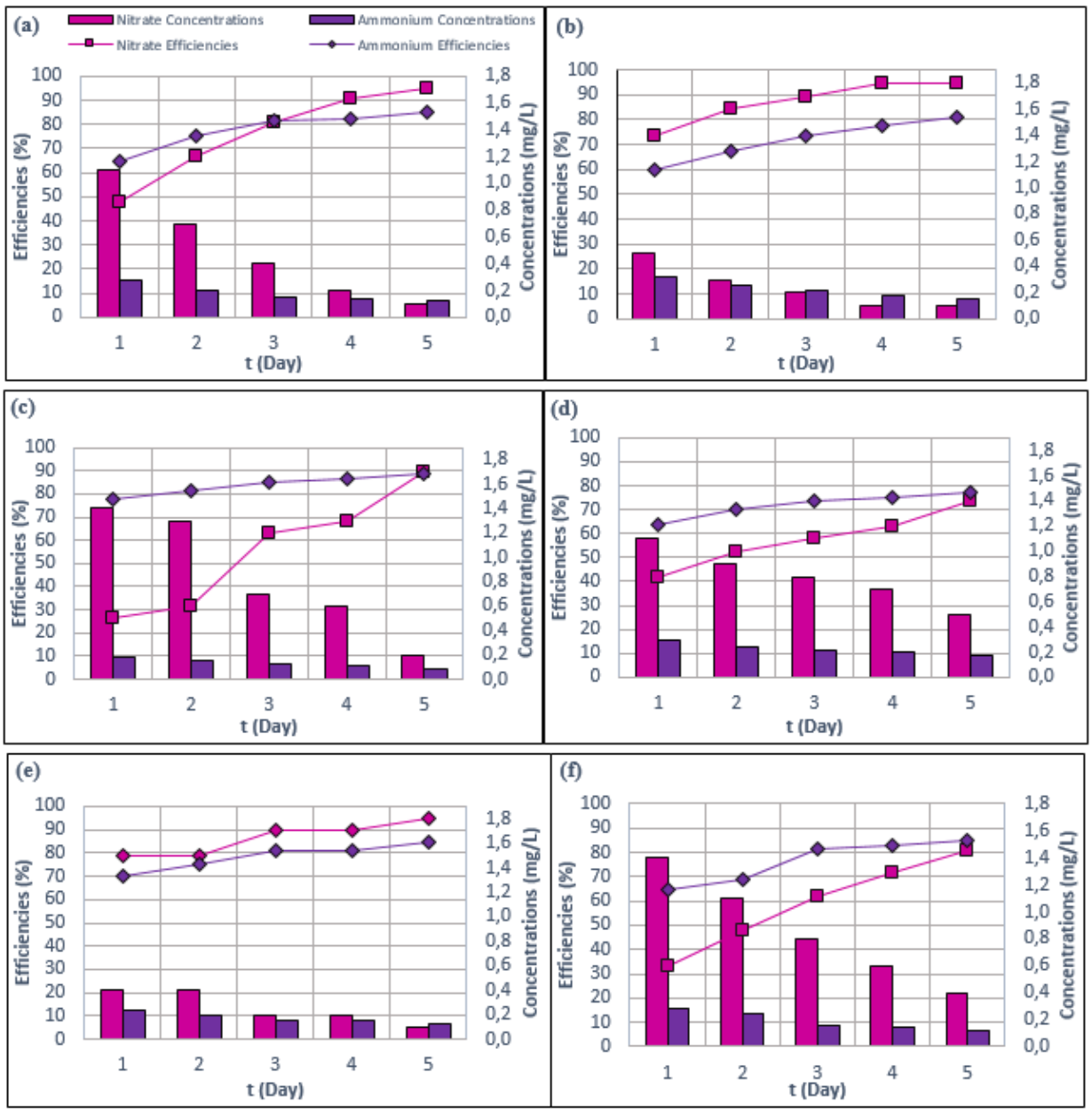

Figure 4. NO3-N and NH4-N levels and removal efficiencies of plant for individual wetland system (a) Micranthemum, (b) Lemna minor, (c) Pistia, (d) Bafra seaweed, (e) Elodea densa, (f) Ceratophyllum.

Regarding the changes in pH values, an increase in pH from 7.20 to 8.94 in 4 days was observed for all plants. Most of the time, the increase of underwater plants, phytoplankton, and algae in the pool water in the spring causes the pH value to increase too much (Ölmez and Saraç, 2009). According to the EC measurements, an increase was observed for all plants in individual wetland systems.

DO levels were found to remain approximately at the same level, except for Elodea densa and Ceratophyllum demersum, which revealed slightly higher DO values when compared with the systems of other plants.

3.1.2. Combined wetland system

As natural systems rely on the effort of various species, plants were combined, and NO_3-N and NH_4-N removal efficiencies were daily monitored as done in the individual wetland system. As can be seen from Figure 5, the NO_3-N removal efficiency achieved at the end of the first day was about 40%, while the NH_4-N removal efficiency approached 51%. These values increased to about 70% and 95% at the end of the 5th day.

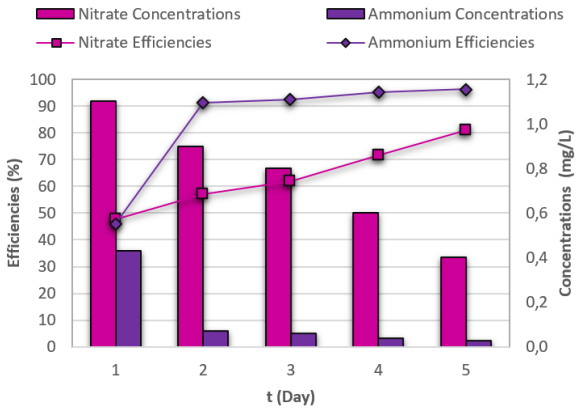

Figure 5. NO_3-N and NH_4-N treatment efficiencies in the common plant system.

3.2. Continuous Flow System Operation

After the evaluation of plant responses and performances in individual and combined wetland systems, the influence of drainage water supply mode was investigated. The combined wetland system was operated under continuous flow mode with the same drainage water at a rate of 12.5 mL/min. A retention time of approximately two days was selected since higher retention times increase the dimensions and thus the investment costs of wetlands. The continuous flow was provided with the help of a laboratory pump. As can be seen from Figure 6, the results of the continuous flow mode were different from the batch mode. Removal efficiencies of both parameters increased with time but remained lower than the efficiencies of batch mode. The NH_4-N removal efficiency increased from about 51% to 88% after 14 days, while the NO_3-N concentration remained high (1.40 mg NO_3-N/L), reaching a removal efficiency of only 26%. The NH_4-N concentration observed after fourteen days was approximately 0.10 mg NH_4-N/L. Results of the continuous flow mode revealed a significant NH_4-N removal, while the ability of plants to remove NO_3-N was very limited. Plants presented better performances in the batch mode.

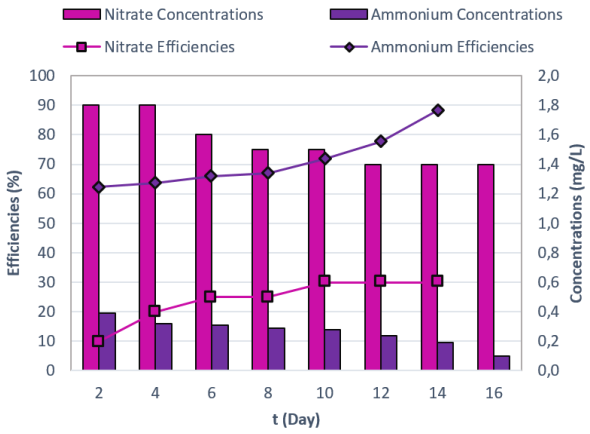

Figure 6. Contaminant removal efficiencies in a continuous flow artificial wetland tank.

Regarding the pH, EC and DO levels of the combined wetland systems under continuous flow mode, it can be stated that the EC values were presenting a decreasing trend while pH remained rather at the same level with minor fluctuations. Surprisingly, the DO values in the system tend to decrease within the system.

4. Discussion

The investigation of treatment methods based on natural ecosystem services, rather than high chemical and technology use is receiving attention. Scientific improvement of natural systems, e.g., wetlands, by supporting its components and/or process combinations during the design stage enables higher efficiencies. Research on the incorporation of advanced treatment systems into environmental systems is increasing. Investments for constructed wetland systems rather focus on pollutant removal from urban and sewage wastewater; however, do not provide sufficient information about plant species and plant treatment yields under salt stress.

Lemna minor, Micranthemum, Pistia, Elodea densa, and Ceratophyllum demersum plants were frequently reported for their ability to reduce nitrogen compounds present in wastewater. Selvarani et al. (2015) mentioned NH_4-N, NO_2, and NO_3-N removal efficiencies up to 98% from different types of wastewaters with Lemna minor. According to the research of Bialowiec et al. (2019) on the Elodea densa plant, a total nitrogen removal efficiency of 52.9% and phosphate removal efficiency of 15.9% were reported. In addition, it was stated that the Elodea densa plant increased the O_2 level in the water with increasing period depending on time (Bialowiec et al., 2019). In a study by Victor et al. (2016) with the Pistia plant, NH_4-N removal from wastewater was determined to be about 58% at the end of the first day, while Gaballah et al. (2019) reported efficiency of about 83%. NO_3-N removal efficiency of 62% was presented for the

Ceratophyllum demersum plant for a retention time of six days (Foroughi et al., 2013). In a detailed study by Teles et al. (2017), the effect of NO_3-N load on the ability of Lemna minor to remove nitrogen compounds was investigated and the decrease in the treatment efficiency of the plant was related to high NO_3-N input.

In the study carried out, the investigation of these plant species for nutrient removal from drainage water with very high salt enabled the evaluation of plants tolerance and future use. Results demonstrated that these plants, other than salt tolerant plant species, have a potential to provide high NH_4-N and regular NO_3-N removal efficiency. The current study also represented the capability of Bafra seaweed, a local plant species, in removing nutrients from water. The effect of water quality (salinity) on the efficiency of wetland systems under different operating conditions (batch and continuous flow mode) was additionally observed. Limitations in the growth rate of evaluated plant species were recorded with respect to salt water exposure over time. Continuous high load of water pollutants are reported to result in decreased plant survivorship causing to disappearance of plants (Wu et al., 2015). Observations, additionally, showed that the number of plants distributed throughout the tank has an influence on removal efficiency. Thus, reduced plant numbers presented lower removal efficiencies. Similarly, in a study carried out by Liu et al. (2017), the lower nitrogen removal efficiency by Lemna minor was attributed to the salinity of water. Results of the continuous flow combined wetland system similarly emphasizes the effect of continuous salt load on the plants, revealing quite low NO_3-N removals when compared with the results of the batch combined wetland system. Wu et al. (2015) stated that the feeding mode (such as batch, intermittent and continuous) may influence the oxidation-reduction conditions, transfer and diffusion rates in wetland systems.

5. Conclusion

Lemna minor, Micranthemum, Pistia, Bafra seaweed, Elodea densa, Ceratophyllum demersum, and Bafra seawood, a local species, were investigated for NO_3-N and NH_4-N removal from agricultural drainage water, with individual and combined plant wetland systems operated under batch and continuous flow mode. The individual plant wetland system aimed to understand each plants potential for salt tolerance and nitrogen removal. NO_3-N concentrations were reduced to 0.10 mg/L NO_3-N (73% efficiency) by Lemna minor, Micranthemum and Elodea densa plants with a retention time of 5 days. The local seaweed plant from Bafra revealed a removal efficiency of 73%. The Pistia plant presented the highest NH_4-N reduction to 0.10 mg NH_4-N/L (88%). The individual wetland system showed that plants having the capacity to remove more NO_3-N provided less NH_4-N removal, while those with a capacity to remove NH_4-N revealed less NO_3-N reduction.

NO_3-N and NH_4-N concentration determined for the combined wetland system in batch mode reduced to 0.40 mg/L NO_3-N (78%) and 0.05 mg/L NH_4-N (97%) at the end of the 5th day. Regarding the continuous flow mode system an increase in removal efficiencies was observed with time. However, continuous load of salty drainage water into the system resulted in especially a reduction in NO_3-N removal efficiency when the 5-day NO_3-N and NH_4-N removal efficiencies were compared with that of batch mode.

In general, it can be concluded that the selection of wetland plant species, residence time and flow rate with respect to contaminant plays a crucial role in wetland performance. Higher residence times yielded higher treatment efficiencies. While salinity did not appear to influence some plants, it had negative effects on others (e.g. Lemna minor), resulting in lower overall. In particular, feeding the salty drainage water continuously into the system caused a decrease in plant number over time. Pistia and Elodea densa plants, seem to have a higher tolerance to salinity, yielding more effective NH_4-N reduction. Overall, plants revealed higher NH_4-N treatment efficiencies in the continuous flow wetland system.

Author Contributions

Concept: İ.G. (50%) and E.B.Ö. (50%), Design: İ.G. (50%) and E.B.Ö. (50%), Supervision: İ.G. (50%) and E.B.Ö. (50%), Data collection and/or processing: İ.G. (100%), Data analysis and/or interpretation: İ.G. (100%), Literature search: İ.G. (50%) and E.B.Ö. (50%), Writing: İ.G. (50%) and E.B.Ö. (50%), Critical review: İ.G. (50%) and E.B.Ö. (50%). Submission and revision. All authors reviewed and approved final version of the manuscript.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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EVALUATION OF DIFFERENT ROOTSTOCKS AND CULTIVARS ON PRUNING WEIGHT IN YOUNG PEAR TREES

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Abstract: This study was carried out to evaluation of the effects on pruning weight (kg plant^{-1}), cumulative pruning weight per plant (kg plant^{-1}), and cumulative pruning weight per hectare (kg ha^{-1}) of 3 quince rootstocks [Quince BA29 (BA29), Quince A (QA), Quince C (MC)] and 3 pear clonal rootstocks (Fox11, OHxF333, Farold 40) and pear seedling rootstocks grafted with 4 standard pear cultivars ('Abate Fetel', 'Deveci', 'Santa Maria', 'Williams') between 2019-2021 years. Rootstocks, cultivars, research years and their interactions significantly affected all examined parameters in the study, except for the interaction of year x rootstock x cultivar. Regarding rootstock averages, the highest pruning weight (kg plant^{-1}) was observed from Fox 11, the lowest was in the BA29, QA, and MC quince rootstocks. Regardless of the cultivar averages, the highest pruning weight was in the 'Deveci', the lowest was in the 'Santa Maria' pear cultivar. The highest pruning weight (kg plant^{-1}) was observed from 'Deveci'/Fox11, and the lowest was in the 'Williams'/QA, 'Williams'/BA29, 'Abate Fetel'/MC, and 'Santa Maria'/MC combinations in terms of rootstock x cultivar interaction. The highest cumulative pruning weight per plant (kg) was determined in the 'Deveci'/Fox11, the lowest was in the 'Williams'/QA combination in terms of rootstock x cultivar interaction. Furthermore, the highest cumulative pruning weight per hectare (kg) was determined in the 'Deveci'/Fox11, the lowest was in the 'Williams'/QA, 'Williams'/BA29, 'Abate Fetel'/MC, 'Santa Maria'/MC, and 'Santa Maria'/Seedling combination in terms of rootstock x cultivar interaction. Except for the pear seedling rootstock, quince clone rootstocks generally had lower all pruning weight traits than pear clone rootstocks in the study. It can be said that the weaker development of quince rootstocks compared to pear rootstocks causes this situation. According to the results of this study carried out on young pear trees, it can be said that quince rootstocks are somewhat advantageous due to less pruning labor and cost.

Keywords: Pear, Rootstock, Cultivar, Cumulative pruning weight, Pruning weight

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1. Introduction

Pear fruit belongs to the *Pyrus* genus of the pome fruit group, related to the *Pomoideae* subfamily and *Rosaceae* family of the Rosales order, which is a crucial fruit species after apple is grown in the temperate climatic regions of the world. Most of the cultivated pear cultivars originate from *Pyrus communis* and *Pyrus serotina*. Türkiye is one of the *Pyrus communis* homeland (Özbek, 1978). World pear production reported 23109219 tons, and Türkiye's pear production ranked fifth in the world with 545.569 tons. China took place in the first position with 16 million tons, Italy in the second with 619470 tons, America in the third with 609628 tons, and Argentina in the fourth position with 600000 tons (FAOSTAT, 2022). In pear orchards, 100 tons of pruning debris are obtained annually. In 2016, 128 thousand tons of pruning residue were obtained from 16000 hectares of pear and apple orchards in Belgium. Pruning residues are now often burned on garden sides, which this incineration process increases the carbon pollution or remains near the orchards that can be the second home of pests and diseases (Boeykens et al., 2018). In order to obtain regular yield and fruit of the high quality from pear trees, pruning is essential and should be done correctly (Larsen and Fritts, 1984; Rom and Carlson, 1987; Jackson, 2003). The pear tree shows specific fruit structures due to the location of the buds on the branches. These buds evaluate in different ways according to their environmental conditions and their importance. Due to the variations in the environmental necessities of cultivars, pear trees require specific pruning methods for high yield and ideal quality fruit production (Jackson, 2003). Pruning varies according to different cultivars and rootstocks' responses and growing strength. Pear cultivars with less vegetative growth, such as 'Hardy', 'Flemish Beauty', 'Anjou', and 'Comice' produce high yield, and 15-20 years old trees form tiny branches that give less fruit. Therefore, regular and conscious pruning is essential for high yield and acceptable fruit quality. However, in the pear orchards without pruning, trees develop severely zigzag, show excessive fruit load, but produce small and poor quality fruit (Gurpinder et al., 2018). In low-yielding pear orchards, vigorous rootstocks and cultivars are the main factors limiting pear production (Maas, 2008; Hawerroth and Petri, 2011; Rufato et al., 2012; Pasa et al., 2016,

2017). *Pyrus* and *Cydonia* species are used as rootstock for pear production (Iglesias and Asin, 2011; North et al., 2015). However, *Pyrus* species as rootstocks have some problems, such as strong growth, heterogeneity, and slow production (Maas, 2008; Massai et al., 2008). Rootstock selection is vital for determining the vegetative development of trees, plant production, garden management, and planting densities (Webster, 2002). Extremely vigorous rootstocks provide excessive vegetative development, reducing the use of light by fruits (Jackson, 2003; Sharma et al., 2009; Clingeleffer et al., 2019). Therefore, pruning of intertwined branches, diseased branches, and dry branches is necessary for benefiting from light and regular fruit formation, and pruning increases the number of high-quality fruits (Sharma et al., 2009). Branch drying, fungal diseases, and other pathogens are minimized when appropriate pruning techniques are used in the orchards (Badrulhisham and Othman, 2017). However, since pruning is costly, rootstocks and cultivars with low vegetative growth are always desired features in pear cultivation. Pruning was reported to be responsible for more than 20% of variable costs in apple 'Gala' orchards in Washington (Gallardo et al., 2009). In addition, pruning is cumbersome and can result in worker falls, cuts, and dangerous injuries (Fathallah, 2010). However, pear cultivars grafted on quince rootstocks show earliness and uniformity of production (North et al., 2015). At the same time, dwarf rootstocks allow good orchard management, high-density planting, and require less pruning (Maas, 2008). In Europe QA, QC, EMH, BA29, Adams 332, and Sydo rootstocks are used (Jackson, 2003). These rootstocks are 40-50% dwarfed, provide early maturation and high yield, and at the same time, they require less pruning (Lombard and Westwood, 1987). The aim of this study was to investigate the effects of BA29, QA, MC, FOX11, FAROLD40, OHF333, and seedling rootstocks on the pruning weights of 'Abate Fetel', 'Deveci', 'Williams' and 'Santa Maria' cultivars.

2. Materials and Methods

This study was carried out in the pear orchard established with 1-year-old saplings in Ondokuz Mayis University Bafra Agricultural Research Center (41°33'50'' N, 35°52'23'' E, and 20 m altitude) in 2018. In the study, dwarf rootstocks (quince) were planted at 1.5x3.5 m (1905 plant ha^{-1}), semi-dwarf and seedling rootstocks were planted at 3.0x3.5 m distances (953 plant ha^{-1}). In the study, 'Deveci', 'Williams', 'Santa Maria', and 'Abate Fettel' pear cultivars grafted on BA29, Quince A, and Quince MC clone rootstock and OHxF333, Fox11, Farold40 pear clone, and seedling rootstocks were used. In the research, the plants were supported by wires on the horizontal arms of 50 cm length, passed from a height of about 50 cm from the ground, and 3.5 m high metal poles with 4 rows of galvanized wire at 80 cm intervals on the main stem. The plants were pruned regularly every year according to the modified leader system. The plants were irrigated with drip irrigation between 15 May and 15 September. Fertilization was done with 15-30-15+ME fertilizer at the beginning of summer and 20-20-20 NPK-containing fertilizer in autumn with drip irrigation. Winter fertilization was done by giving it to the crown projection of the plants with NPK fertilizer containing 15-15-15+Zn in winter. The rows were covered with black ground mulch for weed control, and the rows were regularly processed with a rotovator. The trial area has a soil depth of more than 1 meter and the soil has 2.73-10% clay (low), 13.21-20% silt (medium), 6.5-20% sand (medium), pH 7.5 (slightly alkaline), 0.2-0.3 dS/ m salt (no salt), 0.3-0.5 organic matter (low), 3-6% lime ($CaCO_3$) (low), 0.03-0.06% N (less) and 5-10 ppm P (medium) content. In the district of Bafra, where the research was conducted, the typical Black Sea climate is seen, with cool summers, warm and rainy winters (about 750 - 1000 mm per year). Hot and dry wind blowing from the district's south and southwest directions reduces the humidity. The relative humidity average of Bafra is 73%. Especially in April and May, humidity averages 77 - 79%. Since absolute humidity is directly proportional to temperature, it reaches the highest value of 28% in summer. The highest precipitation in the district is in November, and the least precipitation is observed in May. The average annual precipitation is around 700 mm. The average number of rainy days per year is 100 days (TSMS, 2022).

The research was established in a Randomized Blocks Design with 3 replications and 10 plants per replication in dwarf rootstocks and 5 plants in semi-dwarf and seedling rootstocks. The data obtained were analyzed in the IBM SPSS 21.0 statistical package program, and the differences between the averages were determined at the $P<0.05$ level with the 'Duncan Multiple Range Test (Genç and Soysal, 2018).' the results were presented in Tables and Figures.

3. Results and Discussion

The rootstocks, cultivars, research year, year x rootstock, year x cultivar, and rootstock x cultivar interactions had a significant effect on pear and quince clone rootstocks and pear seedling rootstocks grafted with the 'Deveci', 'Williams', 'Santa Maria' and 'Abate Fetel' pear cultivars on the pruning weight per plant. However, the effect of year × rootstock × cultivars interaction was not statistically significant. In terms of year's average, pruning weight per plant varied between 0.10-0.90 kg $plant^{-1}$. The pruning weight per plant in 2021 (0.90 kg $plant^{-1}$) was higher than in other years. Regarding rootstock average, pruning weight per plant varied between 0.16 kg – 1.07 kg $plant^{-1}$. Fox11 pear rootstock (1.07 kg $plant^{-1}$) had the highest pruning weight per plant among the examined rootstocks, and the lowest was MC, QA, and BA29 quince clone rootstocks (0.16, 0.22, and 0.23 kg $plant^{-1}$). In terms of cultivar averages, pruning weight per plant varied between 0.30-0.68 kg $plant^{-1}$. The highest pruning weight per plant was observed in the

'Deveci' cultivar (0.68 kg plant^{-1}) and the lowest was in the 'Santa Maria' cultivar (0.30 kg plant^{-1}) (Table 1; Figure 1).

The pruning weight per plant varied between 0.07-2.03 kg plant^{-1} in terms of year x rootstock interaction. The highest pruning weight per plant was found in Fox11 rootstock in 2021. In terms of year x cultivar interaction, the pruning weight per plant varied between 0.08-1.26 kg plant^{-1}. The highest pruning weight per plant was determined in the 'Deveci' cultivar in 2021. The pruning weight per plant ranged from 0.07 to 1.59 kg plant^{-1} in terms of rootstock x cultivar interaction.

Table 1. The effects of rootstocks and pear cultivars on the pruning weight (kg plant^{-1}) in pear

Rootstocks	Cultivars	Years			Mean
		2019	2020	2021	
BA29	Abate Fetel	0.06 a	0.22 a	0.42 a	0.23 ef*
	Deveci	0.13 a	0.30 a	0.46 a	0.29 def
	Santa Maria	0.14 a	0.27 a	0.40 a	0.27 def
	Williams	0.10 a	0.11 a	0.12 a	0.11 f
QA	Abate Fetel	0.12 a	0.25 a	0.42 a	0.26 def
	Deveci	0.10 a	0.26 a	0.42 a	0.26 def
	Santa Maria	0.09 a	0.30 a	0.51 a	0.30 def
	Williams	0.04 a	0.06 a	0.10 a	0.07 f
MC	Abate Fetel	0.05 a	0.13 a	0.23 a	0.13 f
	Deveci	0.09 a	0.21 a	0.34 a	0.21 ef
	Santa Maria	0.14 a	0.12 a	0.14 a	0.13 f
	Williams	0.07 a	0.20 a	0.28 a	0.18 ef
Fox11	Abate Fetel	0.09 a	1.29 a	2.56 a	1.31 ab
	Deveci	0.08 a	1.64 a	3.06 a	1.59 a
	Santa Maria	0.11 a	0.36 a	0.57 a	0.35 def
	Williams	0.11 a	1.06 a	1.95 a	1.04 bc
OHxF333	Abate Fetel	0.11 a	0.91 a	1.59 a	0.87 bcd
	Deveci	0.13 a	1.00 a	1.89 a	1.01 bc
	Santa Maria	0.12 a	0.42 a	0.70 a	0.41 def
	Williams	0.15 a	0.81 a	1.41 a	0.79 bcde
Farold 40	Abate Fetel	0.07 a	0.58 a	1.02 a	0.56 cdef
	Deveci	0.08 a	0.89 a	1.57 a	0.85 bcd
	Santa Maria	0.14 a	0.42 a	0.67 a	0.41 def
	Williams	0.18 a	0.84 a	1.54 a	0.85 bcd
Seedling	Abate Fetel	0.09 a	0.51 a	0.93 a	0.51cdef
	Deveci	0.07 a	0.59 a	1.07 a	0.58 cdef
	Santa Maria	0.08 a	0.21 a	0.37 a	0.22 ef
	Williams	0.05 a	0.33 a	0.56 a	0.31 def
Main Effects					
Rootstocks	BA29	0.11 f	0.22 f	0.35 f	0.23 d
	QA	0.09 f	0.22 f	0.36 f	0.22 d
	MC	0.08 f	0.16 f	0.25 f	0.16 d
	Fox11	0.10 f	1.09 bc	2.03 a	1.07 a
	OHxF333	0.13 f	0.78 cd	1.40 b	0.77 ab
	Farold 40	0.12 f	0.68 de	1.20 b	0.67 ab
	Seedling	0.07 f	0.41 ef	0.73 de	0.41 c
Cultivars	Abate Fetel	0.08 f	0.55 cde	1.02 ab	0.55 ab
	Deveci	0.10 f	0.70 bcd	1.26 a	0.68 a
	Santa Maria	0.12 f	0.30 ef	0.48 de	0.30 c
	Williams	0.10 f	0.49 de	0.85 bc	0.48 ab
Years		0.10 c**	0.51 b	0.90 a	
Year		0.001	Year x Cultivar		0.001
Rootstock		0.001	Rootstock x Cultivar		0.001
Cultivar		0.001	Year x Rootstock x Cultivar		0.087
Year x Rootstock		0.001			

*Differences between means with different letters in the same column are significant, **Differences between means with different letters in the same line are significant.

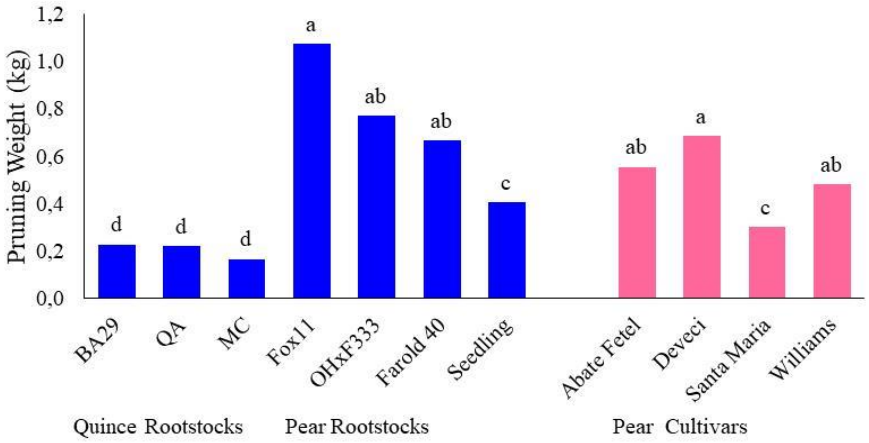

Figure 1. Effects of different rootstock and cultivars on pruning weight per plant (kg) in pear.

In terms of rootstock x cultivar interaction, the highest pruning weight per plant was in the 'Deveci'/Fox11 combination (1.59 kg plant^{-1}), and the lowest was in the 'Williams'/QA, 'Williams'/BA29, 'Abate Fetel'/MC and 'Santa Maria'/MC combinations (0.07, 0.11, 0.13 and 0.13 kg plant^{-1}, respectively). In terms of year x rootstock x cultivar interaction, pruning weight per plant varied between 0.04-3.06 kg plant^{-1} (Table 1). In the study, the pruning weight per plant increased as the years progressed, and it was generally higher in pear rootstocks than in quince clonal rootstocks (Figure 1).

Pruning is necessary to maintain a balanced growth and development in fruit trees. The severity of pruning is essential in keeping vegetative and generative development in balance in fruit trees. In young fruit trees that have not started fruiting, severe pruning usually delays the onset of yield by increasing vegetative development. Pruning is done on trees in physiological balance makes in a way that keeps yield and development in balance (Sansavini and Musacchi, 1994; Jackson, 2003). The study showed significant differences between years in terms of pruning weights per plant. It can be said that this difference is due to the increase in the growth and development of plants from year to year, and the growth vigor of rootstocks and cultivars. The difference in growth in fruit trees is due to the age of the trees and the increase in growth and development (Gercekcioglu et al., 2014). In a study conducted in the field where the study was conducted, pear rootstocks had higher plant growth and development than quince rootstocks (Kurt et al., 2022). It can be said that the difference in the growing strength of the rootstocks also appeared in the pruning weight. The pruning weights of the vigorous growing rootstocks were also high. In modern pear growing, quince clone rootstocks are used for dense planting due to their dwarfing characteristics. Since their growth and development strength are weaker than pear rootstocks, they also need less pruning. Low-density planting in orchards was established with vigorous rootstocks planting fewer saplings per decare than in high-density planting, prolonged pruning time, and increased labor costs (Rom and Carlson, 1987). It was stated that rootstocks with strong growth have higher pruning weights per plant and may need intensive pruning (Jackson, 2003; Giocobbo et al., 2008). Urbina et al. (2003) reported that the effect of years of research on the average pruning weight of 'Williams' pear grafted on different rootstocks was not significant, and they reported that there were significant differences between rootstocks in terms of pruning weight in some research years. Researchers emphasized that the 5-year average pruning weight was 1.7-2.4 kg tree^{-1}. Musacchi et al. (2005) reported that the effects of rootstock and cultivars on pruning weight per plant of 7-year-old 'Abate Fetel', 'Conference' and 'Comice' pear cultivars grafted on MC and Sydo quince clone rootstocks using different pruning systems were significant. They stated that pruning weight per plant was lower in MC than in Sydo. It was emphasized that the pruning weight of 'Abate Fetel' cultivar was the highest, while the 'Conference' cultivar had the lowest. Giacobbo et al. (2008) reported that the pruning weight of the 'Packham's Triumph' pear cultivar grafted on quince and pear rootstocks was higher on strong rootstocks than on weak rootstocks, and they emphasized that the highest pruning weight was in Smyrna and Alongado rootstocks and the lowest in Portugal rootstock. Giacobbo et al. (2010) reported that the effect of rootstocks on the pruning weight of 'Carrick' pear cultivar, which they grafted on 13 different quinces and one pear rootstock, was significant. Clingeleffer et al. (2019) reported that the rootstock x cultivar interaction significantly affected pruning weight. Almeida et al. (2020) cited the effect of important quince rootstocks on the pruning weight of 'Abate Fetel' and 'Rocha' pear cultivars grafted on BA29, EMC, and Adams quince clone rootstocks, and they stated that the pruning weight was higher 'Rocha' pear cultivar. These researchers reported that the pruning costs of poorly growing rootstocks and cultivars and their pruning weights were also low. In the high-density planted orchard, pruning time is prolonged, but the amount and duration of pruning weight per unit tree were less (Rom and Carlson, 1987). McClymont et al. (2021) reported that the pruning weight varied between

2.06-3.23 kg, and also they reported a significant difference between rootstocks in terms of pruning weight. It can be said that the results obtained from the research are compatible with previous studies.

In the study, the cumulative pruning weight per plant varied between 0.49-3.22 kg plant^{-1} regarding rootstock averages and between 0.90-2.05 kg plant^{-1} in cultivar averages. Regarding rootstock averages, the highest cumulative pruning weight per plant was in Fox11 pear clone rootstock (3.22 kg plant^{-1}) and the lowest 0.49 kg plant^{-1} in QA quince clone rootstock. In terms of cultivar averages, the highest cumulative pruning weight per plant was found in 'Deveci' (2.05 kg plant^{-1}) and the lowest in 'Santa Maria' (0.90 kg plant^{-1}) pear cultivars (Figure 2). The highest cumulative pruning weight per plant for rootstock x cultivar interaction ranged between 0.21-4.77 kg plant^{-1}. The highest cumulative pruning weight per plant was determined in 'Deveci'/Fox11 (4.77 kg plant^{-1}), and the lowest was in the 'Williams'/QA (0.21 kg plant^{-1}) (Figure 3).

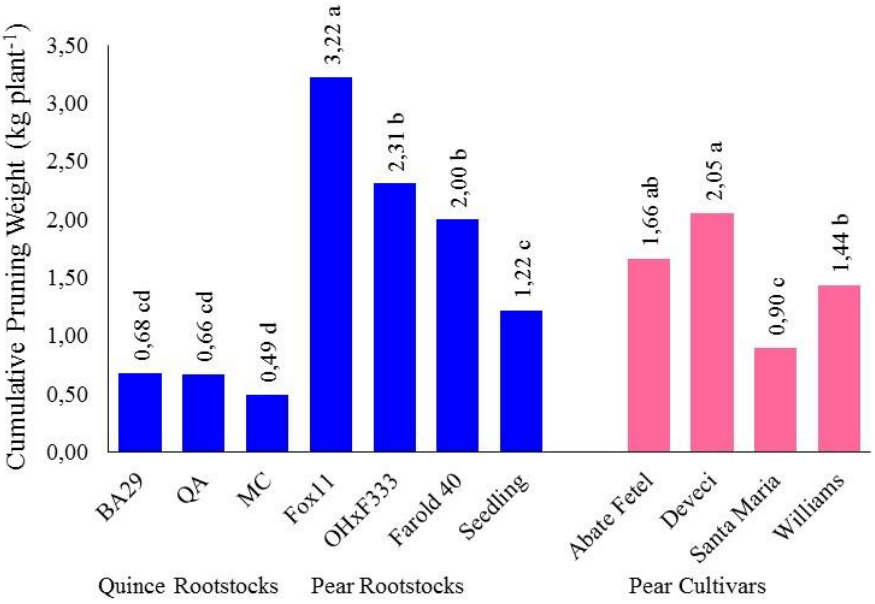

Figure 2. Effects of different rootstock and cultivars on cumulative pruning weight per plant (kg plant^{-1}) in pear.

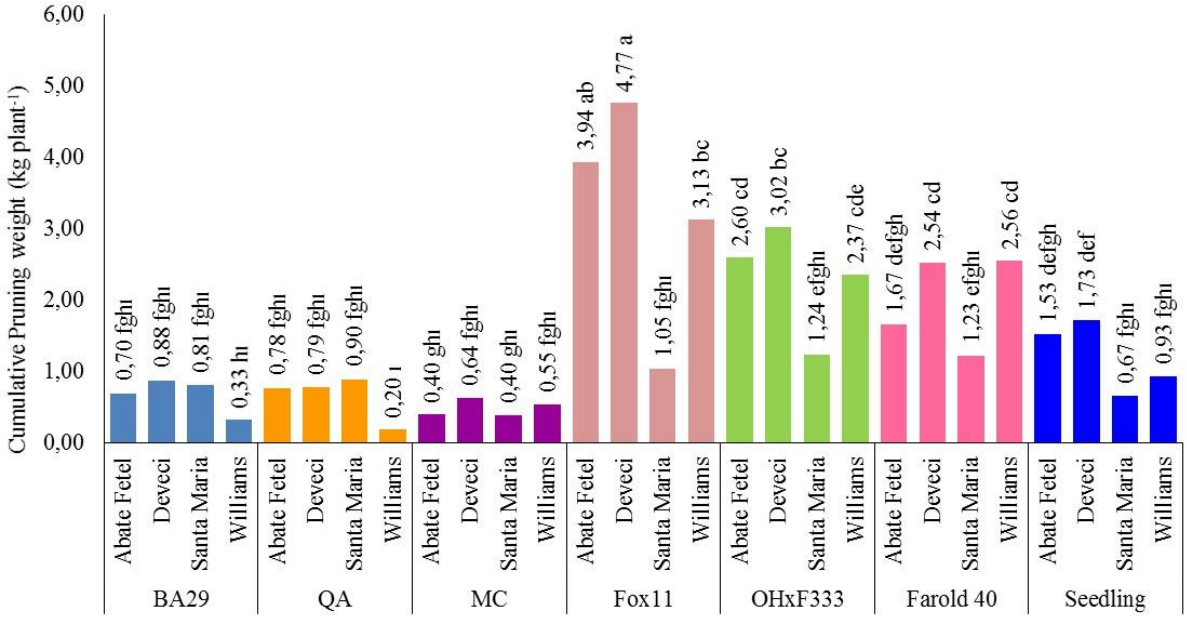

Figure 3. Effects of different rootstocks x cultivars interactions on cumulative pruning weight per plant (kg plant^{-1}) in pear.

The cumulative pruning weight per hectare varied between 942.8-3070.0 kg ha^{-1} in terms of rootstock averages and between 1143.4-2269.0 kg ha^{-1} in cultivar averages. Regarding rootstock averages, the highest cumulative pruning weight per hectare was detected in Fox11 pear clonal rootstock (3070.0 kg ha^{-1}) and the lowest in MC, QA, and BA29 quince clone rootstocks (942.8, 1265.9, and 1299.4 kg ha^{-1}, respectively). In terms

of cultivar averages, the highest cumulative pruning weight per hectare was found in 'Deveci' (2269.0 kg ha^{-1}) and the lowest in Santa Maria (1143.4 kg ha^{-1}) pear cultivars (Figure 4). Regarding rootstock x cultivar interaction, the cumulative pruning weight per hectare varied between 372.43-4544.49 kg ha^{-1}. The highest cumulative pruning weight per hectare was observed from 'Deveci'/Fox11 (4544.49 kg ha^{-1}) and the lowest was found in 'Williams'/QA, 'Santa Maria'/Seedling, 'Williams'/BA29, 'Santa Maria'/MC and 'Abate Fetel'/MC scion/rootstock combinations (372.43, 633.75, 634.50, 654.59 and 754.59 kg ha^{-1}, respectively) (Figure 5).

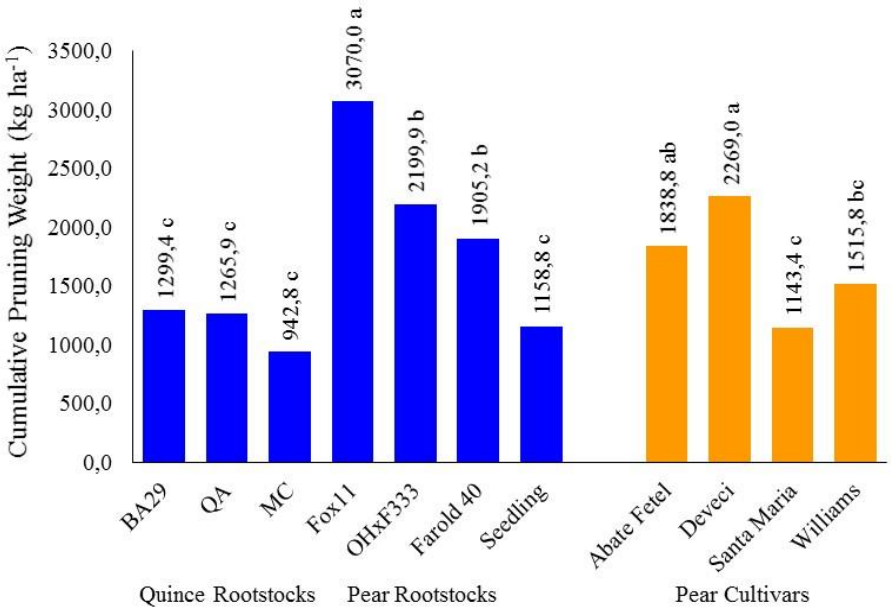

Figure 4. Effects of different rootstock and cultivars on cumulative pruning weight per hectare (kg ha^{-1}) in pear.

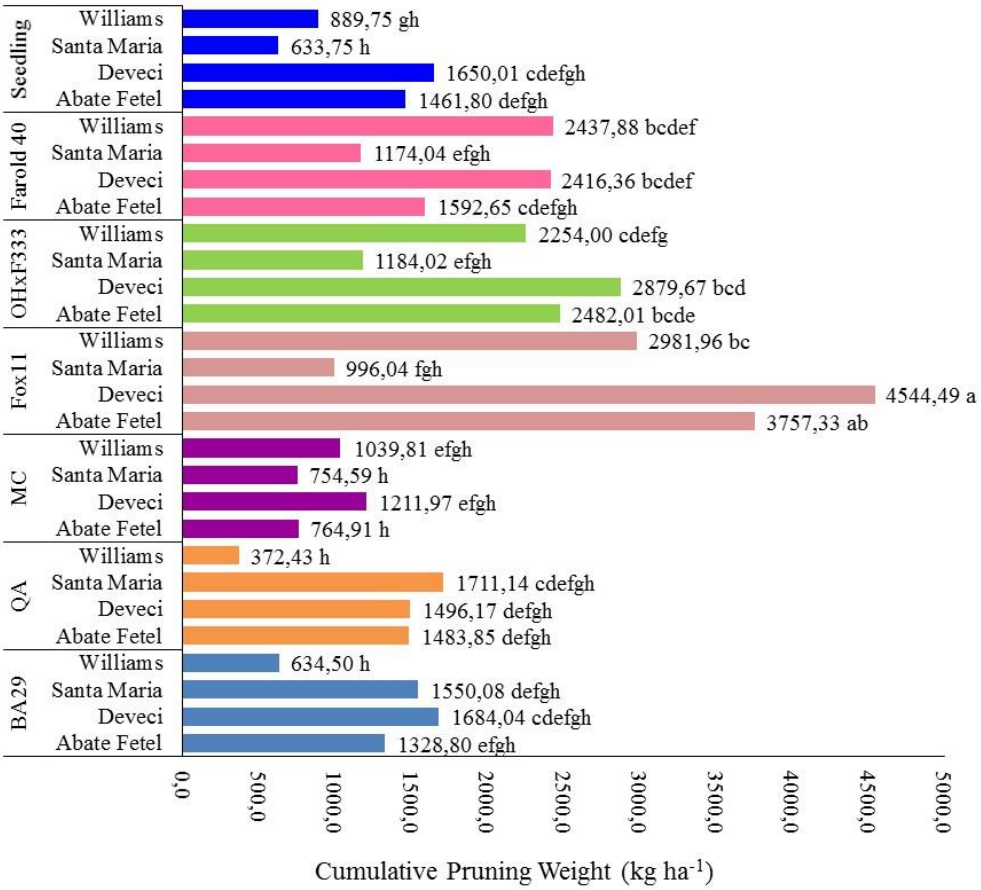

Figure 5. Effects of different rootstocks x cultivars interactions on cumulative pruning weight per hectare (kg ha^{-1}) in pear.

Ozturk and Ozturk (2014), who reported a significant difference between rootstocks in terms of growth and development in the 'Deveci' pear cultivars grafted on different rootstocks, reported that the growing strength of the seedling rootstock was weaker than the other rootstocks. They have emphasized that this is due to the fact that quince rootstocks grow and develop faster than pear seedling rootstocks in the first years after planting. Our results are consistent with the results of the research. The growth characteristics of the cultivars used in the study also affected the pruning weight characteristics. Generally, the pruning weights of the vigorously growing cultivars were also higher. Especially 'Abate Fetel' cultivar, which bears fruit on spur branches, and the 'Deveci' cultivar, which developed strongly, needed more pruning and the pruning weights of these cultivars were higher than the other cultivars. Vigorous trees require more pruning time and labor and yield less fruit per unit of vegetative growth (Rom and Carlson, 1987). The study determined that the 'Santa Maria', which gave the highest yield, developed weaker and therefore had a lower pruning weight. Among the pear cultivars grafted on different rootstocks, it was stated that the cumulative pruning weight per hectare of the 'Abate Fetel', which was spur productive, was higher than the other cultivars (Musacchi et al. 2005). The cultivars that give more yield in fruit trees are weaker than those that give less yield. Since cultivars with high fruit yield spend the nutrients on growth and development, annual shoot growth and pruning weights are low (Rom and Carlson, 1987; Jackson, 2003). It can be said that the results about the pruning weight obtained from the research are compatible with the studies that partially included the rootstocks and cultivars used in this study.

There was a significant difference between rootstocks in terms of growth and development in the 'Deveci' pear grafted on different rootstocks. The growing strength of the seedling rootstock was weaker than the other rootstocks in the first years after planting (Ozturk and Ozturk, 2014). They have emphasized that this is due to the fact that quince rootstocks grow and develop faster than pear seedling rootstocks in the first years after planting due to their root structure. Our results are consistent with the results of the previous research. The growth characteristics of the cultivars used in the study also affected the pruning weight characteristics. Generally, the pruning weights of the vigorously growing cultivars were also higher. Especially 'Abate Fetel', which bears fruit on spur branches, and 'Deveci', which developed strongly, needed more pruning and the pruning weights of these cultivars were higher than other cultivars. Vigorous trees require longer pruning time and more labor and yield less fruit per unit of vegetative growth (Rom and Carlson, 1987). In the study, 'Santa Maria', which gave the highest yield, developed weaker and therefore had a lower pruning weight. Among the pear varieties grafted on different rootstocks, it was stated that the cumulative pruning weight per hectare of the 'Abate Fetel', which was spur productive, was higher than the other cultivars (Musacchi et al. 2005). The cultivars that give more yield in fruit trees are weaker than those that give less yield. Since cultivars with high yield spend the nutrients on fruit growth and development due to that the annual shoot growth and pruning weights are low (Rom and Carlson, 1987; Jackson, 2003). It can be said that the results of the pruning weight obtained from the research are compatible with the previous studies (Urbina et al., 2003; Musacchi et al., 2005; Giocabbo et al., 2008, 2010; Clingeleffer et al., 2019; Almeida et al., 2020).

4. Conclusion

In this study, the effects of some standard pear cultivars grafted on different quince and pear rootstocks on pruning weight, rootstock, cultivar, research years, and their interactions significantly affected the parameters examined. The highest pruning weight properties were determined in Fox11 in terms of the examined rootstocks, and in 'Deveci' in cultivars. The lowest examined pruning weight properties were observed in the MC rootstock in terms of rootstocks and 'Santa Maria' in cultivars. The highest values in terms of all pruning weight-related properties examined in the study were obtained from the 'Deveci'/Fox11 combination. Generally, the pruning weights of pear rootstocks were higher than the quince rootstocks. According to the data for the years 2019-2021, the lowest cumulative pruning weight per plant was determined in the 'Williams'/QA combination. The reason for this is the rootstocks' development vigor and the cultivars' growth characteristics. In addition, graft incompatibility between some quince rootstocks and pear cultivars/varieties can also cause this situation. As a result of this study carried out on young trees, quince rootstocks are recommended due to the low pruning cost and labor. In addition, it is necessary to continue the research for a long time and make detailed examinations to make a final decision about the most proper cultivar/rootstock combination.

Author Contributions

AÖ (45%), ZAF (35%), and YMK (20%) designed the study. AÖ (50%), ZAF (30%), and YMK (20%) data acquisition and analysis. AÖ (34%), ZAF (33%), and YMK (33%) writing up. AÖ (50%), ZAF (30%), and YMK (20%) submission and revision. All authors reviewed and approved the final version of the manuscript.

Conflict of Interest

The authors declared that there is no conflict of interest.

Acknowledgments

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Research Article
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THE EFFECTS OF PHLOEM GIRDLING ON LEAF CELL PHYSIOLOGY AND CHLOROPHYLL BIOSYNTHESIS IN PEACH TREE

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Abstract: Phloem girdling is used for many fruit trees to promote fruit set and quality. Although many studies showed the pomological and biochemical effects of girdling in fruit trees, there is very little information on how girdling affects cell physiology. The current study aimed to characterize the leaf xylem structure, cortex cell division, and expansion affected by trunk girdling during phloem healing in peach. The experiment was carried out on a two-year-old peach cv. Rich May grafted onto Garnem grown in 10 L pots in greenhouse. The girdling was performed on the trunk end of the May. The leaf samples were collected 1, 2, 3 and 4 weeks after treatment (WAT). The study showed that the girdling decreased tree growth, stomatal conductance and stomatal density. Girdling decreased chlorophyll biosynthesis in peach leaves. Furthermore, girdling treatment increased leaf cell expansion, xylem thickness, and xylem conduit number during two weeks. The results have demonstrated that leaf anatomy changed by girdling during phloem healing.

Keywords: Cell division, Cell expansion, Girdling, Phloem, Xylem

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1. Introduction

Carbohydrates are mostly produced in leaves (called sources) and exported to the sink organs such as fruits, and roots (Chai et al., 2021). Fruit quality is closely related to source-sink relationship and photoassimilate distribution between source and sink affects fruit size, color, and soluble solid content (Lo Piccolo et al., 2021). Source-sink balance can be manipulated by girdling treatments. Girdling is defined as surgically removal of a ring of phloem leading accumulation of photoassimilates above the girdle by blocking the phloem transport pathway (Michailidis et al., 2020). Following the girdling, excess sugar accumulates in the leaves leading inhibition of photosynthesis and severe root starvation occurs (Tyagi et al., 2020). This technique is commonly utilized in fruit trees including sweet cherry, peach and mandarin to increasefruit quality (Day and DeJong 1990; Mataa et al., 1998; Michailidis et al., 2020).

Girdling treatment was studied in many fruit trees for many purposes. Lo Piccolo et al. (2021) reported that girdling stimulated anthocyanin accumulation and promoted sugar in red plums. Girdling treatment was performed on 'Aztec Fuji' apple trees and the fruit size, color, and firmness increased (Fallahi et al., 2018). One week after girdling, abscisic acid and gibberellin content was found further in vines (Tyagi et al., 2020). Girdling affected carbohydrates, gas exchanges, and antioxidant activities in olive (Annabi et al., 2019). Furthermore, girdling influenced anatomy of many species (Inoue et al., 1991). Hamada et al. (2009) reported that at ten days after girdling in persimmon, callus formation started in the phloem tissue. The selection of appropriate girdling time is important for fruits. Some researchers stated that girdling at cell expansion period may increase fruit size (Day and DeJong 1990; Chai et al., 2021).

Most studies on girdling have been focused on enhancing fruit quality, anthocyanin accumulation, and assimilate distribution. Although pomological and biochemical effects of girdling in fruit trees have been widely studied, there is very little information on how girdling affects leaf xylem structure, cortex cell division, and expansion. The current experiment aimed to characterize the leaf cell physiology affected by trunk girdling during phloem healing. Furthermore, chlorophyll biosynthesis was evaluated in the present study.

2. Materials and Methods

The experiment was carried out on a two-year-old peach (*Prunus persica* (L.) Batsch) cv. Rich May grafted onto Garnem (*P. dulcis* × *P. persica*) in 2021. The plants were grown in 10 L pots containing substrate and perlite (4:1) in greenhouse. The girdling was performed using a knife at 0.5 m from the ground on the trunk and approximately 10 mm of the bark and phloem were removed from the trunk end of the May (Figure 1.a). The leaf samples were collected 1, 2, 3, and 4 weeks after treatment (WAT). The

experiment was arranged in a randomized plot design with three replicates of four plants per replication.

Rootstock and scion diameters were recorded at 0 and 4 WAT and relative growth rates of rootstock and scion diameters were calculated according to Del Amor and Marcelis (2003) and Aras (2020). Stomatal conductance was measured with a leaf porometer (Li-COR).

The concentrations of chlorophyll a, b and a + b were determined according to Porra et al. (1989). The concentrations of chlorophyll precursors including protoporphyrin IX (Proto IX), Mg-protoporphyrin IX (Mg-Proto IX), and protochlorophyllide (Pchlide) were determined according to the method of Hodgins and Van Huystee (1986) and calculated by the corresponding formulas (Liu et al., 2015). Chlorophyll yield was estimated by chlorophyll a + b/Proto IX (Aras et al., 2021).

For the histological evaluations, the leaves were stored in ethanol 70% and cross sections of the leaf midribs were stained with Toluidine Blue O (for cortical cells) or acid phloroglucin (for xylem) dyes. The samples placed on a slide after staining and visualized with a light microscope (Olympus CX21) coupled to a digital camera (Kameram 5). The cortex, epidermis, and xylem were measured (Aras et al., 2021; 2022). The number of the cortex cell layer was calculated from cortex thickness divided by cortical cell diameter. Cell division was interpreted in terms of the number of the cortex cell layer and cell expansion was explained on behalf of the cortical cell diameter. The stomatal characteristics were measured on the abaxial surface of the leaves. Stomatal length, width, and stomata density were measured. Stomatal area was calculated with the equation of Zhu et al. (2019).

For starch staining, the leaves were boiled in 95% (v/v) ethanol. The depigmented leaves were immersed in Lugol's iodine solution for 15 min. Then leaves were photographed (Wood et al., 1986).

The statistical analyses were performed with the statistical software package SPSS, version 20.0. Data were subjected to two-way ANOVA and were separated by the Duncan's test at a significance level of $P<0.05$.

Figure 1. Girdling treatment (a) and gummosis observation at 2 WAT (b).

3. Results

Girdling treatment affected peach leaves. Gummosis observation was observed in girdling ring at 2 WAT (Figure 1.b). Morphological responses were significantly changed (Figure 2). Relative growth rate of rootstock and scion diameters reduced by 39 and 51%, respectively by girdling treatment. Stomatal movement was influenced by girdling (Figure 3). Stomatal conductance, stomatal density and area significantly increased by girdling at 1 WAT, while stomatal conductance and stomatal density decreased at 2, 3 and 4 WAT.

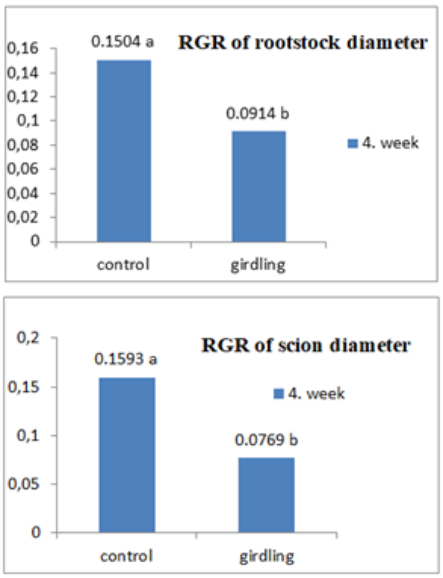

Figure 2. Effect of girdling on relative growth rates of rootstock diameter and scion diameter at 4 WAT.

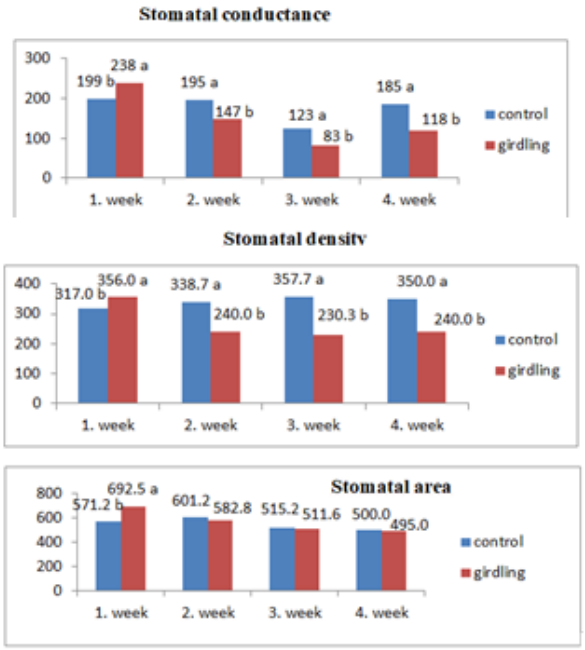

Figure 3. Effect of girdling on stomatal conductance, stomatal density and area.

Girdling affected chlorophyll biosynthesis in peach leaves (Figures 4 and 5). Chlorophyll a, b and a+b did not statistically affect at 1, 2, and 3 WAT, whereas the parameters decreased at 4 WAT. Decline in the concentrations of chlorophyll precursors including Proto

IX, Mg-Proto IX, and Pchlide was found in girdling treatment. Chlorophyll yield significantly decreased by girdling at 4 WAT. Histological responses of the peach leaves affected by girdling treatment were also affected. Cortex thickness of the leaf midrib increased by girdling at 1 and 2 WAT and the increment was found as a result of increase in cortical cell diameter (Figure 6). The results showed that girdling treatment increased cell expansion during 2 weeks. Girdling treatment increased xylem thickness and xylem conduit number during first two weeks (Figure 7). Xylem conduits length was not affected by girdling.

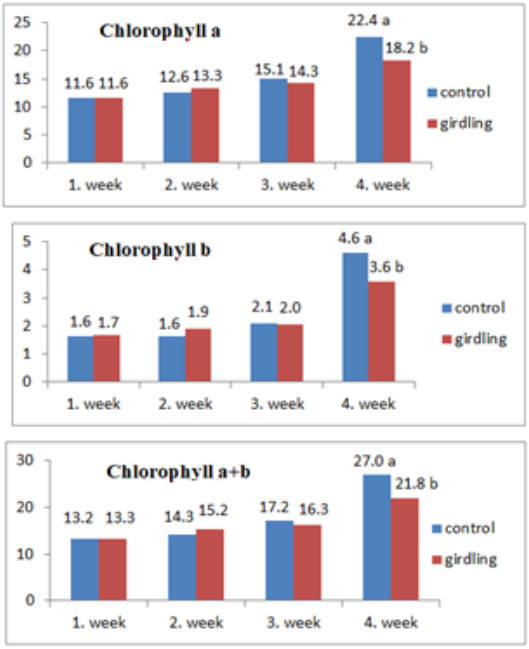

Figure 4. Effect of girdling on chl a, b, a+b concentrations.

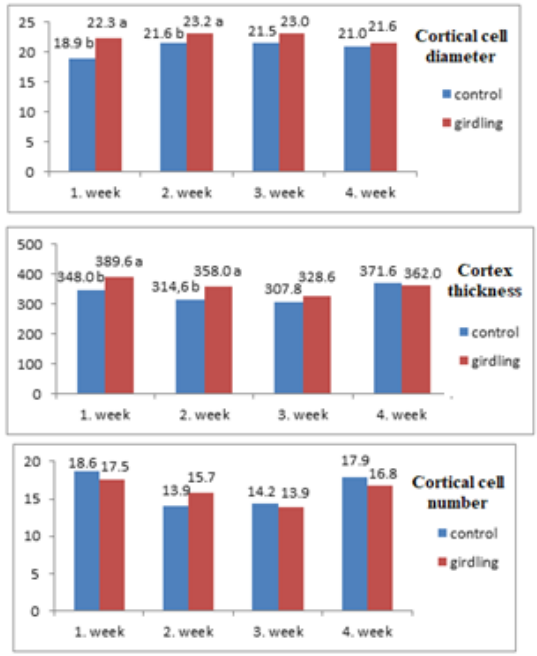

Figure 5. Effect of girdling on Proto IX, Mg- Proto IX and Pchlide concentrations and chlorophyll yield.

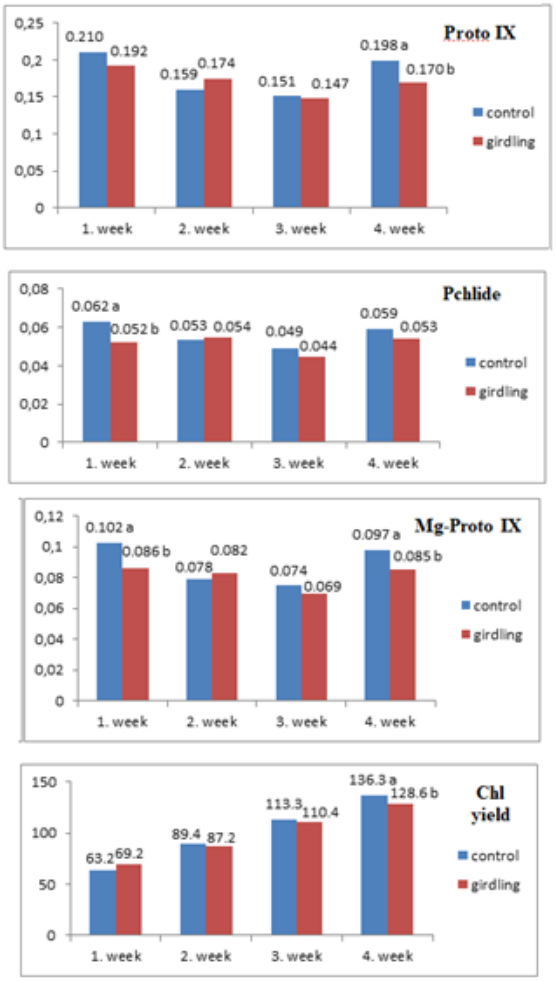

Figure 6. Effect of girdling on cortical cell diameter, cortex thickness and cortical cell number.

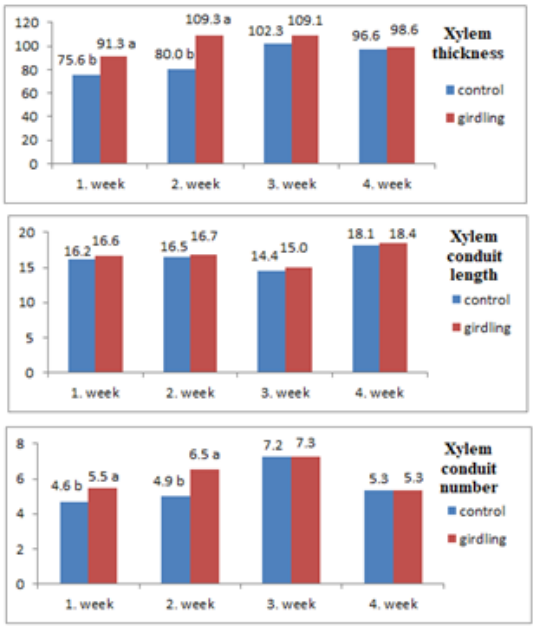

Figure 7. Effect of girdling on xylem thickness, xylem conduits length and xylem conduit number.

The intensity of starch accumulation in leaves was observed by in situ staining with Lugol dye (Figure 8). Girdling at 1 and 2 WAT leaded starch accumulation in the leaves. Starch accumulation was more pronounced in 2 WAT. In 3 and 4 WAT, starch accumulation was not observed.

Figure 8. Effect of girdling on starch accumulation of peach leaves

4. Discussion

The regulation of photosynthesis is linked to the source-sink balance (Matsuda et al., 2011). Girdling leads to photoassimilate accumulation in the leaves by blocking photosynthetic exported products (Lo Piccolo et al., 2021). In the current experiment, the healing response of girdling was observed in leaf midrib cortex and xylem shown in Figure 9.

Girdling treatment decreased tree growth, determined by a logarithmic calculation. Relative growth rates (RGRs) of rootstock and scion diameter decreased by girdling. The parameters were measured under the girdling ring and the decreases in the parameters may show that photoassimilate distribution into roots was hampered by girdling. The decline in plant growth may be linked to loss in photosynthesis and the decrease in photosynthesis is a common phenomenon induced by girdling (Lo Piccolo et al., 2021). Chlorophyll is an important pigment taking pivotal role in photosynthesis. In the current experiment, chlorophyll decreased at 4 WAT by girdling. Reduction in chlorophyll was reported in many studies and the decline may be due to decline in chlorophyll biosynthesis (Guo et al., 2020; Aras et al., 2021; 2022). Chlorophyll is a tetrapyrrole containing Mg and chlorophyll biosynthesis requires the participation of some precursors including Proto IX, Mg-Proto IX, and Pchl (Tanaka, Tanaka 2007). Girdling decreased chlorophyll biosynthesis at 4 WAT by decreasing the concentrations of Proto IX, Mg-Proto IX and Pchl. Chlorophyll yield also decreased at 4 WAT that shows the level of Proto IX driven by chlorophyll (Aras et al., 2021).

Photosynthesis is linked to stomatal conductance (Ainsworth and Rogers, 2007) and girdling may have hampered photosynthesis by decreasing stomatal gas exchange. At 1 WAT, stomatal conductance, density, and area increased by girdling. 2 weeks after girdling, stomatal conductance decreased and the decline was found as a result of decrease in stomatal density. Stomatal area was not affected except at 1 WAT. Girdling led to severe reduction in stomatal formation starting with 2 weeks after treatment. Reduction in stomatal density was reported in barley (Hughes et al., 2017) that improved drought tolerance. Furthermore, decrease in stomatal density promoted water use efficiency in wheat by decreasing water loss (Dunn et al., 2019). Thus, reduction in stomatal density may be a response to girdling in order to uptake further water.

Phloem is a living tissue protects xylem against embolism formation (Hacke and Sperry, 2001) and phloem girdling may induce a direct wounding effect on the xylem (Zwieniecki et al., 2004). Girdling caused gummosis formation leaking from the girdled ring in peach. Gummosis formation is induced by endogenous ethylene hormone (Saniewski et al., 2006) that may show wounding stress effect. Gummosis was observed at 2 WAT (Figure 1.b). Van de Wal et al. (2017) reported that girdling did not hamper xylem functionality in tomatoes. Xylem functionality is very important for water and mineral uptake depending on many factors including tree age, rootstock, and nutritions (Meinzer et al., 2001; Aras, 2021). In the present study, xylem thickness increased at 1 and 2 WAT and increment in number of xylem conduits were attributed to increase in the xylem thickness. The highest increase in the number of xylem conduits was observed at 2 WAT. Xylem consists of lignin (Whetten et al., 1998) and lignin is a phenolic compound (Kleinert, Barth 2008). Tyagi et al. (2020) found that girdling increased phenolics by induction of the phenylpropanoid pathway one week after girdling in grape. The highest increment in xylem thickness was found at 2 WAT and statistical difference was not found in xylem thickness at 3 and 4 WAT between control and girdled peach trees. We consider that phenolic compound formation was induced in 2 weeks after girdling. Girdling can be treated to trees in order to increase xylem functionality.

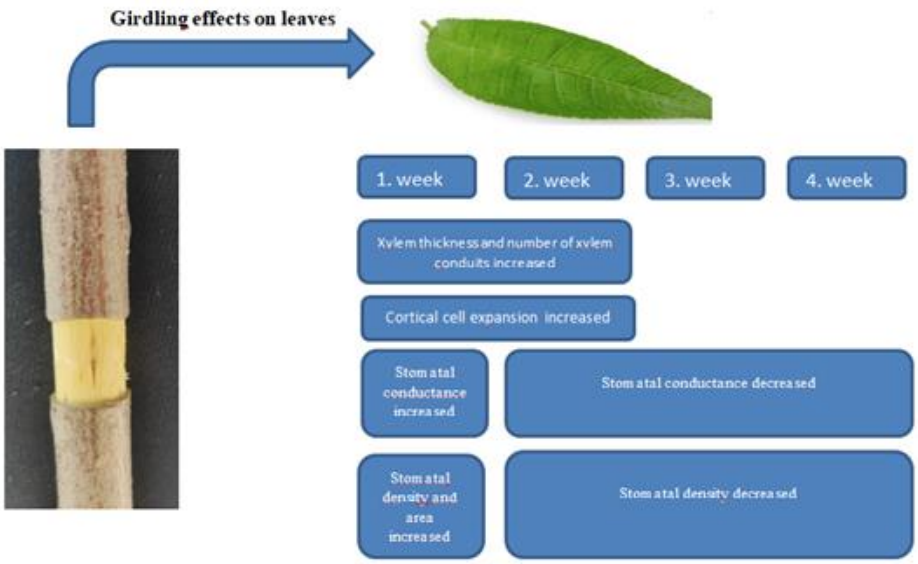

Figure 9. Effects of girdling on peach leaves.

Cortex of leaf midrib was also examined in the current experiment. Cortex plays an important role in mineral acquisition (Malta et al., 2016) and increase in cortex promotes growth of the tissue. Cortex thickness increased at 1 and 2 WAT and the increment was found a result of cell expansion rather than cell division. Leaf expansion consists of two overlapping phases: cell division and expansion (Gonzalez et al., 2012; Boron and Vissenberg 2014). Girdling leaded increments in cortical cell diameter and cortex thickness and cortex cell number did not change. Thus, girdling increased leaf cell expansion and did not alter cell division. Many studies demonstrated that girdling increased fruit growth by driven photoassimilates into fruits (Mataa et al., 1998; Michailidis et al., 2020). In the present study, we did not examine fruit cell physiology, however, we consider that fruit growth improvement is not only a result of the assimilate accumulation but also an increase in cell expansion triggered by girdling. Therefore, we suggest that girdling should be treated to trees during cell expansion period. Day and DeJong (1990) studied effect of different girdling timings in nectarine tree and reported that girdling at the beginning of Stage II was the most effective. Expansion of stone fruits possesses three Stages; cell division (Stage I), cell division+cell expansion (Stage II), and cell expansion (Stage III) (Costa and Vizzotto, 2000). Mataa et al. (1998) found that girdling at early fruit set period may be the best for tree productivity. Thus, the studies prove the results of the current experiment. The present study showed that the healing of the girdling wound was established in 2 WAT. Gummosis formation observed in 2 WAT may be a result of wound healing. Wounded peach shoots induced gummosis formation five days after wounding in a previous study (Li et al., 2015).

In the present study, starch accumulation in the leaves was also evaluated. We suggest that the excess accumulation of starch in leaves may be due to ongoing sucrose production without sucrose export from the leaves due to phloem dysfunctionality. Girdling at 2 WAT induced the starch accumulation in leaves by blocking phloem. Reducing the accumulation of excessive starch is necessary for plants to recover leaf photosynthesis (Lo Piccolo et al., 2020). Starch accumulation was not observed in the leaves of girdling 3 and 4 WAT that may show that phloem healed two weeks after girdling and sugar export from the leaves was succeed. Increase in starch accumulation by girdling was reported in many studies (Onguso et al., 2004; Denaxa et al., 2021).

5. Conclusion

The study showed that the effects of girdling are time dependent. As a result, girdling decreased tree growth, stomatal conductance, and stomatal density. Girdling declined the chlorophyll biosynthesis at 4 WAT by decreasing the concentrations of Proto IX, Mg-Proto IX, and Pchl. Girdling did not cause physical damage to the xylem. At 1 and 2 WAT, girdling increased xylem thickness by increasing the number of xylem conduits. Furthermore, leaf cortical cell expansion increased in two weeks after girdling. Anatomical alterations showed that the healing of the girdling wound was established after two weeks following girdling. The understanding of the process of healing process would contribute to the growers.

Author Contributions

All tasks were done by the single author: S.A. (100%). The author reviewed and approved final version of the manuscript.

Conflict of Interest

The author declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this

study because of there was no study on animals or humans.

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ECONOMETRIC ANALYSIS OF FACTORS AFFECTING THE BUYING OR SELLING AGRICULTURAL LANDS

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Abstract: The aim of this study is to determine the factors affecting the purchase or sale of agricultural lands in certain neighborhoods in the Dulkadiroğlu district of Kahramanmaraş province and the degree of influence of these factors. For this, a study was conducted by obtaining data from the parcel owners in the determined neighborhoods. As a result of the data obtained from the land owners in the areas determined with the help of the questionnaire, a model was established about the factors that affect the purchase or sale of agricultural lands, what kind of benefit or loss it has in the purchase or sale of agricultural lands, and the dependent variable in this model is the independent variables. The econometric test was determined using the Engle Granger two-stage estimation method to reveal the relationship between In line with the answers given by all landowners who participated in the purchase and sale survey, on all lands: 69.68% of the surveyed farmers have tractors and equipment, 94.95% of them have irrigated and flat lands, the number of crops planted is 9, the nearest settlement The average distance to the area is 19 km, and the land purchase or sale prices were determined by a survey. According to the estimation results, the factors that are effective in the purchase and sale of land determined in the survey are the factors whose degree of influence is stated: When purchasing land; 62.54% is the existence of a land investment relationship, 14.74% is the relationship between heirs, 12.35% is livestock activities and 10.35% is the transition from the treasury to private property. 39.57% of land expropriation, 38.25% of financial insufficiency, 8.70% of relations between heirs, 6.06% of immigration, 5.80% of abandonment of agriculture and 1.58% of changes in land productivity affected land owners.

Keywords: Agricultural land prices, Land structure, Tractor and equipment availability, Reason for buying and selling

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1. Introduction

Valuation studies are carried out with various criteria such as insurance, taxation, expropriation, and methods are determined according to the purpose for which it is to be carried out. Knowing the target of the valuation is very important in terms of making the valuation correctly and expressing the results in an efficient way. When it comes to value, it defines four economic factors. Scarcity is in the form of desire, utility and purchasing power (Pirgaip, 2019).

These four economic factors that create the value affect the supply and demand of the real estate. Factors affecting the value can be grouped as external factors (legal legislation, socio-cultural factors and economic factors) and internal factors (scarcity, physical conditions, utility and transferability). The characteristics of the land, human needs and the location of the land are important in determining the land value (Büyükkaracığan, 2021). The market value and values of the land may vary from region to region, as well as the factors affecting the market value and value in each region. Since the factors affecting the land value are individual and objective, it is not easy to evaluate and cluster (Yomralıoğlu, 1993). Population growth, physical characteristics of the land, economic conditions, government policies and regional factors may affect the determination of the current market value of agricultural land (Rehber, 2012). Together with, the current market value and values of the land are used as an important data source in order to make investments in private and public areas, to solve the problems experienced with the land and to create scientific studies (Utkucu, 2007; Öztürk Çoşar et al, 2011).

Many studies have been conducted on the subject of market value and value analysis in agricultural lands in Turkey (Hurma, 2007; Vural et al., 2009; Engindeniz et al., 2009; Karakayacı, 2011; Karakayacı, 2015; Keşli, 2017; Başer et al., 2019). According to these studies, it is possible to scientifically evaluate what kind of effects are in the majority of land purchase or sale with the help of surveys made with our farmers, taking into account the geographical structure of the lands in certain neighborhoods selected in the study prepared to determine what kind of factors affect the determination

of the current value and value of the land. For this, the factors affecting the purchase or sale of land and the degree of impact of these factors are clustered with each other. In addition, it is necessary to determine how these factors affect buying or selling. It is thought that what are the effective factors in the purchase and sale of land for various purposes and that it will contribute to minimizing these factors.

It is expected that the study will guide the determination of the valuation of the farmers in the purchase or sale of land in the determined areas and the extent to which the factors affecting the activities of the farmers in the purchase and sale are affected. With the findings to be obtained in this study, it will enable the determination of the current value based on years, based on realistic and scientific principles, by considering factors such as the location of the land, its structure, dry-wet condition, roughness, distance to the settlement area in the sale or purchase of agricultural land in the title deed transactions in the region. In addition, it is thought that this study will be beneficial for the investment plans to be implemented in the area where the study is applied.

The aim of this study is to try to find the degree of influence of the factors that are effective in the purchase or sale of agricultural lands in selected neighborhoods of the Dulkadiroğlu district of Kahramanmaraş province. For this, with the help of the two-stage estimation method, which is an econometric program, the factor effect degree is selected from the very least to the least and the model is established, and the homogeneity of the model is measured with the two-step method, which is cluster analysis, after the significance level of the model is checked. These data will be obtained from the survey study and the results will be found. The dependent variable in the model to be used; In the survey conducted, it is the purchase or sale of land by the surveyed land owners. The independent variables are plot size of the land, land purchase or sale price, land slope status, irrigation status of the land, average land productivity rate, crops planted on the land, the presence of tractors and equipment used in the land, the distance of the land to the nearest center or settlement area, and for what purpose the land was purchased or is sold.

2. Materials and Methods

2.1. Materials

In this study, it covers a 27-year period in which agricultural lands were bought or sold in 9 neighborhoods in the Dulkadiroğlu district of Kahramanmaraş between the years 1995-2021. The reason for choosing Dulkadiroğlu district as the place where the study will be conducted is primarily the thought that data on the purchase and sale of agricultural land can be partially obtained, and the density of farmers engaged in agricultural work as the first criterion in the selection of neighborhoods in Dulkadiroğlu district. The second criterion is that the land existence and agricultural activities are more common than the other districts. The neighborhoods where the study will be conducted were determined according to these two criteria, and the survey was conducted in 9 neighborhoods determined in Dulkadiroğlu District of Kahramanmaraş Province in 2021. Dulkadiroglu District; A survey was conducted with 630 farmers who were subject to the purchase and sale of agricultural lands in Kapıçam, Tvekkeli, Yeniyurt, Çınarlı, Abbaslar, Alibeyuşağı, Kocalar, Sivricehüyük and Çiğli neighborhoods between the years 1995-2021. Made under the assumption that it has not changed and all the information obtained is given. In the survey conducted in 9 neighborhoods, between the years 1995-2021, a total of 9597000 m^2 decares where the purchase and sale took place was determined, and the total land parcel sizes of the neighborhoods are given in Table 1.

Table 1. The total parcel size of the lands in the neighborhoods

Districts	Land Parcel Size (m^2)
Kapıçam	980500
Tevekkeli	746500
Yeniyurt	1133000
Çınarlı	1395000
Abbaslar	1241000
Alibeyuşağı	1664000
Kocalar	468000
Sivrivehüyük	550000
Çiğli	1419000
Total	9597000

A survey was conducted by randomly reaching as many people as possible and it was determined that 7.93% of the people who participated in the survey were female and 92.07% were male. In addition, the average number of children in the family was determined as 5, and their education level was determined to be 95.33% primary school or literate.

Table 2. Wet-water parcel sizes of the lands in the districts

Districts	Aqueous Land Size (m^2)	Anhydrous Land Size (m^2)
Kapıçam	980500	0
Tevekkeli	746500	0
Yeniyurt	933000	200000
Çınarlı	1286000	109000
Abbaslar	1241000	0
Alibeyuşağı	1664000	0
Kocalar	468000	0
Sivricehüyük	550000	0
Çiğli	1243000	176000
Total	9111500	485000

It has been determined that there is 485000 m^2 of waterless land and 9111500 m^2 of irrigated land. In addition, while Yeniyurt Mahallesi has the most non-

watery land among these neighborhoods, Alibeyuşağı neighborhood has the most irrigated land, and it has been determined that the average ratio of irrigated land productivity and non-watery land productivity is higher than irrigated land (Table 3 and 4).

It has been determined that the crops planted in this region are wheat, barley, cotton, beet, corn, chickpea, watermelon, pepper and cucumber, respectively. Wheat, barley and chickpea cultivation is common in dry lands, while planting of the other listed products is common in irrigated lands. In addition, it has been determined that two crops are planted annually on irrigated lands, while one crop is planted on non-watery lands. Since the geographical shape of the lands is partially uneven in the region where the lands are located, the structure of the lands is flat and the soil is generally normal or slightly stony soil. It has also been learned that all of the lands that have been sold or bought without water are slightly hilly, and the irrigated ones are flat lands, and the distance of the lands to the nearest center or settlement area: 16-18 km on average in Kapıçam district, 19-21 km on average in Tevekkeli district, on average in Yeniyurt district. 19-20 km, an average of 19-22 km in Çınarlı district, an average of 25-27 km in Abbaslar district, an average of 24-25 km in Alibeyuşağı district, an average of 20-22 km in Kocalar district, an average of 20-23 km in Sivricehüyük district and an average of 16 km in Çiğli district. It has been concluded that it is -18 km.

Table 3. Average productivity of the lands in the districts

Districts	Irrigated Land Average Productivity
Kapıçam	Sugar beet=4000-8000 kg Corn=700-1000 kg Cotton=300-500 kg Wheat=400-500 kg Barley=300-400 kg Cucumber=300-500 kg
Tevekkeli	Sugar beet=4000-8000 kg Corn=700-1000 kg Cotton=300-500 kg Wheat=400-500 kg Barley=300-400 kg Cucumber=300-500 kg
Kocalar	Sugar beet=6000-12000 kg Corn=1000-1500 kg Cotton=500-900 kg Wheat=600-700 kg Barley=500-600 kg Cucumber=400-700 kg Pepper=300-500 kg
Alibeyuşağı	Sugar beet=6000-12000 kg Corn=1000-1500 kg Pamuk=500-700 kg Wheat=500-600 kg Barley=400-500 kg Cucumber=300-400 kg Pepper= 450-500 kg
Sivricehüyük	Sugar beet=4000-7000 kg Corn=800-1000 kg Pamuk=300-500 kg Wheat=500-600 kg Barley=400-500 kg Cucumber=300-400 kg Pepper=250-400 kg
Abbaslar	Sugar beet=10000-13000 kg Corn=1000-1700 kg Pamuk=400-600 kg Wheat=700-800 kg Barley=400-500 kg Chickpeas=300-400 kg
Çınarlı	Sugar beet=7000-12000 kg Corn=1000-1500 kg Cotton=500-700 kg Wheat=500-600 kg Barley=400-500 kg Cucumber=350-400 kg Pepper=400-500 kg; Chickpeas=300-400 kg
Yeniyurt	Sugar beet=4000-8000 kg Corn=800-1000 kg Cotton=400-600 kg Wheat=400-500 kg Barley=300-400 kg Cucumber=300-500 kg Pepper=250-300 kg Chickpeas=250-300 kg
Çiğli	Sugar beet=6000-12000 kg Corn=1000-1500 kg Cotton=500-700 kg Wheat=500-600 kg Barley=400-500 kg Chickpeas=300-400 kg

Table 4. Average productivity of the lands in the districts

Districts	Average Productivity of Dry Land
Çınarlı	Wheat=300-400 kg Barley=250-300 kg Chickpeas=200-300 kg
Yeniyurt	Wheat=300-400 kg Barley=200-300 kg Chickpeas=200-300 kg
Çiğli	Wheat=300-400 kg Barley=250-300 kg Chickpeas=200-300 kg

In the survey study, the average percentages of tractor and equipment availability are given in Table 5. It has been found that 69.68% of the land owners have tractors and agricultural equipment, and the remaining 30.32% do not have tractors and equipment. While the reasons stated in the survey are among the factors affecting the purpose for which the land owners buy or sell land, 36.53% have investment relations, 48.07% transition of the land from the treasury to private property, 13.47% relations between heirs, and %36.5 in Kapıçam Mahallesi. While 1.93 of them bought land due to livestock activities; Land was sold 67.56% due to financial inadequacy, 8.10% due to relations between heirs, 21.62% due to expropriation of the land and 2.70% due to immigration. While purchasing land in Tevekkeli Neighborhood, 44.12% due to the existence of investment relations, 50% due to relations between heirs and 5.88% due to livestock activities; 46.15% of the land was sold due to financial inadequacy, 15.35% to quit agriculture, 23.12% to relations between heirs and 15.38% to immigration. While purchasing land in Kocalar Mahallesi, 63.63% of them have investment relations and 36.37% of them are due to livestock activities; 63.63% of the land was sold due to financial insufficiency, 9.10% to quit agriculture and 27.27% to migration. While purchasing land in Sivricehüyük Neighborhood, 60% due to the existence of investment relations, 30% due to relations between heirs and 10% due to livestock activities; the land was sold 40% due to financial insufficiency, 50% due to relations between heirs and 10% due to immigration. While purchasing land in Yeniyurt Mahallesi, 64.30% due to investment relations, 17.85% due to relations between heirs and 17.85% due to livestock activities; 60.72% of the land was sold due to financial inadequacy, 12.53% to quit agriculture, 14.25% to relations between heirs and 12.50% to immigration. While purchasing land in Çiğli Neighborhood, 72% is due to investment relations, 12% due to relations between heirs, 4% due to the transition of the land from the treasury to private property and 12% due to livestock activities; 29.54% of the land was sold due to financial insufficiency, 52.30% due to expropriation of the land, 4.54% to abandon agriculture, 6.81% due to relations between heirs and 6.81% due to immigration. While purchasing land in Alibeyuşağı District, 73.34% due to investment relationship and 26.66% due to livestock activities; Land was sold due to financial inadequacy of 73.34%, leaving agriculture 13.33% and migration 13.33%. While purchasing land in Çınarlı Mahallesi, 80.77% due to investment relationship and 19.23% due to livestock activities; 13.07% of the land was sold due to financial insufficiency, 83.02% due to expropriation of the land, 1.30% to abandon agriculture and 2.61% to migration. While purchasing land in Abbaslar Neighborhood, 82.87% due to the existence of investment relations, 11.42% due to relations between heirs and 5.71% due to livestock activities; 63.90% of the land was sold due to financial insufficiency, 8.33% to quit agriculture, 11.11% to relations between heirs and 16.16% due to changes in land productivity.

Table 5. The presence of tractors and equipment in the districts

Districts	Number of People Who Participated in the Survey	Number of Own Tractors and Equipment	Number of Those Without Tractors and Equipment
Kapıçam	89	52	37
Tevekkeli	60	30	30
Yeniyurt	60	40	20
Çınarlı	179	113	66
Abbaslar	71	71	0
Alibeyuşağı	60	51	9
Kocalar	22	17	5
Sivricehüyük	20	20	0
Çiğli	69	45	24
Total	630	439	191

2.2. Methods

2.2.1. Cluster analysis

Cluster analysis is a method that provides classification by gathering the units examined in a study into groups determined according to their closeness, explaining the common features of the units and making general definitions about these classes (Kaufman and Rousseuw, 1990). Cluster analysis analyzes the available data with determined methods and divides them into groups with unknown labels. The clusters resulting from this process

show a high level of intra-cluster homogeneity and inter-cluster heterogeneity (Kantardzic, 2003). Cluster analysis is a multivariate statistical analysis method used to group individuals or objects according to their similarities (Tatlıdil, 1996). Clusters created as a result of clustering analysis are more similar to the units in the same cluster than the units in the other cluster. Cluster analysis is a group of methods that make up the data matrix and show the natural communities to sub-cluster the unidentified units that are similar to each other (Romesburg, 1984). As a result of the cluster analysis, each unit in the cluster is very similar to the other units in the cluster according to a predetermined criterion. Thus, high homogeneity within clusters and high heterogeneity between clusters are provided in the clusters formed. If the grouping is successful, when the clusters are placed on the graph geometrically, the units within the cluster are very close to each other, while the units in different clusters are far from each other. Social sciences, medicine, agriculture etc. It is a method that has a relationship with other multivariate analyzes such as cluster analysis, multivariate analysis of variance, logistic regression analysis, and multidimensional scaling, which are widely used in engineering sciences. Clustering can often be encountered in our normal lives. For example, students in a classroom can be counted as a cluster. Many similar examples can be given. Before biologists can make a meaningful definition between various animal species, they need to group animal species correctly. In short, it is inevitable to encounter a clustering problem in a research (Everitt, 1974). Cluster analysis has been used as a general data reduction technique to develop large data sets. The purpose of cluster analysis is to group the unclassified data according to their similarities and help the researcher to obtain appropriate information (Çelik, 2013). Clustering is a test that brings together similar areas in the same class and dissimilar areas in a different class (Guha, 2000). Cluster analysis is a method used for research and identification purposes as well as being useful in statistical fields. It has been foreseen as a useful research technique in terms of seeing the effect of many variables and observation subjects on the units (Doğan, 2008). In cluster analysis, real or standardized data are used. In addition, while the assumption of normality of values in the multivariate statistical method is not important in cluster analysis, the normality of distance values is considered sufficient (Tatlıdil, 2002). The choice of clustering processor and solution techniques is vital for the successful use of analysis (Punj and Stewart, 1983). Clustering methods; they are methods that make use of distance, similarity or diversity matrix to classify values or data as homogeneous and heterogeneous among themselves (Özdamar, 2018). The most used clustering methods are; hierarchical and non-hierarchical methods (Yılmaz, 2011).

Two-step method

The two-step method is an algorithm created to test big data classes. The algorithm, on the other hand, clusters the observations in the classes using the approach preference. Compared to ordinary cluster analysis methods, it allows for both categorical and continuous features. Also, this method can automatically select the most compatible class for itself. The method proceeds with the following steps: pre-classification, outlier resolution, and final classification domain (Schiopu, 2010). In the pre-classification phase, it scans the data record one by one and decides whether the existing record will join one of the previously created classes or starts a new classification based on the distance criterion. In the classification phase, the subclasses formed before the classification are classified according to the required number of classes (Ceylan et al., 2017). The method uses two types of interval measures: log-likelihood and Euclidean distance (Schiopu, 2010). Two-ward method is also a hybrid classification technique that is formed by combining hierarchical methods from non-hierarchical classification methods and Ward method from K-means methods. Since the two-ward algorithm provides even more similar clusters in itself, it has been used by many people in various studies (Ceylan et al., 2017). In this study, 5 data sets were created and 3 clusters were determined and analyzed.

2.2.2. Engle-granger (EG) two-stage estimation method

The stages of EG cointegration analysis, which is defined as revealing the long-term relations of the linear combinations of the time series, which are not mentioned as stationary alone, with the stationarity processes at a sufficient stability are as follows:

Stage 1

In order to see the effect of shocks in the economic system, the model is estimated by using the level values of the variables by the classical least squares method (Equation 1).

$$Y_t = a_0 + a_1 X_t + u_t \quad (1)$$

Stage 2

Whether the error terms obtained from the estimated regression are stationary or not is determined by applying the Augmented Dickey Fuller (ADF) or Dickey Fuller (DF) test (Equation 2, 3 and 4).

$$\Delta u_t = \beta u_{t-1} \quad (2)$$

$$\Delta u_t = \alpha + \beta u_{t-1} \quad (3)$$

$$\Delta u_t = \alpha + \beta u_{t-1} + \delta_t \quad (4)$$

$H_0 : \beta = 0$ (There is no cointegration relationship between the variables).

$H_1 : \beta < 0$ (There is a cointegration relationship between the variables).

H_0 If the hypothesis is rejected and the error term is determined to be stationary, the stationary error term is substituted in the error correction model (Equation 5 and 6):

$$\Delta y_t = \alpha_1 + \alpha_y e_{t-1} + \sum \alpha_{11}(i)\Delta y_{t-i} + \sum \alpha_{12}(i)\Delta x_{t-i} + u_{yt} \quad (5)$$

$$\Delta x_t = \alpha_2 + \alpha_x e_{t-1} + \sum \alpha_{21}(i)\Delta y_{t-i} + \sum \alpha_{22}(i)\Delta x_{t-i} + u_{xt} \quad (6)$$

Here e_{t-1} is the error correction term. The above illustration is a representation of the Vector Autoregressive (VAR) model and the coefficients of the equation can now be estimated with the Least Squares Method (KEKK). Special cases of finding a cointegration relationship with the Engle-Granger method;

1. $y_t \sim I(1)$, $x_t \sim I(0)$ and $u_t \sim I(1)$ ise y_t and x_t variables are not cointegrated.
2. $y_t \sim I(1)$, $x_t \sim I(1)$ and $u_t \sim I(0)$ it could be y_t and x_t variables, only $[\beta, -1]$ When it is a cointegrating vector, it is cointegrated.
3. $y_t \sim I(0)$, $x_t \sim I(0)$ and $u_t \sim I(0)$ When it does, the problems about cointegration don't make much sense.
4. $y_t \sim I(0)$, $x_t \sim I(1)$ and $u_t \sim I(1)$ ise y_t and x_t variables are not cointegrated (Engle and Granger 1987).

In this study, it was determined that the series were stationary after taking the first differences in the model established.

3. Results and Discussion

In this study, the land purchase-sale price, the irrigation status of the land, the tractor and equipment availability of the farmers who buy or sell the land, and the land for what purpose, in 9 neighborhoods determined in the Dulkadiroğlu District of Kahramanmaraş Province between 1995-2021. The aim is to establish a model related to the factors affecting the purchase or sale and to specify the degree of influence with the cointegration method and to test the homogeneity of the model established with the two-ward method, which is a clustering method. In the applied analysis results, the values in the models established in Figure 1 are homogeneous. In addition, in the application of the Engle-Granger method in determining the degree of influence of the independent variables in the model, the stationarity of the dependent and independent variables in the model was tested with the help of the Dickey-Fuller (DF) method to test the stationarity of the series, and it was determined that the series were stationary after the first differences were taken. Since the probability value is less than 0.05 in Table 6, the series is stationary. In addition, when the Argument Dickey-Fuller (ADF) test statistic is taken into the absolute value, the value of 14.2478 is determined to be stationary since the absolute values of the crystal test values are greater than the sum of 8.8658.

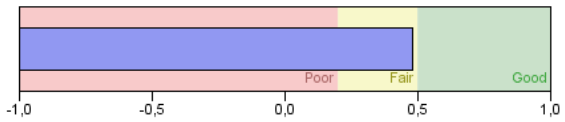

Figure 1. The result of the Two-Step method (Silhouette measure of harmony and separation)

In the application of the Engle-Granger method in determining the degree of influence of the independent variables in the model, the stationarity of the dependent and independent variables in the model was tested with the help of the Dickey-Fuller (DF) method in order to test the stationarity of the series, and after the first differences were taken, the stationarity results of the series are given in Table 6.

Table 6. The stationarity result of the dependent variable after taking the first difference

Augmented Dickey-Fuller Fullness Test Statistics	
%1 Level	-3.440702
%5 Level	-2.865999
%10 Level	-2.569203
t	-14.24784
Probability	0.0000

Since the probability value is less than 0.05 in Table 7, the series is stationary. In addition, Argüment Dickey-Fuller (ADF) test statistic shows that the series is stationary since the sum of absolute values of crystal test values is greater than 8.8755, with a value of 21.6281 in absolute value. Since the probability value is less than 0.05 in Table 8, the series is stationary. In addition, Argüment Dickey-Fuller (ADF) test statistic shows that the series is stationary when the absolute value of the crystal test values is greater than 8.8759, with a value of 12.8532 in absolute value. Since the probability value is less than 0.05 in Table 9, the series is stationary. In addition, Argüment Dickey-Fuller (ADF) test statistic is 13.1978 in absolute value, since the absolute values of crystal test values are greater than the sum of 8.8759, the series is stationary. Since the probability value in Table 10 is less than 0.05, the series is stationary. In addition, when the Argument Dickey-Fuller (ADF) test statistic is taken into the absolute value, the series is stationary because the absolute value of 16.3577 crystal test values is greater than the sum of 8.8756. Table 11. The probability value is less than 0.05. This refers to the stationarity in the error terms level value and the independent variables in the established model affect the dependent variable in the long run. There is cointegration in the values at the level. These series are cointegrated series. It was understood that H_0 was not accepted in the hypothesis given while establishing the model. In the survey conducted with the land owners who were the subject of purchase and sale between 1955 and 2021, there is the degree of influence of the factors in the purchase or sale of land.

Table 7. The stationarity result of the independent variable X_{1it} after taking the first difference

Augmented Dickey-Fuller Fullness Test Statistics	
%1 Level	-3.440584
%5 Level	-2.865946
%10 Level	-2.569175
t	-21.62817
Probability	0.0000

Table 8. The stationarity result of the independent variable X_{2it} after taking the first difference

Augmented Dickey-Fuller Fullness Test Statistics	
%1 Level	-3.440736
%5 Level	-2.569211
%10 Level	-2.569211
t	-12.85328
Probability	0.0000

Table 9. The stationarity result of the independent variable X_{3it} after taking the first difference

Augmented Dickey-Fuller Fullness Test Statistics	
%1 Level	-3.440719
%5 Level	-2.866006
%10 Level	-2.569207
t	-13.19785
Probability	0.0000

Table 10. The stationarity result of the independent variable X_{4it} after taking the first difference

Augmented Dickey-Fuller Fullness Test Statistics	
%1 Level	-3.440668
%5 Level	-2.865984
%10 Level	-2.569195
t	-16.35776
Probability	0.0000

After the stationarity test of the series obtained from the data in the established model, the degree of influence of the independent variables on our dependent variable was determined using the Engle-Granger (Co-Integration) method;

Table 11. Engle-Granger (Co-Integration) result

Augmented Dickey-Fuller Fullness Test Statistics	
%1 Level	-3.440600
%5 Level	-2.865954
%10 Level	-2.569179
t	-9.817367
Probability	0.0000

According to the results of the research, in line with the answers given by all land owners who were included in the survey, in all lands: 69.68% of the surveyed farmers have tractors and equipment, 94.95% of them have irrigated and flat lands, the number of crops planted is 9, the nearest settlement The average distance to the land area is 19 km, and the land purchase or sale prices were determined by a survey, and among the factors that were effective in the purchase and sale of land determined in the survey, the factors whose degree of influence were stated: when purchasing land; 62.54% is the existence of a land investment relationship, 14.74% is the relationship between heirs, 12.35% is livestock activities, and 10.35% is the transition from the treasury to private property. 39.57% of land expropriation, 38.25% of financial insufficiency, 8.70% of relations between heirs, 6.06% of migration, 5.80% of abandonment of agriculture and 1.58% of changes in land productivity have affected land owners.

In order to determine the current value of agricultural lands, the region where the land is located and the capitalization rate must be directly proportional. However, since the capitalization rate varies from region to region and even from land to land, this rate should be taken separately for each region in scientific studies to be carried out. Researchers who will make appraisals should have a good grasp of the regional conditions and analyze them well, as well as have necessary and sufficient knowledge of the economic and technical aspects of agricultural production. In addition, in the determination of the current values of agricultural lands, these and similar transactions are carried out more quickly and easily with the market value maps obtained by using Geographic Information Systems (GIS).

While determining the current value of agricultural lands, various factors have effect sizes and the degree of impact of each factor differs from region to region. For this reason, it is very important to determine the factors affecting the market value in determining the current market value of agricultural lands. Since all factors cannot be taken into account in determining the current market value, fair market value cannot be obtained. In the article or thesis work to be done after that; The fact that the people in the surveyed area were not fully reached and the information was not given in full, and the fact that the land purchase or sale in each neighborhood was not coincided with each year as a result of the determined years, was determined to cause disruption in the analyzes applied to the data should be preferred and it was foreseen that the existence of land expropriation should be paid attention to.

In this study, first of all, a model was established with the help of the factors that are effective in buying and selling by making use of the survey conducted in 9 selected neighborhoods in Kahramanmaraş Dulkadiroğlu district.

H_0: In the survey conducted with the land owners who were the subject of purchase and sale between the years 1995-2021, there is no effect of the factors in the

purchase or sale of land.

H_1: In the survey conducted with the land owners who were the subject of purchase and sale between the years 1995-2021, there is the degree of influence of the factors in the purchase or sale of land.

The hypotheses are stated and the model is established.

This model given in Equation 7:

$$Y_{it}=\beta_0 +\beta_1 X_{1it} + \beta_2 X_{2it} + \beta_3 X_{3it} +\beta_4 X_{4it} +u_{it} \quad (7)$$

Y_{it}: Land purchase and sale of the surveyed farmers between 1995 and 2021.

X_{1it}: Land purchase and sale prices of the surveyed farmers between 1995 and 2021.

X_{2it}: Irrigation status of the lands of the surveyed farmers.

X_{3it}: Tractor and equipment assets of surveyed farmers between 1995 and 2021.

X_{4it}: The purpose for which the surveyed farmers buy or sell land.

First of all, using the two-step method, which is a cluster analysis, the homogeneity of the data was tested with 5 inputs and 3 clusters.

While determining the current values of agricultural lands in various regions of Turkey, the effect sizes of the factors that are effective may differ from region to region. As a matter of fact, the most important factors affecting the fair value of agricultural lands are; He demonstrated with an econometric model that inflation and land rent are effective on agricultural land prices. He stated that changes in government programs would also affect land prices (Belongia, 1985). Factors affecting agricultural land prices; classified as those with agricultural characteristics and others, and the factors they took into account; they examined land rent, government payments and land properties (Dunford et al., 1985). In a study conducted in some villages of Ankara province, he explained the methods used in the valuation of agricultural lands. In addition, he examined the issue of the presence of some factors related to value in field lands in the province of Ankara. As a result, he determined that the average sales prices and capitalization interest rates of the lands differ according to the types of businesses (Vural, 1991). In a study conducted in Erzurum and Erzincan provinces, the values of agricultural lands and various factors affecting these values were discussed. As a result of the statistical study, the aesthetic location and slope for the irrigated lands of Erzurum province and the landform value for the barren lands were determined as the factors affecting the value. For the irrigated lands of Erzincan province, aesthetic location, fragmentation and landform were found to be effective factors (Birinci, 1997). In a study conducted in the Ereğli district of Konya province, 4 villages out of 87 village settlements defined 5% of the campus-targeted sampling system. In these 4 village settlements, 894 agricultural enterprises constitute the data set in the study. The data in the research consists of data obtained through questionnaires from 64 agricultural enterprises. Useable capitalization rates have been determined according to the variety of land based on agriculture in the Ereğli district of Konya province. In the study area, 2.19% of the enterprises are operated by tenancy, 1.67% by shareholding and 96.14% by property. While 22.81% of the enterprises have dry agricultural lands, 77.19% of them have irrigated agricultural lands. The capitalization rate in the study area was determined as 7.38% in orchards, 5.62% in irrigated agricultural lands and 6.63% in dry agricultural lands (Tanrivermis et al., 2004). In the research conducted in the Bogota region of Colombia, located in the south of the American country, they conducted a research on the high-speed train transit network, which is thought to have an effect on the land values in the region. As a result of the research, it was determined that there was an increase of 14% in the appraisal of the lands in the region (Rodriguez et al., 2009). In a study conducted in the Keskin district of Kırıkkale, plot size, land rent, distance to the village center, distance to the province and district road, and the distance to the nearest railway and water source (Koç, 2011). In a study conducted in the Menemen district of İzmir, the soil quality was determined as the outbuilding status and parcel size in the land (Öztürk et al., 2013). In a study conducted in the Lâdik district of Samsun, the probability of stoniness, slope, irrigation rate, yield and the distance of the land to the nearest residential area were determined (Baser et al., 2016). In a study conducted in Kemalpasa district of Izmir, soil structure and fertility were determined as road and transportation conditions, irrigation conditions, land location and size (Karaca et al., 2016). In another study conducted in İzmir, the quality of the parcel in Bergama district, the productivity of the parcel in Bayındır district, the application of rotation in the parcel in Tire district, the proximity of the parcel to the village center in Torbalı district, and the mode of operation or saving of the parcel in Ödemiş district were determined (Öztürk et al., 2017). In a study conducted in Evren district of Ankara, soil structure, shape and slope were determined as land irrigation status, width and productivity (Bayramoğlu et al., 2021).

Author Contributions

Concept: İ.G. (40%), E.Y. (30%) and M.Ş. (30%), Design: İ.G. (40%), E.Y. (30%) and M.Ş. (30%), Supervision: İ.G. (40%), E.Y. (30%) and M.Ş. (30%) , Data collection and/or processing: İ.G. (40%), E.Y. (30%) and M.Ş. (30%), Data analysis and/or interpretation: İ.G. (40%), E.Y. (30%) and M.Ş. (30%), Literature search: İ.G. (40%), E.Y. (30%) and M.Ş. (30%), Writing: İ.G. (40%), E.Y. (30%) and M.Ş. (30%), Critical review: İ.G. (40%), E.Y. (30%) and M.Ş. (30%). Submission and revision. All authors reviewed and approved final version of the manuscript.

Conflict of Interest

The authors declared that there is no conflict of interest.

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FLOWER TYPE AFFECTS THE TIMING OF EMBRYO DEVELOPMENT IN NATIVE PRIMULA VULGARIS

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Abstract: In this study, pin and thrum flowered wild primrose (*Primula vulgaris= Syn: Primula acaulis*) populations, which are naturally distributed in Black Sea Region in Türkiye, were cultivated and their pollen viability, pollen tube growth and embryo development stages were investigated. As a result of the study, pollen viability in pollen of the pin flower type was 83.10%, while the *in vitro* germination rate was 69.43%. The viability and *in vitro* germination rates of pollen of thrum flower type were 84.91% and 67.92%, respectively. As a result of the squash preparation examinations, it was observed that the pollen tubes of both types started to germinate on the stigma on the 1 DAP (Day after pollination). On 4 DAP, it was determined that pollen tubes penetrated to the ovule. Embryo developmental stages examinations showed that, zygote formation occurred on 6 DAP in the pin flower type; while on the 20 DAP, the embryo was in the heart stage and finally on the 40 DAP, it was seen that it formed a mature cotyledonary stage embryo. Endosperm in the thrum type started to develop faster than the pin type and formed on the 6 and 7 DAP. In the thrum flower type, the proembryo formed on 10 DAP, but embryo formation could only be seen on 30 DAP. In the ovary examinations performed on the 40 DAP, some embryos were in the cotyledon stage while some were still in the globular or heart stage. It was also determined that no embryos were found in some ovules.

Keywords: Fertilization, Germination, Histology, Primrose, Pollen viability

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1. Introduction

The genus *Primula* is taxonomically classified in the family Primulaceae and is naturally grown in the temperate climate zone of Europe, South America, Asia and North Africa. (Smith and Forrest, 1929; Jia et al., 2014). The genus is the largest genus of the family but there is no consensus about the number of the species. It is indicated that there are about 400 or more than 500 species in the genus (Zhang and Kadereit, 2004; Yankova-Tsvetkova et al., 2021). Additionally, several new species have been identified in the last decade (Xu et al., 2019). Many species have become popular ornamental plants because attractive flowers bloom in early spring (Jia et al., 2014). Native primroses grown in high altitudes are used by the local people for the treatment of skin wounds as well as their visual beauty (Uce and Tunçtürk, 2014). Prapajati et al. (2003) reported that extracts were obtained from the leaves and rhizomes of *P. veris* L., *P. vulgaris* Huds. and *P. elatior* (L.) Hill widely used since ancient times as a diuretic, antispasmodic, analgesic, antipyretic, expectorant, cough suppressant, sedative, relieving insomnia and in the treatment of colds, acute and chronic bronchitis.

P. vulgaris (Syn: *P. acaulis*) is one of the common primroses and its chromosome number is 22 (2n =22) (Cocker et al., 2018). The species is represented by two subspecies, namely *P. vulgaris* subsp. *vulgaris* (Syn: *P. acaulis* subsp. *acaulis*) and *P. vulgaris* subsp. *sibthorpii* (*P. acaulis* ssp. *rubra*) in Türkiye. The yellow and white flower colors appear in *P. vulgaris* subsp. *vulgaris*, while white flowers and dark purple to pink flower color appear in *P. vulgaris* subsp. *sibthorpii*. Both subspeciesshare the same habitat at altitudes from 500 to 850 m along the eastern Black Sea coast of the country (Gündoğan et al., 2019). *P. vulgaris* exhibits heteromorphic flower types that prevent self-fertilization. The sporophytic self-incompatibility with heteromorphic flower development originating from S alleles is common in primroses (Li et al., 2011; Li et al., 2015; Keller et al., 2016), making these species interesting for researchers. Although observations on the existence of heteromorphic flowers date back, Charles Darwin was the first to realize the importance of this reproductive system. The distylous primroses have been studied since then (Cocker et al., 2018). Distylous primroses have two different flower structures called

'Pin' and 'Thrum'. In the pin flower structures, the stigma is positioned at the mouth of the corolla tube, while the stigma in the thrum flower structure is located close to the flower base and the anthers are located at a higher level (Cahalan and Gliddon, 1985; Li et al., 2011). Long-styled flowered (pin) plants have homozygous s allele (ss), whereas short-styled flowered (thrum) plants have heterozygous dominant S allele (Ss) (Bateson and Gregory, 1905). When the flower structure is observed from outside, it can be understood which flower structure the plant has. The fertilization does not occur between flowers with the same flower structure. However, thrum plants with homozygous S allele (SS) can be rarely obtained from crossing thrumxthrum (Webster and Gilmartin, 2006).

Researchers have conducted many studies on the members of the genus *Primula* to understand this heteromorphic flower structure and its genetic structure. However, a limited number of studies have been carried out on embryo development in pin and thrum flower of primroses. Therefore, it was aimed to reveal whether there is an effect of flower structure on embryo development in primrose after reciprocally crossing pin and thrum flowers.

2. Materials and Methods

2.1. Plant Material

Wild *P. vulgaris* plants were collected from the campus area of Ondokuz Mayıs University, Samsun, Türkiye in the autumn of 2019 and cultivated in pots (2.5 L) containing peat: perlite: sand in the ratio (1:1:1 v/v/v) in an unheated greenhouse. Two different populations were created with at least 20 plants considering flower structure and populations were labeled as pin and thrum. The plants were irrigated once a week in the cool season and twice a week in summer. The cultivated plants started to bloom in January 2020. At the end of January, with the increase of flowering in the plants, crossing studies were started for histological analysis.

2.2. Pollen Collection and Hand Pollination

The emasculation process was carried out in flowering plants within the population. Unopened flower buds were carefully emasculated with forceps to avoid any injury of the stigma just prior to their opening. All previously opened flowers and small immature buds were removed and the emasculated flowers were covered with cotton bags to avoid free pollination. Anthers separated from flower buds and they were placed in separate Petri dishes according to flower type (pin or thrum) (Figure 1a). Anthers brought to the laboratory environment were kept overnight at room temperature for dehischence (Figure 1b). One day later, at anthesis, the emasculated flowers were pollinated with fresh pollen by hand using a small brush and covered with cotton bags immediately (Karabıyık and Eti, 2020). During hand pollination (Figure 1c), crossing was carried out with pollen obtained from thrum flowers in cases where the pin flower type was used as the mother (i.e. pinxthrum) and with pollen obtained from plants with pin flower type when the thrum flower type was the mother (i.e. thrumxpin).

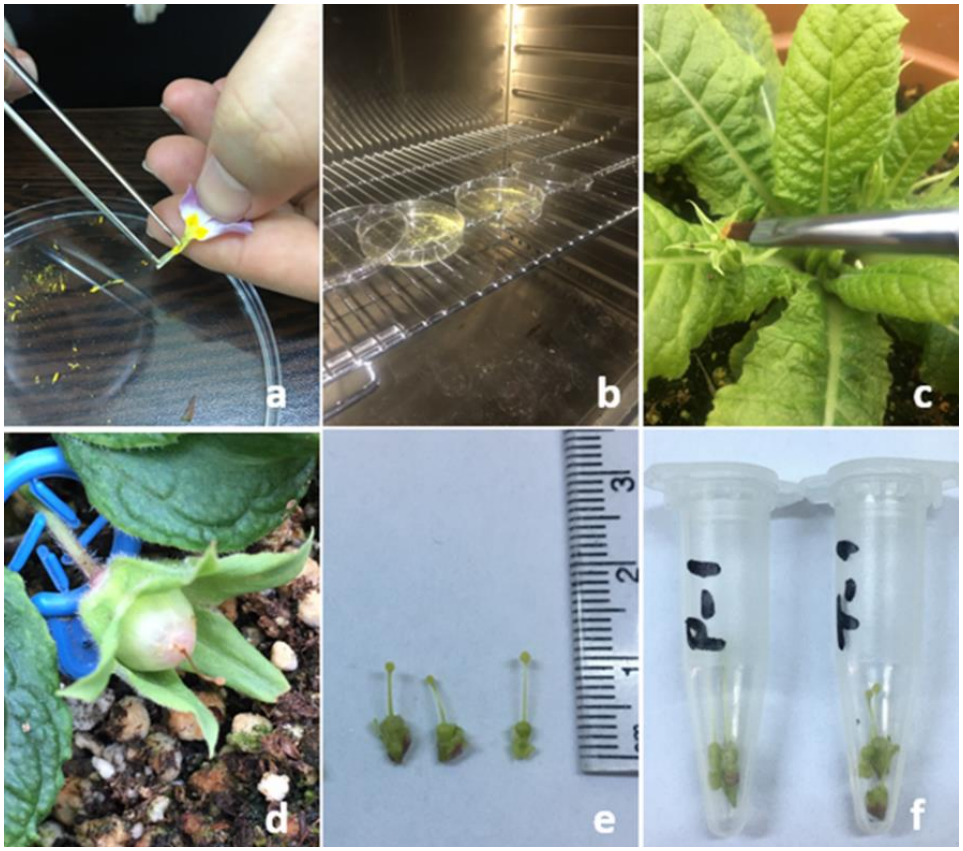

Figure 1. Hand pollination and sample collection in primroses (a: isolation of anthers, b: dehiscence of anthers, c: hand

pollinations, d: pollinated pistils at 40 DAP, e-f: harvesting samples).

2.3. Pollen Viability and *in vitro* Pollen Germination

To obtain fresh pollens, 30 flowers were collected one day before anthesis from each type and brought immediately into the laboratory. The anthers were removed and left to dehiscence at room temperature throughout the night. Pollen viability rates were tested with 1% 2,3,5 Triphenyltetrazolium Chloride. Fresh pollen grains were dispersed homogeneously on a TTC drop with a brush. Then, the top of the drop was covered with a coverglass. In the test, dark red colored pollens were recorded as 'viable' and colorless or very light pinkies as 'non-viable'. The pollen viability rate was obtained by calculating the total value of viable pollens. For each flower type, pollen viability was recorded in 4 slide replications by counting at least 100 tetrads for each replication.

Pollen germination rates were tested with "agar in petri" method with medium consisting 10% sucrose, 1.0 mM $CaCl_2$, 0.16 mM boric acid, and 7g/L agar (Grouh et al., 2015). Pollens which has a pollen tube greater than its diameter was recorded as germinated. Germinated pollens were used for calculating the germination percentage. For each type, germination was recorded in 3 petri dish replications by counting at least 100 tetrads for each replication.

2.4. Experimental Design and Statistical Analysis

Pollen viability and *in vitro* pollen germination tests were designed according toa randomized plot design. TTC test was carried out with 4 replications for each flower type. The germination test was carried out in 3 replications, one repetition for each petri dish. The arc-sine transformation was applied to the percentage values obtained before statistical analysis. All data were subjected to analysis of variance with the JMP (version 8.00) and the significance levels of the means were compared with the LSD ($P<0.01$) test (Genç and Soysal, 2018).

2.5. Histological analysis

Pollen tube growth and embryo developmental stages were investigated after reciprocal crossing between pin and thrum flowering plants according to Tütüncü and Mendi (2020). Emasculated flower buds were pollinated and collected in 24 h intervals until 7th day after pollination (DAP) to scan pollen germination on the stigma, pollen tube growth through the style and penetrating to the ovule. In addition, for paraffin sectioning studies, pollinated pistils were harvested on 10, 20, 30 and 40 DAP to determine zygote/embryo developmental stages. The samples (Figure 1d-e) were fixed immediately in FPA-70 (formaldehyde-propionic acid-alcohol) until examination (Figure 1f).

Pollen tube germination on the stigma, polen tube growth through the pistil and polen tube penetration to the ovules were monitored on squash preparations of pistils, previously softened in 8N sodium hydroxide for 5-7 h, stained with 0.1% aniline blue in 0.1 N K_3PO_4 and observed under a fluorescence microscope (Olympus BX51, Tokyo, Japan) equipped with a U-MWU filter (Olympus, Tokyo, Japan). Pollen tube growth rate was determined as percentage of the style traversed by the longest pollen tube in each pistil by a digital micrograph system (Olympus DP72 camera, Tokyo, Japan) (Karabiyik, 2022).

Paraffin sectioning method was used for determining embryo formation in pistils. Five samples from all fixed ovaries of flower buds and pollinated pistils were dehydrated in ethanol and tert-butanol series and embedded into paraffin (Johansen, 1940). Then the samples were blocked on a wood block and sectioned with a rotary microtome (Leica RM2135, Leica, Wetzlar, Germany) at 10µ. All preparations were stained with 0.125% hematoxylin buffered with $KMnO_4$ and mounted in Entellan (Karabıyık and Eti, 2020). Preparations were observed with fluorescence microscope (Olympus BX51, Tokyo, Japan) equipped with a U-MWU filter (Olympus, Tokyo, Japan) and photos were obtained by a digital micrograph system (Olympus DP72, Tokyo, Japan).

3. Results and Discussion

3.1. Viability and Germination Rates of Pollens

The results showed that, there was no statistically significant difference between pollen viability and *in vitro* pollen germination rates in terms of flower types. According to this, pollen viability in pollen of pin flower type was 83.10%, while *in vitro* germination rate was 69.43%. Pollen viability and in vitro pollen germination rates in the thrum flower type were 84.91% and 67.92%, respectively (Figure 2).

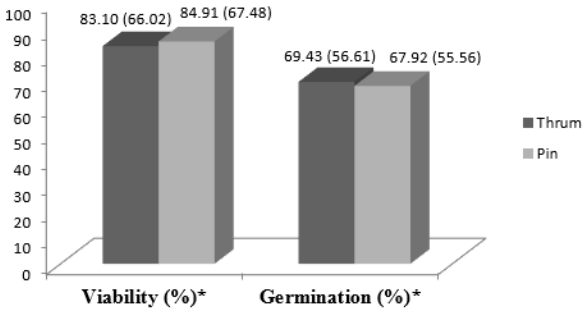

Figure 2. Percentage of pollen viability and *in vitro* pollen germination (*the difference between means was not statistically significant. Arc-sine transformed values are indicated in the parentheses).

Determining pollen viability and *in vitro* pollen germination are significant parameters for the reproductive biology of species. However, they are affected by genotype and environmental factors. In the present study, *in vitro* pollen germination was lower than pollen viability and no statistical differences were found between viability and germination rates of thrum and pin flower structures. In contrast, Aronne et al. (2021) reported that the pollen viability rate of thrum flower was significantly higher than that of the pin flower type

in *P. paliurni*. However, Yankova-Tsvetkova et al. (2021) reported that pollen sterility (unviability) in pin flower structure in *P. bayernii* was found by Gachechiladze (1993), while fertile (viable) pollen grains in both pin and thrum flower formed in their study.

3.2. From Pollen Tube Growth to Embryo Development

Flower samples were taken daily from first to the seventh day after pollination (DAP) on the primrose plant. As a result of the aniline blue staining, it was observed that the pollen tubes of both types started to germinate on the stigma on the 1 DAP (Figure 3a). In the examinations made on the samples of 2 DAP, it was determined that the pollen tubes progressed in style (Figure 3b). Pollen tubes in pin flower type reached to the ovary on the 3 DAP, and some of them penetrated the ovules. The thrum type determined that the pollen tube continued to elongate in style on 3 DAP, with a maximum elongation of 72% and entered the ovules on 4 DAP (Figure 3c).

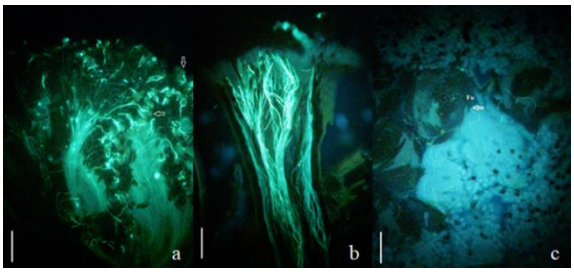

Figure 3. Pollen tube development and penetrate to the ovule. a: in the samples of 1 DAP, pollen tubes start to germinate on the stigma (white arrows) and enter the style (scale bar: 100µm), b: progress of pollen tube in style (scale bar: 100µm), c: entrance of pollen tube into ovule (white arrow) (Fu: Funiculus, scale bar: 50µm).

It was observed that many ovules were tied with a short funiculus by wrapping the placenta. This structure is called free central placentation, also observed in previous studies in *P. veris* (Webster and Gilmartin, 2003; Yankova-Tsvetkova et al., 2021) and *P. vulgaris* (Webster and Gilmartin, 2003). As a result of the examinations, when the sections of the first and second days of both flower types were examined, the ovule was seen to have an anatropous type. The embryo sac has 8 nuclei and it has been determined that antipodes are located in the chalaza part, and synergids and egg cells are located in the micropylar part (Figure 4a). These specialized nuclei could not be imaged together due to the cross-section direction and depth difference. In this context, it can be stated that the embryo sac is polygonum type. Most flowering plants have polygonum type embryo sacs, a common feature of the Primulales (Johri et al., 1992). In ovule imaging, unlike many other species, the nucellus did not develop and there were two well-developed integuments. The undeveloped nucellus, which is common in gamosepalous species, is called 'tenuinucellate'. Tenuinucellate ovule structure was also reported in *P. algida* and *P. amoena* by Akhalkatsi et al. (1998). This structure results from the failure of the differentiated archespore cell to divide into two during the initial formation of the ovule and it is a general feature of the Primulales (Johri et al., 1992). Since tapetum cells do not form, nucellus tissue does not form, and a rather large embryo sac is formed in the middle of the ovule (Figure 4b). In addition, the ovules were surrounded by the epidermis structure rich with oxalate crystals, and a similar structure was found between the inner integument and the embryo sac (Figure 4b).

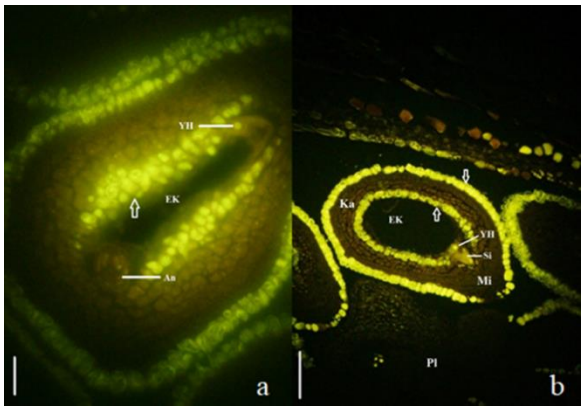

Figure 4. Ovule structure of the pin flower type. a: egg cell and antipodes (Scale bar: 20µm). b: the epidermis structure surrounding the integuments in the ovule of the seed (white arrows) (Scale bar: 50µm) An: Antipodes, EK: Embryo Sac, Ka: Chalaza, Mi: Micropil, Pl: Placenta, Si: Synergids, YH: Egg cell).

It was determined that the first pollen tube reached the ovule on 3 DAP in pin flower. Additionally, residues of pollen tubes were found in ovule samples belonging to 3 and 4 DAP in sectioning examinations (Figure 5a). Again, on the third and fourth days, the embryo sac was highly developed and even the micropylar part of some ovules began to elongate. It was observed that synergids and egg cells showed fluorescence inside ovules at this period which means that the pollen tube penetrated to the ovule via synergids and fertilized the egg cell (Figure 5b).

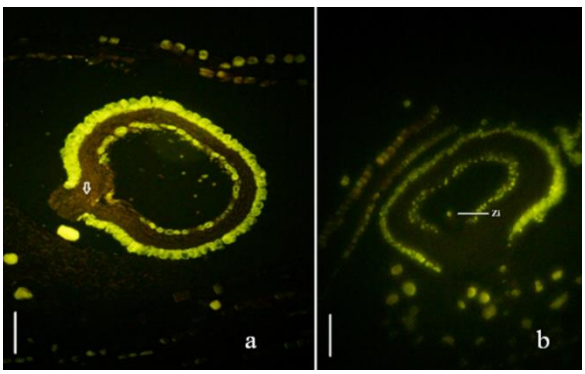

Figure 5. The ovule of the post-fertilization stage. a: pollen tube residue (white arrow) displayed on 3 DAP (Scale bar: 50µm), b: On the fourth day, the zygote (Scale bar: 50µm). Zi: Zygote.

In the samples obtained on the sixth day, a proembryo was formed where the structure thought to be a zygote was located. This cell clump was determined to proliferate adherent to the integuments (Figure 6a). In the examinations made on the 10th-day samples, it was observed that the proembryo became rounded (Figure 6b and Figure 6c), and the endosperm structure began to develop in the lower part of this structure (Figure 6b).

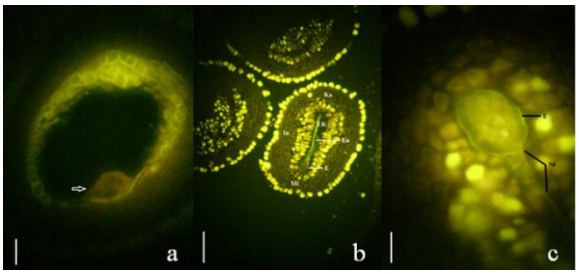

Figure 6. Embryo formed on the 6 and 10 DAP of the pin type and the developing endosperm. a: proembryo (white arrow) formed in the embryo sac on 6 DAP (Scale bar: 20 µm). b: close-up view of embryo and endosperm (Scale bar: 10 µm) c: suspensor, embryo and endosperm were developing on 10 DAP (Scale bar: 50 µm). E: Embryo, EK: Embryo Sac; En: Endosperm; In: Integuments; Ka: Chalaza; Mi: Micropyle; Su: Suspensor.

After this stage, the embryo continued to develop and that the secondary nuclei of the endosperm were also formed. It was then observed that the embryo, which was at the heart stage by 20 DAP (Figure 7a), formed a mature embryo that reached the cotyledon stage at 40 DAP. In addition, it was determined that embryo development was not the same size in all ovules on this stage. In this context, while some embryos did not cover half of the ovules (Figure 7b), some of them were quite larger (Figure 7c). It was also observed that the endosperm was not completely consumed and some of it remained during the period when the embryo gained its full size.

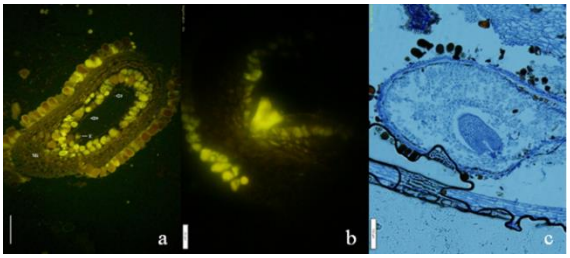

Figure 7. Embryo and endosperm development in pin flower type. a: embryo and endosperm secondary nuclei formed on day 20 (Scale bar: 50µ). b: heart stage embryo from day 20 (scale bar: 20µ). c: embryo and endosperm at the cotyledonous stage at day 40 Scale bar: 100µ). E: Embryo; Mi: Micropyle.

In the thrum flower type, it was determined that fertilization took place on the 4 and 5 DAP (Figure 8a), and pollen tube residues were also formed on the 5 DAP in some ovules. Endosperm in the thrum flower type started to develop faster than the pin type and formed on the 6 and 7 DAP and endosperm tissue was formed on the 10 DAP (Figure 8b). However, embryo development was not as fast as in the pin type; the proembryo started to form on the 10 DAP, but the embryo formation could only be seen on the 30 DAP. In the ovary examinations performed on the 40 DAP, it was determined that some embryos were in the cotyledon stage while some were still in the globular or heart stage and no embryos were found in some ovules. Woodell (1960) reported that inhomogeneous embryo developmental stages and the final size of the seeds arose from environmental differences in legitimate reciprocal crosses between *P. vulgaris* and *P. veris*. This unhomogeneous embryo development is thought to be due to pollenizer quality. As a result of the examinations, there were developing and non-developing ovules in both types and the size difference between them is quite high. When the spare pin flower samples on 20 DAP were examined, the ovules were closer to each other, while the difference between the sizes of the ovules was higher in the other samples on 20 DAP. This may have resulted from either the amount of pollen during pollination or the pollination period. The same situation was also observed in the thrum type.

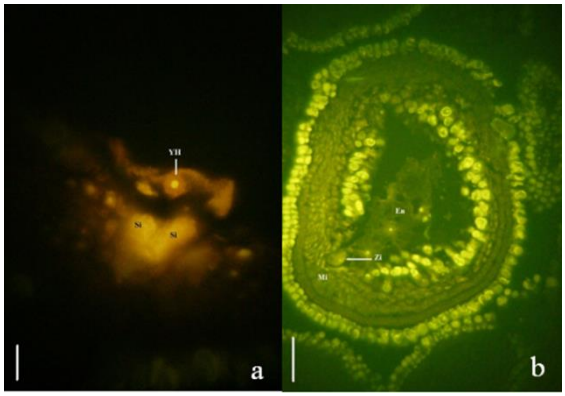

Figure 8. Fertilization and endosperm development in Thrum type. a: fluorescence of synergids during fertilization and prominence of the egg cell. (Scale bar: 20 µ). b: zygote and endosperm formation on day 10 (Scale bar 50 µ). En: Endosperm, Mi: Micropyle, Si: Synergids, YH: Egg cell.

4. Conclusion

At present study, pollen viability, in vitro pollen germination, in vivo pollen tube growth, fertilization and embryo developmental stages were illustrated by histological analysis after legitimate reciprocal crosses between pin and thrum flowers in wild *P. vulgaris* plants. No differences were found in pollen viability and *in vitro* pollen germination rates between flower structures and general pattern of the reproductive biology of *P. vulgaris* were similar with the members of the genus. However, timing of fertilization and embryo developmental stages in the pin and thrum flowers were slightly varied that may have resulted from the amount of pollen during

pollination, the pollination period or environmental stress during flowering period. Unhomogeneous development of the embryo will most likely result with different sizes of seeds and different seed germination abilities. Therefore, whether asynchronous development of embryos in inter-morph flowers affects sustainability of fragmented *P. vulgaris* populations over the years and seasons should be investigated.

Author Contributions

Concept: M.T. (35%), Ş.K. (35%) and B.S. (30%), Design: M.T. (50%) and Ş.K. (50%), Supervision: M.T. (100%), Data collection and/or processing: M.T. (25%), Ş.K. (25%), N.B. (25%) and B.S. (25%), Data analysis and/or interpretation: M.T. (25%), Ş.K. (25%), N.B. (25%) and B.S. (25%), Literature search: M.T. (25%), Ş.K. (25%), N.B. (25%) and B.S. (25%), Writing: M.T. (50%) and Ş.K. (50%), Critical review: M.T. (35%), Ş.K. (35%) and N.B. (30%). Submission and revision. All authors reviewed and approved final version of the manuscript.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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Research Article
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ANALYZING SOME RELATIONSHIPS BETWEEN PHYSICAL-MECHANICAL AND THERMAL PARAMETERS OF BIO-BRIQUETTES PRODUCED FROM PERSIMMON PRUNING RESIDUES

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Abstract: Turkey imports a considerable part of its energy means from abroad. This creates a huge burden on the national economy. Turkey can be considered as poor in fossil fuel resources, but rich in renewable energy sources. Biomass is one these renewable energy sources which comes from various agricultural production lasting whole year due to four seasons richness of the climate in Turkey. In this study, solid cylindrical briquettes produced from persimmon tree pruning wastes under 2 different moisture contents (12% - 15%), with 3 different particle sizes (3-5-8 mm) and under 4 different briquetting pressures (80 - 120 - 160 - 200 MPa) were analyzed for its solid fuel properties. The lowest lower heating value was found as 18.50 MJ/kg where the highest lower heating value was 18.90 MJ/kg. The lowest ash content was obtained as 1.73% at 15% moisture content, 3 mm particle size under 200 MPa briquetting pressure while the highest was 2.47% at 12% moisture content, 5 mm particle size under 120 MPa briquetting pressure. The relationships between some physical-mechanical and thermal parameters of the produced briquettes were also analyzed.

Keywords: Briquette, Energy, Agricultural waste, Solid bio-fuel, Persimmon pruning

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1. Introduction

Energy is of great importance for our country as it is in the world. Energy is a necessity needed by humanity in order to live a modern life. Where, how and at what costs this need is met is a fundamental factor that determines the way that countries gain superiority over each other today. Countries rich in energy resources make large amounts of financial, technological and military investments in order to protect their existing energy resources, and countries with insufficient energy resources to access energy resources. In addition, the proximity of a country to energy resources and hosting energy corridors means that the strategic position of that country and its bargaining power in the international arena increase (Yılmaz, 2012).

Turkey, when analyzed in terms of overall energy use 78% and of fossil fuel sources and its derivate 92% dependent to foreign sources. For this reason, it has a risky economical structure even effected by tiny fluctuations in energy markets (Acar et al, 2016). Thus, the current account deficit highly affects by this uncertain fluctuation. The search for new energy sources has gained a big momentum considering that existing fossil fuels will run out in the near future. The increasing need for energy and the search for new energy resources has increased the importance of renewable energy sources.

Renewable energy sources are; solar energy, wind energy, geothermal energy, biomass energy, wave energy, hydraulic energy, tidal energy, hydrogen energy, etc. are resources that are constantly available and cannot be consumed (Özalp, 2019). The industrialization efforts of developed countries and developing countries, the developments in technology and the gradual increase in the population have caused a rapid increase in energy need and thus in energy prices. When examined from the perspective of our country, it concentrates on resources such as natural gas and the big stress posed by international forces led by price increases on the economy, is a major problem in Turkey's development (Demirel ve Gürdil, 2018).

Despite being a poor country in terms of fossil fuels sources Turkey has advantage of having four seasons throughout the year that makes her rich in renewable energy sources. Renewable energy sources are an endless source of energy. One of the most important features of them is that they help to protect the

environment by reducing carbon dioxide emissions. Besides, they contribute to the decrease dependency on abroad in energy and decrease in unemployment with the increase of various job opportunities. Biomass as one of the renewable energy sources, should be taken into account due to its residual potential in recent years. It is an important factor in preventing environmental pollution, too (Demirel ve Gürdil, 2018). According to 2014 data, agricultural production is carried out in approximately 20-21 million hectares of land in our country. Approximately 60 million tons of residue and waste produced just from crop production. However, it is estimated that 15-20 million tons of this amount can be used as biofuel raw material (Acar ve ark, 2016).

For example, cultivated areas in the Black Sea region constitute 20% of the region. Tea, hazelnut, paddy, corn, wheat, sunflower and various fruits are the leading products. A significant amount of renewable and non-fossil-based solid fuel can be obtained from the wastes of these products such as; straw, stalk, hazelnut or rice husk, pruning residues etc. Thus, waste that is left to the environment, burned randomly and not economically utilized will be evaluated as pellet fuel and contribute to the economy of the region (Dok, 2014).

Biomass energy is the largest primary energy source in the world after coal and oil, and more than half of the world's population uses biomass as the primary energy source (Öztürk and Ekici, 2016). Biomass is defined as the source of organic matter that is the origin of living organisms and is formed as a result of green plants' storage by converting solar energy into chemical energy through photosynthesis. Within the scope of biomass energy technology; wood (energy forests, wood residues), oilseed crops (sunflower, rapeseed, soy, safflower, cotton, etc.), carbohydrate crops (potato, wheat, corn, beet, etc.), fiber crops (flax, hemp, hemp, sorghum, etc.), plant wastes (branches, stalks, straw, roots, bark, etc.), animal wastes, and urban and industrial wastes are evaluated. Biomass is a strategic energy source that can be renewed, can be grown anywhere, provides socio-economic development, environmentally friendly, fuel for vehicles and also electricity can be produced from that and can be obtained. Biomass is used in energy technology by directly burning or by increasing the fuel quality through various processes, and producing alternative biofuels (easily transportable, storable and usable fuels) with properties equivalent to existing fuels. Many liquid, solid or gaseous biofuels are obtained from biomass by physical processes (size reduction-crushing and grinding, drying, filtration, extraction and aggregation) and transformation processes (biochemical and thermochemical processes) (Karaosmanoğlu, 2006). For example, wastes generated as a result of tree pruning in orchards and in vineyards and wastes in forest areas are collected in one place and burned or left to decompose. Using modern biomass energy will be very useful the country's economy and for the minimization of environmental pollution.

2. Materials and Methods

This work is carried out in the laboratories of Ondokuz Mayıs University Faculty of Agriculture, Department of Agricultural Machinery and Technologies Engineering. Persimmon pruning waste is used as a material. The wastes were dried down to 12-15 % moisture content under natural conditions. The material was first ground with a hammer mill and the particle sizes were reduced to 3mm, 5mm and 8mm (Figure 1). The ground material was then briquetted with no adhesive material inside with a hydraulic type briquetting machine under 80, 120, 160 and 200 MPa pressures (Figure 2). The specific mass of the material before briquetting varied between 140-150 kg/m^3.

Figure 1. Ground material.

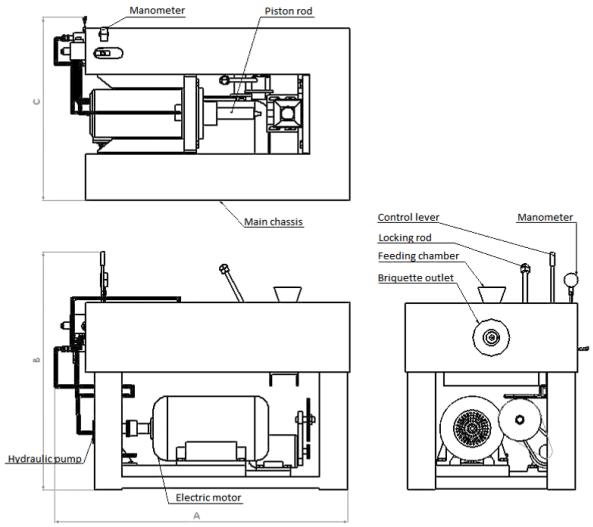

Figure 2. Hydraulic briquetting machine (A: 1280 mm; B: 1155 mm; C: 740 mm).

The density of the briquettes was determined by stereometric method and varied between 950-1115 kg/m^3. Storage and transportation costs of materials can be reduced after compression. The lower calorific values of the samples were determined using a calorimeter device according to the EN 14918 standard. The ash content of the materials was determined according to the EN 14775 standard. The mechanical durability (tumbler index) test of the briquettes was performed according to the EN 15210-2 standard. For shatter resistance of the briquettes, the briquettes were weighed and recorded before the test. Then the briquettes were dropped 10 times from a certain height (1-1.8 m) on a solid ground

and their weight was recorded again. Shatter resistance was then calculated as a percentage (%) of mass loss.

In order to test the suitability of the measured values to the analysis of variance, the normality analysis was performed with the Kolmogorov-Smirnov single-sample test, and it was determined that the data had a normal distribution (P> 0.05) and the variance homogeneity was evaluated with the Levene test and the variances were homogeneous (P> 0.05). Then it is understood that variance analysis can produce reliable results. Duncan multiple comparison test was used to compare the means. Analyzes were made in SPSS 20.0 software with OMU license. Trials were carried out in three replications.

3. Results and Discussion

Persimmon pruning wastes first chopped by axe then dried down to 12-15% moisture content and grinded to 3 mm, 5 mm, 8 mm particle sizes, and then it was briquetted in a hydraulic type briquetting machine under 80, 120, 160 and 200 MPa pressures. Briquette density, firmness, lower heating values, ash content of the briquettes were determined.

The below works are done:

- Thermal properties were compared with some other fossil fuels.
- Relations between briquetting pressure and briquette density were analyzed.
- Relations between briquetting pressure and firmness were analyzed.
- Relations between briquette density and firmness were analyzed.
- Relations between briquette density and ash contents were analyzed.

Relations between the analyzed variables were evaluated and also expressed in graphs. Best fit in the curves were obtained with linear equation 1 as follows.

$$y=ax+b \quad (1)$$

3.1. Comparison of Heating Value and Ash Contents

Lower heating values and ash contents of the briquettes are given in Table 1 and 2. Ash content and heating value of some other fossil fuels are given in Table 3.

Average heating value and ash content of briquettes at 12% moisture content are 18.69 MJ/kg and 2.28%, respectively. Those values were 18.64 MJ/kg and 1.89% at 15% moisture content. The heating values of the briquettes are said to be compatible with some other fossil fuels. They were higher than brown coal and wood, but lower than other coals, fuel oil and diesel fuel (Table 3). From this point of view, it can be concluded that the produced briquettes can be used as solid biofuel also keeping in mind that the briquettes have higher heating value than the wood (2500 cal/g) (Gürdil et al., 2014).

It's also seen that the briquettes had lower ash contents than some of the selected fuels (Table 3.) This is also a good indicator for a biofuel material. It is considered that briquettes produced from pruning wastes can be used as an alternative energy source in solid fuel combustion systems in terms of their thermal values and ash contents.

Dok et al. (2014) in their research on finding suitable mixture values for pellet production from some agricultural wastes with high energy value, revealed that agricultural wastes can be used by making pellets or briquettes in every area where coal is used, either alone or by mixing them. Besides, considering that 90% of lignite coal mined in our country has a calorific value below 3000 kcal/kg (Dok, 2014), it can be said that agricultural wastes are a quality fuel source. For example, paddy stalk and paddy husk, which have the lowest calorific value among the wastes obtained from field crops, are around 3500 kcal/kg. As can be seen, the calorific value, moisture content, ash and flue gas emission values of agricultural wastes are of higher quality than lignite coal, so can be used instead of coal.

Table 1. Lower heating values and ash contents at 12% moisture content

PS (mm)	P (MPa)	LHV (MJ/kg)	AC (%)
3	120/160	18.73	2.20
5	120	18.71	2.47
8	200	18.63	2.17

PS= particle size, P= pressure, LHV= lower heating value AC= ash content.

Table 2. Lower heating values and ash contents at 15% moisture content

PS (mm)	P (MPa)	LHV (MJ/kg)	AC (%)
3	200	18.90	1.73
5	200	18.53	1.85
8	200	18.50	2.10

PS= particle size, P= pressure, LHV= lower heating value AC= ash content.

Table 3. Heating values and ash content of some fuels (Erdoğmuş et al., 2016)

Material	LHV (MJ/kg)	AC (%)
Hazelnut husk residue	17.72	10.65
Soma coal	23.03	27.3
Zonguldak coal	25.54	14.3
Brown coal	11.51	19.10
Fuel oil	40.61	0.1
Diesel	42.70	<0.01

LHV= lower heating value (MJ/kg), AC= ash content (%).

3.2. The Effect of Briquetting Pressure on Briquette Density

The effect briquetting pressures (80, 120, 160 and 200 MPa) on briquette densities concerning the different

particle sizes (3, 5 and 8 mm) and moisture contents (12 and 15%) are given in figure, below.

When the graphs are examined:

- Briquetting pressure and briquette density values showed linear variation at both moisture contents.
- When the R2 values for both moisture values compared, it is seen that the 12% moisture values are higher.
- When the graphs formed by briquette pressure and briquette density values for both moisture values are examined, it is seen that the curves for 5mm grinding fineness of 12% moisture are higher.
- For the graphs at 12%;
- All the curves showed a polynomial fluctuation and briquetting densities varied between 700 and 1600 kg/m3.
- The highest briquette densities depending on briquetting pressure recorded at 5 mm particle sized briquettes.
- The equations of the curves showed a reliable relation due to high R2 values.
- For the graphs at 15%;
- Best fit for the curves achieved at linear function and briquetting densities varied between 700 and 1000 kg/m3.
- The highest briquette densities recorded at 5 mm particle size.
- The equations of the curves showed a reliable relation due to high R2 values.

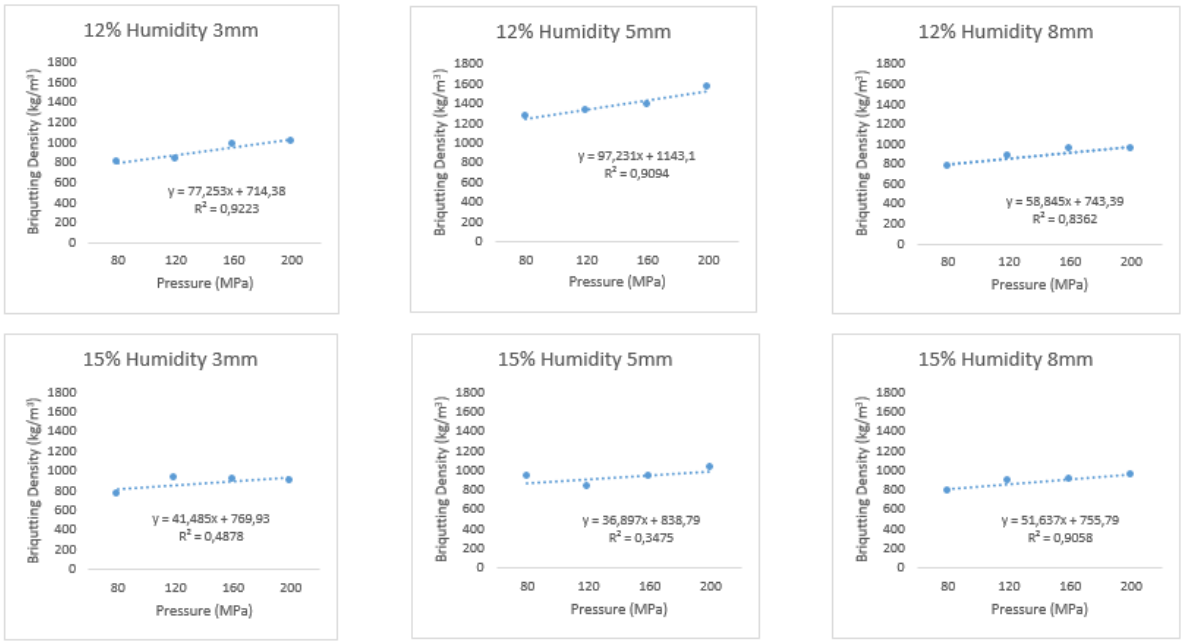

Figure 3. Effect of briquetting pressure on briquette density

3.3. The Effect of Briquetting Pressure on Firmness

The effect briquetting pressures (80, 120, 160 and 200 MPa) on firmness values of the briquettes concerning the different particle sizes (3, 5 and 8 mm) and moisture contents (12 and 15%) are given in Figure 4.

When the graphs are examined:

- Briquetting pressure and firmness values showed a linear variation at both moisture contents, that firmness values increased when the briquetting pressures increased.
- Firmness values changed in between 500 and 6000 N at all samples. The briquettes produced with 12% moisture content had higher firmness values than the ones at 15%.
- Highest firmness value was obtained at 12% moisture content with 5 mm particle size where the lowest was at 15% moisture content with 3 mm.

3.4. The Effect of Briquette Density on Firmness

The effect of briquette density obtained at different briquetting pressures on firmness values of the briquettes concerning the different particle sizes (3, 5 and 8 mm) and moisture contents (12 and 15%) are given in Figure 5.

When the graphs are examined:

- There was a linear relation between briquetting density and firmness values of the briquettes.
- The briquettes produced with 12% moisture content had higher firmness values than the ones produced with 15%.
- Highest firmness value was obtained at 12% moisture content with 5 mm particle size where the lowest was at 15% moisture content with 3 mm.

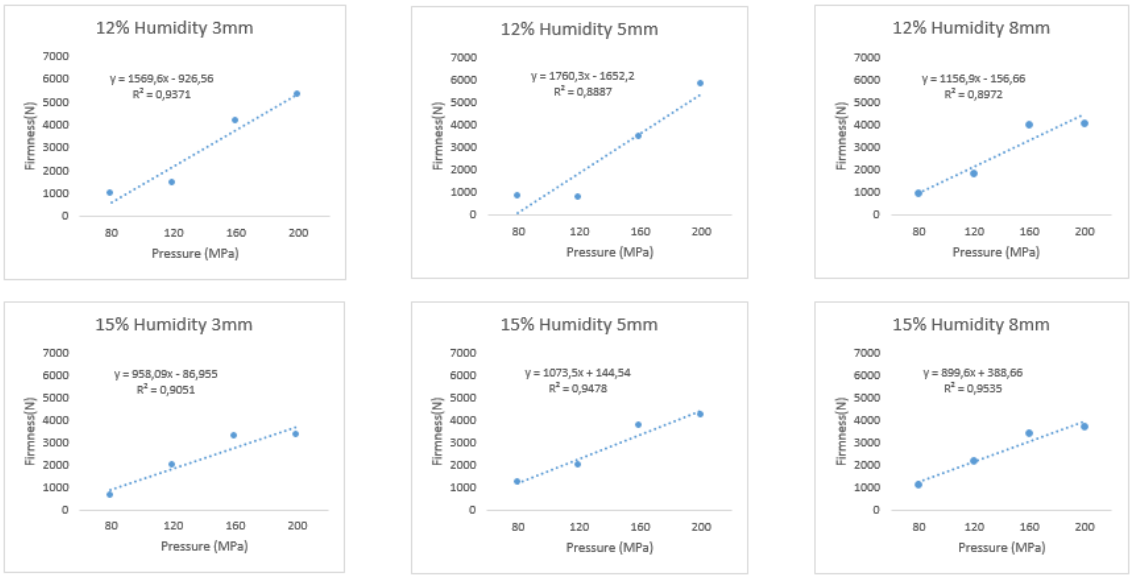

Figure 4. Effect of briquetting pressure on firmness

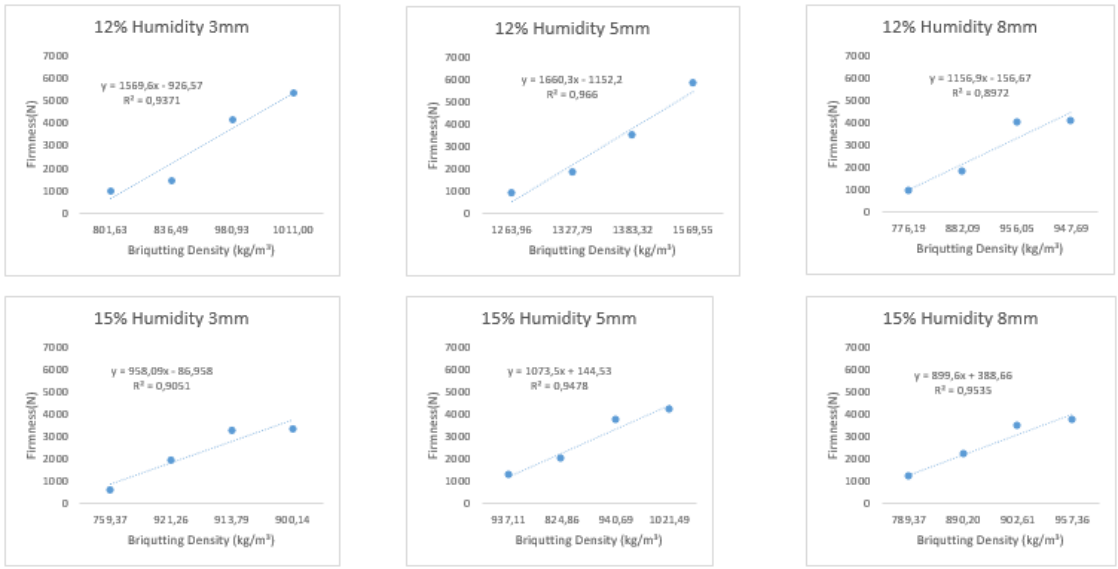

Figure 5. Effect of briquette density on firmness

3.5. **The Relation Between Briquette Density and Ash Content**

The relations between briquette densities obtained at different briquetting pressures and particle sizes under two different moisture contents and their ash contents after burning were examined. The results showed that they had a polynomial relationship between each other and the parameters of their equation are given below in Table 4 and Table 5.

When the relations between the analyzed variables were evaluated and expressed in graphs. Best fit in the curves were obtained with second degree polynomial equation 1 as follows.

$$y=ax^2+bx+c \quad (1)$$

Table 4. Parameters of equations at 12%

PS (mm)	a	b	c	R^2
3	0.025	-0.221	2.7	0.90
5	0.1875	-0.9665	3.7175	0.94
8	-0.1875	0.7665	2.1325	0.95

PS= particle size

Table 5. Parameters of equations at 15%

PS (mm)	a	b	c	R^2
3	0.1575	-1.0205	3.3425	0.88
5	-0.1	0.48	1.535	0.98
8	-0.3825	1.9275	0.5825	0.85

PS= particle size

Conclusion

Solid cylindrical briquettes produced from persimmon tree pruning wastes under 2 different moisture contents (12% -15%), with 3 different particle sizes (3mm-5mm-8mm) and under 4 different briquetting pressures (80 - 120 - 160 - 200 MPa) were analyzed for its solid fuel properties. The lowest lower heating value was found as 18.50 MJ/kg where the highest lower heating value was 18.90 MJ/kg. The lowest ash content was obtained as 1.73% at 15% moisture content, 3 mm particle size under 200 MPa briquetting pressure while the highest was 2.47% at 12% moisture content, 5 mm particle size under 120 MPa briquetting pressure. Relations between briquetting pressure and briquette density, briquetting pressure and firmness, briquette density and firmness, briquette density and ash contents along with their thermal properties were analyzed. As a result, the briquettes produced from pruning wastes of persimmon orchards can be accepted as an alternative solid bio-fuel source.

Author Contributions

Concept: G.A.K.G. (50%) and B.D. (50%), Design: G.A.K.G. (50%) and B.D. (50%), Supervision: G.A.K.G. (50%) and B.D. (50%), Data collection and/or processing: G.A.K.G. (50%) and B.D. (50%), Data analysis and/or interpretation G.A.K.G. (50%) and B.D. (50%), Literature search: G.A.K.G. (50%) and B.D. (50%), Writing: G.A.K.G. (50%) and B.D. (50%), Critical review: G.A.K.G. (50%) and B.D. (50%). Submission and revision. All authors reviewed and approved final version of the manuscript.

Conflict of Interest

The authors declared that there is no conflict of interest.

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Research Article
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PHYSICAL-MECHANICAL PARAMETERS OF HAZELNUT HUSK BIO-BRIQUETTES PRODUCED BY A HORIZONTAL PRESSING BRIQUETTING MACHINE

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Abstract: Türkiye has a big potential of hazelnut residues, especially the husks that is not used for any purposes. It is estimated that 200.000 tons of husk residue is produced per year after hazelnut harvesting. Biomass became very popular since it is alternative source of energy and provides employment facilities in rural areas. Briquetting is the most widely used waste compaction technology. A hydraulic type briquetting machine with horizontal course was manufactured to convert this particular idle residue into biofuels. Some physical and mechanical properties of briquettes produced under different pressures with different particle sizes and having differ-ent moisture contents were determined.

Keywords: Hazelnut, Residue, Biomass, Briquetting

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1. Introduction

Biomass is the second biggest source of primary energy on the world after coal and petroleum, and more than half of the world's population uses this energy (Bapat, et al., 1997; Öztürk and Ekinci, 2016). Biomass defined as different materials of biological origin mainly plant material and animal wastes (Sampson, et al., 1993; Trebbi, 1993), used primarily as domestic energy source is naturally abundant and present a renewable energy opportunity that could serve as an alternative to fossil fuel. Considerable work has been conducted on the use of energy in agri-culture with respect to efficient and economic use of energy for sustainable production (Öztürk, 2016). Comprehensive studies have been performed on energy use in different agri-cultural products (Ahmad et al., 1991; Bobobee, 1992; Dash and Das, 2000; Elbatawi and Mohri, 1999; Hetz, 1992; 1998; Joshi et al., 1992; Mahapatra et al., 2003; Singh and Singh, 1992). Utilization of agricultural residues is often difficult due to their uneven and trouble-some characteristics (Gürdil et al., 2014). The process of compaction of residues into a product of higher density than the original raw material is known as densification. Densification has aroused a great deal of interest in developing countries all over the world lately as a technique for upgrading residues as an energy source (Bhattacharya, et al., 2002). Briquetting is the most widely-used waste compaction technology (Biath and Ondruska, 2012). High-density, compressed biomass simplifies the logistics of handling and storage, improves biomass stabil-ity, facilitates the feeding of solid biomass fuels into energy utilization devices and offers higher energy density, cleaner burning solid fuels that in some cases can approach the heating value of coals (Klass, 1998).

The objective of study is to develop and manufacture a farm scale hydraulic briquetting ma-chine with horizontal pressing course in order to convert agricultural residues into energy materials. For this reason, one of the most important agricultural products of Türkiye the hazelnut residues were used in this research. Briquetting parameters for hazelnut husks were deter-mined under two different briquetting pressures (80 MPa and 160 MPa) with two different particle sizes (2 to 5 mm and 7 to 10 mm) and at M10(8-10%), M12(12-15%) moisture con-tents.

Materials and Methods

A hydraulic type briquetting machine (Figure 1) with a horizontal pressing course (Figure 2) manu-factured for briquetting the hazelnut husk residues. Briquetting pressure range of this machine is adjustable from 0 to 320 MPa by a manometer on it. The pump of the machine has a tank of 25 dm^3 capacity of hydraulic oil with a 1.2

$m^3.s^{-1}$ flow rate. Stroke of the piston is 310 mm and the velocity of the stroke is adjusted to 10 mm.s-1. Machine dimensions are 1280×1155×740 (A×B×C) mm (Figure 3). Operation of the machine is controlled by a start-stop button embedded on it. Hydraulic pump functions by a 15 kW powered 3-phase electrical engine with a star delta starter. The mold for the briquette was not heated. As a support block for the pressing a rectangle shaped metal plate is placed at the end of the course having 125×105×30 mm dimensions. Movement of this plate is done manually.

Figure 1 Hydraulic type briquetting machine.

Figure 2. Horizontal pressing course.

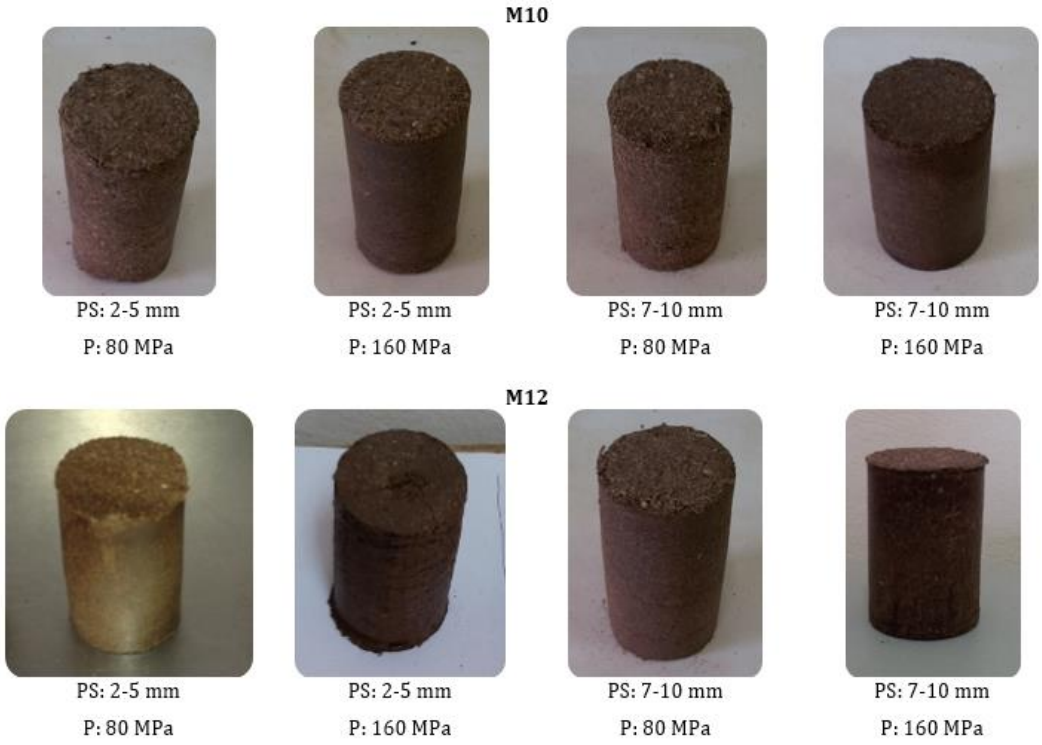

Figure 3. Briquettes produced from hazelnut husks.

The residues were first dried in normal conditions under the sun and their moisture contents were decreased down to 8-10 %. Then the dried material was ground by a knife-hammer mill till the required particle sizes were obtained (2-5; 7-10 mm). Their moisture contents were con-trolled again and they were briquetted under 80 and 160 MPa briquetting pressures.

The chosen briquetting pressure showed that the briquettes were enough solid and durable both physically and in shape. This working pressures also matches with the studies defined in Križan et al. (2015), Zhang and Guo (2014) and Sun et al. (2014). Feeding of material was done batch wise during the briquetting process in order to avoid occlusion. The material prepared for briquetting was poured into the cylindrical mold and they were squeezed by a piston in the mold and the briquettes were obtained. Full cylindrical shape briquettes having 50 mm diameter and 80 to 110 mm varying lengths were produced by this process.

Results and Discussion

The physical-mechanical properties of the briquettes are given in Table 1. The highest briquette volume mass achieved (1198.56 ± 2.89 kg.m-3) at 160 MPa with 2-5 mm particle size, whereas the lowest was achieved at 80 MPa with 7-10 mm particle size (962.18 ± 2.20 kg.m-3). The results showed that briquette volume mass were significantly affected by briquetting pressure and particle size ($P<0.01$). Tumbler index is an indicator of resistance of briquettes against the forces they face during loading, discharging, transporting procedures. Thus it is an indicator of solidness of briquettes (Zhang and Guo, 2014; Niedziolka, et al., 2015). Shatter Index tests the resistance of bri-quettes against impacts during loading and discharging processes. Tumbler and Shatter index-es of briquettes increased with increases in briquetting pressure and decreased with increases in particle size.

Physical mechanical parameters of hazelnut husk briquettes at M12 are given in Table 2. The highest Tumbler Index (91.96 ± 0.22) was achieved at M12 briquettes with 2-5 mm parti-cle sizes at 160 MPa briquetting pressure whereas the lowest was 44.7 ± 1.00 % achieved at 80 MPa briquetting pressure with the material having 7 to 10 mm particle size in M10 bri-quettes. The difference between the Tumbler Indexes of the briquettes at different briquetting pressures was found to be statistically significant ($P<0.01$). The results of Tumbler Index tests showed that the main abrasion and breakdowns realized at the both ends and at the middle part of the briquettes. The reason for that can be the batch squeezing procedure depending on the material feeding which ends up with layered structure. The breaking mainly occurred in that layer borders.

The difference between the Shatter Indexes of the briquettes at different briquetting pressures was found to be statistically significant ($P<0.01$). The tests showed again that all the breakings and split ups happened at the both ends and in the middle of the briquettes due to batch squeezing of the material. The highest and lowest Shatter Indexes were 97.58 ± 0.35 % and 76.20 ± 3.80 % achieved at M12-160 MPa briquetting pressure with 7-10 mm particle size and at M10-80 MPa briquetting pressure with 7-10 mm particle size, respectively.

After all the tests the physical-mechanical parameters of the briquettes produced under 160 MPa briquetting pressure and with two different particle sizes at both moisture contents were found to be suitable for hazelnut husk agricultural residue according to the standards given in EN 14961-1, EN 14961-2 and EN 15210-2.

Converting the available and unused agricultural residues into energy sources is important. Because, this process would eliminate agricultural wastes without harming the environment and it will contribute to the economy of the country since it has potential to create employ-ment opportunities in rural areas (Demirel ve Gürdil, 2014). Besides, when considering the total heating value potential of hazelnut husk residue that is 1,278,405 GJ (Karaca and Gürdil, 2017) in Black Sea Region in Türkiye this huge idle amount of energy must be converted to energy source in benefit of farmers or people living in rural areas.

Table 1. Physical mechanical parameters of hazelnut husk briquettes at M10

M (%)	P (MPa)	PS (mm)	Briquette volume mass (kg.m^{-3})	Tumbler Index (%)	Shatter Index (%)	Water intake capacity, (%) (2 min)	Air moisture resistance (%)
8-10	80	2-5	1012.79 ± 1.51	60.18 ± 0.76	83.04 ± 3.11	37.00 ± 0.57	5.90 ± 0.10
		7-10	962.18 ± 2.20	44.7 ± 1.00	76.20 ± 3.80	40.11 ± 0.88	7.88 ± 0.56
	160	2-5	1198.56 ± 2.89	84.88 ± 0.69	91.65 ± 1.13	27.59 ± 1.09	7.06 ± 0.22
		7-10	1159.04 ± 2.91	83.75 ± 0.59	95.08 ± 0.91	48.18 ± 1.43	5.62 ± 0.14

M= moisture content, P= pressure, PS= particle size

Table 2 Physical mechanical parameters of hazelnut husk briquettes at M12

M (%)	P (MPa)	PS (mm)	Briquette volume mass (kg.m^{-3})	Tumbler Index (%)	Shatter Index (%)	Water intake capacity, (%) (2 min)	Air moisture resistance (%)
12-15	80	2-5	1115.39 ± 1.51	78.72 ± 1.76	95.82 ± 2.14	48.00 ± 0.40	6.68 ± 0.14
		7-10	950.12 ± 2.20	75.6 ± 1.02	86.23 ± 2.90	52.21 ± 0.78	7.89 ± 0.42
	160	2-5	1041.46 ± 6.19	91.96 ± 0.22	95.20 ± 1.13	17.02 ± 0.39	0.76 ± 0.26
		7-10	1031.07 ± 6.25	88.65 ± 0.55	97.58 ± 0.35	12.72 ± 0.32	1.93 ± 0.09

M= moisture content, P= pressure, PS= particle size

Conclusions

In this study a particular hydraulic type briquetting machine with a horizontal course was de-signed and developed for the briquetting of hazelnut husk agricultural residues in order to be evaluated as solid biofuel. Effect of different particle sizes (2-5 mm and 7-10 mm), moisture contents (M10 and M12) were analysed on the physical-mechanical parameters of briquettes which are produced under 80 MPa and 160 MPa pressures by horizontal pressing. The results showed that the horizontal pressing by hydraulic type briquetting machine is very suitable for briquetting of hazelnut husk agricultural residues and especially at 160 MPa briquetting pres-sure at both moisture contents when the physical-mechanical parameters are concerned. These kinds of researches will help to improve the design and function of briquetting machines for the future and by this way for the energy deficiency of the world by converting agricultural residues to energy sources.

Author Contributions

Concept: B.D. (50%) and G.A.K.G. (50%), Design: B.D. (50%) and G.A.K.G. (50%), Supervision: B.D. (50%) and G.A.K.G. (50%), Data collection and/or processing: B.D. (50%) and G.A.K.G. (50%), Data analysis and/or interpretation: B.D. (50%) and G.A.K.G. (50%), Literature search: B.D. (50%) and G.A.K.G. (50%), Writing: B.D. (50%) and G.A.K.G. (50%), Critical review: B.D. (50%) and G.A.K.G. (50%). Submission and revision. All authors reviewed and approved final version of the manuscript.

Conflict of Interest

The authors declared that there is no conflict of interest.

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SPIRULINA (*Arthrospira Platensis*) EXTRACT PROMOTES MOTILITY, MICROSCOPIC, AND ANTIOXIDATIVE PARAMETERS OF RAM SEMEN DURING REFRIGERATED STORAGE

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Abstract: This study investigated the effect of spirulina ethanolic extract (SEE) on the quality of ram semen during low-temperature liquid storage and the relationship between sperm features. Ejaculates were collected from five *Djallonké* rams, pooled, extended with Tris-egg yolk (TEY) enriched with 0 (control), 20 (SEE20), 40 (SEE40), or 80 µg/mL (SEE80) of SEE to reach the concentration of 200×10^6 spz/mL, and stored at 4 °C for 72 h. Extended semen samples were assessed for total motility, progressive motility, sperm motion characteristics, viability, membrane integrity, and morphology at 6, 24, 48 and 72 h of storage. Moreover, malondialdehyde (MDA), nitric oxide (NO), superoxide dismutase, (SOD) and catalase (CAT) levels were measured at 72 h of storage. The enrichment of TEY with SEE at 40 and 80 µg/mL, improved sperm total motility at 48 and 72 h of storage ($P<0.05$). Also, all SEE-treated samples evidenced higher progressive motility in comparison to the control at 48 and 72 h ($P<0.05$). SEE80 group showed the highest percentages of viability (76.26±0.90%) and membrane integrity (58.19±1.50%); whereas, SEE40 demonstrated the lowest percentage of morphological abnormality (18.14±1.01%) at 72 h of storage. SEE did not influence NO levels; however, at 40 µg/mL, it reduced MDA concentration and improved SOD and CAT activities ($P < 0.05$). Total motility was positively correlated to progressive motility ($r=0.69$, $P<0.01$), viability ($r=0.91$, $P<0.01$), and membrane integrity ($r=0.49$, $P<0.05$); while, morphological abnormality was negatively correlated to the other sperm parameters. Furthermore, MDA was negatively correlated to total motility ($r=-0.91$, $P<0.01$), progressive motility ($r=-0.70$, $P<0.01$), and viability ($r=-0.91$, $P<0.01$), and positively correlated to morphological abnormality ($r=0.70$, $P<0.01$). An entirely opposite figure was recorded for SOD. Overall, the results indicated that SEE, especially at 40 µg/mL, can protect ram semen against liquid storage-associated damages. Furthermore, positive and negative correlations exist between semen parameters.

Keywords: Liquid storage, Ram semen, Spirulina, Semen quality

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1. Introduction

Liquid semen storage represents a practical and affordable alternative to freeze-thawing especially in developing countries where farmers are smallholders, mainly relying on extensive practices, and more prone to loss of production as a consequence of global warming. Liquid semen storage consists of the reduction of the metabolism of sperm cells thereby extending their shelf life. Coupled with artificial insemination (AI), this assisted reproductive tool allows the acceleration of lambing program and the dissemination of genetics of high merit, prevents disease transmission associated to natural mating (Baldassarre and Karatzas, 2004; Shi et al., 2020), and can therefore considerably improve food security thereby assisting with poverty alleviation and household income security.

However, like freeze-thawing process, liquid semen storage is associated to the decrease of sperm quality which arises as a consequence of oxidative stress (Gundogan et al., 2011). Indeed, following semen collection, sperm cells are exposed to *in vitro* conditions and inherent sources of stress (light, temperature, dilution, and pressure to name a few), leading to the overproduction of reactive oxygen species (ROS). Furthermore, the dilution process reduces semen antioxidant capacity (Bilodeau et al., 2000) in which superoxide dismutase (SOD) and catalase (CAT) play essential roles. Besides, ram spermatozoa are devoid of

synthesis ability as they loss their antioxidant-rich cytoplasm during the differentiation step of spermatogenesis, hence they cannot compensate the deficient antioxidant capacity (Bucak et al., 2007; Eslami et al., 2017). When ROS surpass the detoxifying capacity of semen, oxidative stress occurs. In excess, ROS attack membrane phospholipids, proteins, carbohydrates, DNA, and respiration process, leading to the loss of membrane functionality, DNA integrity, mitochondrial activity, motility, antioxidant activities, and increase of lipid peroxidation (LPO) (Peris-Frau et al., 2020; Kameni et al., 2021), hence the deleterious effects on sperm quality. Moreover, the abundance of polyunsaturated fatty acids (PUFAs) in cellular membrane makes ram spermatozoa particularly vulnerable to LPO, thus the accumulation of toxic products such as malondialdehyde (MDA) during semen storage (Eslami et al., 2017; Zarei et al., 2021). Therefore liquid semen storage suffers from insufficient antioxidant capacity to prevent the generation and/or scavenge excess ROS, maintain homeostasis, and ultimately prevent the loss of sperm quality.

To face this limitation, enrichment of semen extenders with antioxidant compounds from natural origin has shown to be effective in reducing storage-associated damages of semen and consequent gain in fertility after AI (Allai et al., 2016; Abadjieva et al., 2020).

Research directed towards the filamentous cyanobacterium blue-green alga *Arthrospira platensis* commonly known as spirulina, which is an abundant source of bioactive molecules, has provided evidence of its robust antioxidant capacity (Kannan et al., 2014) and its beneficial effect against oxidative stress (Karadeniz et al., 2008; Sorelle et al., 2020). Spirulina belongs to the substances that are listed by the US Food and Drug Administration under the category Generally Recognized as Safe and its typical composition as percentage dry weight can be summarized as: 50% -70% protein, 15 - 25% carbohydrates, 6 - 13% lipids, 4.2 - 6% nucleic acids, and 2.2 - 4.8% minerals (Belay, 2002). Additionally, spirulina possesses PUFAs and other potent antioxidants such as carotenoids, vitamins (B and E), spirulans, C-phycocyanin which is particularly accountable for the antioxidant activity, and allophycocyanin (Estrada et al., 2001). These chemicals makes this alga a rich source of biomolecules with strong antioxidant potential.

With this background, the objectives of the present study were to assess the effect of the supplementation of Tris-egg yolk (TEY) extender with different concentrations of spirulina ethanolic extract (SEE) on ram sperm motility, motion characteristics, viability, membrane integrity, and morphology of the *Djallonké* breed during liquid storage at 4 °C for different time intervals (6, 24, 48, and 72 h). Moreover, levels of MDA, an indicator of LPO, nitric oxide (NO), SOD, and CAT were evaluated at 72 h of storage. The correlations between sperm quality parameters, LPO, NO, SOD, and CAT were also investigated.

2. Materials and Methods

2.1. Preparation of The Spirulina Ethanolic Extract

Spirulina pellets were harvested on the shores of the Lake Chad, sun-dried, and mechanically milled. The resulting powder was used to prepare SEE, according to the procedure described by Baghshahi et al. (2014) with minor modifications. Briefly, 400 g of spirulina powder was added to 2 L of ethanol 96% and soaked at 4 °C in the dark. The soaked material was stirred every 12 h. After 72 h, the mixture was filtered using qualitative filter paper (Whatman 113V, England). The filtrate was evaporated to total dryness by vacuum distillation on a rotary evaporator at 45 °C and the resulting extract stored in the dark at 4 °C.

2.2. Animals and Semen Collection

Five healthy Djallonké rams of proven fertility, aged 2.5 - 3 years, weighing 38±2 kg were used in this study. The animals were housed at the Teaching and Research Farm of the University of Dschang with appropriate balanced diet: commercial concentrate (0.5 kg/ram/day) and *ad libitum* access to good quality hay, water, and mineral blocks. Ejaculates were collected once a week for 6 consecutive weeks using electroejaculator. Immediately after collection, each ejaculate was transferred to a water bath (37 °C) and instantly assessed for semen colour, volume, concentration, and mass motility. Only ejaculates with colour score ≥ 3, volume ≥ 0.75 mL, concentration ≥ 2.5×10^9 spz/mL, and mass motility score ≥ 3 were included in the study. Semen samples that met the above-mentioned characteristics were pooled to minimize individual variation and processed for extending.

2.3. Extender Preparation and Semen Processing

The TEY extender consisted of 2.666 g Tris, 0.44 g glucose, 1.398 g citric acid in 100 mL distilled water, and egg yolk 12% (v/v). To prevent bacterial growth, penicillin and streptomycin (0.05 mg/mL) were added to freshly prepared TEY. Pooled semen sample was divided in equal aliquots and extended at 37 °C using TEY supplemented with 0 (control), 20 (SEE20), 40 (SEE40), or 80 µg/mL (SEE80) of SEE to reach the concentration of 200×10^6 spz/mL and stored at 4 °C for 72 h. Extended semen samples were assessed for sperm quality at 6, 24, 48 and 72 h of storage.

2.4. Semen Evaluation

2.4.1. Assessment of sperm motility

Sperm motility and motion characteristics were assessed by computer-assisted sperm analyser (CASA) with a warmed stage at 37 °C (Sperm Analyze Vista, version V1.12 Maya, Guangzhou, China).

The extended samples were further diluted using Tris-based extender without egg yolk to 25×10^6 spz/mL at 37 °C. A semen sample (10 µL) was placed on a warmed slide and covered with a cover slip. For each sample, 4 – 5 fields per drop were analysed at 200 × and a minimum of 200 spermatozoa were evaluated as described by Eslami et al. (2017). The semen variables included in the analysis were total motility (TM, %), progressive motility (PM, %), curvilinear velocity (VCL, µm/s), straight line

velocity (VSL, µm/s), average path velocity (VAP, µm/s), linearity (LIN, %), and straightness (STR, %).

2.4.2. Assessment of sperm viability and morphology

Sperm viability was evaluated with eosin-nigrosin staining (Evans and Maxwell, 1987). Thin smears, made in duplicate, were prepared by mixing 10 µl of semen (diluted at 25 × 10^6 spz/mL with Tris-based extender without egg yolk) with 20 µL of eosin-nigrosin (eosin-Y 1.67 g, nigrosin 10 g, sodium citrate 2.9 g, dissolved in 100 mL distilled water) on a warm slide (37 °C) and immediately spread with another slide. After air drying, the viability was assessed by counting a minimum of 200 cells from 3-4 different fields with bright-field microscopy (400×). Spermatozoa showing partial or complete purple colour were considered non-viable and only spermatozoa showing white colour, indicative of strict exclusion of the stain were considered to be alive.

The same slides were used to determine sperm morphology; with similar microscopic settings. A minimum of 200 sperm cells per slide were examined and morphological abnormalities included head, midpiece, and tail defects (Zarei et al., 2018).

2.4.3. Assessment of sperm functional membrane integrity

Sperm functional membrane integrity was assessed following the principle described by Revell and Mrode (1994). Extended semen sample (20 µL) was mixed with 200 µL of pre-warmed (37 °C) 100 mOsm hypoosmotic solution (9 g fructose, 4.9 g trisodium citrate per litre of distilled water) which was prepared daily and kept at 4 °C. The mixture was incubated at 37 °C for 60 min. After incubation, the sample was gently mixed. Smears were realized in duplicate. A drop (15 µL) of the treated mixture was smeared on a pre-warmed slide and air-dried. A minimum of 200 spermatozoa were counted in 4 - 5 different microscopic fields at 400 × magnification. Spermatozoa with swollen or coiled tails were considered to have functional membranes; whereas, sperm cells showing no swollen or coiled tails were considered to have defective plasma membranes.

2.4.4. Measurement of lipid peroxidation

At the end of the storage period (72 h), semen samples were centrifuged at 550 g for 10 min and the pellet was discarded. The supernatant was again centrifuged at 550 g for 10 min and finally at 3000 g for 30 min. The resulting supernatant, considered as the medium, was used to evaluate oxidant and antioxidant profiles.

Lipid peroxidation was determined in extended semen sample by measuring the amount of MDA in medium as per the procedure described by Kodjio et al. (2016). Briefly, equal volumes (500 µL) of 1% orthophosphoric acid solution and precipitating mixture (1% thiobarbituric acid + 1% acetic acid) were gently mixed with 100 µL of medium in glass tubes. Subsequently, tubes were incubated at 100 °C for 15 min, cooled, and centrifuged at 1000 g for 10 min. Finally, the absorbance of the upper layer was read against the blank at 532 nm wavelength. The MDA levels were expressed in nmol/g of protein in the medium.

2.4.5. Measurement of nitric oxide

The NO content was evaluated according to the Griess reaction (Griess, 1879). An aliquot of medium (350 µL) was mixed with 350 µL of 1% sulphanilamide (prepared in 1% orthophosphoric acid) in glass tube and the mixture was incubated in the dark at room temperature for 5 min. Afterwards, 350 µL of 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride (prepared in 10 mM Tris) was added to the preparation which was again incubated for 5 min in similar conditions as previously. Finally, the absorbance of the preparation was read at 530 nm. NO amounts in the samples were calculated using a standard curve developed for this purpose and expressed in µmol/g of protein.

2.4.6. Assessment of superoxide dismutase activity

The SOD activity was evaluated according to the principle described by Misra and Fridovich (1972), assaying the auto-oxidation and illumination of adrenalin at 480 nm for 1.5 min. One unit total SOD activity was considered as the amount of protein causing 50% inhibition of adrenalin auto-oxidation. The total SOD activity was expressed as units per milligram (U/mg) of protein.

2.4.7. Assessment of catalase activity

The activity of CAT was evaluated according to the principle described by Sinha (1972). When heated in the presence of hydrogen peroxide, dichromate in acetic acid is reduced to chromic acetate with the formation of perchromic acid as an unstable intermediate. The absorbance of the chromic acetate produced, which reflects the hydrogen peroxide not degraded by CAT in the sample, is then recorded at 570 nm. The concentration of hydrogen peroxide in the medium was determined using a standard curve developed for this purpose. The CAT activity was expressed in units per gram (U/g) of protein, one unit being equivalent to one mM of hydrogen peroxide degraded per minute.

2.4.8. Measurement of total protein content

Total protein content was determined using Chronolab commercial kit, strictly following the manufacturer directives. The kit is based on the Biuret method. In alkaline medium, proteins form with copper salts, an intensive violet-blue complex with maximum absorbance at 540 nm. The intensity of the colour is proportional to the total protein concentration in the sample.

2.5. Statistical Analysis

The experiment was conducted in 6 replicates and statistical analyses of the data were performed using R statistical package, version 4.2.0. One-way ANOVA was used to determine the difference among means at each time point. Changes in different variables over time were evaluated using repeated measure ANOVA to reveal time effect in each group. In case of significant difference, the Tukey post hoc test was used to separate means. The results were reported as mean ± standard error of the mean and values of $P<0.05$ were considered statistically significant. Pearson correlations between parameters were evaluated by combining data for all treatments.

3. Results

3.1. Sperm Motility and Kinematics

The effects of SEE concentration and storage period on sperm motility and kinematic parameters are depicted in Table 1. Independently of the treatments, the total and progressive motility of ram sperm decreased steadily with increasing storage time. Semen samples treated with 40 and 80 µg/mL evidenced significantly (P<0.05) higher TM in comparison to the control sample at 48 and 72 h of refrigerated storage. Likewise, all SEE treated semen samples demonstrated higher PM in comparison to the control at 48 and 72 h of refrigerated storage (P<0.05). Kinematic parameters VCL, VSL, and VAP showed a decreasing trend over time. Both SEE20 and SEE80 treated semen samples showed significantly (P<0.05) higher VSL at 72 h and VAP at 24 h in comparison to the respective control. On the other hand, SEE neither influence LIN nor STR of semen samples, both parameters showed to be constant during storage (Table 2).

Table 1. Percentages of total and progressive motility of spermatozoa in ram semen stored at 4 °C for 72 h in Tris-egg yolk extender supplemented with spirulina ethanolic extract

Parameters	Treatments	Storage duration (h)			
		6	24	48	72
TM	Control	80.49 ± 1.88^{Aa}	76.49 ± 1.11^{Ba}	69.86 ± 1.37^{Bb}	66.53 ± 0.93^{Bb}
	SEE20	82.54 ± 1.67^{Aa}	79.73 ± 0.94^{ABab}	77.24 ± 1.19^{Ab}	70.96 ± 1.55^{ABc}
	SEE40	84.83 ± 1.16^{Aa}	78.97 ± 1.57^{ABb}	79.40 ± 1.43^{Ab}	71.79 ± 1.25^{Ac}
	SEE80	84.94 ± 1.06^{Aa}	82.83 ± 0.90^{Aa}	77.42 ± 1.14^{Ab}	74.62 ± 0.90^{Ab}
PM	Control	62.49 ± 1.26^{Ba}	60.55 ± 1.18^{Ba}	51.39± 0.92^{Bb}	43.94 ± 0.90^{Cc}
	SEE20	66.78 ± 1.18^{ABa}	63.15 ± 0.84^{ABab}	61.36 ± 1.24^{Ab}	53.72 ±0.71^{Ac}
	SEE40	66.33 ± 1.15^{ABa}	63.32 ± 2.63^{ABa}	60.64 ± 1.68^{Aa}	48.50 ± 1.28^{Bb}
	SEE80	69.38 ± 1.37^{Aa}	68.75 ± 1.29^{Aa}	60.82 ± 1.38^{Ab}	53.96 ± 0.96^{Ac}

TM= total motility, PM= progressive motility, SEE20, SEE40, and SEE80= 20, 40, and 80 µg of spirulina ethanolic extract per mL of extender, $^{A, B, C}$ values with different superscripts indicate significant differences (P<0.05) within groups at each time point. $^{a, b, c}$ values with different superscripts indicate significant differences (P<0.05) within groups over storage time.

Table 2. Kinematic parameters of spermatozoa in ram semen stored at 4 °C for 72 h in Tris-egg yolk extender supplemented with spirulina ethanolic extract

Parameters	Treatments	Storage duration (h)			
		6	24	48	72
VCL	Control	109.08 ± 2.88^{Aa}	97.70 ± 5.78^{Aa}	71.40 ± 3.79^{Ab}	62.46 ± 2.83^{Ab}
	SEE20	112.18 ± 3.45^{Aa}	100.88 ± 3.27^{Aa}	83.46 ± 3.76^{Ab}	72.73 ± 2.95^{Ab}
	SEE40	109.85 ± 3.27^{Aa}	97.55 ± 3.16^{Aa}	75.03 ± 3.27^{Ab}	68.23 ± 3.11^{Ab}
	SEE80	111.18 ± 4.55^{Aa}	99.89 ± 4.71^{Aa}	83.60 ± 2.89^{Ab}	74.46 ± 3.89^{Ab}
VSL	Control	25.20 ± 1.19^{Aa}	19.09 ± 1.28^{Ab}	17.41 ± 1.63^{Ab}	14.95 ± 1.31^{Bb}
	SEE20	23.65 ± 1.01^{Aa}	22.12 ± 1.01^{Aa}	20.10 ± 1.05^{Aa}	20.29 ± 1.01^{Aa}
	SEE40	23.05 ± 1.58^{Aa}	21.74 ± 1.63^{Aa}	20.98 ± 1.92^{Aa}	18.32 ± 1.38^{ABa}
	SEE80	24.52 ± 1.82^{Aa}	23.98 ± 1.42^{Aa}	22.24 ± 1.53^{Aa}	21.11 ± 1.21^{Aa}
VAP	Control	36.86 ± 1.03^{Aa}	28.26 ± 1.36^{Bb}	24.39 ± 0.98^{Ab}	19.90 ± 0.96^{Ac}
	SEE20	34.58 ± 1.34^{Aa}	30.36 ± 1.23^{Aba}	24.26 ± 1.46^{Ab}	22.18 ± 1.02^{Ab}
	SEE40	33.64 ± 1.65^{Aa}	30.32 ± 1.61^{ABab}	26.59 ± 1.87^{Abc}	22.45 ± 1.41^{Ac}
	SEE80	35.02 ± 1.53^{Aa}	33.84 ± 1.46^{Aa}	27.75 ± 1.51^{Ab}	24.11 ± 1.22^{Ab}
LIN	Control	26.34 ± 3.22^{Aa}	26.81± 2.78^{Aa}	22.62 ± 1.95^{Aa}	23.32 ± 1.48^{Aa}
	SEE20	27.45 ± 1.89^{Aa}	23.11 ± 1.62^{Aab}	21.26 ± 1.28^{Ab}	21.26 ± 1.16^{Ab}
	SEE40	26.99 ± 3.20^{Aa}	26.66 ± 2.84^{Aa}	22.01 ± 2.06^{Aa}	21.24 ± 1.82^{Aa}
	SEE80	27.96 ± 2.38^{Aa}	26.18 ± 2.12^{Aa}	25.09 ± 1.79^{Aa}	22.16 ± 1.26^{Aa}
STR	Control	67.85 ± 1.83^{Aa}	69.54 ± 1.41^{Aa}	65.23 ± 1.18^{Ba}	70.91 ± 1.67^{Aa}
	SEE20	68.85 ± 2.03^{Aa}	68.31 ± 2.43^{Aa}	69.98 ± 1.51^{ABa}	71.61 ± 1.58^{Aa}
	SEE40	68.65 ± 1.96^{Aa}	70.72 ± 1.86^{Aa}	71.50 ± 1.27^{Aa}	69.78 ± 1.37^{Aa}
	SEE80	69.61 ± 1.55^{Aa}	71.64 ± 0.92^{Aa}	69.63 ± 1.48^{ABa}	68.30 ± 1.26^{Aa}

VCL= curvilinear velocity (µm/s), VSL= straight line velocity (µm/s), VAP= average path velocity (µm/s), LIN= linearity (%), STR= straightness (%), SEE20, SEE40, and SEE80= 20, 40, and 80 µg of spirulina ethanolic extract per mL of extender, $^{A, B}$ values with different superscripts indicate significant differences (P<0.05) within groups at each time point, $^{a, b, c}$ values with different superscripts indicate significant differences (P < 0.05) within groups over storage time.

3.2. Sperm Viability and Morphology

Irrespective of the treatments, sperm viability decreased over time, with significantly (P<0.05) lower viability at 48 and 72 h compared to the corresponding values at 6 h (Table 3). However, enrichment of TEY extender with SEE preserved sperm viability at 6, 48, and 72 h of cooling storage where treated semen samples with SEE at 40 and 80 µg/mL demonstrated significantly higher viability in comparison to the control sample (P<0.05). The percentage of morphological abnormality gradually increased as the storage period progressed, especially in the control sample where values were significantly (P<0.05) higher from 24 h to 72 h in comparison to corresponding one at 6 h (Table 3). The incorporation of SEE at 20 and 40 µg/mL in Tris-based extender significantly (P<0.05) reduced sperm abnormality at 48 and 72 h, respectively.

3.3. Sperm Membrane Functional Integrity

As shown in Table 3, except at 48 h, the percentage of spermatozoa with functional membrane was significantly (P<0.05) higher in SEE80 group compared to the corresponding control values throughout the storage period; however, other SEE treated samples did not show any significant difference (P>0.05) compared to the control. Changes of spermatozoa membrane functionality over time revealed significant (P<0.05) decrease when comparing values for consecutive time points, especially in the control and SEE80 groups. Conversely, SEE20 and SEE40 groups revealed no difference in membrane functionality for consecutive time points.

3.4. Lipid Peroxidation and Nitric Oxide Content

As depicted in Figure 1a, only samples of SEE20 and SEE40 groups showed significantly (P<0.05) lower MDA level compared to the control. Conversely, no significant difference among treatment groups was observed for NO level (Figure 1b).

Table 3. Percentages of sperm viability, membrane functional integrity, and abnormal morphology in ram semen stored at 4 °C for 72 h in Tris-egg yolk extender supplemented with different concentrations of spirulina ethanolic extract

Parameters	Treatments	Storage duration (h)			
		6	24	48	72
Viability	Control	81.25 ± 1.11^{Ba}	78.62 ± 1.23^{Aab}	75.25 ± 0.94^{Bb}	70.22 ± 0.55^{Bc}
	SEE20	84.00 ± 1.07^{ABa}	82.37 ± 0.86^{Aab}	79.37 ± 1.16^{ABb}	73.78 ± 0.64^{Ac}
	SEE40	86.12 ± 1.38^{Aa}	82.12 ± 2.59^{Aa}	80.94 ± 1.93^{Aab}	74.37 ± 1.25^{Ab}
	SEE80	88.37 ± 1.25^{Aa}	84.51 ± 0.81^{Aab}	81.25 ± 1.11^{Ab}	76.26 ± 0.90^{Ac}
Membrane functional integrity	Control	63.99 ± 0.43^{Ba}	60.59 ± .080^{Bb}	56.89 ± 0.85^{Ac}	51.91 ± 1.15^{Bd}
	SEE20	65.43 ± 1.41^{Ba}	62.23 ± 1.73^{Bab}	59.82 ± 1.11^{Abc}	56.72 ± 1.06^{ABc}
	SEE40	62.89 ± 1.08^{Ba}	59.47 ± 0.92^{Ba}	57.61 ± 1.38^{Aab}	52.94 ± 2.36^{ABb}
	SEE80	73.21 ± 1.02^{Aa}	67.53 ± 1.58^{Ab}	60.86 ± 1.21^{Ac}	58.19 ± 1.50^{Ac}
Abnormal morphology	Control	9.69 ± 0.85^{Aa}	14.00 ± 0.79^{Ab}	18.19 ± 0.68^{ABc}	23.64 ± 0.58^{ABd}
	SEE20	10.94 ± 0.92^{Aa}	12.58 ± 0.62^{Aab}	15.69 ± 0.45^{Cb}	20.22 ± 1.15^{BCc}
	SEE40	11.98 ± 0.87^{Aa}	13.31 ± 0.94^{Aab}	16.02 ± 0.70^{BCbc}	18.14 ± 1.01^{Cc}
	SEE80	12.61 ± 0.96^{Aa}	14.56 ± 0.86^{Aa}	20.15 ± 0.69^{Ab}	27.02 ± 0.97^{Ac}

SEE20, SEE40, and SEE80= 20, 40, and 80 µg of spirulina ethanolic extract per mL of extender, $^{A, B, C}$ values with different superscripts indicate significant differences (P<0.05) within groups at each time point, $^{a, b, c, d}$ values with different superscripts indicate significant differences (P<0.05) within groups over storage time.

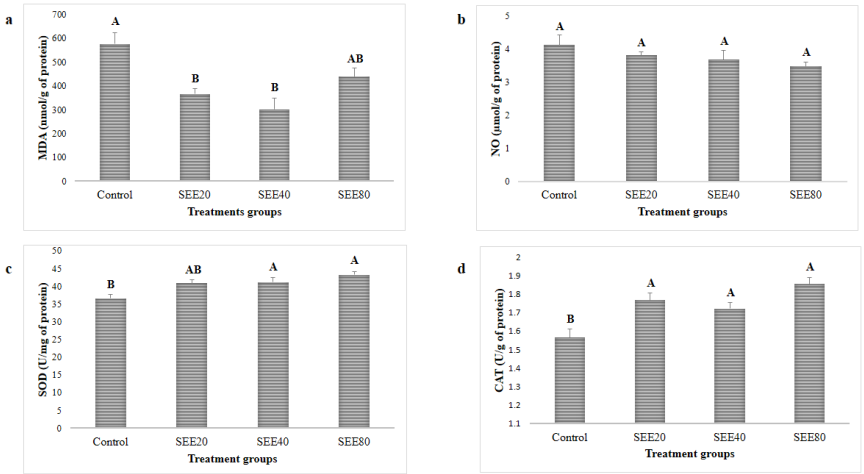

Figure 1. MDA (nmol/g of protein) (a), NO (µmol/g of protein), SOD (U/mg of protein), and CAT (U/g of protein) values after 72 h of liquid storage at 4 °C of ram semen extended in Tris-egg yolk supplemented with different concentrations of spirulina ethanolic extract (SEE20, SEE40, and SEE80: 20, 40, and 80 µg of spirulina ethanolic extract per mL of extender, $^{A, B}$ values with different superscripts indicate significant differences (P<0.05) among groups.

3.5. Superoxide Dismutase and Catalase Activities

Semen samples treated with SEE at 40 and 80 µg/mL showed significantly higher (P<0.05) SOD activity in comparison to the control (Figure 1c). Likewise, compared to the control, the enrichment of TEY with the different concentrations of SEE significantly increased (P<0.05) CAT activity (Figure 1d).

3.6 Correlations between ram Sperm Parameters after 72 h of Liquid Storage at 4 °C in Tris-Egg Yolk Extender

As evidenced in Table 4, sperm TM was positively correlated to PM (r= 0.69, P<0.01), VCL (r=0.62, P<0.01), VSL (r=0.49, P<0.05), viability (r=0.91, P<0.01), and membrane integrity (r=0.49, P<0.05) and negatively correlated to morphological abnormalities (r=-0.65, P<0.01). In the same way, PM was positively correlated to VCL (r=0.50, P<0.05), VSL (r=0.48, P<0.05), and viability (r=0.81, P<0.01) and negatively correlated to morphological abnormalities (r=-0.61, P<0.05). Viability was positively correlated to membrane integrity (r=0.48, P<0.05), whereas morphological abnormality was negatively correlated to viability and membrane integrity (r=- 0.68, r=-0.56, respectively, P<0.01).

3.7 Correlations of Sperm Parameters with Oxidative Stress Indicators after 72 h of Liquid Storage at 4 °C in Tris-egg Yolk Extender

The relationship of sperm parameters with oxidative stress indicators are summarized in Table 5. MDA was negatively correlated to TM (r=- 0.91, P<0.01), PM (r=-0.70, P<0.01), VCL (r=-0.49, P<0.05), VSL (r=-0.67, P<0.01), and viability (r=-0.91, P<0.01) and positively correlated to STR (r=0.45, P<0.05) and morphological abnormality (r=0.70, P<0.01). SOD was positively correlated to TM (r=0.66, P<0.01), PM (r=0.52, P<0.05), VSL (r=0.70, P<0.01), VAP (r=0.51, P<0.05), viability (r=0.55, P<0.05), and membrane integrity (r=0.52, P<0.05) and negatively correlated to morphological abnormality (r=0.74, P<0.01). Moreover CAT was positively correlated to membrane integrity (r=0.59, P<0.01).

Table 4. Correlations (r) among ram sperm parameters after 72 h of liquid storage at 4 °C of ram semen in Tris-egg yolk

	PM	VCL	VSL	VAP	LIN	STR	Viab	SFM	Abn
TM	0.69[a]	0.62[a]	0.49[b]	0.40	-0.24	-0.312	0.91[a]	0.49[b]	-0.65[a]
PM		0.50[b]	0.48[b]	0.24	-0.15	0.17	0.81[a]	0.44	-0.61[a]
VCL			0.31	0.58[a]	-0.32	-0.55[b]	0.64[a]	0.54[b]	-0.99[a]
VSL				0.42	0.48[b]	0.06	0.53[b]	0.32	-0.35
VAP					-0.004	-0.11	0.39	0.30	-0.57[a]
LIN						0.48[b]	-0.14	-0.18	0.33
STR							-0.37	-0.19	0.51[b]
Viab								0.48[b]	-0.68[a]
SFM									-0.56[a]

TM= total motility (%), PM= progressive motility (%), VCL= curvilinear velocity (µm/s), VSL= straight line velocity (µm/s), VAP= average path velocity (µm/s), LIN= linearity (%), STR= straightness (%), Viab= viability (%), SFM= spermatozoa with functional membrane (%), Abn= morphological abnormality (%), [a] correlation is significant at the 0.01 level, [b] correlation is significant at the 0.05 level.

Table 5. Correlations (r) of ram sperm parameters with oxidative stress indicators after 72 h of liquid storage at 4 °C of ram semen in Tris-egg yolk

Oxidative stress parameters	Sperm parameters									
	TM	PM	VCL	VSL	VAP	LIN	STR	Viab	SFM	Abn
MDA	-0.91[a]	-0.70[a]	-0.49[b]	-0.67[a]	-0.35	0.26	0.45[b]	-0.91[a]	-0.43	0.70[a]
NO	-0.21	-0.35	-0.19	-0.41	-0.35	0.13	0.27	-0.28	-0.12	0.42
SOD	0.66[a]	0.52[b]	0.36	0.70[a]	0.51[b]	-0.41	-0.37	0.55[b]	0.52[b]	-0.74[a]
CAT	0.38	0.14	0.04	0.26	0.30	-0.29	-0.18	0.33	0.59[a]	-0.25

TM= total motility (%), PM= progressive motility (%), VCL= curvilinear velocity (µm/s), VSL= straight line velocity (µm/s), VAP= average path velocity (µm/s), LIN= linearity (%), STR= straightness (%), Viab= viability(%), SFM= spermatozoa with functional membrane (%), Abn= morphological abnormality (%), MDA= malondialdehyde (nmol/g of protein), NO= nitric oxide (µmol/g of protein), SOD= superoxide dismutase (U/mg of protein), CAT= catalase (U/g of protein), [a] correlation is significant at the 0.01 level, [b] correlation is significant at the 0.05 level.

4. Discussion

The important loss of semen quality during liquid storage limits the usage of stored semen to short time interval and hence represent a drawback for efficient application of assisted reproductive techniques such as AI and consequent improvement of production. The results of the current study indicated that sperm quality parameters decline steadily throughout the storage period. However, SEE at appropriate concentration and varying with the duration of storage, can effectively reduce the decline of TM, PM, VCL, VSL, VAP, percentages of viable spermatozoa, spermatozoa with functional

plasma membrane, and spermatozoa with normal morphology. SEE can inhibit LPO, and stimulate SOD and CAT activities during low temperature liquid storage. Moreover, there were significant positive and negative correlations between sperm and oxidative stress parameters.

This study demonstrated that enrichment of TEY with SEE can enhance the preservation of sperm TM, PM, and sperm motion characteristics VSL and VAP during refrigerated storage. The results reported herein are consistent with previous findings regarding the supplementation of extenders with antioxidant compounds from natural origin during storage at low temperature (Allai et al., 2016; Wen et al., 2019) and cryopreservation (Merati and Farshad, 2020), but contrary to other (Taşdemir et al., 2020). The comparison of the results of sperm kinematics obtained from different experiments is challenging considering the variety of sperm concentrations in the samples and diluents used (Câmara et al., 2011).

Motility has been documented as one of the essential sperm parameters for fertility (Kasimanickam et al., 2011), especially in AI procedures that require sperm cells to move within the reproductive tract of the females to reach the ovum. Effective semen storage relies on reversible decrease in motility and metabolic activity of sperm cells following cooling at lower temperatures; however, exposure of sperm cells to artificial conditions amplifies the generation of ROS which normally arises as a consequence of aerobic conditions where live sperm cells are involved (Agarwal et al., 2005). As the ROS accumulate and reach a critical concentration, oxidative stress occurs and provokes an irreversible loss of motility, inhibition of fructolysis and respiration in sperm cells (Salamon and Maxwell, 2000), hence the decrease over time in sperm motility and motion characteristics as observed in the present study. Additionally, motility, which is an energy-dependent function, is particularly associated to mitochondrial activity and therefore may also decrease as a consequence of insufficient supply of energy from mitochondria which impairment drives to adenosine triphosphate (ATP) depletion. In fact, sperm mitochondrion is particularly sensitive to cooling process and this sensitivity results in disturbance in ATP transport with consequent reduction in motility (Zarei et al., 2021).

Interestingly, all SEE treated samples showed superior PM in comparison to the control sample from 48 h onwards. Progressive motile sperm cells represent the spermatozoa fraction that can effectively move within the female reproductive tract once insemination is performed. Therefore, by reducing the loss of PM, SEE may improve the fertilization rate of chilled semen. Enrichment of extenders with SEE at 80 µg/mL beneficially affected VSL and VAP respetively at 72 h and 24 h. The preservation of these sperm attributes can be ascribed to the capacity of the bioactive components present in SEE to inhibit the generation and/or scavenge ROS in excess. Particularly SEE bioactive components may inhibit the mitochondrial outer membrane enzyme monoamine oxidase that catalyses the oxidative deamination of biogenic amines, producing a large amount of H_2O_2 that contributes to an increase in the steady state concentrations of reactive species within both the mitochondrial, matrix and cytosol (Cadenas and Davies, 2000). In this way, SEE may restore the balance between the amounts of ROS produced and scavenged, and consequently preserve the metabolic activity of sperm cells. It is well known that spirulina is a rich source of bioactive ingredients among which vitamin E which is considered as an essential component of the sperm antioxidant defence system, hence one of the major protector against oxidative stress and LPO (Yousef et al., 2003). SEE, thanks to the presence of vitamin E which is liposoluble, may have inhibited the peroxidation of PUFAs abundant in ram sperm membrane.

Viability, as assessed by dye exclusion, allows to discriminate the necrozoospermia from the total lack of motility associated to structural deficiencies in the tail zone (Chemes and Rawe, 2003). The results of this study evidenced the beneficial influence of SEE especially at 40 and 80 µg/mL on sperm viability during storage. Natural herbs cladodes (*Opuntia ficus indica*) and green tea (*Camellia sinensis*) used as additives to semen extenders improved viability (Allai *et al*., 2016; Mehdipour *et al*., 2016). During semen storage, the accumulation of ROS above the detoxifying capacity of spermatozoa leads to peroxidative damage of membrane proteins, phospholipids, and PUFAs (Peris-Frau et al., 2020; Kameni et al., 2021), hence the loss of membrane integrity and subsequent cell death. This phenomenon is particularly prominent in ram because of the abundance of PUFAs in sperm plasma membrane (Bucak *et al*., 2007). The improvement of sperm viability in the present study may be essentially linked to the bioactivity of phycocyanin which has been documented as the compound mainly responsible for the antioxidant activity of spirulina thanks to its strong radical scavenging properties (Piñero Estrada et al., 2001). Phycocyanin and other chemicals present in spirulina may have scavenged the ROS generated in excess, hence reducing ROS detrimental action on sperm membrane constituents, inhibiting LPO, and ultimately preserving sperm viability as noticed in this investigation. Furthermore, SEE may have inhibited the release of cytochrome C from the mitochondria to the cytosol, release which is the initiation point of the apoptosis cascade (Silva, 2006). Besides improving the antioxidant defence of spermatozoa, SEE may have strengthen the levels of phosphoinositide-3 kinases which have been documented as potent stimulators of several anti-apoptotic effectors, hence preventing cell death (Oudit et al., 2004).

The results of the current work indicated that, SEE at 80 µg/mL improved sperm functional membrane integrity at 6, 24, and 72 h. This observation may be associated to

the capacity of SEE to inhibit the generation of free radicals and their negative action on lipid bilayer interactions and proteins' anchorage to the bilayer, ultimately preventing the loss of physiological function.

For satisfactory results following AI in small ruminants, the threshold of 15% has been suggested as the maximum critical percentage of sperm morphological defects (Rehman et al., 2013). In this study, as the storage was extended, the percentages of sperm morphological abnormalities increased. This result is in accordance with previous reports (Gundogan et al., 2011; Gheller et al., 2018). However, enrichment of extenders with SEE at the intermediate concentration (40 µg/mL) showed to be effective at reducing sperm morphological defects. While many studies have reported no effect following extender supplementation with antioxidant compounds on sperm morphology (Amini et al., 2019; Zarei et al., 2021), arguing that morphology is mainly related to spermatogenesis, others have highlighted positive effects (Allai et al., 2016; Rateb, 2018). It seems as the dynamics of sperm morphology expands beyond the scope of spermatogenesis, and morphology alteration might also be prevented thanks to the antioxidative potential of SEE. Moreover, the nature, chemical composition and incorporation level of the antioxidant compound coupled with the variety of methodologies used to assess sperm morphology may account for the discrepancy observed.

Recent investigations have evidenced the increase of the antioxidant capacity of semen and the inhibition of LPO following addition of antioxidants from natural origin to extenders during semen storage (Wen et al., 2019, Taşdemir et al., 2020). Likewise, the enrichment of TEY extender with SEE increased SOD and CAT levels and inhibited MDA production during cooling storage at 4°C. Under low LPO rates (sub-toxic conditions), cells initiate their maintenance and survival through intrinsic antioxidant defence systems or signalling pathways activation that up-regulate protein antioxidants resulting in an adaptive stress response. On the other hand, under medium or high LPO rates (toxic conditions), the magnitude of oxidative stress exceeds repair capacity, and the cells induce apoptosis or necrosis (Ayala et al., 2014). Therefore, by moderating the rate of LPO as observed in the present work, SEE may have created sub-toxic conditions that favour the increase of enzymatic antioxidant activities and the preservation of sperm quality.

The correlation analysis revealed that TM was positively related to PM, VCL, VSL, viability and sperm membrane functionality. Similar relations have been established in earlier reports (Câmara et al., 2011; Singh et al., 2014). The positive correlations among sperm quality parameters reported herein may be linked to the involvement of sperm membrane in the maintenance of these parameters. On the other hand and as reported by Gupta and Singh (2018), morphological abnormality was negatively correlated to other sperm quality parameters. Impaired morphology, especially at the level of the tail which is the spermatozoon structure in charge of locomotion, may lead to decrease motility. In addition, morphology impairment may alter normal metabolism with reduction of energy production and accumulation of toxic products, and subsequent loss of sperm motility, viability, and membrane integrity.

MDA was negatively correlated with sperm TM, PM, viability and positively correlated to morphological abnormality; whereas, the totally inverse figure was observed for the SOD. These results are consistent with previous findings (Kadirve et al., 2014). Alvarez and Storey (1992) reported that semen samples with highest viability after freeze-thawing were characterized by high SOD activity and a strong correlation between loss of SOD activity and loss of motility and membrane integrity, and concluded that the loss of sperm quality might be at least partly mediated by SOD. However, Lone et al. (2018) observed no correlation between the SOD and semen parameters following freeze-thawing of buffalo semen.

5. Conclusion

In conclusion, SEE at 40 µg/mL can improve ram semen storage by reducing the loss of sperm motility, viability, morphology, and membrane integrity during chilled storage at 4 °C for up to 72 h. Moreover, SEE can inhibit LPO and stimulate enzymatic antioxidant SOD and CAT at 72 h of storage. Significant positive and negative correlations exist between sperm and oxidative stress parameters. It will be worth to assess the underlying mechanism(s) of the SEE protection of semen during storage and conduct AI trial with SEE treated semen.

Author Contributions

Concept: S.L.K. (100%), Design: S.L.K. (100%), Supervision: S.L.K. (50%) and F.N. (50%), Data collection and/or processing: S.L.K. (25%), A.B.N.D. (25%), T.T.T. (25%) and F.D.T.B. (25%), Data analysis and/or interpretation: F.M. (100%), Literature search: A.B.N.D. (35%), T.T.T. (35%) and F.D.T.B. (30%), Writing: S.L.K. (100%), Critical review: S.L.K. (50%) and F.N. (50%), Submission and revision: S.L.K. (50%) and F.N. (50%). All authors reviewed and approved final version of the manuscript.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

The experimental procedures used in the present study were reviewed and validated by the local Animal Care and Ethics Committee of the Department of Animal Science, University of Dschang, in accordance with the Code of Ethics of the EU Directive 2010/63/EU for animal experiments as amended by Regulation (EU) 2019/1010.

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THE EFFECTS OF CLIMATE CHANGE ON ANIMAL NUTRITION, PRODUCTION AND PRODUCT QUALITY AND SOLUTION SUGGESTIONS

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Abstract: This article has been prepared to examine the effects of heat stress on livestock nutrition, yield and product quality, and to reveal strategies for adaptation and mitigation of climate change. Global climate change is primarily caused by greenhouse gas emissions, which result in warming of the atmosphere. Therefore, soil, air, water pollution and reductions in biodiversity may occur. At the same time, climate change can directly and indirectly affect livestock and animal nutrition. Heat stress results from inability to dissipate enough heat to maintain homeothermy of the animals. High ambient temperature, relative humidity and radiant energy compromise ability to dissipate heat of the animals. Ruminants, pigs and poultry are susceptible to heat stress due to their species-specific characteristics such as their metabolic rate and growth, high yield levels, rumen fermentation, sweating disorder and skin insulation. The indirect effects of climate change on livestock are changes in crop and forage production and quality, decrease in pasture/rangeland quality as a result of decrease in biodiversity and decrease in water availability. The direct effects are on the feed and water consumption, growth, milk, meat, egg, wool/hair and honey yield and product quality of the animals. These effects are primarily the result of a combination of temperature and increase in atmospheric carbon dioxide concentration, variation in precipitation, and relative humidity. Heat stress can cause significant losses in animal production, some of these may be immediate and some may be delayed. Animals under heat stress can decrease feed consumption to reduce metabolic heat. The decrease in feed consumption may cause a decrease in the growth rate of animals, decrease in milk, meat, egg, wool/hair yield and quality. The rations of animals can be manipulated to mitigate the negative effects of climate change.

Keywords: Climate change, Heat stress, Animal nutrition, Production, Product quality, Solution suggestions

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1. Introduction

The combination of elements such as temperature, air movement, barometric pressure, radiation, humidity, precipitation and ionization creates the climate. In many parts of the world there can be differences in climatic zones, the reason for these differences is latitude, winds, evaporation conditions, water availability, altitude, proximity to mountains, etc. are factors (West, 2003). The climate system is under the influence of its own internal dynamics and may change over time depending on the changes in external factors affecting the climate. Climate change is a natural phenomenon that has occurred throughout history. In recent years, the term 'climate change' has mostly been used to describe changes in the Earth's climate. These changes started from the pre-industrial period (from about 1850) primarily by human activities. The use of fossil fuels, which causes a relatively rapid increase in the CO_2 concentration in the world's atmosphere, and the removal of forests are the biggest reasons for these changes (Pachauri et al., 2014).

Climate change includes not only rising average temperatures, but also extreme weather events, changing wildlife populations and habitats, sea level rise, changes in river flows and water levels, and many other effects. While precipitation is increasing in some parts of the world, others are experiencing severe droughts, increasing the risk of wildfires, crop loss and drinking water shortages (IPCC, 2021). The human population is expected to increase from 7.2 to 9.6 billion by 2050, and this corresponds to a population increase of 33% (UN, 2013). The demand for animal products is also increasing for the adequate nutrition of the increasing human population. Therefore, even as average global temperatures increase and precipitation patterns change, the increasing demand for food must be met. The impact of extreme climatic conditions and seasonal fluctuations on feed quality and quantity is considered a compelling source of influence on animal welfare. Animals grazing in poor pasture conditions in arid and semi-arid regions are exposed to malnutrition. In extremely hot environments, the productivity of animal decreases due to the lack of

sufficient and quality feed (Naqvi et al. 2015). In a dry and warm environment, animals expend a lot of energy because they have to walk long distances to find food and water. Thus, in addition to many other factors, the quality of animal products is probably most affected by feed quantity and quality. Therefore, most of the research has focused on improving the quality of animal products through manipulations in animal nutrition (Babinzski et al., 2011). Animal nutrition strategies should be developed and solutions found to reduce the negative effects of global climate change on animal nutrition.

2. Global Climate and Global Warming

The global climate is the interconnected system of everything humans do, with the sun, earth and oceans, wind, rain, snow, forests, deserts and savannas. Very important changes in the global climate are occurring. Climate change refers to long-term changes in temperatures and weather patterns. These changes can be natural, such as changes in the solar cycle. But since the 1800s, humans primarily burning fossil fuels such as coal, oil and gas, cutting and burning forests and producing lime have been the most important drivers of climate change. Climate change is primarily caused by greenhouse gas emissions, which result in warming of the atmosphere. It is a term often used interchangeably with climate change, as one of the most important measures of global climate change is global warming. Global warming refers to the increase in average global temperatures associated with significant impacts on people, wildlife and ecosystems around the world (IPCC, 2021). Three important gases, mainly CO_2, CH_4 and N_2O are shown as the main causes of global warming and climate change. The ones that increase greenhouse gas emissions the most are CH_4 (%44), N_2O (%29) and CO_2 (%27) (IPCC, 2013). The livestock sector contributes to 14.5% of global greenhouse gas emissions (Gerber et al., 2013). In addition, the increase in the level of water vapor in the atmosphere contributes to global warming. Climate change and heat stress affect livestock health and productivity, natural resources, quantity and quality of feed, and animal production through loss of biodiversity (Chapman et al., 2012).

2.1. Temperature and Relative Humidity

Animals are adversely affected by the harmful effects of extreme weather conditions. The effects of climatic extremes and seasonal fluctuations on feed quantity and quality can affect livestock welfare, leading to reductions in production and reproductive efficiency (Sejian et al., 2013). The quantity of heat stress is generally related to relative humidity. The increase in relative humidity with the ambient temperature exceeding the normal limits (5-25 °C) adversely negatively affects the thermoregulation ability of the animal. As the relative humidity increases, the lower the temperature at which a dairy cow shows signs of heat stress. For example, the most suitable environmental conditions for dairy cattle; it is defined as an ambient temperature of 13-18 °C and a relative humidity of 60-70% (McDowell, 1972). This relationship between ambient temperature and relative humidity has led to the development of the Temperature-Humidity Index (THI) (Table 1).

Table 1. Temperature and humidity index values for animals (NADIS, 2017)

C	20	30	40	50	60	70	80	90	100
22	66	66	67	68	69	69	70	71	72
24	68	69	70	70	71	72	73	74	75
26	70	71	72	73	74	75	77	78	79
28	72	73	74	76	77	78	80	81	82
30	74	75	77	78	80	81	83	84	86
32	76	77	79	81	83	84	86	88	90
34	78	80	82	84	85	87	89	91	93
36	80	82	84	86	88	90	93	95	97
38	82	84	86	89	91	93	96	98	100
40	84	86	89	91	94	96	99	101	104

Green= heat stress moderate, Yellow= heat stress acute, Red= deadly, THI= temperature humidity index.

According to the Table 1, when the THI value is 71 and below, the animals are not affected by heat stress, when the THI reaches 72, the cows show signs of moderate heat stress, if the THI is between 72-79, some yield losses may occur, and when the THI is 80 and above significantly affected by the heat stress of the animal.

2.2. Thermoneutral (Comfort) Zone for Animals

The heat produced during the digestion, excretion and metabolism of nutrients is called "temperature increase". The temperature range in the immediate environment where a healthy adult animal can maintain its normal body temperature without needing to use energy outside of its normal basal metabolic rate is called the "thermoneutral zone" (Metze, 2016). In other words, the range of environmental conditions in which an animal can regulate its heat excretion with minimal effort is defined as the "Thermo neutral zone". In a thermoneutral environment, the animal's heat production is minimal and therefore dietary energy can be used efficiently for production (growth, meat, milk, eggs, etc.). The thermoneutral zone in cattle differs according to race, age, sex, reproduction and lactation status, and milk yield and milk composition. The thermoneutral zone for dairy cattle is between 13.9 °C and 27.2 °C, within this range the changes in core body temperature are minimal. The thermal comfort zone (TCZ) represents the range of ambient temperatures at which an animal feels thermally comfortable (Figure 1) (Curtis, 1981).

Homeotherms (warm-blooded) have zones of optimum temperature for production, where no additional energy is spent above the HP requirement to warm or cool the body. The upper critical air temperature for dairy cows has been reported to be 25 to 26°C (Berman et al., 1985).

3. Heat and Cold Stress in Animals

The heat dissipation capacity of animals due to high temperature and humidity is exceeded and there is an increase in body temperature beyond physiological

limits, this situation is called heat stress (Ronchi et al., 1997). Extreme heat in the summer can adversely affect grazing animals and cause nutritional imbalances. In the arid and semi-arid tropics, insufficient and poor quality feed is the most important factor in animal malnutrition.

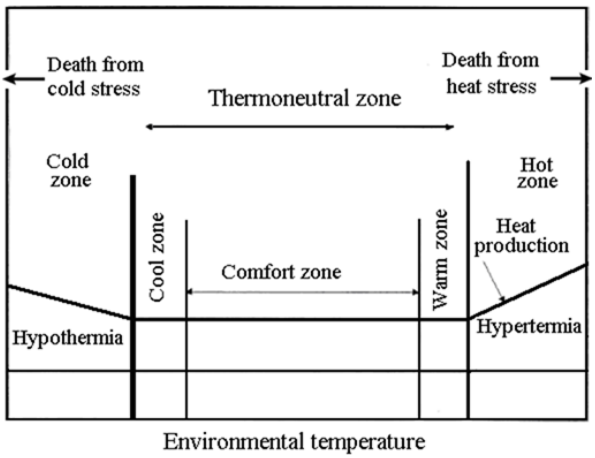

Figure 1. Thermoneutral Zone for dairy cattle (Curtis, 1981).

Cold stress (low critical temperature) is the animal body's response to low temperatures when the ambient temperature falls below the thermal neutral zone. Cold stress is the opposite of heat stress (upper critical temperature). If the environmental temperature falls below the thermal neutral zone of the animal, its body's metabolic rate increases (Metze, 2016). Cold stress can also be critical for more on rangeland grazing animals. As cold stress causes an increase in basal metabolic rate, it also causes an increase in additional energy requirements. Thus, in ruminants; rumination, digestive system activity, feed and fluid passage rate increases and digestion ends in a shorter time. This rapid digestion results in a reduction of energy from feed consumption (Navratil et al., 2015).

4. The Effects on Animal Nutrition of Climate Change

According to the studies and reports, it is understood that the average temperature of the world has increased and will continue to increase, and this will have a significant impact on agricultural production. Heat stress has direct and indirect effects that negatively affect the health, nutrition and productivity of animals. The direct effects are primarily due to increased temperatures and the frequency and intensity of heat waves. The most important direct impact of climate change on animal production is due to heat stress. These environmental conditions cause metabolic disturbances, oxidative stress, infections and impaired immune system in animals and may adversely affect animal health (NRC, 1981; Gaughan et al., 2009). Indirect effects are those related to changing the availability and quality of feed raw materials and drinking water (Lacetera, 2019). Drought caused by climate change affects water availability, quantity and quality of forage production, rangeland vegetation diversity, this, in turn, leads to reduced feed consumption, malnutrition, reduced body weight, reduced reproductive efficiency and weakened resistance to diseases of animals (Naqvi et al., 2015).

4.1. Effect on Rumen Metabolism of Climate Change

4.1.1. Cattle

High ambient temperature in summer can have a significant impact on rumen physiology, which in turn affects the nutritional status of animals. Heat stress may cause deterioration in rumen physiology, decrease in rumination, rumen activity and reticulo-rumen motility, metabolic disorders, slowdown of digestive transit rate and decrease in production performance (Nardone et al., 2010; Yadav et al., 2013). It has been determined that when Holstein heifers are exposed to ambient temperatures ranging from 20°C to 33°C, the rumen pH decreases (Tajima et al., 2007), and the rumen microbial population of cows changes at high ruminal temperatures (Yadav et al., 2013; Correia Sales et al., 2021). Microbial change in the rumen is important in determining the digestibility of fibrous feeds, feed transition rate, growth rate and feed consumption of ruminants. It is known that the degradability of roughage in the rumen depends on the presence of microorganisms (bacteria, protozoa and fungi) in the rumen (Özel and Sarıçiçek, 2009). The residence time of the feeds in the rumen varies according to the temperate and tropical regions, this suggests that the potential degradability of feeds may be affected by climate and ambient temperature.

Since heat stress causes an increase in lactic acid concentrations in ruminants, it reduces the animal's production, energy availability and lowers the rumen pH, the rumen water content rises, and accordingly the osmotic pressure of the rumen fluid decreases (Yadav et al., 2013). The electrolyte concentration (K and Na) in the rumen fluid of the cow under heat stress decreases, which causes loss of Na in the urine and K loss from the skin, feed consumption is reduced and the rumination period is shortened (Sammad et al., 2020), due to the shortening of the rumination period, the secretion of saliva, which acts as a buffer, also decreases (Meneses et al., 2021). It was determined that the ammonia nitrogen (NH3-N) concentration of ruminants exposed to heat stress also decreased significantly. This indicates that heat stress can also affect rumen fermentation and digestion of dietary proteins and the metabolism of other nitrogenous compounds (Cai et al., 2019).

In heat stress, the acetate ratio in the rumen decreases and the butyrate ratio increases (Cai et al., 2019). The reduction in acetate may be the result of the reduction in the presence of acetobacter (Zhao et al., 2019), in addition, total volatile fatty acids (VFA) and propionate concentrations in the rumen of animals under heat stress are significantly reduced. Concentrated feed consumption increases significantly during heat stress,

this causes an increase in propionate production (Uyeno et al., 2010). Decreased feed consumption and changes in the amount and/or activity of microorganisms in the rumen are important factors affecting VFA production (Özel and Sarıçiçek, 2009). It is also stated that changes in VFA concentrations in the rumen may be due to the increase in water consumption of the animal and the high water content in the rumen (Cai et al., 2019). Although the rumen contains the greatest number and diversity of bacteria, ciliate protozoa that affect the production of VFAs can account for about half of the rumen microbial biomass. In addition, heat stress may cause changes in the amount of other microorganisms such as anaerobic fungi, archaea and protozoa (Özel and Sarıçiçek, 2009). While acetic acid, one of the volatile fatty acids produced in the rumen and transported to the bloodstream, is mainly used in lipid synthesis, the level of propionic acid affects the protein content of milk. The decrease in feed consumption due to high ambient temperatures causes a decrease in sulfur uptake, which negatively affects the methionine synthesis of rumen bacteria. Decreased microbial protein synthesis or inhibition of methionine synthesis, which has a very important role in meeting the protein needs of ruminants, causes a significant decrease in milk protein (Sarıçiçek, 2007).

4.1.2. Buffalo

Omran et al. (2011) determined that the adaptation process of the rumen is significantly affected when the buffalo is exposed to heat stress in the laboratory environment at 40°C and 25°C. Rumen movements in buffaloes are slower than in cattle, and the rate of passage of digested content through the rumen is lower (Sarıçiçek, 2007). According to Wang et al. (2012), heat stress changed the rumen fermentation of water buffalo, in particular, it caused a decrease in the concentration of acetic and butyric acid, which are the precursors of fatty acids and total cholesterol. Rumen fermentation provides a dynamic response to changes in rumen microorganisms. Among the microorganisms (archaea, fungi, protozoa and bacteria) in the rumen of the buffalo under heat stress, the most changes were seen in bacteria. While heat stress did not have a significant effect on the alpha diversity of rumen bacteria, visible changes in the β-diversity of the bacterial community were detected (Wang et al., 2022).

4.1.3. Goat

It decreased rumen pH level and rumen fermentation (Castro-Costa et al., 2015), decreased rumen VFA production (Choubey and Kumar, 2012), acetate and acetate/propionate ratios and increased butyrate production (Tajima et al., 2007) of Murciano-Granadina dairy goats exposed to heat stress. Hirayama et al., (2004) stated that Saanen goats exposed to heat (35 °C) had a decrease in plasma acetate and VFA concentrations compared to those kept in thermoneutral conditions (20 °C), which was caused by the reduction in feed consumption and the change in the rumen microbial population. Pragna et al. (2018) determined that rumen acetate concentrations decreased in Osmanabadi and Malabari goats exposed to heat, whereas there was no change in Salem goats. This suggests that there may be differences between varieties against heat stress.

4.2. Effects on Feed Consumption of Climate Change

The relationship between the increase in ambient temperature and body temperature and dry matter consumption (DMT) has been explained (West, 2003). The terms appetite and hunger need to be explained to explain the animal's desire to consume food. appetite; the desire to eat a previously tried and admired food, hunger; It is the state of saturation felt by the animal as a result of the animal's inability to get the nutrients it needs and insufficient nutrition (mechanical and physiological) (Sarıçiçek, 2007). Ghrelin is a hormone secreted by ghrelinergic cells in the gastrointestinal tract when the stomach is empty, it increases hunger and gastrointestinal motility (Pearce et al., 2014). Decreased appetite and feed intake due to heat stress may result in a decrease in metabolic body weight, and as a result, the animal may enter physiologically negative energy balance, and then is decrease in body condition score of the animal (Rhoads et al., 2009).

4.2.1. Cattle

Since there is a significant reduction in feed consumption under heat stress, nutrient deficiency occurs due to the interaction between stress and feeding. The reason of decrease in feed consumption may also be the result of the longer filling of the digestive tract and the decrease in the passage rate of digestion (Rana et al., 2014). Animals under heat stress reduce feed consumption to lower metabolic heat. In a hot environment, the ability to lose heat is limited when the air temperature is above the upper critical temperature, therefore, livestocks reduce feed consumption to maintain thermal balance, thus reducing the temperature rise (Kadzere et al., 2002). Studies show a strong negative correlation between rectal temperature and feed consumption in pigs, poultry and dairy cows during heat stress. High ambient temperature causes hyperthermia in the body, this reduces the activity of the appetite center in the medulla oblongata. Therefore, feed consumption decreases proportionally with the increase in ambient temperature (Brown-Brandl et al., 2001).

Feed consumption starts to decrease at an average temperature of 25-26 °C in lactating cows, and this decrease is faster at temperatures above 30 °C (NRC, 1989). Since there is an inverse relationship between high temperature and DMI, it was observed that feed consumption was suppressed after one day from the increase in high (29 ^{o}C, 50% RH) temperature (Spiers et al., 2004). According to NRC (2001), DMI decreases by 40% at 40 ^{o}C ambient temperature. In beef cattle, feed consumption starts to decrease at an ambient temperature of about 30 °C with <80% RH or 27 °C with >80% RH (Bernabucci et al., 2010).

4.2.2. Buffalo

The fact that the buffalo has dark skin and sparse hair

causes the sun's rays to be absorbed at a high level (Dayal et al. 2017). In addition, the underdeveloped sweat glands of buffalo weakens their heat dissipation capacity, making them highly susceptible to global warming (Bombade et al., 2018). With the increasing effects of climate change, the heat stress problem for buffalo is likely to worsen. Heat stress in buffalo starts at THI 72, THI above this cannot maintain the heat balance, which causes a decrease in DMI and an increase in respiratory rate (Gu et al., 2018).

Buffalo are also very sensitive to cold stress and cannot maintain their internal temperatures in the thermo-neutral zone. According to Omran and Fooda, (2013), under environmental conditions in Medill Egypt there may be THI>87 for Friesians, while this threshold is THI>91 for buffaloes, this may be dangerous for Frisians. The best THI for production in both species is lower than 68, but at this threshold also Buffalo are under cold stress. Under native Egyptian conditions, Friesians are more tolerant of cold but more sensitive to heat than buffaloes.

4.2.3. Goat

It is known that due to the lip structure and foraging habits of goats, they can be satisfied with limited feed especially in arid and semi-arid regions and have the ability to evaluate low quality feeds (Sarıçiçek, 2007). Although goats are more resistant to heat stress, they are affected when exposed to temperatures above their comfort zone. While the upper critical temperatures for goats at the maintenance level were determined as 25°-30 °C', the critical temperature was not determined for growing and lactating goats (Gupta and Mondal, 2021). Goats looking for forage in open areas for most of the day are sensitive to heat stress. Feed consumption decreases in goats under heat stress, which leads to a decrease in yield. The reason for the decrease in feed consumption is the reaction of the animal to reduce the metabolic heat, since the heat increase resulting from the metabolism of nutrients is the most important source of heat production (Sejian et al., 2018).

4.2.4. Sheep

It has been observed that ad libitum roughage consumption decreases in sheep exposed to heat stress as in other species, especially when high humidity is present during the day (temperature 32 °C and relative humidity 98%). In addition, due to high heat stress, the maintenance requirements of sheep increase by 30% (NRC, 2007), and live weight loss occurs as feed consumption will not be sufficient to meet daily energy requirements (Hamzaoui et al., 2013).

4.2.5. Pig

It is stated that pigs cannot tolerate high temperatures up to 36 °C and high temperatures cause loss of appetite (Babinszky et al., 2011). The increase in the relative humidity with the upper critical temperature causes the animal to reject the feed, and with the increase in the relative humidity, the daily feed consumption of a 60-70 kg pig can decrease by 80-150 g (Huynh et al., 2005).

4.2.6. Poultry

Since the critical temperature for poultry is 30 °C, they can compensate for the energy loss up to this temperature. However, at temperatures above 30 °C, feed and energy consumption decreases, production decreases rapidly and mortality increases (Daghir, 2009). In severe heat stress (34-35 °C), feed consumption may decrease by approximately 30-50%. In addition, decreased activity of trypsin, chymotrypsin and amylase at high ambient temperatures, possibly, reduces the digestibility of nutrients in poultry (Hai et al., 2000). Chicks exposed to low ambient temperature (20 °C) cluster to maintain optimum temperature, thus, they cannot consume enough feed as they reduce the frequency in the feeder and drinker. Feed consumption of 4-6 week old broilers at 32 °C was reduced by 24% compared to that at 22 °C. In addition, in case of heat stress, broilers may refuse feed at a rate of 50% depending on age (Gonzalez-Esquerra and Leeson, 2005).

4.3. Impact on Water Consumption of Climate Change

Water is absolutely necessary for life, animals need water; water loss while animals meet their water needs from feed and metabolic water and drinking water; provided by the skin and respiratory, fecal and urinary tracts (Sarıçiçek, 2007). Problems related to the availability of water will affect the water needs of animals, the growth of forage crops and the livestock sector using the crops grown. Approximately 8% of global human water use belongs to the livestock sector. While the water requirements of heat-stressed animals increase by two to three times compared to thermoneutral conditions (Nardone et al., 2010), some species, such as camels and Bedouin goats, have water requirements of up to 30-40% of live weight loss (Cain et al., 2006).

4.3.1. Cattle

Since the comfort zone for dairy cows is between 5 and 25 °C, at a temperature of 26 °C or higher, the thermoregulatory capacity of the animals is exceeded and they enter heat stress. Dairy cattle show signs of severe dehydration when they lose 12% of their body weight of water. While the water requirement of a maintenance cow is 4-6 Lt per kg DM consumed under normal conditions (Sarıçiçek, 2007), the water consumption for a dairy cow under heat stress increases by 1.2 kg/°C of the minimum ambient temperature (West, 2003). Drinking water, which is the most important nutrient of dairy cattle, also has the feature of regulating heat distribution. Since 87% of milk consists of water, lactation cows have high water needs depending on their body weight, DMI and yield levels. Meat production is also affected when the body water content of beef cattle decreases by 10% (Herrero, 1998).

4.3.2. Buffalo

Since water buffalo is sensitive to heat and cold stress, the most important nutrient in hot climates is water. Water intake is closely related to DMI and milk yield. Water consumption increases in hot conditions, so it is

important to always have plenty of water (Omran et al., 2013). Buffaloes consume 4-6 Lt of water for each kg of DM consumed for maintenance (Sarıçiçek, 2007), water consumption increases when the temperature increases, water consumption of buffalo calves in summer increases approximately twice the daily consumption or three times per metabolic weight (Ashour, 1990).

4.3.3. Goats

The thermoneutral zone for goats is 12–24 °C in the hot regions of the world. Water needs of goats in heat stress conditions have high (Salama et al., 2012). To alleviate the effects of heat stress, goats should be provided with plenty of fresh, cold water. A goat needs 4 - 8 liters of water on average per day. Young and lactating females need more water than adults. The reason for the higher of water consumption in Alpine goats compared to Saanen goats is probably to dissipate more heat released as a result of rumen fermentation, this shows that there is a difference between breeds in terms of water/DMI ratio (Kadzere et al., 2002). It is also suggested that this difference may be due to the difference in the hair color of the goats: the skin temperature in black areas in Holstein cows is higher than in whites, which causes increased sweating in black areas compared to whites (Da Silva and Maia, 2011). Thus, it explains why Alpine goats with dark brown hair drink more water/kg DMI than white Saanen goats.

4.3.4. Sheep

Sheep consume 2-3 Lt water/kg DM at temperatures between 0 and 15 °C, and this rate triples at temperatures above 20 °C. In addition, water consumption in sheep is 9-11% of total body weight in winter and 19-25% in summer (Khan and Ghosh, 1989). In general, thirst for three days or more negatively affects feed consumption of sheep as a result of increased milk production, body weight, chewing movements during rumination, respiratory rates and rectal temperatures (Aganga et al., 1990).

4.3.5. Pig

The most suitable ambient temperature for the optimum production performance of growing pigs is between 18-21 °C (Ingram, 1965). Pigs need to consume more water to reduce the effect of heat stress and to lose evaporative heat through respiration. Cold drinking water provides the greatest heat removal. A baby pig can drink 4-5 liters of water per day. Pigs at the beginning of fattening should be given 4 liters of water, 12 liters for growing pigs, 20 liters for pigs at the end of fattening, 24 liters for pregnant pigs, and 20 to 35 liters per day for lactating pigs. As a general rule, pigs should be provided with drinking water at 10% of their body weight, but pigs under heat stress typically drink six times more water than normal (Zaake et al., 2020).

4.3.6. Poultry

At high temperatures, the water holding capacity of the body is significantly reduced, as the poultry excessive water consumption causes the electrolyte balance to change (Borges et al., 2003). Poultry consume approximately 1.6–2.0 times more water than the feed they consume (El Saidy et al., 2015). The thermoneutral temperature for seven-day-old broilers ranges from 28 to 35 °C, high temperatures above this cause hyperthermia and dehydration, feed consumption decreases and this causes growth to slow down. The water consumption of broilers exposed to acute heat stress is also high (Egbuniwe et al., 2018).

5. The Effects on Animal Production of Climate Change

Animals must be in a thermal comfort zone in order to perform their physiological functions. When the environmental temperature rises above the upper critical temperature, animals begin to be exposed to heat stress. Heat stress affects bioenergy of animal and has a negative impact on welfare. Heat stress changes the physiology of animals, causing a decrease in performance, yield and product quality. Under heat stress, feed consumption, digestibility of poor quality feeds, and absorption of nutrients decrease. In such a case, the animal may have to benefit from less nutrients for its maintenance and productivity needs. This causes a decrease in yields such as milk, meat, fleece/angora, and eggs (West, 2003).

5.1. Effects on Growth Performance of Climate Change

Growth is defined as a coordinated increase in body appearance over a given period of time. The volumetric and numerical increase of the cell, that is, the increase in live weight, adequate nutrient intake, is genetically and environmentally controlled by hormones and enzymes (Sarıçiçek, 2007). Heat stress negatively affects growth performance, body weight and daily weight gain.

The negative effects of high temperature on growth performance are the result of a decrease in anabolic activity and an increase in tissue catabolism. The decrease in anabolism is caused by the inadequacy of essential nutrients, especially ME, which is necessary for maintenance and body weight gain, due to the decrease in feed consumption (Morrison and Lofgreen, 1979).

5.1.1. Cattle

Heifers produce less metabolic heat compared to cows, so they are expected to be less affected by heat stress. However, although the maintenance requirements of cattle are higher in hot weather, their appetite has decreased, and because they are fed with low quality roughage, their growth slows down, causing them to be smaller in size (NRC, 1981). Holstein, Brown Swiss and Jersey heifers from one year to thirteen months were grown in chambers at constant temperatures of 10 °C and 26.7 °C to determine the effect of temperature. It has been determined that Holstein heifers in a 26.7 °C environment are weaker, and although they gain more weight in a cool environment, it takes longer to reach the desired weight in a warm environment (Johnson and Ragsdale, 1959). When the comfort conditions (10 °C) and heat stress conditions (35 °C) of Jersey, Brown Swiss

and Holstein heifers were compared, it was determined that nitrogen retention decreased by 25.4% and 49.0%, and the nitrogen balance was positive in Holstein calves and negative in cows (Kamal et al., 1970).

5.1.2. Buffalo

The summer-born calves of Egyptian buffaloes are significantly weaker at weaning compared to calves born in other seasons, Egypt (Marai et al., 2009) and in Murrah and Surti Indian buffaloes (Bhavsar et al., 1974), it was determined that the daily live weight gain from birth to weaning was significantly affected by the birth season. The lowest daily weight gain between birth and weaning was determined in calves born in summer. This may be due to the adverse effects of exposure to moderate to very severe heat stress (during the summer) of the fetus in the last period of pregnancy (Marai et al., 2009).

5.1.3. Goat

Although live weight is an important parameter to explain the growth variability of goats, changes are also observed in the growth parameters of goats under heat stress (Habibu et al., 2016). Pragna et al., (2018), who determined that the live weight loss in Osmanabadi breed was higher than Malabari and Salem back goat breeds, stated that the decline in growth differed between different goat breeds and that heat stress had a negative effect on the body condition score of the animals.

5.1.4. Sheep

Although sheep are one of the most resistant species to high environmental temperatures, climate change has a great impact on their growth characteristics. Heat stress reduces appetite and causes a decrease in feed consumption, slowing of rumen functions and a decrease in growth rate.

Since high temperatures also reduce the rangeland quality, it causes the sheep grazing on the rangeland to not get enough nutrients, and as a result, their daily live weights decrease. According to Marai et al. (2007), when sheep are exposed to high temperatures (30-40 °C, 40% relative humidity) in the early embryonic stage, a significant reduction in total cell number, cell size, and placentome size was observed compared to thermoneutral temperatures (18-20 °C, 30% RH). During placental growth, fetal development slowed down due to temperature, feed consumption of animals decreased, water consumption and survival requirement increased. Temperature stress caused to slow down of growth performance, and energy demand increased at higher temperatures (Pérez-Barbería et al., 2020). When THI increased from 60-65 to 72-75 in Sarda sheep in a semi-arid-tropical environment, there was a 20% reduction in production performance, while the body condition scoring of the sheep also changed (Sejian et al., 2010). In addition to low precipitation in spring and summer, high temperatures can directly affect the productivity of sheep and lambs.

5.1.5. Pig

Since pigs are sensitive to heat stress, high temperatures cause loss of appetite and reduced growth (Quiniou et al., 2000). When sows are exposed to temperatures above 25 °C during lactation, milk yield decreases due to reduced feed consumption, which reduces the chance of piglets surviving. Pigs with high body weight are more sensitive to heat stress than those with low body weight (Renaudeau et al., 2011).

5.1.6. Poultry

Broilers are also sensitive to the increase in ambient temperature during the growing period, metabolic heat production also increases as their feed consumption is high. When the ambient temperature exceeds the upper limit of the thermonotral zone, this heat load must be distributed in order to maintain the body temperature of the animal, but because chickens do not have sweat glands, they do not have the capacity to dissipate heat, in this case the animal enters heat stress, feed consumption decreases and growth rate decreases (Lara and Rostagno, 2013).

5.2. Effects on Milk Yield and Quality of Climate Change

Milk yield of animals is highly affected by climate change. Increase in temperature and humidity reduces milk production, negatively affects the quantity and quality of milk, and shortens the lactation period of animals.

5.2.1. Effects on milk yield of climate change

Cattle: The effect of heat stress on milk yield performance of dairy cattle is negative. The decrease in milk yield occurs when the average daily temperature rises above 26 °C. Changes in environmental factors such as ambient temperature, relative humidity, wind speed and solar radiation cause stress in lactating cattle, and heat stress negatively affects both the quantity and quality of milk. It was determined that milk yield decreased by 33% at 35 °C and 50% at 40 °C (Rhoads et al., 2009). When THI is in the range of 72-80, 80-90 or 90-98, the effect of heat stress is respectively; mild, moderate or severe. Both increased ambient temperature (from 25 to 32 °C) and increased THI (from 73 to 82) have a negative effect on dry matter consumption and milk yield of cows (Lopez et al., 1991). There is a positive relationship between milk yield and feed consumption and heat production. In addition, during long heat stress periods, there is a loss of body weight and condition in dairy cows (Baumgard et al., 2006). According to West, (2003), milk yield decreased by 0.88 kg/day for each unit increase above THI 72, which is accepted as heat stress in Holstein cows, and in milk production potential of dairy cows exposed to climate change-related heat wave conditions showed a 10-14% reduction. Bernabucci et al. (2010) also determined that there is a 0.27 kg decrease in milk production for each unit increase in THI. In addition, exposure of cows to heat stress in the dry period affects mammary gland development before birth and causes a decrease in milk yield in the next lactation (Tao et al., 2011).

Buffalo: When the environment in which the buffalo lives exceeds THI 75, the productivity, fertility and health

of the animal are significantly affected, which in turn causes a decrease in milk yield, a 1 °C increase in rectal temperature under heat stress conditions reduces the performance of dairy cows (Amamou et al., 2019). Yadav et al. (2022), in their study to determine the effect on milk yield of buffaloes of THI 79.88, 80.57 and 85.36 in May, June and July, they found a significant decrease in milk yield only in THI 85.36 in July. The negative effect of sudden temperature change (cold or heat wave), on milk yield of buffaloes was seen not only on the next day but also on the day(s) after or after the extreme event. Return to normal milk yield took 2-5 days in individual buffaloes. While the milk production performance of buffalo is less affected when THI is 75, lactation period is shortened by a few days (3-7 days) in hot summer months when THI is higher than 80 (Upadhyay et al., 2007). Buffalo experience greater heat stress when behavioral traits such as shelter-seeking, rolling over, and/or submerging are inhibited. Also, lactating buffaloes are exposed to severe heat stress with increased heat production in summer months when heat stress combined with high milk production and lactation stress.

Goat: It was determined that especially in July, steaming and rolling increase milk yield, and rolling is more effective in terms of milk yield (Yadav et al., 2016). As THI value increased in dairy goats, milk yield decreased and each 1 unit increase in THI caused a 1% decrease in milk yield (Salama et al., 2014). Differences were determined between goat breeds in terms of resistance to heat stress. In comparative studies conducted with Alpine and Nubian goat breeds, it was observed that the production performances of goat breeds adapted to heat stress were relatively higher. In similar environmental conditions (34 °C temperature and 25% relative humidity; THI = 79), the milk yield of Nubian goats native to tropical regions was preserved, while the milk yield of Alpine breeds decreased when the temperature increased from 27 °C to 34 °C (Brown et al., 1988).

Sheep: The contribution to milk production of sheep is quite low compared to cows and goats. High temperature and humidity are the main factors on the productivity of sheep in tropical and subtropical regions. However, it has been reported that the effect on milk yield of cold stress in Manchega sheep is more negative compared to heat stress (Ramon et al., 2016). In addition, there was a 20% decrease in milk yield in Comisana sheep exposed to temperatures above 35 °C (Sevi et al., 2001). Similarly, there was a 15% reduction in milk yield when Sarda sheep were exposed to maximum ambient temperatures above 21-24 °C, and a 20% reduction when minimum temperatures were increased from 9-12 °C to 18, and a 30% decrease was determined when the maximum and average temperatures are higher than 21 °C – 24 °C and 15 °C – 21 °C (Peana et al., 2007).

5.2.2. Effects on milk quality of climate change

Cattle: Metabolic heat production during lactation can reduce the resistance of cattle to high ambient temperature, resulting in a reduction in milk yield as well as a change in milk composition. The default neutral temperature range for dairy cattle is –5 to 25 °C (Knizkova et al., 2002). Since there is a relationship between high milk production and high heat production, it was determined in a study that milk yield and composition may change depending on the decrease in ambient temperature, and especially protein and milk dry matter levels may decrease (Bickert and Mattiello, 2016). While heat stress causes a decrease in milk fat, protein and short-chain fatty acids, and an increase in long-chain fatty acids in milk (Kadzere, et al., 2002), increasing heat load index caused a decrease in lactose, protein and fat levels in milk (Van Laer et al., 2015). Likewise, Bernabucci et al., (2015) recorded a significant decrease in milk fat content in summer (3.20 g/100 g) compared to values in winter (3.80 g/100 g) and spring (3.61 g/100 g).

In milk, there are two groups of proteins whose chemical structures and physical properties are known: caseins and whey proteins. While caseins αS1-, αS2-, β- and κ-CN represent approximately 80% of the total milk protein, the other 20% consists of whey proteins, mainly β-LG and α-LA (Farrell et al., 2004). A decrease in milk yield and milk protein fraction was observed in cattle exposed to heat stress, and there was a decrease in milk casein, which was suggested to be due to the direct effect of heat stress. It has been determined that milk casein consists of several fractions as αs1, αs2, β, κ and γ caseins, and there is an increase in αs1 casein and a decrease in αs2 casein in the milk of cows under heat stress (Cowley et al., 2015). Similarly, Bernabucci et al., (2015) determined that the milk casein content was higher in winter (2.75 g/100 g) and spring (2.48 g/100 g) compared to summer (2.27 g/100 g). They determined that milk was lower in terms of αs casein (αs1 + αs2) and higher in terms of κ casein compared to other seasons, whereas β casein was similar. In addition, it was observed that heat stress caused a decrease in total protein, fat, casein, lactose, lactalbumin, short and medium chain fatty acids, IgG and IgA in Holstein heifers in the first four lactations (Nardone et al., 1997). There are few studies on the effect of heat stress on milk mineral content. It has been stated that all mineral content of milk is significantly affected by the season (Poulsen et al., 2015), seasonal differences are important on the mineral content of milk, and cows exposed to heat stress in summer have lower milk ash and phosphorus content (Mariani et al., 1993).

Buffalo: Buffalo milk contains higher levels of DM, fat, protein, calcium and P compared to cow's milk. The ratio of albumin and globulin is higher in milk protein (Sarıçiçek, 2007). The hot and humid climate affects the quality as well as the quantity of buffalo milk. Habeeb et al. (2000) determined that buffalo produced higher quality milk in winter (February) than in summer conditions (in July), and milk production, milk DM, fat, protein, lactose content and milk quality decreased in July at high ambient temperature. Omran et al. (2017)

determined that milk yield, milk fat, protein, lactose, total DM, lean DM content at THI 58.64 (January-March, late pregnancy) decrease significantly compared to THI 69.33 (April-June, postpartum), and reported that buffalo is more sensitive to cold stress.

Goat: There was a decrease in milk protein and protein fractions of goats under heat stress (Hamzaoui et al., 2013). The decrease in feed consumption due to heat stress also decreased feed protein consumption, and thus, insufficient amino acids required for milk protein synthesis caused a decrease in milk protein level (Salama et al., 2014). In addition, another reason for the decrease in milk protein under heat stress may be the change in the rumen environment with high water consumption and the decrease in rumen microbial protein synthesis (Hamzaoui et al., 2013). Fat content in milk of dairy goats also decreased under heat stress conditions.

In a study conducted with Saanen, Anglo-Nubian and Alpine breed goats, it has been observed that goats raised in tropical climates have lower milk yield, some milk components such as milk fat and total DM, compared to those reared in temperate climates due to high air temperature and malnutrition (Juaréz, 1986). The protein and casein levels in milk of goats under heat stress decreased by 12.5% and 11.5%, respectively, compared to those in a thermo-neutral environment.

In addition, dairy goats under heat stress conditions were also found to produce less fat milk (Hamzaoui et al., 2013). Similarly, fat and protein contents were found to be significantly lower in the milk of temperate Alpine and Nubian goats in summer (Brown et al., 1988).

Sheep: Sheep milk is of high quality, suitable for cheese and yoghurt production, due to its high DM, protein and fat content. Sheep milk has higher calorie content as it contains more fat and non-fat dry matter compared to goat and cow milk (Sarıçiçek, 2007). Sheep are most affected by climate change, especially heat stress, and the amount and quality of milk also changes. It has been stated that there is a positive relationship between milk properties and relative humidity, and when RHI is > 23, milk yield and milk fat and protein content of sheep decrease (Finocchiaro et al., 2005). It has been determined that when Comisana sheep are exposed to sunlight, saturated fatty acids such as caproic, capric, lauric, myristic and stearic acids increase (3-18%) and the oleic, linoleic and linolenic acid contents decrease (2-9%) in milk (Sevi et al., 2002).

5.3. Effects on Meat Yield and Quality of Climate Change

Meat has an important place among the proteins of animal origin in human nutrition. Animal proteins contain essential amino acids that cannot be synthesized in the human body. Meat and meat products are the most valuable protein sources with high biological value for humans due to their amino acid composition. Meat production resources; although there are cattle, buffalo, sheep, goats, pigs, poultry, there are differences in the world countries in terms of meat production and consumption.

Cattle: Beef cattle are breeds that are bred for meat yield, they are breeds with high feed efficiency and more weight gain. Nutrition level affects the quality of meat, muscle, bone and adipose tissue of the carcass (Sarıçiçek, 2007). One of the main reasons for the decrease in meat production in beef cattle is heat stress. Cattle are under heat stress when the ambient temperature is above 26-28 °C, the relative humidity is above 50% and the wind speed is below 5 km/h. High-weight, thick-haired and darker beef cattle are more vulnerable to heat stress. In addition, global warming may reduce body size, carcass weight and fat thickness in ruminants (Nardone, 2000). Changes in climatic conditions, increase in temperatures and decrease in precipitation may cause a decrease in the CP and digestible organic matter content of the feed, and the botanical structure of the pasture may weaken due to drought and water deficiency (Naqvi et al., 2015). In addition to all these negativities, the decrease in feed consumption can lead to a decrease in growth, that is, it can lead to muscle loss. Pasture has a positive effect on meat quality. It is known that the meat of beef cattle fed with pasture plants is richer in n-3 fatty acid and conjugated linoleic acid content than those fed with concentrated feed (Scollan et al., 2006). Extreme weather events reduce the growth performance (weight gain, feed consumption and feed efficiency), especially of cattle calves kept outdoors, thus decreasing the fattening and slaughter weight, less intramuscular fat and meat juiciness and tenderness and lower meat quality (Keane and Allen, 1998).

Buffalo: The body temperature of the buffalo is around 37-39 °C, the intense vascularity in the skin causes the heat to be absorbed and spread, sweat glands are less compared to cattle. This situation causes them to enter heat stress. The buffalo in the heat, cannot ruminate, becomes grumpy and irritable, and their stress increases even more (Sarıçiçek, 2007). The meat yield of buffalo is relatively higher than beef, but it has less fat, less saturated fatty acid content and lower calories than beef (Di Luccia et al., 2003). The nutritional value of buffalo meat is higher than beef due to its much lower fat and cholesterol content. It contains almost all essential amino acids, vitamins A and B necessary for human health, it is also an important source of iron, copper, zinc and selenium (Rebak et al., 2010). The average carcass yield of buffaloes is around 42%. Summer-born calves of Egyptian buffaloes are significantly weaker than calves born in other seasons (Marai et al., 2009). Similar results are found for Egyptian buffalo (Chawla and Tripathi, 1994) and Indian buffalo (Peeva and Vankov, 1994) and other buffalo species. It has been determined that the daily live weight gain from birth to weaning is significantly affected by the birth season in Egyptian (Marai et al., 2009), Murrah and Surti Indian buffaloes (Bhavsar et al., 1974). The lowest daily weight gain between birth and weaning was determined in calves born in summer. Omran, (2013), in his study on the meat

quality (fiber diameter, fiber area, intramuscular fat) of buffalo calves exposed to heat stress in laboratory conditions (40 °C and 25 °C) for 6 months, it has been determined that the protein and ash content of the meat is higher, the lipid content is lower, the meat color is clear, the softness and taste test is excellent, the connective tissue and cooking loss are lower in the calves kept at 40 °C compared to those kept at 25 °C. Similarly, in another study, buffalo meat exposed to heat stress was found to have higher protein and ash content, lower lipid, lighter color, tender as veal, and excellent flavor. It has been stated that cooking loss is reduced due to low fat and moisture content, and heat stress does not affect the quality of meat (Rebak et al., 2010).

Goat: Goat meat is of higher quality for the human diet than other red meat, due to its lean, low content of saturated fatty acids and cholesterol. In a comparative study between Baladi and Damascus goat breeds, the decrease in body weight of Damascus goats under heat stress was greater (2.85% vs. 3.33%) (Helal et al., 2010). Pragna et al., (2018) also reported that the body weight and body condition score of Osmanabadi goats exposed to heat stress were significantly lower compared to Malabari and Salem Black breeds. Heat stress also reduced meat quality in goats. Kadim et al. (2006) determined that three Oman goat breeds had higher meat pH value and cooking loss after transport stress at high ambient temperatures. In the meat of animals exposed to heat stress and high ambient temperatures, the final pH value and shear force, cooking loss and drip loss were higher, crispness and water holding capacity were lower, so it was seen that heat stress affects eating and organoleptic properties (Rana et al., 2014; Kadim et al., 2004). It has also been stated that the meat of sheep and goats exposed to heat stress has higher odor, taste, water holding capacity and is susceptible to spoilage by microorganisms (Salama et al., 2014).

Sheep: Sheep are small ruminants kept in extensive conditions. Sheep meat is an important function of body weight. The average carcass yield of sheep is around 16 kg (FAOSTAT, 2014). As a result of global climate change affecting the amount and quality of feed, energy and protein intakes of sheep decrease, thus slowing down growth and causing a decrease in carcass weight (Gowane et al., 2017). On the contrary, it was observed that the Indian domestic sheep breed Malpura exposed to heat stress conditions showed no decline in growth compared to sheep kept in thermoneutral conditions (Sejian et al., 2010).

Climate change, especially high temperature and high relative humidity, has a significant impact on meat quality. According to Gregory (2010), the meat quality of sheep is more endangered in summer than in other seasons. Rana et al. (2014) stated that high temperatures will cause dehydration in thirsty animals and may affect meat quality by darkening meat color through shrinkage of myofibrils, and there will be less weight loss during cooking due to dryness of meat. Heat stress can also affect the usefulness and organoleptic quality of meat from both sheep and goats. Due to the high pH in the meat of sheep under heat stress, the color of the meat is darker (Kadim et al., 2006), the shearing force and cooking loss are higher, and the water holding capacity is lower (Archana et al., 2018). It has been determined that the pH value of the meat of Somali and Merino Sheep is higher in the hot season (5.77) than in the cold season (5.60) (Kadim et al., 2007). Similarly, the meat color of Barbados Black Bally lambs at 20 °C and 30 °C was adversely affected by heat stress. The meat color of lambs at low temperatures was brighter than those kept at high temperatures (Jallow and Hsia, 2014). In a comparative study of the effects of heat stress between Dorper and Poll Dorset × Merino/Border Leicester lambs, while the water holding capacity of Dorper lambs exposed to heat stress was lower compared to lambs in thermoneutral conditions, no negative effect was observed in crossbred lambs (Zhang, et al., 2020).

Kadim et al. (2008) found that the L*, a* and b* values of Somali Merino sheep fed in a heat stress environment (35 °C, 47% RH; 6 months) were significantly lower than those fed in the cool season (21 °C, 59% RH). Similarly, Al Amria et al. (2021), in their study examining the effects of seasonal ambient temperature and humidity on sheep and beef quality characteristics, showed that L*, a* and b* color measurements of sheep and beef meats in the hot season were significantly darker than those in the cold season. Thus, it was stated that high temperature increase (>40ºC) causes heat stress in sheep and cattle, which increases muscle pH and affects other properties of meat.

Pig: The decrease in feed consumption in pigs causes a decrease in daily live weight gain. When pigs are exposed to hot periods of 30-33 °C, it is seen that although they can compensate for growth, they cannot compensate for high temperatures of 36ºC and above (Babinszky et al., 2011). Similarly, pigs exposed to high temperatures above 25 °C have adverse effects on meat production and their feed consumption is reduced by 5-6 times compared to those exposed to 18-25 °C (lactation period), and also, the reduction in growth, carcass weight and feed consumption of larger pigs was quite evident (Nardone et al., 2010).

Studies highlight the fact that high temperatures not only slow down growth, but also change body composition and therefore can degrade the nutritional value and quality of pork. Prolonged heat stress (30-33 °C) reduced protein content in meat of growing and fattening pigs (Kerr et al., 2003). Rinaldo and Mourot, (2001) found that large white pigs (35 to 94 kg) reared in tropical climates had lower feed consumption, lower daily weight gain, leaner carcass, higher pH, lower water loss, compared to those raised in optimal climates, and a decrease in fat content on the entire back was observed. Heat stress in pigs caused decrease in growth, in feed efficiency, in carcass quality (increase in fat, decrease in protein accumulation) and reproductive performance

(Mayorga et al., 2019). Heat stress in pigs accelerates muscle glycolysis, significantly lowers muscle pH and redness (a*), increases drip loss and L* value, less protein is stored in the bodies of fattening pigs growing in hot environments, so the decrease in protein/fat ratio reduces meat quality (Gao et al., 2020).

Poultry: When poultry are exposed to heat stress, body weight gain, feed consumption, carcass weight, protein and muscle calorie content decrease (Tankson et al., 2001). High temperatures caused a decrease in protein synthesis and an increase in protein catabolism of chickens. Thus, there was a decrease in body and muscle tissue protein and a greater increase in fat level under heat stress (Gonzalez-Esquerra and Leeson, 2005). Although poultry consume more water in case of heat stress, the body's water holding capacity is significantly reduced due to the change in electrolyte balance (Borges et al., 2003). Carcass and meat quality characteristics such as crispness and color are critical to consumer acceptance. The effects of heat stress on meat quality in broilers were investigated and it was revealed that high temperature causes lower pH, denatured muscle protein and drip loss, and an increase in L* value and shear force. Exposure to heat probably accelerates the breakdown of glycogen, increasing lactic acid production due to glycolysis in the muscles, which leads to a decrease in pH (Hao and Gu, 2014). Water holding capacity is one of the important sensory properties of meat. Studies have shown that heat stress causes a decrease in body weight gain, rapid decreases in muscle pH and water holding capacity, an increase in protein and lipid oxidation of meat, dripping and cooking loss, Warner-Bratzler shearing force is significantly reduced, pale, tender, exudative It has been stated that meat is an important problem that causes economic losses in poultry meat production (Petracci et al., 2015; Wang et al., 2009). Zhang et al., 2012).

The other poultry: Heat stress also appears to affect the meat quality of other poultry. Meat of heat-stressed turkeys at 32-38 °C for 4 weeks before slaughter was found to have a pale flesh color and increased drip loss and reduced cooking loss after 24 hours compared to meat kept in thermoneutral conditions (McKee and Sams, 1997). In quail meats exposed to heat stress, drip loss increased compared to those left to the optimal temperature (Remignon et al., 1998). Similarly, heat stress caused an increase in cooking loss in rabbit meat (Zeferino et al., 2013).

5.4. Effects of Climate Change on Wool and Mohair Production and Quality

5.4.1. Sheep

Climate change caused a significant decrease in wool production between 1990 and 2000 (FAO STAT, 2010). Climate change has several potential effects on the quantity and quality of wool produced. Most of the impacts will occur indirectly, through changes in rangeland conditions, rather than the direct impact of climate. There are very few studies on the effect of climate change on wool/mohair quality. Wool production in lambs is completely related to nutrition. Extreme increases in temperature in the tropics and subtropics have reduced the wool yield (Gowane et al., 2017). In addition to the impact of climate change on wool production, sheep's nutrition, health, quality of rangeland grazing and water availability are also effective. Their deficiency also affects wool production. For example, the change in precipitation affects the amount and quality of feed, which in turn affects the feeding of the sheep. Thus, besides the wool yield, the quality characteristics of the fiber such as fineness and length are also affected by climate change. The decrease in precipitation and the increase in temperature caused the fleece fiber diameter to increase from thin to thick. However, as a result of insufficient feeding, length and strength decreased, and insufficient feeding of lambs also caused the deterioration of follicles (Harle et al., 2007). Jolly and Lyne (1970) found that by raising subcutaneous temperatures from 37 °C to 42 °C, an increase in temperature stimulates fleece growth, while a 5°C increase in subcutaneous temperature increases wool growth, but if the temperature increases too much, They stated that wool growth decreases, and if the temperature rises much more, wool growth stops.

5.4.2. Goat

Angora goats' most important yields are mohair and they depend on natural pastures for feeding. Natural pastures must have sufficient rainfall for plants to grow. Decreased precipitation or drought has a negative effect on the growth of plants in pastures, which causes goats to consume less and poor quality feed, resulting in a decrease in angora yield (De Waal, 1994). In addition, it is stated that there is a positive relationship between annual lint yield (kg) and precipitation (mm) in goats (Ng'ambi et al., 2009). Sarıçiçek (2021) found that the nutrient content and digestibility of the plants were lower, and the cell wall components were higher, especially in July and August, in low quality rangeland, as a result of this, it was determined that the mohair quality of Angora goat kids and cebich decreased, and there was a decrease in the mohair mineral (Ca, Mg, S) content.

5.5. Effects on Egg Yield and Quality of Climate Change

Poultry need a warm climate in the first days of their life (32-38 °C), but the optimal temperature decreases rapidly with age by 2.5-3.0 °C per week (FASS, 2010). The critical temperature for poultry is 30 °C. Since the feed conversion rate is better and the basal metabolic rate is lower in poultry up to this temperature, it can compensate for the energy loss that occurs as a result of the decrease in feed consumption (Esminger et al., 1990). In the case of heat stress, feed consumption decreases in poultry, and the digestibility of nutrients decreases due to decreased activity of trypsin, chymotrypsin and amylase (Hai et al., 2000). The decrease in feed consumption due to heat stress causes decrease in live weight, malnutrition, decrease in egg production and egg

weight, and decrease in growth rate in broilers (Tankson et al., 2001; Nardone et al., 2010). Researchers have determined that heat stress reduces egg production and egg weight (Emery et al., 1984), feed consumption reduces feed efficiency (Muiruri and Harrison, 1991), and egg or albumin weights (Wolfenson et al., 1979). In addition, it was determined that the eggshell quality decreased shortly after the hen was exposed to heat stress, and that acute heat stress significantly reduced feed consumption of all poultry. It has been reported that there is a significant interaction between egg weight, egg surface area, yolk index, yolk weight and white weight and the relative weights of various organs except gizzard (Ghoname et al., 2022). In warmer conditions, egg quality may be adversely affected, egg weight, shell weight and thickness (Mashaly et al., 2004), egg shell breakability may increase with decreasing egg specific gravity (Yahav et al., 2000). The decrease in feed consumption due to high environmental temperatures causes a decrease in the presence of Ca required for shell synthesis of the chicken (Nidamanuri et al., 2017). During heat stress, poultry lose a lot of CO_2 by panting. Since CO_2 is required for Ca-carbonate in eggshell formation, eggshell formation is compromised and egg production may decrease, as well as nutritional deficiencies (Mashaly et al., 2004). For the deposition of calcium carbonate in the eggshell, there must be approximately 4 to 12 times higher Ca in the shell gland fluid than in the serum, and this Ca must be obtained from the digestive tract, blood and bones. Approximately 2-2.5 g of Ca is taken from the blood by the shell gland and transferred to the egg for 15 hours (Kim et al., 2012).

5.6. Effects on Honey Production and Quality of Climate Change

It is stated that sudden weather changes caused by global climate change will cause great damage to the development of plants, especially during flowering. As average monthly temperatures increase, flowers may bloom earlier in the spring, creating a potential mismatch in seasonal timing between when flowers produce pollen and when bees are ready to feed on that pollen. As temperatures increased, North American and European wasp populations decreased, bees died when exposed to high temperatures in the southern regions, and their populations decreased in the northernmost regions (Turner, 2019). The increase in the duration and severity of summer drought may cause the drying and extinction of many plant species, thus changing the flowering periods of many plant species. Loss of natural flora may also cause loss of bee colonies (Potts et al., 2010).

Due to climate change, there is a decrease in bees at very high rates. In addition, the symbiotic interactions of bees and the flowering plants they pollinate are also affected (Belsky and Joshy, 2019). Climate change is cited as one of the causes of colony deterioration, especially in the most sensitive bioclimatic regions such as the Mediterranean regions. Adverse conditions significantly affect the evolution of bee populations, honey and pollen reserves, increase food stress for bees, and also affect the pollen spectrum and commercial properties of honey (Flores et al., 2018). Continuous high temperatures (>40-45 °C) cause significant losses in the colony. The highest foraging activity takes place in the temperature range of 12-25 °C, and no activity is observed at <7 °C and >43 °C. While the increase in extreme temperatures leads to an increase in colony losses, years with spring seasons characterized by very low precipitation and extreme temperatures will become more frequent in the future, which may lead to increased winter mortality rates (Soroye et al., 2020). Heat stress affects the growth and development of bees, as well as pollen services and foraging activities; these effects may not be exactly the same in different bee species (Willmer and Stone, 2004). Global warming also negatively affects honey bees used in plant pollination (Rader et al., 2013). Extreme temperatures put pressure on bee colonies due to the interactive relationship with honey bees and ecology (Biesmeijer et al., 2006). In addition, it is stated that each honey bee breed develops at its own pace, moving from one region to an unknown region or any change in climate has a negative effect on the development of bees, since the effect of rain on honey harvest for honey bees is known, it has been determined that environmental changes directly affect honey bee development, for example, some flowers such as Acacia flowers are no longer attractive to honeybees as they dilute the nectar when washed with rain (Conte and Navajas, 2008). Extreme temperatures can also cause plants not to produce enough pollen and nectar in the spring, which may cause honeybees to leave the hive (Kevan, 1999). Honeybees at lower altitudes remain active throughout the year, while at higher latitudes they go through a period of complete inactivity due to very long winters. This indicates that activity decreases significantly with increasing altitude due to colder months (Delgado et al., 2012).

6. Suggestions for Solutions on Animal Nutrition against the Effects of Global Climate Change

Climate change can cause environmental temperature rise and extreme weather conditions in certain parts of the Earth. Climatic conditions determine the energy and nutrient metabolism of livestocks. Since heat production is high after feed intake, livestocks reduce their feeding activities at high ambient temperatures, which has significant consequences on feed consumption. To maintain nutrient intake, increase the nutrient density of the ration, or restore homeostasis, the composition of the ration can be manipulated in hot weather so that the feed contributes less to the temperature increase, reducing the overall heat production of the animals. Here, the precautions to be taken in terms of feeding are emphasized.

6.1. Dry Matter Consumption

DMI decreases in summer months when the weather gets warmer. Decreased DMI in hot weather will result in undernutrition as it means less nutrients are taken. Therefore, it is necessary to balance the dry matter (DM) amount of their rations. For best DMI in a temperature stress environment, either the feeding interval should be reduced or the energy density of the ration should be increased.

6.2. Ration Protein Concentration

Crude protein (HP) and crude cellulose (HS) have low availability and higher temperature increases because of higher satiety thermogenic reactions. Excess rumen degradable protein reduces ME by 7.2 kcal/g N. Excess protein in the ration will require additional energy to excrete ammonia and urea, which will be released as a result of metabolism, through the kidneys. In most cases, the cow's amino acid needs can be balanced while reducing HP consumption, by selecting appropriate protein sources and by careful use of rumen-conserved amino acids (AAs).

a) When cows are exposed to heat stress, rumen degradable protein should not exceed 61% of ration HP and total protein (NRC, 2021) should not be more than 100 g N/d. One hundred grams of N is equivalent to approximately 3.1% CP in this ration, assuming 20 kg DMI/day. Optimizing protein that is not broken down in the rumen increases milk yield in hot climates.
b) High quality protein sources should be used in the rations of farm animals.

6.3. Ration Cellulose Content

For normal rumen physiology in ruminants, a source of cellulose must be present in the diet. However, in order to reduce the effect of heat stress, it is necessary to give ruminants and high quality roughage sources with digestibility. Otherwise, the temperature increases will increase due to the low availability of CF.

a) Consumption of diets with high CF content increases metabolic heat production in ruminants. Normally, high-fiber rations cause mechanical satiety and decrease feed consumption. DMI decreases when the ration NDF concentration is in the range of 27 to 35%, so an increase in NDF in hot environments causes a further decrease in DMI.
b) Since rations with high fiber content increase heat production, a ration with low fiber and easily soluble carbohydrates should be prepared. The CF content in the ration should be reduced appropriately.
c) Feed quality and digestibility: The composition of the feed affects the digestibility. Factors such as plant type, variety, nutrient content, harvest time, maturity and storage methods affect feed quality and digestibility. As the plant matures, the structural carbohydrate content of the feed increases and the easily soluble carbohydrate content decreases. To reduce heat stress, harvesting the roughage at the right time, depending on the type, is important to maximize the amount of nutrients provided by the roughage and its digestibility.

6.4. Concentrated Feed Level of Rations

In summer, the most limiting nutrient for dairy cows, especially in lactation, is usually energy consumption. Since roughage increases heat production, it further increases heat stress when the ambient temperature is high. Roughage should be reduced and the concentrated feed level of the ration order to increase the energy density should be increased. The aim is to reduce fiber consumption, to increase the energy density of the diet with more concentrated feed.

6.5. Adding Fat to the Ration

Fat should be added to increase the energy value of the rations and to reduce the roughage content. Since the energy content of fat sources (of vegetable and animal origin) is much higher than other nutrients and concentrated feeds (about 2-2.5 times more than carbohydrates), the addition of fat increases the energy density of the ration. One of the advantages of adding fat is that high-fat diets generate less heat, the other is that it improves the digestibility of certain amino acids and the addition of fat can change the amino acid ratio. Increasing the ration fat content also increases milk production in dairy cows under heat stress. Normally, the ration fat content (based on DM) is around 3-5%. With the addition of oil seeds, this rate can be increased by 2-3%, but it should not exceed 7-8%.

6.6. Roughage Can be Wetted

When roughage consumption decreases in hot weather, adding some water to dry roughage such as hay and hay given to cattle may cause an increase in feed consumption.

6.7. Increasing Feeding Frequency in Ruminants

Increasing the frequency of feeding helps to minimize the daily fluctuation in rumen metabolites and increase the efficiency of feed use in the rumen. Feeding should be at least two meal in a day, the number of meals can be increased to 4-5 in order to reduce heat stress, feeding should be done little by little at meals.

6.8. Poultry Ration

The correct ration should be formulated to meet the needs of the birds against heat stress. The protein content of the broiler diet should be reduced: Since protein has the highest temperature rise in broilers, low protein diets should be given to reduce heat production, but the dietary amino acid composition should be adjusted. It is necessary to use high quality protein and amino acids (eg methionine and lysine) to prevent the negative effects of heat stress. Methionine and lysine are essential to make up for deficiencies caused by consuming low-quality protein. This also applies to pigs.

6.9. Poultry Should Be Fed Wet

In a hot environment, wet feeding refreshes the animal and helps to improve the live weight and daily live weight gain.

6.10. Feeding Time in Poultry

Feeding times in poultry should be in the morning or evening or the night feeding concept should be followed. It is known that night feeding can improve eggshell quality. Thus, 1/3 of the ration to hens should be given early in the morning and 2/3 in the afternoon.

6.11. Feed Restriction

Early feed restriction may be beneficial as heat stress has a negative impact on broiler growth and immunity.

6.12. Cold Water Supply

There is a relationship between water consumption, DMI and milk yield. Generally, DMI and milk yield decrease in hot weather, but water consumption increases. Water temperature also affects water consumption. In hot seasons, the temperature of the water should be between 13-18 °C. Giving chilled drinking water to dairy cows increases milk yield as it absorbs heat and lowers body temperature.

6.13. Mineral Supplementation Should Be Done

With the decrease in feed consumption of animals, increase in sweat and urine amount in hot weather, some minerals (K, Na) are excreted, blood acid-base balance deteriorates and blood bicarbonate and buffering capacity decrease. Due to the urinary excretion of electrolytes, the need for minerals increases in hot weather. Suitable salts (ammonium chloride, sodium and potassium bicarbonate, sodium and potassium hydrocarbonate, potassium sulfate, etc.) may give good results for the diets of poultry, pigs and ruminants.

6.14. Some Additives Can Be Used

The use of some additives to increase the usefulness of feeds in hot conditions may give positive results.

a) Anti-oxidant vitamins (A, C and E) should be supplemented.
b) Essential micronutrient supplementation consisting of mineral blends and antioxidants may give a better result in improving the reduction in milk production caused by heat stress.
c) Enzyme use: Adding different enzyme supplements to the ration can improve the digestibility of nutrients such as amino acids, carbohydrates, and Ca and P. However, appropriate enzymes (phytase, xylanase, β-glucanase, etc.) should be used according to the composition of the feed.
d) Adding probiotic-based lactobacillus strains to poultry exposed to high temperatures can help balance intestinal microorganisms.
e) Additives that increase feed digestibility: The use of additives such as buffers, probiotics, prebiotics, niacin and yeast can improve the digestibility of feeds by increasing rumen fermentation.

6.15. Reducing Methane Production

Starch-rich diets increase propionic acid production and reduce methane production in the rumen. For this reason, methane can be reduced by increasing the ratio of concentrated feed in the ration, using vegetable and marine oils, oilseeds or essential oils and ionophores. Physical processing of feed, such as chopping, grinding and steam treatment, also improves forage digestibility and reduces enteric methane production in ruminants. The use of antimethanogenic plants in the diet is the most important solution. Increasing animal productivity is often a good strategy to reduce greenhouse gas emissions from livestock production systems. This is because high-yielding animals produce less methane than low-yielding animals.

6.16. For Bees

Various and flower-rich plants for pollinators should be planted in pastures, mixed and arable farmlands, burned forests, roadsides, gardens of houses, plant diversity should be ensured by planting wildflower seed mixtures rich in nectar and pollen, and bees should be able to collect pollen. Grow more flowers, shrubs and trees that provide nectar and pollen for bees and other pollinators throughout the year.

6.17. Cooling

In a temperature environment, the animals can be ventilated and cooled by using sprinklers or fans. In order to reduce heat stress in buffaloes, a pond, swamp, etc. water source or fountain system should be established.

6.18. Animals Should Be Provided With Good Shading

With the high body temperature produced by ruminants and pigs, direct sunlight especially in the hot season brings too much heat load to the animal and becomes harmful. Therefore, shade should be provided.

7. Conclusion

In parallel with the increase in the human population, the global demand for animal products is also increasing. However, climate change causes negative effects on animal production and productivity. The increase in temperatures due to global climate change causes heat stress and significantly affects the physiology, metabolism and productivity of animals. In addition to the increasing temperature, the change in precipitation regimes and the increase in drought negatively affeccetvelt the amount and quality of feed materials. Decreased quality of rangeland will cause malnutrition of grazing animals. This is especially important for ruminants. There is a very close relationship between the energy metabolism of animals and ambient temperature and animal performance and the quality of their products. As a result of insufficient feed consumption and lack of energy, there will be a decrease in animal products such as milk, meat, meat, eggs, hair / fleece and their quality will decrease. The studies carried out so far on this subject can help the livestock sector in the fight against climate change. In order to reduce greenhouse gas emissions caused by animals, it is imperative to make changes in the ration. Feeding strategies should be implemented to adapt to climate change and mitigate its effects, and as a result, quality and safe animal products should be produced for human nutrition without increasing the environmental burden of production. For this, there is a need for more studies on feeding in

different geographical regions and on different animal species.

Author Contributions

All tasks were done by the single author; B.Z.S. (100%) and the author reviewed and approved the manuscript.

Conflict of Interest

The author declare that there is no conflict of interest.

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