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TARIM ve DOĞA DERGİSİ

İÇİNDEKİLER

ARAŞTIRMA MAKALESİ - RESEARCH ARTICLE

KAHRAMANMARAŞ SÜTÇÜ İMAM ÜNİVERSİTESİ
TARIM ve DOĞA DERGİSİ

KAHRAMANMARAŞ SÜTÇÜ İMAM ÜNİVERSİTESİ
TARIM ve DOĞA DERGİSİ

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Computational Approaches for Drug Repurposing in Alzheimer's Disease

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ABSTRACT

Alzheimer's disease is a progressive age-related brain disorder. It causes gradual memory loss, changes in personality traits, confusion, impaired thinking, and mood changes Acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) inhibitors have been chosen for the treatment of Alzheimer's disease. Dual cholinesterase inhibitors have become a new hotspot in the investigation of anti-Alzheimer's drugs. The current study was designed to identify inhibitors for both AChE and BuChE enzymes using computational approaches to accelerate the process of identifying an effective treatment for Alzheimer. From the available drugs, we selected families of the aspirin and imatinib. After the adoption of molecular docking, we found that fendosal from aspirin group and Flumatinib from the Imatinib group are the most active compounds. The docking scores for fendosal was -8.160 kcal/mol against AChE while Flumatinib had -9.433 kcal/mol and -9.541 kcal/mol scores with BuChE and AChE, respectively. The 10 ns molecular dynamics simulation for fendosal and flumatinib against AChE and BuChE was performed to evaluate the drug's ability to remain stable within the binding sites of AChE and BuChE with the aid of RMSD and RMSF plots. These results revealed that Flumatinib and fendosal are good inhibitors for both BuChE and AChE, which could be used in vivo and in vitro studies to improve outcomes.

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Alzheimer Hastalığında İlacın Yeniden Kullanım İçin Hesaplamalı Yaklaşımlar

ÖZET

Alzheimer hastalığı, ilerleyici, yaşa bağlı bir beyin hastalığıdır. Kademeli hafıza kaybına, kişilik özelliklerinde değişikliklere, kafa karışıklığına, düşünme bozukluğuna ve ruh hali değişikliklerine neden olur. Alzheimer hastalığının tedavisi için asetilkolinesteraz (AChE) ve butirilkolinesteraz (BuChE) inhibitörü seçilmiştir. Çift kolinesteraz inhibitörleri, anti-Alzheimer ilaçlarının araştırılmasında yeni bir etkin nokta haline geldi. Bu çalışma, Alzheimer için etkili bir tedavi tanımlama sürecini hızlandırmak için hesaplama yaklaşımlarını kullanarak AChE ve BuChE enzimlerine yönelik inhibitörlerin belirlemesi için tasarlanmıştır. Mevcut ilaçlardan aspirin ve imatinib aileleri seçilmiştir. Moleküler yerleştirmenin benimsenmesinden sonra, aspirin grubundan fendosalın ve Imatinib grubundan flumatinib'in en etkin bileşikler olduğu bulunmuştur. Fendosal için kenetlenme skorları AChE'ye karşı -8,160 kcal/mol iken Flumatinib BuChE ve AChE ile sırasıyla -9,433 kcal/mol ve -9.541 kcal/mol skorlarına sahip olmuştur. AChE ve BuChE'ye karşı fendosal ve flumatinib için 10 ns moleküler dinamik simülasyonu, RMSD ve RMSF grafiklerinin yardımıyla ilacın AChE ve BuChE'nin bağlanma bölgeleri içinde stabil kalma kabiliyetini değerlendirmek için yapılmıştır. Bu sonuçlar, Flumatinib ve fendosal'ın hem BuChE hem de AChE için iyi bir inhibitör olduğunu ortaya koymuş olup sonuçların geliştirilmesi için in vivo ve in vitro çalışmaların kullanılabilir.

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INTRODUCTION

Alzheimer's disorder is the most prominent dementia condition and its global incidence has increased in recent years. Alzheimer's disease (AD) is described as synaptic dysfunction, oxidative stress, neuroinflammation, mitochondrial dysfunction, and disruption of the blood-brain barrier (Mendiola-Precoma et al., 2016). In 2021, more than 55 million people worldwide suffered from AD and dementia leading to a large number of deaths, but the data in 2020 cannot be stated as a precise number due to the COVID-19 outbreak (Gauthier et al., 2021). The disease has most frequently been observed in individuals over the age of 65, while about 4-5 percent of cases are early-onset. Down syndrome is a common form of early-onset dementia. Adults with down syndrome, after the age of 40, consistently display oncoming cognitive decline and dementia superimposed on their baseline cognitive limitations. They gather amyloid, neurofibrillary tangles, and cell depletion similar to sporadic AD (Gauthier et al., 2021). Alzheimer's disease is an age-related neurodegenerative disorder that leads to a rapid decline of physical, cognitive, and behavioral abilities (Jellinger, 2010).

Pathophysiologically, Alzheimer's disease is marked by the extracellular accumulation of Aß protein in amyloid plaques and the generation of neurofibrillary tangles resulting from the hyperphosphorylation of the tau protein linked with cellular microtubules (Brunton et al., 2011). Abnormalities in acetylcholine (ACh) and butyrylcholine (BCh) levels, which act as neurotransmitters, have been observed in the brains of patients with Alzheimer's disease. Inhibiting acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE)enzymes degrade ACh and BCh neurotransmitters and so it is accepted as one of the therapy choices for AD (Kim et al., 2018). Especially in patients with Alzheimer's disease, cholinesterase inhibitors are thought to i) enhance cognition and indirectly aid function and behavior, ii) improve cognition and overall outcome, and iii) ameliorate some stabilization of function during this time (Birks and Evans, 2015). Cholinesterase is a family of esters that degrade choline-based esters and many of which act as neurotransmitters (Colović et al., 2013). Thus, it is either of the two enzymes that stimulate the hydrolysis of these cholinergic neurotransmitters, like acetylcholine breakdown into choline and acetic acid (Colović et al., 2013). As part of the evaluation of the effects of aspirin and iminatip family compounds on cholinesterase enzymes, it is known that Alzheimer's disease affects many neurotransmitter systems, especially ACh and neurotransmission deficiency (Corbett et al., 2012).

Acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) inhibitor have been chosen for the treatment of Alzheimer's disease (Colović et al., 2013). Dual cholinesterase inhibitors have become a new hotspot in the investigation of anti-Alzheimer's drugs. Related families of aspirin and imatinib drugs are commonly used in the market. Our aim is to determine more economical and rapid anti-Alzheimer's inhibitors by evaluating the interactions amid commonly used drug groups with cholinesterase target structures ACh and BCh. Based on this main purpose, the effects of aspirin and imatinib family drugs as anti-Alzheimer inhibitors were assessed by using computational approaches to accelerate the process of an effective treatment and identifyng.

MATERIALS and METHODS

Molecular docking analysis

Molecular docking studies of the selected compounds from aspirin and imatinib families with AChE and BuChE were carried out with AutoDock Vina (Trott and Olson, 2010). The targeted protein structures (PDB ID: 1GQS for AChE and 4XII for BuChE) were obtained via the RCSB PDB database, and were prepared before the docking, including water removing, adding hydrogen atoms and missing residues and charges with help of Autodock Tools 1.5.6. The related ligands were optimized using the DFT/B3LYP/6-31G(d) basis set in Gaussian09 (Gaussian 09, Revision E.01, 2009). Autodock Tools was also utilized to define the grid box with the dimensions of 40 × 40 × 40 sizes. During the docking procedure, 100 conformations for each ligand were left flexible, while the protein was held rigid. At end of the docking processes, the docking conformations were ranked and determined the fendosal and flumatinib compounds according to their lowest binding energies, for each target

Molecular dynamics simulation

Molecular dynamics (MD) simulation is now considered a decision-making step in the computational investigations of drug discovery (Al-Khafaji and Taskin Tok 2021a). In the present study, two ligands obtained from the docking were subjected to the ligand-free and ligand-based simulations of the target enzymes via MD simulation on a 10-ns time scale. We used ‹GROMACS 2018.1 package'

(Abraham et al., 2015) to operate MD simulations. The three-point transferable intermolecular potential (TIP3P) was selected as solvent and the charge of the targets was adjusted by adding (Na^+ or Cl^- ions). The energy was reduced by using the steepest descent algorithm at a tolerance value of 1000 kJ/mol nm. In the next phase, the amount of substance's volume, temperature, moles, pressure, and temperature ensembles counterpoised the complexes with position restraint on molecules of each target protein for 0.1 ns. The particle-mesh Ewald (PME) was appointed for dealing with nonbonded interactions (Essmann et al., 1995). Now the MD simulation was carried out to determine stability for 10 ns, with no restraint on the protein molecules or ligands.

Both fendosal and flumatinib were carried out by MD simulation to determine stability for 10 ns without any restraint on the protein. The related ligands also were compared to the docking of both fendosal and flumatinib againt BuChE and AChE. The results of MD simulations give us the root mean square deviation (RMSD) of AChE and BuChE backbone atoms and also measure the root-mean-square fluctuation (RMSF) values for the assessment of chosen comparable drugs against BuChE and AChE..

Statistical Analyses

Shapiro-Wilk test was used to test the normality of data. Multiple comparisons were performed using one-way variance analysis, ANOVA or the Kruskal-Wallis test. Student t-test and Mann-Whitney U test were used for normal and non-normally distributed data in comparison of treatment groups, respectively. Results are expressed as mean ± SEM or median ± interquartile ranges (Q1 and Q3). A cut off level 0.05 was used for the statistical importance of the results. OriginPro 2021b program was used for statistical analysis (OriginPro, Version 2021b. OriginLab Corporation, Northampton, MA, USA.).

RESULTS

Results of Aspirin family

Results of the binding energy of the Aspirin family and ref ligand against to BuChE and ref ligand are summarized in Table 1. Diflunisal, fendosal, and salsalate had the lower binding energy against BuChE, which indicates that they have higher docking scores against BuChE. Salicylic acid had a higher binding energy against BuChE, which refers that it has the lowest docking score against BuChE. The fendosal displayed a significant interaction than the rest of chosen drugs against BuChE because it had the lowest binding energy, the highest docking score (Table 1), and the suitable RMSD value.

A docking comparison of aspirin family against AChE are given in Table 1. The same approach was carried out for the same compounds (Aspirin, Diflunisal, Fendosal, Phosphosal, Salicylic acid, Salsalate and reference ligand.) against acetylcholinesterase as a target. Fendosal had the lower binding energy against AChE, which indicates that it has the highest docking score against AChE. Salicylic acid had higher binding energy against AChE, which indicates that it has the lowest docking score against AChE. Fendosal significantly has stronger interaction than those of chosen remain drugs against AChE because it has the lowest binding energy, the highest docking score, and the suitable RMSD value, as given in Table 1.

Results of Imatinib Family

Results of the binding energy of ref ligand and the Imatinib family against to BuChE are given in Table 2. Imatinib, Flumatinib, and Nilotinib had the lower binding energy against BuchE, which indicates that they have higher docking scores against BuchE. Mocetinostat had the higher binding energy against BuchE. This shows that it's has the lowest docking score against BuchE. The Flumatinib significantly has stronger interaction than those of chosen comparable drugs against BuChE because it has the lowest binding energy, the highest docking score (Table 2), and the suitable RMSD value. The binding energy of ref ligand acetylcholinesterase AChE = -8.16 kcal/mol and the binding energy of AChE results with Imatinib family were exhibited in Table 2 . Imatinib, Flumatinib, and Nilotinib indicated the lower binding energy against AChE, which means that they have the highest docking scores against AChE. Mocetinostat had the higher binding energy against AChE and the lowest docking score against AChE. Flumatinib significantly has stronger interaction than those of chosen remain drugs against AChE because it has the lowest binding energy, the highest docking score and the suitable RMSD value. Results revealed that Flumatinib has the highest affinity to act as a dual inhibitor against AChE and BuChE (Table 2).

Docking results of the selected medicinal group was higher than the docking scores of the ref ligands when compared with the native ligand, diflunisal, fendosal, and salsalate. It is important to note that fendosal had significantly stronger interaction than that of chosen comparable drugs against BuChE (Table 1). A comparision of the selected Aspirin family drugs against the AChE for targeting Alzheimer's showed that fendosal has a binding affinity higher than the native ligand of AChE (Table 1). The rest drugs of the selected group had a binding affinity less than the ref ligand. Molecular docking results revealed that flumatinib, imatinib, and nilotinib have good binding affinities to interact with both AChE and BuChE as dual targets for targeting Alzheimer's.

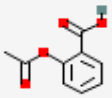
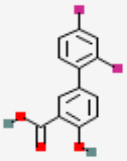
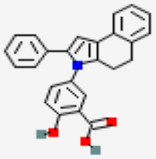
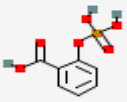
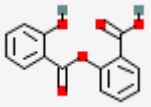
Molecular dynamics simulation results

The impact of ligand-protein interactions on the

dynamics of a biological system is crucial in drug discovery. The RMSD was used to explore the influence of fendosal and flumatinib drugs upon the stability of AChE protein and compare it with the apo form of AChE. We used Gromacs to perform the MD simulations of 10 ns for four drug-protein systems besides apo AChE protein. RMSD fluctuations for apo and holo forms were measured and presented in Figure 1. It was apparent that the AChE protein's backbone has indistinguishable-stable RMSD values (Figure 1) when NadR in apo, NadR-flumatinib, and NadR- fendosal forms. Fendosal had a similar fashion of RMSD backbone atoms when compared with apo AChE form. The RMSD values for flumatinib showed little fluctuations.

The RMSD average values of BuChE- fendosal were lower than the BuChE-flumatinib (Figure 2). Both of them fluctuated nearly to the RMSD values of BuChE apo form. According to the dynamic results in Figure 2, fendosal rather than flumatinib has RMSD values closer to the BuChE apo form, and therefore fendosal compound has priority.

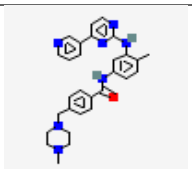
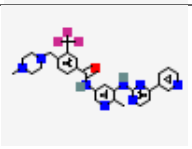
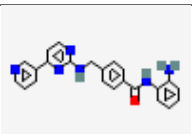
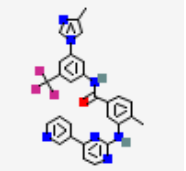
Table 1. Docking results of aspirin family against BuChE and AChE.
Çizelge 1. Aspirin ailesinin BuChE ve AChE'ye karşı kenetlenme sonuçları

No	Name of ligand *Ligandın adı*	PubChem CID *PubChem KID*	Structure of ligand (2D) *Ligandın yapısı (2D)*	Molecular Formula *Moleküler formül*	against BuChE (kcal/mol) *BuChE'ye karşı (kcal/mol)*	against AChE (kcal/mol) *AChE'ye karşı (kcal/mol)*
1	Aspirin *Aspirin*	2244		$C_9H_8O_4$	-5.340	-5.340
2	Diflunisal *Diflunisal*	3059		$C_{13}H_8F_2O_3$	-5.991	-5.990
3	Fendosal *Fendosal*	40821		$C_{25}H_{19}NO_3$	-7.836	-8.240
4	Fosfosal *Fosfosal*	3418		$C_7H_7O_6P$	-5.470	-5.631
5	Salicylic acid *Salisilik asit*	338		$C_7H_6O_3$	-4.610	-4.730
6	Salsalate *Salsalate*	5161		$C_{14}H_{10}O_5$	-6.560	-6.0130
7	Ref ligand *(ref ligandı)*	-	-		-5.490	-8.160

Root mean square fluctuation (RMSF) values explored the effect of fendosal and flumatinib upon the flexibility of AChE and BuChE backbone atoms were presented in Figure 3 and Figure 4, respectively. The binding of fendosal and flumatinib to the AChE conserved the binding site regions and whole proteins from fluctuation (Figure 3). Especially in the range of 2500-3000, 4500-5500 and 7000-8000, fluctuations in AChE backbone atoms are quite evident in the phendosal compound. On the other hand, Fendosal

and flumatinib increased the flexibility of BuChE in different regions. This indicates that both selected drugs effectively block the binding site of BuChE from different sites.

Table 2. Docking results of Imatinib family against BuChE and AChE.
Çizelge 2. Imatinib ailesinin BuChE ve AChE'ye karşı kenetlenme sonuçları

No.	Name of ligand *Ligandın adı*	PubChem CID *PubChem KID*	Structure of ligand *Ligandın yapısı*	Molecular Formula *Moleküler formül*	against BuChE (kcal/mol) *BuChE'ye karşı (kcal/mol)*	against AChE (kcal/mol) *AChE'ye karşı (kcal/mol)*
1	Imatinib *Imatinib*	5291		$C_{29}H_{31}N_7O$	-9.100	-9.390
2	Flumatinib *Flumatinib*	46848036		$C_{29}H_{29}F_3N_8O$	-9.433	-9.541
3	Mocetinostat *Mocetinostat*	9865515		$C_{23}H_{20}N_6O$	-8.0700	-7.980
4	Nilotinib *Nilotinib*	644241		$C_{28}H_{22}F_3N_7O$	-9.570	-9.340
5	Ref ligand *Ref ligandı*	-	-		-5.490	-8.160

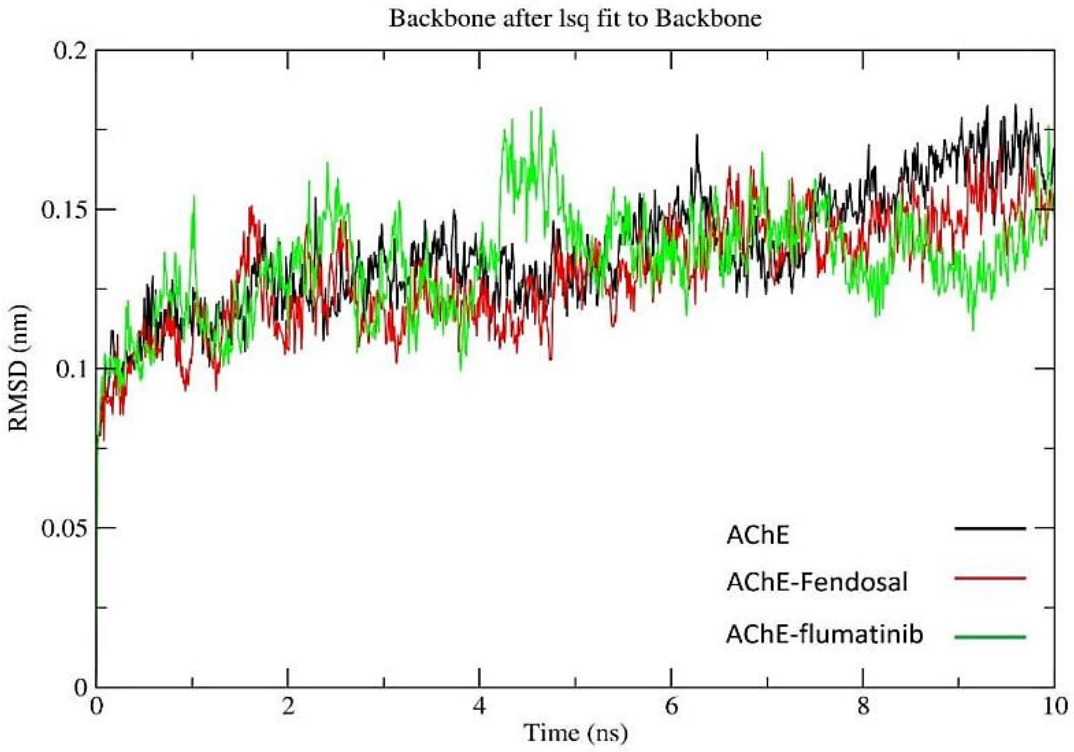


Figure 1. Analysis of RMSD of AChE backbone atoms
Şekil 1. AChE omurga atomlarının RMSD analizi

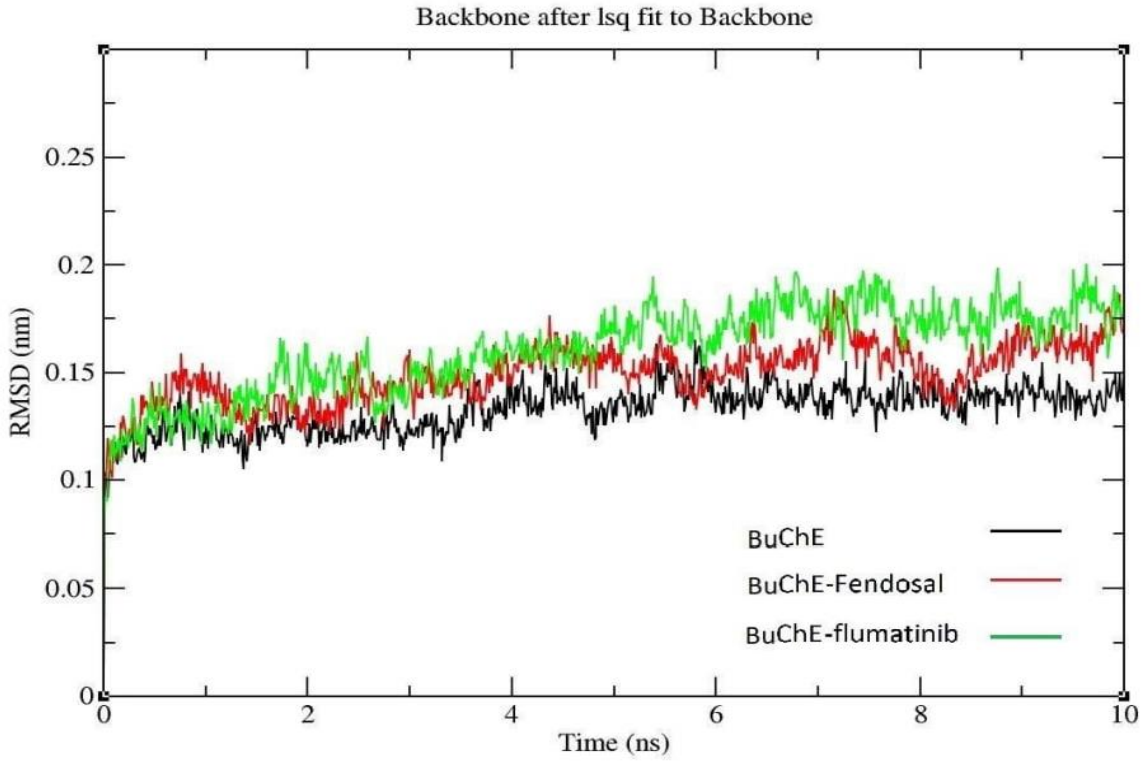


Figure 2. Analysis of RMSD of BuChE backbone atoms.
Şekil 2. BuChE omurga atomlarının RMSD analizi

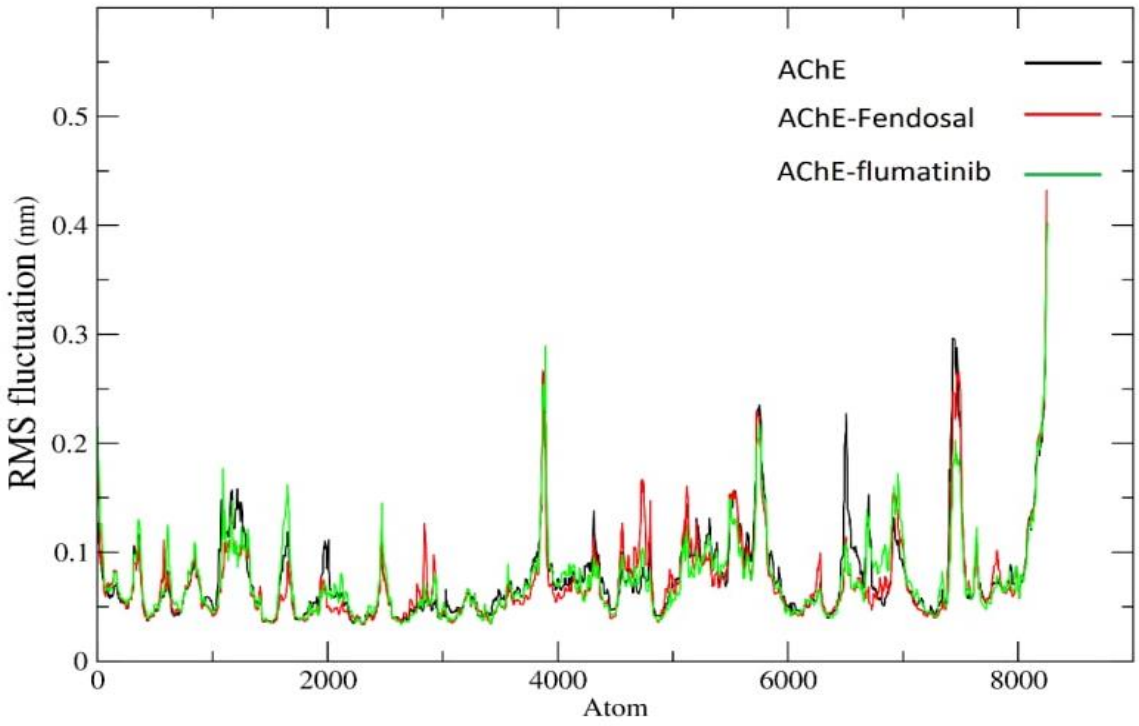


Figure 3. RMSF evaluations of AChE backbone atoms.
Şekil 3. AChE omurga atomlarının RMSF değerlendirmeleri

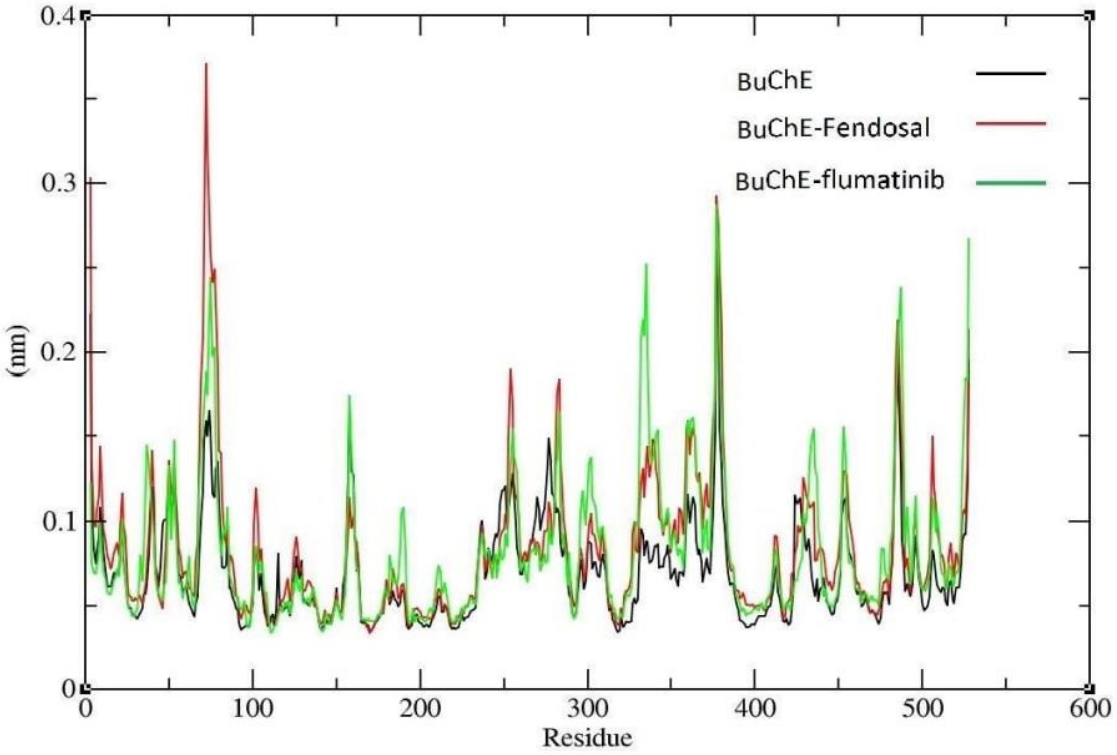


Figure 4. RMSF evaluations of BuChE backbone atoms.
Şekil 4. BuChE omurga atomlarının RMSF değerlendirmeleri

DISCUSSION

Alzheimer's illness is a retrogressive brain complication of unknown etiology, which is the most popular kind of dementia. Alzheimer's generally develops in middle age or old age, rises in gradual memory damage, defective thoughts, disorder, and personality and mood shifts (Wittenauer and Smith, 2013; Birks and Evans, 2015). The magnitude of computational tools has been investigated to identify effective treatment in a short time and the most promising targets are AChE (Al-Khafaji et al., 2021b) and BuChE as promising targets to stop or reduce the progression of Alzheimer's. We took the advantage of the possibility of Food and Drug Administration (FDA) available medicines that could be warheads to inhibit the AChE and BuChE proteins. Inhibition of cholinesterase in approximately 50 % of Alzheimer's patients stabilizes the cognitive function at a steady level during a 1-year period of treatment (Giacobini, 2003). The growing evidence revealed that both AChE and BuChE are significant proteins in the development and progression of AD (Greig et al., 2002).

Results of the present study indicated that Aspirin and imatinib have biological activities against Alzheimer's. The most interesting finding was that the fendosal compound has the binding affinities toward AChE and BuChE better than native ligands of both AChE and BuChE. Where the docking score of fendosal against BuChE (-7.836 kcal/mol) was smaller than the docking score of AChE's ref ligand (-5.490 kcal/mol). Further, fendosal had -8.240 kcal/mol which is lower than the ref ligand of AChE. This shows that only fendosal can act as a dual inhibitor from the relative drugs.

Contrary to this result, in the imatinib family, Nilotinib showed the best binding tendency with a binding energy value of -9.570 kcal/mol against BuChE, while Flumatinib compound took its place as the compound with the best interaction tendency against the AChE target with a binding energy of -9.541 kcal/mol. Based on docking scores, we nominated flumatinib and fendosal for further investigation by running a molecular dynamics simulation.

One interesting finding is the RMSD of protein of apo AChE's backbone has a similar fashion of fluctuation of AChE-fendosal and AChE-flumatinib. This reflects that both fendosal and flumatinib have a relaxed mode to bind efficiently with AChE. Whereas the binding of fendosal and flumatinib with BuChE increased the fluctuations in RMSD averages. Another remarkable notice was that the RMSF of the protein's backbone is more flexible when compared to an apo form of BuChE. It is possible to use fendosal and flumatinib in two ways: either alone or in a combined way. These findings suggest the potential using of nominated medicines against Alzheimer's in a short time. As well as the declared results are

noteworthy in at least showing the clinicians to adopt these safe remedies to suspend the evolution of Alzheimer's and secondly suggesting available drugs as a powerful therapy.

CONCLUSION

Based on molecular docking and molecular dynamics studies, the most prominent findings in the estimation of the interactions and activities of compounds selected from the aspirin and imatinib family against AChE and BuChE enzymes are that Fendosal compound exhibits strong inhibitory properties for both targets, while Flumatinib compound is more active against AChE enzyme than BuChE target. The other important result of this study is that Fendosal and Flumatinib can form stable interactions within the binding sites of AChE and BuChE with the help of MD simulation analysis. Comprehensively, both respective drugs show computationally good affinity for inhibition of AChE and BuChE enzymes. The results may suggest Fendosal and Flumatinib for extra clinical studies to evaluate inhibitory activities against AChE and BuChE enzymes.

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Statement of Conflict of Interest

The authors declare that there are no conflicts of interest.

Author's Contributions

The contribution of the authors is equal.

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Evaluation of Toxic Effects of *Dictamnus albus* L. Extracts on PC-12 and SHSY-5Y Cell Lines and Investigation of Antioxidant Activity

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ABSTRACT
This study investigated the antioxidant and cytotoxic properties of the *Dictamnus albus* L. plant grown in Türkiye. The aerial parts and roots of the plant were evaluated qualitatively in terms of chemical content. Total phenol and flavonoid amounts were calculated by spectrophotometric methods, antioxidant activity was tested with DPPH and ABTS radical scavenging activity assay. In addition, cell viability determination in PC-12 and SHSY-5Y cell lines was evaluated by performing MTT (3-4,5-dimethyl-thiazolyl-2,5-diphenyltetrazolium bromide) test. According to the results, both parts of the plant gave negative results in the tannin, cyanogenetic glycoside, anthraquinone, cardiac glycoside, and anthocyanoside identification tests, while they gave positive results in the alkaloid, coumarin, saponin, carbohydrate identification tests. While the total amount of phenol was calculated as 77.13 ± 5.73 mg_{GAE} $g_{exracts}$-1 in the extract prepared with methanol from the aerial parts of the plant (D.A Herba), it was calculated as 43.81 ± 9.49 mg_{GAE} $g_{exracts}$-1 in the extract prepared from the roots (D.A Root). The total flavonoid content could only be calculated in the D.A Herba extract (19.11 ± 0.16 mg_{CA} $g_{extract}$-1). Although the DPPH radical scavenging effect of the extracts was higher in D.A Herba extract, the ABTS radical scavenging effects were found similar in both D.A Herba and D.A Root extracts. According to the toxicity test, D.A Root extract reduced the viability below 50% (43.17 ± 3.44%) at 500 µg mL-1, but D.A Herba extract was found to be more toxic at the same concentration with 19.53 ± 0.183% in the PC-12 cell line. However, D.A. Herba and D.A.Root extract increased cell proliferation in the SHSY-5Y cell line at 3.25 µg/mL concentrations with 122.87±6.29 and 112.78±7.00%, respectively. The results suggest that *D. albus* may be a promising candidate for the new phytopharmaceuticals due to its neuroprotective effects.

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Dictamnus albus L' a Ait. Ekstrelerin PC-12 ve SHSY-5Y Hücre Hatlarında Toksik Etkilerinin Değerlendirilmesi ve Antioksidan Aktivitesinin Araştırılması

ÖZET
Bu çalışmada, Türkiye'de yetişen *Dictamnus albus* L. bitkisinin, antioksidan ve sitotoksik özellikleri araştırılmıştır. Bitkinin topraküstü ve kök kısımları kimyasal içerik bakımından kalitatif olarak değerlendirilmiştir. Toplam fenol ve flavonoit miktarı spektrofotometrik yöntemlerle hesaplanmış, antioksidan aktivitesi DPPH ve ABTS radikalini süpürücü aktivite deneyi ile test edilmiştir. Ayrıca PC-12 ve SHSY-5Y hücre hatlarında canlılık tayini MTT (3-4,5-dimetil-tiyazolil-2,5-difeniltetrazolyum bromür) testi yapılarak değerlendirilmiştir. Sonuçlara göre; bitkinin her iki kısmı da tanen, siyanogenetik glikozit, antrakinon, kardiyak glikozit ve antosiyanozit teşhis deneylerinde negatif sonuç verirken, alkaloit, kumarin, saponin, karbonhidrat teşhis deneylerinde pozitif sonuç vermiştir. Bitkinin topraküstü kısımlarından metanol ile hazırlanan

Biyoloji

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PC-12
Rutaceae
SHSY-5Y

ekstresinde (D.A Herba) toplam fenol miktarı 77,13 ± 5,73 mg_{GAE} g_{ekstre}-1 olarak hesaplanırken, köklerinden hazırlanan ekstresinde (D. A Root) ise 43,81 ± 9,49 mg_{GAE} g_{ekstre}-1 olarak hesaplanmıştır. Toplam flavonoit içeriği ise yalnızca D.A Herba ekstresinde hesaplanabilmiştir (19,11 ± 0,16 mg_{CA} g_{ekstre}-1). Ekstrelerin DPPH radikalini süpürücü etksi D.A Herba ekstresinde daha yüksek bulunurken, ABTS radikalini süpürücü etkileri her iki ekstrede de benzer bulunmuştur. Toksisite deneyi sonuçlarına göre, PC-12 hücre hattında 500 µg mL-1'de D.A Root ekstresi canlılığı % 50'nin altına düşürmüş (% 43,17 ± 3,44), ancak D.A Herba ekstresi aynı konsantrasyonda %. 19,53 ± 0,183 ile daha toksik etkili bulunmuştur. Ancak, D.A. Herba ve D.A.Root ekstresi SHSY-5Y hücre hattında 3,25 µg mL-1 konsantrasyonlarda sırasıyla % 122,87 ± 6,29 ve 112,78 ± 7,00 canlılık oranı ile hücre proliferasyonu arttırmıştır. Sonuçlar *D. albus* bitkisinin nöroprotektif etkileri sebebiyle yeni fitofarmasötiklerin için potansiyel adaylar olabileceğini düşündürmektedir.

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INTRODUCTION

Dictamnus albus L. is known as "Purple Dittany" or "Burning Bush", are shrub-shaped plants that have been grown as ornamental plants for centuries. The plant is belonging to the Rutaceae family and has a woody stem at the base, pinnate leaves, and large purplish-pink and white zygomorphic flowers. The whole plant is covered with aromatic glandular hairs with flammable essential oils (Compton and Akeroyd, 2019). *D. albus*, has a natural and wide distribution area almost all over the world throughout South and Central Europe, temperate Asia, and the Himalayas (Nissar et al., 2021). *D. albus*, which is accepted as a polymorphic species due to its distribution in a wide geographical area, has been tried to be distinguished by taxonomists (Compton and Akeroyd, 2019). Many researchers have accepted this plant as the only species in the world. All *Dictamnus* species, which are called different species in the literature, have been accepted as synonyms of *Dictamnus albus* (Plantlist 2021).

Dictamnus albus, which is generally known as an attractive decorative plant, has a wide variety of therapeutic properties due to its bioactive components (Gnatiuk 2019). The investigations contain various information that the plant is used in the cure of diseases by people in different countries traditionally. In Türkiye; flowering branches of *D. albus* are used as stomachic, stimulant, tonic and antipyretic and local names of the plant are "Akgirit otu or Gazel otu" (Baytop 2009). The plant, known under two different names as *Dictamnus dasycarpus* Turcz and *Dictamnus angustifolius* G. Don ex Sweet. in traditional Chinese medicine and "Cortex Dictamni" has been used in rheumatism, bleeding, itching, jaundice, chronic hepatitis, and skin diseases for centuries (LV et al., 2015). Indians have traditionally used the plant for its emmenagogue and abortive effects. It has been used in Israel to treat hypertension and ocular ailments such as cataracts, conjunctivitis, and diabetic retinopathy. In Bulgaria and Korea, people use this plant as a sedative, antispasmodic, diuretic, and it is stated to have anti-cancer effects. It is reported to be utilized as a tea mixture in the treatment of mental disorders such as neurasthenia, hysteria, and schizophrenia in Serbia. In addition, it is known that especially the root bark of the plant is used in the treatment of leprosy, cough, and amenorrhea (Nissar et al., 2021).

The main chemical components of *Dictamnus* species were determined as limonoids, alkaloids, sesquiterpenes, flavonoids, coumarins, terpenoids, and steroidals. Among these compounds, especially limonoids and quinoline alkaloids were identified as specific components of *Dictamnus* species. The biological activities of these active constituents are quite diverse. Limonoids and quinoline alkaloids are significant compounds with anticancer, anti-inflammatory, antimicrobial, antioxidant, antiviral, immunosuppressing, neuroprotective, and vascular-relaxing properties (Gao et al.,2021).

This study carried out various bioactivity tests of extracts prepared from roots and aerial parts of *D. albus* grown in Türkiye. The total phenol and total flavonoid amount of the extracts were analyzed by spectrophotometric methods. The toxicity profile of the plant was evaluated on PC-12 and SHSY-5Y cell lines and their antioxidant activities were tested

using radical scavenging methods.

Fundamentally, this research aims to evaluate the neuroprotective effects of the extracts of the *Dictamnus albus* plant grown in Türkiye, used for its therapeutic properties in different areas of the world, and to measure the antioxidant activity. In addition, different parts of the plant (root and herba), which traditionally used root bark in the treatment, were evaluated in terms of bioactivity for the first time by us. The obtained data are important in terms of comparing the secondary metabolites contained in the same species studied from different regions of the world and the resulting bioactivity tests and guiding the studies. Our preliminary research on this plant, which is accepted as a single species in the whole world and spreads in Türkiye, contains promising results.

MATERIAL and METHODS

Plant Material

Dictamnus albus was collected from Mecidiye/Keşan area from Edirne. The collected specimens were identified by Dr. Necmettin Güler and plant material is being stored in Ankara University Pharmacy Herbarium under voucher number AEF 30894. The roots and aerial parts of the plant were fragmented into two parts and dried under shade at room temperature.

Extraction of Plant Materials

After the root and aerial parts of the plant were roughly pulverized, they were placed in a shaker water bath to prepare methanol extracts. Plant materials to which methanol was added for 3 days were filtered at the end of each day and the filtrates were combined. Then the solvents were removed in vacuo and the extracts were obtained. All the extracts were lyophilized as powder and stored at -20°C until used in experiments.

Total Phenol Content

The total phenol content of the extracts was measured by revising the methods of Re et. al (1999). The samples were prepared by mixing extracts (50 µL), distilled water (3.95 mL), Folin Ciocalteu reagent (250 µL), and 20% Na_2CO_3 (750 µL), and kept in a water bath at 25 °C for 2 hours. The samples' absorbance was finally read at 760 nm and gallic acid was used as the reference to calculate the total phenol content (Singleton et al.,1999).

Total Flavonoid Content

The total flavonoid content of the extracts was measured by revising the methods of Singleton et al. with the colorimetric aluminum chloride assay. 4 mL distilled water was added to the 1 mL extract. A 0.3 mL solution of $NaNO_2$ (5%) was added to the mixture. After 5 minutes, 0.3 mL of a 10% $AlCl_36H_2O$ solution was added. Then 2 mL of 1 M NaOH was added, and the total volume was made up to 10 mL with distilled water. The test sample's absorbance was read at 510 nm. Catechin was used as the reference standard to calculate the total flavonoid content (Zhishen et al.,1999).

Phytochemical Analysis

Qualitative analyzes of the plant prepared from the roots and aerial parts were made according to the standard specified methods (Tanker and Sakar 1991). The presence of alkaloids, flavonoids, carbohydrates, coumarins; tannins, saponins, cyanogenetic glycosides, cardiac glycoside, anthraquinone, and anthocyanoside in the root and aerial parts of *D. albus* were evaluated.

Antioxidant Activity Assays

The antioxidant activity of the *D. albus* extracts was determined using DPPH radical scavenging assay (Gyamfi et al. 1999). In a test tube, the extract was mixed with 450 mL of Tris-HCl buffer and 1.0 mL of 0.1 mM DPPH after being dissolved in the proper quantity of MeOH. The extracts were measured at 517 nm after 30 minutes of incubation at room temperature in the dark. Butylated hydroxytoluene (BHT) was used as a positive control.

The antioxidant activities of the extracts were also measured by another method, $ABTS^{+\bullet}$ radical scavenging activity. 7 mM ammonium ABTS salt was dissolved in water and treated with 2.45 mM potassium persulfate. A dark blue solution was obtained by keeping this mixture at room temperature for 12-16 hours. The solution was then diluted with ethanol to have an absorbance of 0.7 at 734 nm. To measure the reaction kinetics, radical solution (990 µL) was added to the extract (10 µL) and absorbance was taken at 734 nm for 30 minutes at 1-minute intervals. Results were expressed in terms of antioxidant capacity equivalent to Trolox (Re et al., 1999).

Cell Viability Assay

PC-12 (ATCC, CRL-1721™ Passage no:8) and SHSY-5Y (ATCC, CRL-2266™ Passage no:5) were purchased from American Type Culture Collection (Manassas, VA, USA). Cells were seeded into a 75 cm2 flask using Roswell Park Memorial Institute Medium (RPMI-1640 30-2001™) and Dulbecco's Modified Eagle's Medium (DMEM 30-2002™) with 10% fetal bovine serum (FBS 30-2020™) and 1% penicillin-streptomycin (Gibco-Invitrogen, Grand Island, NY, USA) respectively. Then cells were grown in the incubator (at 37°C in an atmosphere supplemented with 5% CO_2) to reach the appropriate

density.

A total of $1x10^5$ cells per well were seeded in 96-well plates. Extracts were prepared by dissolving in the medium containing 1% dimethyl sulfoxide (DMSO) at a 4 mg mL^{-1} concentration. Cells were treated with varying doses of extract (7.8; 15.6; 31.25; 62.5; 125; 250; 500; 1000; and 2000 µg/mL) after 24 hours of incubation and allowed to re-incubate for another 24 hours. The medium was then removed and MTT (5 mg mL-1 of stock in PBS) was added. Cells were incubated with MTT dye for an additional 2 hours. At the end of the incubation period, absorbances (solution in each well) were measured at 570 nm in a microplate reader.

Statistical analysis

All statistical analyzes were made with the SPSS 12 (SPSS for Windows, 12.0, SPSS Inc. Chicago, IL, USA) statistical program. Analysis of variances was applied according to the ANOVA procedure. According to the results of Levene statistics applied to test the homogeneity of variance, Dunnet T3 test was used in the experiments where the assumption was not provided for the variable($p<0,05$). In the experiments where the assumption was provided, the analysis was made with the Tukey test($p>0,05$). IC50 values were calculated using a nonlinear regression algorithm.

RESULTS AND DISCUSSION

D. albus, which has a wide distribution area and is represented by a single species all over the world, has various biological activities related to its natural compounds. The plant is used especially in the treatment of various diseases in Chinese Folk Medicine. Cortex Dictamni has been used for the treatment of rheumatism, bleeding, itching, jaundice, chronic hepatitis, and skin diseases in China (Lv et al., 2015). The plant contains primarily limonoids, furoquinoline alkaloids, flavonoids, coumarins, sesquiterpene, and sesquiterpene glycosides (Chang et al., 2002). Due to these bioactive compounds, the plant has valuable medicinal properties such as anti-inflammatory, anti-tumor, antibacterial, and immune-regulating functions (Cao et al., 2022).

In the present study, qualitative analyzes were carried out to have preliminary information about the secondary metabolites of the root and aerial parts of *D. albus* species grown in Türkiye. The presence of alkaloids, flavonoids, carbohydrates, coumarins, tannins, saponins, cyanogenetic glycosides; cardiac glycoside; anthraquinone, and anthocyanoside in the parts of plants was tested qualitatively. These analyzes are based on visual observation of color change or precipitate formation after the addition of certain reagents. Phytochemical analysis results are given in Table 1. According to the results, the same results were obtained according to the diagnostic reactions performed on both the aerial parts and root parts of the plant (except for the flavonoid detection reaction). The presence of alkaloids, coumarins, carbohydrates, and saponins was detected in the aerial parts and root of the plant, while tests for cyanogenetic glycosides, cardiac glycosides, anthraquinones, and anthocyanosides gave negative results. The flavonoid identification reaction gave positive results only in the aerial parts. This result is thought to be related to the inability to quantitatively calculate the total amount of flavonoids in root extracts (Table 2). In a study conducted in Indonesia, the phytochemical content of *D. albus* roots was evaluated in terms of alkaloid, flavonoid, carbohydrate, coumarin, saponin, tannin, triterpenoid, and steroids, and similar results were obtained with our analysis. However, while the presence of saponin in the roots of the plant was not detected in the study, the saponin identification reaction gave a positive result in our study (Rohim et al., 2018).

Table 1. Phytochemical analysis of *D. albus* aerial parts and roots
Çizelge 1. *D. albus* topraküstü kısımları ve köklerinin fitokimyasal analizi

Parts of Plant	Alk*	Flav*	Carb*	Coum*	Tan*	Sap*	Cyano gly*	Card. Gly*	Anth*	Antho*
Aerial parts	+	+	+	+	-	+	-	-	-	-
Root	+	-	+	+	-	+	-	-	-	-

*Alk= alkaloid; Fla= flavonoid; Carbo= Carbohydrate; Cou= coumarin; Tan= tannin; Sap= saponin; Cyano Gly=Cyanogenetic glycosides; Card. Gly=Cardiac glycoside; Anth=Anthraquinone; Antho=Anthocyanoside

The total phenol and flavonoid content of the plant was studied by preparing methanol extracts. Total phenol and flavonoid contents of the extracts were calculated as equivalent to gallic acid and catechin, respectively. The total phenol content of the plant was calculated as 77.13 ± 5.73 mg_{GAE} $g_{extract}$-1 in the D.A. Herba extract and 43.81 ± 9.49 mg_{GAE} g_{extrac}t-1 in the D.A. Root extract. However, the total flavonoid

content in D.A Herba was also higher than in D.A Root (Table 2). In a study in which the content analysis and antioxidant capacity of some plants belonging to the Rutaceae family were determined, the total flavonoid content of the ethanol and methanol extract prepared from the aerial parts of the *D. albus* species was examined. According to the results of this study, the total flavonoid content in the methanol extract of the aerial parts of the *D. albus* collected from Serbia (7.27±0.12 mgrutin g-1) was lower than the methanol extract of the aerial parts of the *D. albus* plant collected from Türkiye (19.11 ± 0.16 mg_{CA} $g_{extract}$-1) (Pavlović et al. 2018). It is known that changes in the physiological activities of the plant significantly affect its chemical content, depending on the environment in which it lives. In addition, different methods applied and different concentrations are also reflected in the results obtained.

Table 2. Total phenol and total flavonoid contents of *D. albus* extracts
Çizelge 2. *D. albus ekstrelerinin toplam fenol ve flavonoit içeriği*

Extracts	Total phenol [$mg_{GAE}/g_{extract}$]	Total flavonoid [$mg_{CA}/g_{extract}$]
D.A. Herba	77.13 ± 5.73	19.11 ± 0.16
D.A. Root	43.81 ± 9.49	n.d*

The data are presented as mean ± standard error (n=3), (*n.d= not determination)

Many studies have shown that plants have significant antioxidant activity due to the natural chemicals they contain (Kähkönen et al. 1999, Karatoprak et al. 2017). In addition, studies show that many different methods are used to determine the antioxidant capacity of secondary metabolites with different chemical properties in plant extracts (Alam et al. 2013, Yucel et al 2017, Borjan et al. 2020). Thus, by applying different methods, more detailed information about the antioxidant effects of plants can be obtained.

The antioxidant capabilities of *D. albus* extracts were measured using DPPH• and $ABTS^{•+}$ radical scavenging effects in this research. The extracts' radical scavenging effects were investigated at concentrations ranging from 0.5mg mL-1 to 4mg mL-1. According to the DPPH• radical scavenging method, it was determined that D.A Herba extract at 4mg mL-1 concentration showed higher activity (56.86 ± 2.4%) than D.A Root extract (34.56 ± 2.77%). Furthermore, the antioxidant capacity of the extracts was compared with BHT, one of the most known synthetic antioxidants. For BHT, the % inhibition was found to be 85.4 ± 3.02 at 4mg mL-1 concentration. (Table 3). In other words, although the extracts are not as effective as BHT, they show moderate antioxidant capacity In a recent study, the antioxidant capacity of the parts of the *D. dasycarpus* Turcz (root whisker, core stem, and leaf) other than the root bark, which is known to be used in treatment, were evaluated. According to the results obtained, the highest DPPH radical scavenging effect was detected in the leaves of the plant (IC50:.0.133±0.23 µg mL-1), while the weakest effect was calculated in the root extracts (IC50: 3.681±0.56 µg mL-1) (Cao et al., 2022). Similarly, in this study, root extracts were found to be less effective than herba extracts (Tables 3 and 4). In a study investigating the antioxidant capacity of the *D. albus* from Indonesia, the inhibition was found to be 82%, especially at 50µg mL-1 of the root extract and it was stated that it had a high antioxidant effect (Rohim et al., 2018). This data obtained by Rohim et. al. is quite different when compared to our study. This dissimilarity may be due to differences in the extraction methods and test procedures. In addition, since plants may show different physiological characteristics according to the regions where they grow, they may have different chemical contents. In another study, the IC50 values of methanol and ethanol extracts prepared from the aerial parts of the plant were calculated as 59.80 ± 1.53 µg mL-1 and 76.48 ± 2.30 µg mL-1, respectively, and it was recorded to be moderately effective compared to rutin (Pavlović et al. 2018).

Table 3. DPPH• radical scavenging activity of *D. albus* extracts
Çizelge 3. *D. albus ekstrelerinin DPPH● radikali süpürücü aktiviteleri*

	%Inhibition			
Samples	4 mg/mL	2 mg/mL	1 mg/mL	0.5 mg/mL
D.A. Herba	56.86 ± 2.4^{e}	40.89 ± 2.71^{d}	$29.56 \pm 2.12^{b,c}$	$24.99 \pm 3.18^{a,b}$
D.A. Root	$34.56 \pm 2.77^{c,d}$	$26.19 \pm 3.17^{a,b}$	$23.32 \pm 5.57^{a,b}$	19.25 ± 2.83^{a}
BHT	85.4 ± 3.02^{f}	82.3 ± 3.01^{f}	78.8 ± 3.8^{f}	66.0 ± 4.5^{g}

Another frequently used method to measure antioxidant activity is ABTS radical scavenging activity assay. According to the results obtained with this method, when the extracts were compared with the positive control BHT, D.A Herba extract and D.A Root extract showed similar antioxidant effects at 4 mg mL-1, but not as effective as BHT. However, it was determined that the D.A root extract showed very weak antioxidant activity compared to D. A Herba extract in the concentration range of 0.5mg mL-1 - 2 mg mL-1 (Table 4).

Table 4. ABTS•+radical scavenging activity of *D. albus* extracts
Çizelge 4. *D. albus ekstrelerinin ABTS•+radikali süpürücü aktiviteleri*

	TEAC* (mmol/L/Trolox)			
Samples	**4 mg/mL**	**2mg/mL**	**1mg/mL**	**0.5mg/mL**
D.A. Herba	2.53 ± 0.00^{a}	$2.52 \pm 0.01^{*}$	$2.19 \pm 0.19^{+}$	1.58 ± 0.13^{I}
D.A. Root	2.52 ± 0.07^{a}	$2.13 \pm 0.18^{**}$	$1.35 \pm 0.14^{++}$	0.064 ± 0.06^{II}
BHT	2.93 ± 0.2^{b}	$2.57 \pm 0.8^{***}$	$2.55 \pm 0.9^{+++}$	2.50 ± 0.1^{III}

Values presented as mean ± standard errors (n = 3), with statistical analyses performed using the Tukey comparison test. Same lower case letter (a–b), and the same symbols (*–***), (+–+++), and (I–III) are not significantly (p>0.05) different.

Values presented as mean ± standard errors (n = 3), with statistical analyses performed using the Tukey comparison test. The same lower case letter (a–g) is not significantly (p>0.05) different.

Neurotoxic effects of extracts prepared from *D. albus* species grown in Türkiye were investigated on PC-12 and SHSY-5Y cells. Studies show that SHSY-5Y and PC-12 cell lines are widely used in the investigation of neuronal cytotoxicity (Siddiqui et al. 2021; Sharma et al., 2021; Zhang et al 2022). SHSY-5Y cells are used in neuronal differentiation, metabolism, neurodegenerative and neuroadaptive mechanisms, neurotoxicity, and neuroprotective research. PC-12 cells have similar properties to neuroblasts and neurons. PC-12 cells are frequently used to investigate cellular events such as proliferation, differentiation, cell viability, and apoptosis, as well as mechanisms involving neuronal repair, neuroprotection, and neurotoxicity (Wang et al., 2019).

D. A. Herba was applied to SHSY-5Y and PC-12 cells at varying concentration ranges (3.25-1000 µg mL-1), and cell viability was observed. Following, it was determined that D. A. Herba was toxic to SHSY-5Y cells at high concentrations, while it increased the proliferation of cells at low concentrations. In SHSY-5Y cells, it was observed that the viability decreased below 50% in the concentration range of 250-1000 µg mL-1, while an increase in cell viability was observed in the concentration range of 3.25-31.25 µg mL-1 (Figure 1). D. A. Herba is seen to be highly toxic, especially at a concentration of 1000 µg mL-1 (8.116 ± 1.98%).

In the PC-12 cell line, it was determined that D.A Herba showed high toxicity in the concentration range of 250-1000 µg mL-1 and reduced the viability below 50%. No toxic effect of the extract was observed in the concentration range of 125 and 3.25 µg mL-1 (Figure 2). As a result, it was determined that D.A Herba at concentrations of 1000 and 500 µg/mL was significantly toxic to both PC-12 and SHSY-5Y cell lines.

To evaluate the toxic effect of D. A Root extract on SHSY-5Y and PC-12 cells, it was applied for 24 hours under the same conditions. According to the results obtained, D. A Root extract was found to have a toxic effect on the SHSY-5Y cell line in the concentration range of 125-1000 µg mL-1 and it was observed that it reduced the viability below 50%. At the lowest concentration (3.25 µg mL-1), the extract increased cell proliferation (112.784 ±7.07%) (Figure 3).

When the effects of D.A Root extract on the PC-12 cell line were evaluated, it was observed that it significantly inhibited cell viability at a concentration of 1000 µg mL-1 (11.504 ± 1.44). However, no significant toxic effect on cells was observed in the concentration range of 3.25-250 µg mL-1 (Figure 4). In particular, D.A root extract reduced the viability to below 50% (47.931 ± 0.79) at 125 µg mL-1 concentration in the SHSY-5Y cell line, while the viability was 76.260±0.68% at the same concentration in the PC 12 cell line.

As a result, when the toxicity of the extracts on PC-12 and SHSY-5Y cells is compared in terms of IC50 values, it can be observed that D. A. Herba extract has less toxic effects on both cell lines, especially at decreasing concentrations (Table 5).

Many studies have been carried out to investigate the neuroprotective effects of this plant in the literature. (Jeong et al., 2010; Choi et al., 2011; Sun et al., 2015; Yoon et al., 2010). However, the toxicity of the extracts of *D. albus* grown in Türkiye on PC-12 and SHSY-5Y cells was investigated by us for the first time. Therewithal, studies have generally focused on limonoids isolated from the plant and identified as major active components. Sun et al. (2015) determined that six limonoids (dictangustones A-F) isolated from the root bark of *D. angustifolius* (Sym:

D. albus) were nontoxic to SH-SY5Y cells and showed considerable neuroprotective effect against oxidative stress-induced neuronal death. Furthermore, limonoids extracted from the plant have been shown to exhibit considerable neuroprotective action in rat cortical cells against glutamate-induced neurotoxicity (Yoon et al., 2008). It was also noted that obakunone

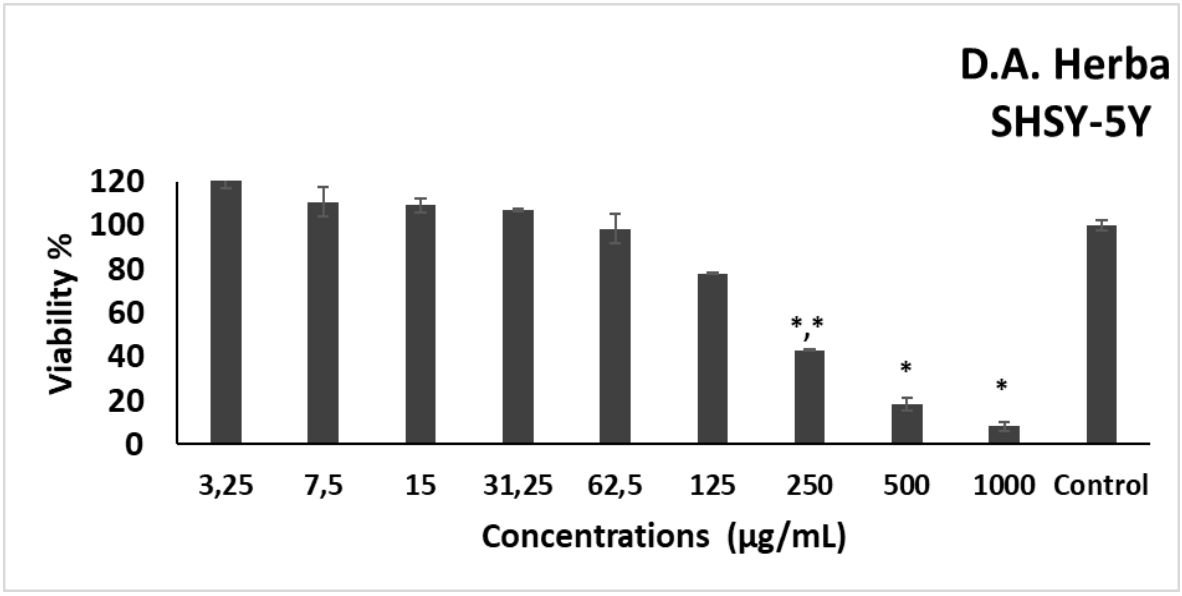

Figure 1. Toxicity profile of D. A herba extract in SHSY-5Y cell line. Values are presented as mean ± standard errors (n = 3), with statistical analysis performed using the Dunnett T3 comparison test.* p<0.001; ** p<0.01.

Şekil 1. *D. A Herba ekstresinin SHSY-5Y hücre hattında toksisite profili. Değerler, Dunnett T3 karşılaştırma testi kullanılarak gerçekleştirilen istatistiksel analiz ile ortalama ± standart hatalar (n = 3) olarak sunulmuştur.* p<0,001; ** p<0.01.*

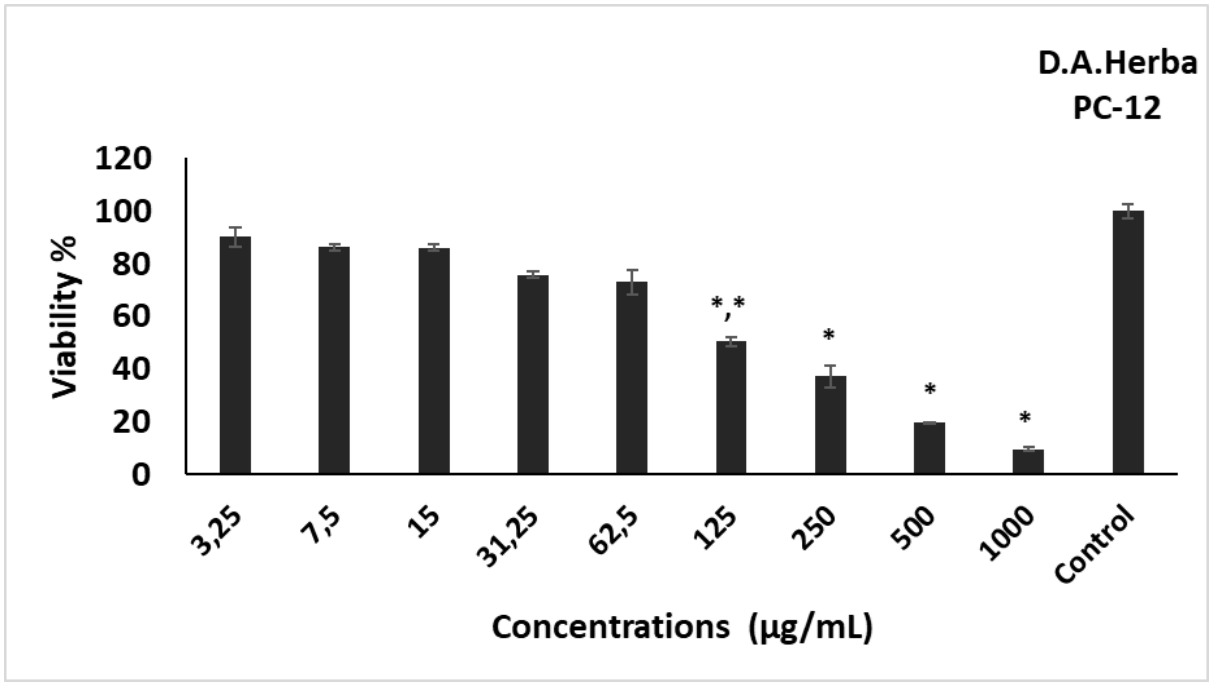

Figure 2. Toxicity profile of D. A Herba extract in a PC-12 cell line. Values are presented as mean ± standard errors (n = 3), with statistical analysis performed using the Dunnett T3 comparison test. * p<0.001; ** p<0.01.

Şekil 2. *D. A Herba ekstresinin PC-12 hücre hattında toksisite profili. Değerler, Dunnett T3 karşılaştırma testi kullanılarak gerçekleştirilen istatistiksel analiz ile ortalama ± standart hatalar (n = 3) olarak sunulmuştur. * p<0,001; ** p<0.01.*

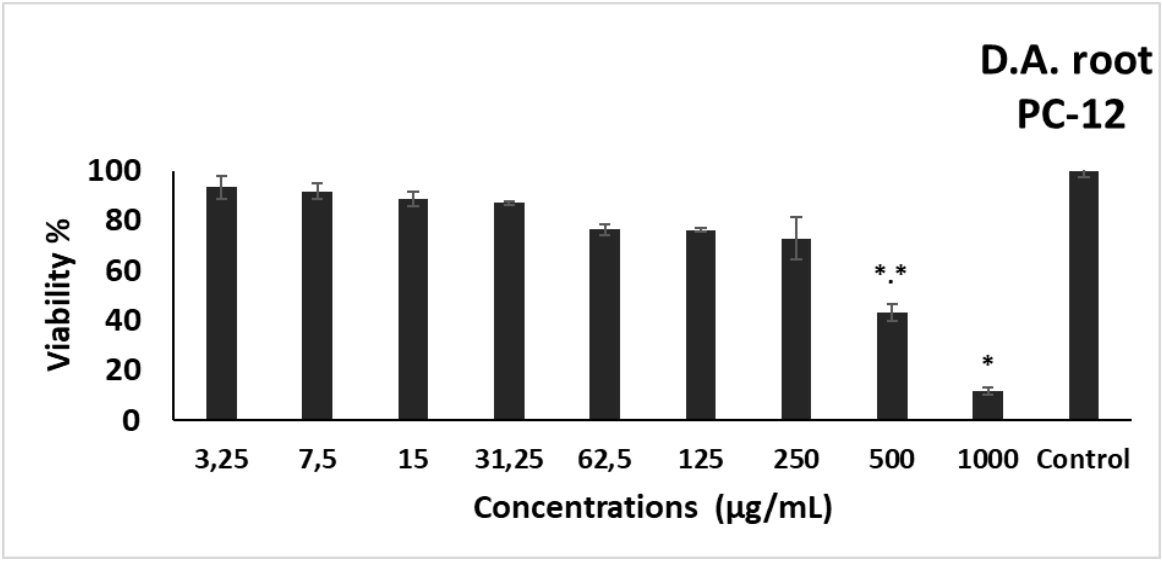

Figure 3. Toxicity profile of D. A Root extract in SHSY-5Y cell line. Values are presented as mean ± standard errors (n = 3), with statistical analysis performed using the Dunnett T3 comparison test. * p<0.001; ** p<0.01; *** p<0.05.

Şekil 3. *D. A Root ekstresinin SHSY-5Y hücre hattında toksisite profili. Değerler, Dunnett T3 karşılaştırma testi kullanılarak gerçekleştirilen istatistiksel analiz ile ortalama ± standart hatalar (n = 3) olarak sunulmuştur. * p<0,001; ** p<0.01.*

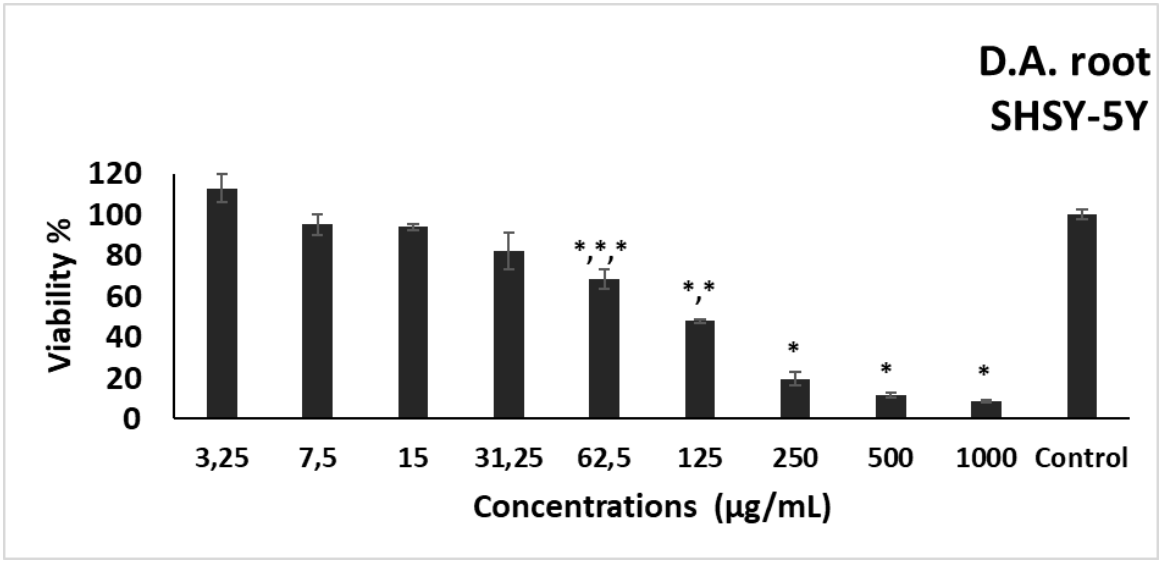

Figure 4. The toxicity profile of D. A Root extract in PC-12 cell line Values is presented as mean ± standard errors (n = 3), with statistical analysis performed using the Dunnett T3 comparison test. * p<0.001; ** p<0.01.

Şekil 4. *D. A Root ekstresinin PC-12 hücre hattında toksisite profili. Değerler, Dunnett T3 karşılaştırma testi kullanılarak gerçekleştirilen istatistiksel analiz ile ortalama ± standart hatalar (n = 3) olarak sunulmuştur. * p<0,001; ** p<0.01.*

Table 5. The IC50 value of D.A. Herba and D.A. Root extracts in SHSY-5Y and PC-12 cell lines were determined by the MTT assay

Çizelge 5. *D.A. Herba ve D.A. Root ekstrelerinin SHSY-5Y and PC-12 hücre hatlarında MTT deneyi ile belirlenen IC50 değerleri*

	IC50 (µg/mL) SHSY-5Y	PC-12
D.A. Herba	171.85±18.22	484.79±5.92
D.A. Root	92.12±2.48	167.26±1.76

isolated from this plant was preventive against glutamate-induced oxidative damage in mouse hippocampal HT22 cells (Jeong et al., 2010).

According to the results, although the extracts did not show a high antioxidant effect compared to the positive control, it was found to be moderately effective depending on the concentration. When the toxicity was evaluated, it was observed that the extracts showed neuroprotective effects on SHSY-5Y and PC-12 cells, especially at decreasing concentrations.

CONCLUSION

This research, which is a preliminary study for *D. albus* growing in Türkiye, is important in terms of determining its neuroprotective and neurotoxic effects as a result of the data obtained. The data will guide the planning of future studies. Thus, *D. albus*, which is a polymorphic species and spread all over the world as a single species, can be compared with taxa grown in other regions. This medicinal plant and its active ingredients can be evaluated in detail.

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Author's Contributions

The contribution of the authors is equal.

Statement of Conflict of Interest

The authors have declared no conflict of interest.

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Yulaf (*Avena sativa* L.) Tanelerinin Ultrasonik Destekli Etanolik Ekstraksiyonunun Toplam Antioksidan Kapasitesi, Radikal Süpürücü Aktivitesi ve Yağ Asidi Kompozisyonunun Belirlenmesi

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ÖZET
Bu çalışmada yulaf tanelerinin ultrasonik destekli ekstraksiyonu ile elde edilen ekstraktların toplam antioksidan kapasitesi, serbest radikal süpürme aktivetisinin belirlenmesinin yanı sıra yulaf ekstraktının yağ asidi profili ve toplam tokoferol içeriğinin belirlenmesi amaçlandı. Bu amaçla, bakır iyonları (Cu^{2+}) azaltıcı antioksidan güç testi (CUPRAC) metoduna göre yulaf taneleri ekstraktlarının toplam antioksidan kapasitesi belirlendi. Aynı zamanda, 1,1-difenil-2-pikril-hidrazil (DPPH•) yöntemine göre yulaf ekstraktlarının radikal süpürme kapasitesi araştırıldı. Yulaf ekstraktının yağ asidi komposizyonu GC-FID analizlerine göre belirlendi. Toplam tokoferol içeriği ise spektrofotometrik olarak belirlendi. Yulaf taneleri ekstraktının CUPRAC metoduna göre toplam antioksidan kapasitesi 1.5 ± 0.03 mmol TR /100g-yulaf taneleri ekstraktı olarak hesaplandı. Yulaf ekstraktının DPPH• radikali süpürme aktivitesi ise 2.02 ± 0.01 mmol TR /100 g – yulaf ekstraktı olarak hesaplandı. Yulaf özleri, güçlü antioksidan ve radikal süpürme yeteneği sergiledi. GC-FID sonuçları yulaf ekstraktının doymamış yağ asitlerince zengin olduğunu (Oleik asit, %43; Linoleik asit, %34) ortaya çıkardı. Yulaf taneleri ekstraktlarının toplam tokoferol içeriği ise 1376.3 ± 16 mg/kg-yulaf ekstrakt olarak bulundu.

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CUPRAC metod
Yağ asidi profili
Ultrasonik destekli ekstraksiyon

Determination of Total Antioxidant Capacity, Radical Scavenging Activity and Fatty Acid Composition of Ultrasonic Assisted Ethanolic Extraction of Oat (*Avena sativa* L.) Grains

ABSTRACT
In this study, it was aimed to determine the total antioxidant capacity and free radical scavenging activity of the extracts obtained by ultrasonic assisted extraction of oat grains, as well as to determine the fatty acid profile and total tocopherol content of oat extracts. For this purpose, we determined the total antioxidant capacity of oat grains extracts according to the copper ions (Cu^{2+}) reducing antioxidant capacity (CUPRAC) test. At the same time, the radical scavenging capacity of oat extracts was investigated by the 1,1-diphenyl-2-picryl-hydrazil (DPPH•) method. Fatty acid composition of oat extract was determined via GC-FID analysis. Total tocopherol content was determined spectrophotometrically. The total antioxidant capacity of the oat grain extract was calculated as 1.5 ± 0.03 mmol TR $100g^{-1}$-oat grains extract by CUPRAC method. The DPPH• radical scavenging activity of the oat extract was calculated as 2.02 ± 0.01 mmol TR 100 g^{-1} – oat extract. Oat extracts exhibited potent antioxidant and radical scavenging ability. GC-FID results revealed that oat extract was rich in unsaturated fatty acids (Oleic acid, 43%; Linoleic acid, 34%). Total tocopherol content of oat oil extracts was 1376.3 ± 16 mg kg^{-1}-oat extract.

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GİRİŞ

Yulaf (Avena sativa L.) , Poaceae familyasına aittir ve tek yıllık bir bitkidir. Dünya çapında ılıman ve serin subtropikal koşullarda yaygın olarak bir bahar ürünü olarak yetiştirilmektedir (Zakirullah ve ark., 2017). Mükemmel büyüme alışkanlığı, kaliteli yem, yüksek yem verimi, harika lezzet, iyi rejenerasyon kapasitesi ve yüksek protein içeriği nedeniyle genellikle hayvan yemi olarak kullanılır (Saleem ve ark., 2015; Kumar ve ark., 2017). Başlangıçta tıbbi amaçlar için kullanılmış ve daha sonra önemli bir gıda tahılı haline gelmiştir. Yulaf, besin değeri nedeniyle eski zamanlardan beri önemli bir ürün olarak kabul edilmektedir (Martínez Villaluenga ve Peñas, 2017). Bugün yulaf hâlâ sağlıklı tam tahıl olarak bilinir (Singh ve ark. 2013; Stewart ve McDougall, 2014), yüksek konsantrasyonlarda doymamış yağlar (Carlson ve ark., 2019) ve kalp sağlığını destekleyen ß-glukanlar içerir (Newell ve ark., 2012). Yulaf, hipokolesterolemik etkileri, glisemik kontrolü ve bağırsak mikrobiyotası üzerindeki olumlu etkisi ile popülerdir ve aynı zamanda diğer tahılların çoğuna kıyasla son derece yüksek protein içeriğine sahiptir (Carlsson-Kanyama ve González, 2009).

Yulaf tanesi sağlık açısından önemli doymamış oleik ve linoleik yağ asitlerinden oluşan önemli bir yağ içeriğine sahiptir. Esansiyel yağ asitleri olarak da bilinen linoleik ve linolenik asitler insan ve diğer memelilerde yaşam için mutlak gerekli olan ve vücutta sentezlenemediğinden diyetle alınması gereken çoklu doymamış yağ asitleridir (Çakmakçi ve Kahyaoğlu, 2012). Bu yağ asitlerinin dokuların sağlıklı gelişmesi ile; organların dengeli çalışmasına yardımcı olmalarının yanı sıra vücudun biyokimyasal ve fizyolojik aktiviteleri üzerinde dengeleyici, yapıcı ve onarıcı görevler üstlendiği bilinmektedir (Gogus ve Smith 2010).

Yulaf, aynı zamanda antioksidan aktivite gösteren birçok bileşiğin kaynağıdır. E vitamini (tokoller), fitik asit, fenolik bileşikler ve avenantramidler yulafta en bol bulunan antioksidanlardır. E vitamini olarak da bilinen tokoferoller yemeklik yağlarda yaygın olarak bulunur ve güçlü antioksidan aktivite sergiler (Fritsche ve ark., 2017). E vitamini, ciltte en bol bulunan antioksidandır (Graf, 2005). Kişisel bakım kozmetik ürünlerinde E vitamini ve türevleri sıklıkla kullanılmaktadır. Çünkü topikal olarak uygulanan E vitamini cildi serbest radikal hasarından ve güneşin zararlı ışınlarından korunmasında büyük rol oynar (Godic ve ark., 2014). Ayrıca yulaf tanelerinde flavonoidler ve steroller de mevcuttur (Peterson, 2001). Bu antioksidanlar, çekirdeğin dış katmanlarında yoğunlaşmıştır. Yulaf özütlerinin antioksidan aktivitesini değerlendirmek için çeşitli in vitro yöntemler kullanılmıştır (Kilci ve Gocmen, 2014). Bu yöntemlerden biri olan, Cupric İndirgeyici Antioksidan Kapasitesi (CUPRAC) yöntemi, besin bileşenleri, sentetik antioksidanlar, C ve E vitaminleri dahil olmak üzere birçok farklı bileşeni uygulamak için basit ve çok yönlü bir antioksidan kapasite yöntemidir (Apak ve ark., 2004). Bu reaktif, kararlı, ucuz, kolay ulaşılabilen, hidrofilik ve lipofilik antioksidanlara cevap verebilen bir reaktiftir. CUPRAC reaktifi, diğer kromojenik reaktiflere (örn. ABTS, DPPH) göre daha kararlı ve erişilebilir avantajlara sahip olduğu rapor edimiştir (Çelik ve ark., 2008). DPPH• yöntemi ise kısa sürede birçok numuneyi barındırabilmesi ve düşük konsantrasyonlarda aktif maddeleri tespit edecek kadar hassas olması nedeniyle, meyve-sebze suları veya ekstraktlarının antiradikal aktivitelerini taramak için yaygın olarak kullanılmaktadır (Zou ve ark. 2004). Ayrıca, testin kullanımı basittir ve test maddelerinin termal bozunmasını önleyen ortam sıcaklığında gerçekleşmesi avantajdır (Peterson,2001).

Serbest radikaller canlı hücreleri parçalayan, yaşlanmaya ve hastalıklara neden olan yıkıcı moleküllerdir. Serbest radikaller, eşleşmemiş elektrona sahip moleküllerdir. Organizmadaki serbest radikallerin çoğu moleküler oksijenden oluşan radikallerdir. Moleküler oksijen, diradikal doğası nedeniyle oldukça reaktif oksijen türleri (ROS) oluşturma eğilimindedir. ROS, normal oksijen metabolizması sırasında küçük miktarlarda oluşan süperoksit radikali ($O2^{\cdot-}$), hidrojen peroksit (H2O2) ve hidroksil radikalidir ($OH^{\cdot}$). ROS nükleik asitler, lipidler, proteinler ve karbonhidratlar dahil olmak üzere önemli biyomoleküllere zarar verebilir ve buna bağlı olarak, mutasyonlara yol açabilecek DNA hasarına neden olabilir. (Ak ve Gülçin, 2008). Benzer şekilde reaktif azot türleri (RNS) de, DNA, proteinler ve lipidler gibi farklı biyomolekülleri oksitleyerek epitel hücre hasarına ve ölümüne yol açabilir (Boukhenouna ve ark., 2018). Reaktif azot türlerinin başlıcaları ise (RNOS); nitrik oksit radikali ($NO^{\cdot}$), peroksinitrit radikali ($ONOO^{\cdot}$) ve azot dioksit radikalidir ($NO_2^{\cdot}$). Süperoksit radikalinin beyindeki nöronal ve endoteliyal nitrik oksit sentaz (NOS) aracılığı ile sürekli oluşan ve bir gaz radikal olan nitrik oksit ile girdiği reaksiyon sonucu peroksinitrit

oluşur (Beckman ve ark., 1990). $ONOO^-$ güçlü bir oksidandır, NO ve $O_2^{\cdot -}$ 'den daha toksiktir (Keynes ve Garthwaite, 2004).

Yeni ekstraksiyon yöntemlerinden biri olan Ultrason Destekli Ekstraksiyon (UDE), biyoaktif bileşiklerin farklı kaynaklardan geri kazanılması için yeni ve basit bir tekniktir (Carreira-Casais ve ark., 2021). Ultrason gücünün yoğunluğu, numune moleküllerinde ekstra titreşim üretir ve hedef bileşiklerin katıdan sıvı çözücü fazına geri kazanılmasını kolaylaştırır (Okur ve ark., 2019). UDE, ekstraksiyon süresini kısaltmak, ekstraksiyon verimini yükseltmek ve ekstraktın kalitesini iyileştirmek için bitki bileşenlerinin ekstraksiyonu için sıklıkla kullanılmıştır (Toma ve ark., 2001). Bu bitkilerden yulafla ilgili ekstraksiyon çalışmaları, Maserasyon (Bryngelsson ve ark., 2002), sokslet ekstraksiyonu (Biel ve ark., 2014; Kouřimská ve ark., 2018), hızlandırılmış solvent ekstraksiyonu (Yang ve ark., 2019) ve ekstrüzyon işleme (Liu ve ark., 2020) gibi yöntemleri içerir. Bununla birlikte, literatürde, ulstrasonik destekli ekstraksiyon yönteminin yulaf üzerinde, ekstrakt verimi, yağ asidi profili, toplam tokoferol içeriği ve toplam antioksidan aktivitesi gibi özellikleri bakımından ne gibi bir değişiklik yapacağına ilişkin araştırmalar sınırlıdır.

Bu çalışmada; yulaf tanelerinin ultrasonik destekli etanolik ekstraksiyonunun CUPRAC metoduna göre toplam antioksidan kapasitesi, DPPH metoduna göre serbest radikal süpürme aktivitesi belirlenmesi amaçlanmıştır. Çalışmanın diğer bir amacı ise, yulaf tanelerinin ultrasonik destekli ekstraksiyonundan yüksek verimlilikte ekstraktlar elde edilmesi ve elde edilen ekstraktların yağ asitleri profilinin ve toplam tokoferol içeriğinin belirlenmesidir.

MATERYAL ve METOD

Bitki materyali

Mevcut çalışmada kullanılan yulaf taneleri yerel bir satıcıdan temin edildi. Örnekler ayrıca Doç. Dr. Sabri Erbaş tarafından da teşhis edilip, Isparta Uygulamalı Bilimler Üniversitesi Ziraat Fakültesi Endüstri Bitkileri laboratuvarına YLF32-2022 fiş numune numarası ile bırakıldı.

Kimyasallar

Bakır(II) klorür dihidrat ($CuCl_2.2H_2O$), Neocuproine (Nc:$C_{14}H_{12}N_2$), 2,2′-Bipiridin ($C_{10}H_8N_2$), DL-α-Tokoferol, DPPH•(2,2-difenil-1-pikrilhidrazil: $C_{18}H_{12}N_5O_6$) ve Troloks (TR), Sigma Chemical Co.'dan (St. Louis, MO, ABD) satın alındı. Amonyum asetat (NH_4Ac) ve Demir(III) klorür heksahidrat ($FeCl_{3.6}H_2O$) Merck (Darmstadt, Almanya)' ten satın alındı. Mutlak etanol (EtOH, %99), ISOLAB Laborgeräte GmbH (Eschau, ALMANYA)'den satın alındı.

Çözeltilerin hazırlanması

10 mM $CuCl_2$ çözeltisi, 0.4262 g $CuCl_2.2H_2O$' nun az miktarda distile suda çözünmesi ve distile su ile 250 mL' ye seyreltilmesiyle hazırlandı. 1.0 M amonyum asetat tamponu (pH 7.0), 19.27 g NH_4Ac'nin az miktarda distile su içinde çözünmesi ve 250 mL' ye seyreltilmesiyle hazırlandı. 7.5 mM Neocuproin (Nc) çözeltisi, 0.078 g Nc' nin %96 etanol içinde çözünmesi ve etanol ile 50 mL' ye seyreltilmesiyle günlük olarak hazırlandı. DPPH• çözeltisi (0.2 mM), 7.88 mg DPPH' nin %96' lık etanol içinde çözünmesi ve etanol ile 100 mL'ye seyreltilmesiyle hazırlandı. %0.2' lik FeCl3.6H2O çözeltisi; 0.2 g Demir(III) klorür heksahidrat etanolde çözünmesi ve %95' lik etanolle 100 mL' ye seyreltilmesiyle hazırlandı. %0.07' lik 2,2'-bipiridin çözeltisi; 70 mg 2,2′-Bipiridin'in %95' lik etanol ortamında çözünmesi ve etanol ile 100 mL' ye seyreltilmesiyle hazırlandı.

Ultrasonik - Destekli Ekstraksiyon (UDE)

Yulaf tanelerinin ultrasonik destekli etanolik ekstraksiyonu Zhang ve ark. (2009) tarafından önerilen yönteme göre gerçekleştirildi. Öncelikle nemden arındırılmış yulaf taneleri ekstraksiyondan önce kahve değirmeni (Sinbo SCM 2934-Türkiye) ile öğütülerek hazırlandı. 10 g öğütülmüş yulaf, kapalı bir şişede 100 mL %96' lık etanol ortamında ekstraksiyona tabi tutuldu. Ekstraksiyon için ultrasonik banyo sistemi (Power sonic 180, 40 kHz frekans ve maksimum 150 W, iç ölçü: 300 mm × 150 mm × 100 mm) kullanıldı. Ultrasonik ekstraksiyon, aşağıdaki deneysel koşullar altında gerçekleştirildi: sıcaklık: 50 °C; zaman: 45 dakika; katı/çözücü oranı: 1:10 (w/v) ve maksimum ultrason gücü: 40 kHz ve 150 W güç. Toplanan ekstraktlar Whatman No: 1 filtre kağıdından süzüldü ve çözücüler döner bir buharlaştırıcı (IKA RV 10 digital, IKA, Almanya) ile 55 °C' de vakum altında buharlaştırılarak uzaklaştırıldı. Ekstraksiyon prosedürü paralel olarak üç kez gerçekleştirildi. Ekstraksiyon verimi aşağıdaki eşitlik (1) kullanılarak hesaplandı.

% Verim= (Ekstrakt ağırlığı / kuru örnek ağırlığı) x 100 (1)

Yağ asitlerinin GC analizi

Etanolik yulaf taneleri ekstraktlarından, Fathi-Achachlouei ve Azadmard-Damirchi (2009) tarafından bildirilen yönteme göre yağ asidi metil esterleri (FAME' ler) hazırlandı. Kısaca, 0.5 mL hekzan içinde çözünmüş yulaf taneleri ekstraktı (yaklaşık 20 mg) içeren bir tüpe metanol içinde 2 mL 0.01 M NaOH ilave edilip, 10 dakika boyunca 60°C' deki su banyosunda tutuldu. Daha sonra metanol içinde bor triflorür (metanol içinde %20 BF_3) ilave edilip, numuneler 60°C' deki su banyosunda 10 dakika daha bekletildi. Numune, akan su altında soğutuldu. 2 mL %20 (a/h) sodyum klorür ve 1 mL hekzan ilave edildi. Tamamen karıştırıldıktan sonra,

Fame'leri içeren hekzan tabakası, santrifüjleme ile ayrıldı ve daha sonra GC ile analiz edildi. Esterleşmiş yağ asitlerinin (FAME) toplandığı üst fazdan 1 µL çekilerek gaz kromatografisi (GC-FID) cihazına enjekte edildi. Yağ asitlerine ilişkin kromatogramlar elde edilerek yağ asitlerinin oranları belirlendi. GC-FID cihazının çalışma koşulları şu şekildedir (Önder ve ark., 2022): Kolon Teknokroma TR-CN100 (100 m × 0.25 mm, 0.20 µm), enjektör sıcaklığı 250 °C, detektör sıcaklığı 250 °C, akış hızı (psi) 10, taşıyıcı gaz N_2 (40 mL dk^{-1}), enjektör kapasitesi 1.0 µL'dir. Fırın sıcaklığı 140 °C'de 10 dakika bekledikten sonra 240 °C'ye dakikada 3 °C'lik artışla yükseltilip, bu sıcaklıkta 10 dakika bekletildi. Elde edilen kromotogramlardaki pikler ticari standart yağ asidi metil ester karışımına (Sigma, Supelco® 37 Component FAME Mix) göre isimlendirildi (Erdoğan ve Homan Gökçe, 2021).

Toplam tokoferol analizleri

Toplam tokoferol içeriği, Wong ve ark., (1988) tarafından açıklanan yönteme göre belirlendi. 10 mL'lik bir deney tüpüne, 0.2 g etanolik yulaf ekstraktı tartıldı. Üzerine toluen (5 mL) ilave edildikten sonra, 3.5 mL 2,2'-bipiridin (%0.07) ve 0.5 mL $FeC1_3.6H_2O$ (%0.2) ilave edilip, çözelti %95 etanol kullanılarak 10 mL'ye tamamlandı. 1 dakika sonra 520 nm'de absorpsiyon, referansa karşı spektrofotometrede belirlendi (Referans çözelti örnek yerine toluen içeriyordu ve diğer adımlar aynıdı). Benzer şekilde α-tokoferolun 50, 100, 150, 200 ve 250 µg mL^{-1} konsantrasyonları hazırlanarak yukarıda açıklanan yönteme göre kalibrasyon grafiği oluşturuldu. Ekstraktaki toplam tokoferoller aşağıdaki eşitlikten (2) hesaplandı ve sonuçlar numunenin gram başına miligram [mg α-tokoferol/g] cinsinden α-tokoferol eşdeğerleri olarak ifade edilmiştir.

Toplam tokoferoller (ppm)= (Ae - Ab) / (M * W) (2)

burada Ae= 10 mm hücrede numune absorpsiyonu, Ab= 10 mm hücrede boş absorpsiyon, M= a-tokoferol kalibrasyonu için absorbansa karşı ağırlık grafiği ve W= numunenin ağırlığı (g).

Toplam antioksidan kapasitenin belirlenmesi

Etanolik yulaf ekstraktlarının toplam antioksidan kapasitesi, Apak ve ark., (2006) tarafından geliştirilen CUPRAC metoduna göre belirlendi. Yulaf ekstraktının CUPRAC metoduna göre toplam antioksidan kapasitesinin belirlenmesi için 0.420 g yulaf ekstraktı tartıldı ve üzerine 10 mL etanol ilave edildi ve 4 kat etanolle seyreltilerek son nihai çözeltiden 0.5 mL örnek alınarak aşağıdaki formül uygulandı. Her test 3 paralel olarak hazırlandı. Kısaca, cam bir tüpe sırasıyla 1 mL bakır(II) çözeltisi (10 mM), 1 mL neocuproin çözeltisi (7.5 mM) ve 1 mL amonyum asetat tamponu (1 M, pH 7) eklendi. Daha sonra, 4 kat etanol ile seyreltilmiş ekstrakt çözeltisinden 0.5 mL ve 0.6 mL distile su eklenerek son hacim 4.1 mL' ye tamamlandı. Toplam 4.1 mL hacimde hazırlanan çözeltiler oda koşullarında 30 dakika bekletildi. Bu süre sonunda örnek içermeyen referans çözeltisine karşı absorbans değerleri, bir UV-Vis spektrofotometrede (UV-1280, Shimadzu, Japan) 450 nm' de ölçüldü.

Referans Çözeltisi: 1 mL Cu(II) + 1 mL Nc + 1 mL NH_4Ac + 1.1 mL H_2O
Numune Çözeltisi: 1 mL Cu(II) + 1 mL Nc + 1 mL NH_4Ac + 0.5 mL numune çözeltisi + 0.6 mL H_2O.

Saf troloks için kalibrasyon eğrisi orijinden geçen bir çizgi olduğundan, nihai solüsyondaki bitki ekstraktı numunesinin troloks eşdeğer molar konsantrasyonu, gözlemlenen absorbansın troloks için ε 'ye bölünmesiyle bulunabilir (optik küvet kalınlığı= 1 cm). Troloksa eşdeğer antioksidan kapasitesi (TEAC), tüm seyreltmeler göz önünde bulundurularak orijinal ekstreye kadar izlenebilir ve milimol TR/g -kuru madde birimi cinsinden bir kapasite bulmak için alınan bitki numunesinin başlangıç kütlesiyle orantılıdır (Çelik ve ark., 2008).Yulaf ekstraktının toplam antioksidan kapasitesi aşağıdaki eşitliğe (3) göre mmol troloks/g- yulaf ekstrakt TEAC eşdeğeri olarak hesaplandı.

$$\text{TAC (mmol TR g}^{-1}\text{ extract)} = \frac{A}{\varepsilon_{TR}} \text{ x } \frac{V_m}{V_s} \text{ x } S_f \text{ x } \frac{V_E}{m} \quad (3)$$

Burada; A: 450 nm'de ölçülen numune absorbansı; ε_{TR}: CUPRAC yönteminde TR bileşiğinin molar absorpsiyon katsayısı: 1.67×10^4 L mol^{-1} cm^{-1} (Çelik ve ark., 2010); V_m: CUPRAC yöntemi ölçüm çözeltisinin toplam hacmi (4.1 mL); V_s: Örnek hacmi (mL); S_f: Seyreltme faktörü (seyreltme yapılmayacaksa bu faktör "1" olarak alınır); V_E: Hazırlanan ekstraktın hacmi (mL); m: Ekstraksiyon işleminde alınan numune miktarı (g).

Serbest radikal süpürücü (SRS) kapasitenin belirlenmesi

Yulaf taneleri ekstraktının serbest radikal süpürme kapasitesi, Bener ve ark., (2022) tarafından bildirilen DPPH metodolojisi izlenerek değerlendirildi. Özetle, DPPH testinin uygulanması şu şekildeydi: bir tüp içinde, 1 mL numune ekstraktı, 1 mL etanol (%99) ve 2 mL DPPH• (0.2 mM) çözeltisi ile karıştırıldı. DPPH• ilavesinden 30 dakika sonra 515 nm'de etanole karşı absorbans kaydedildi. Ekstrelerin serbest radikal süpürücü aktivitelerini hesaplamak için düzeltilmiş absorbans değerleri (Δ_A) kullanıldı. Δ_A aşağıdaki eşitlikten (4) hesaplandı:

$$\Delta_A = A_{DPPH} - (A_E - A_0) \quad (4)$$

A_{DPPH}, örnek içermeyen DPPH• reaktifinin absorbansı; A_E: numune ekstraktının absorbansı; A_0: DPPH• reaktifi içermeyen çözeltinin absorbansı.

Serbest radikal süpürücü aktivite, troloksun standart

eğrisine dayalı olarak troloks eşdeğeri (mmol TR g^{-1} ekstrakt) olarak ifade edildi. DPPH• radikalini süpürme yeteneği, aşağıdaki eşitlik (5) kullanılarak hesaplandı:

$$\text{SRS (mmol TR g}^{-1}\text{ ekstrakt)} = \frac{\Delta_A}{\varepsilon_{TR}} \text{ x } \frac{V_m}{V_s} \text{ x } S_f \text{ x } \frac{V_E}{m} \quad (5)$$

burada ε_{TR}: DPPH• yönteminde TR bileşiğinin molar absorpsiyon katsayısı (2.168×10^4 L mol^{-1} cm^{-1}); V_s, numune hacmi; V_m, metodun toplam hacmi (4 mL); S_f, seyreltme faktörüdür (gerektiğinde); V_E, özüt hacmidir ve m, ekstraktın kütlesidir.

TR bileşiğinin standart kalibrasyon eğrisi, konsantrasyona karşı absorbans olarak oluşturuldu (1.25×10^{-5} - 6.25×10^{-5} M), ve Troloks için DPPH• yönteminin molar absorptivitesi (ε), ilgili kalibrasyon çizgisinin eğiminden bulundu ($y= 2.168 \times 10^4 c + 1.130$; R^2: 0.9991).

BULGULAR ve TARTIŞMA

Ekstraksiyon verimliliği

Yeni ekstraksiyon yöntemlerinden biri olan ultrasonik destekli ekstraksiyon (UDE), farklı kaynaklardan biyoaktif bileşiklerin geri kazanılması için yeni ve basit bir tekniktir (Vinatoru ve ark., 1997). Ultrason gücünün yoğunluğu, numune moleküllerinde ekstra titreşim üretir ve hedef moleküllerin katıdan sıvı çözücü fazına geri kazanılmasını kolaylaştırır (Samaram ve ark., 2015). Bu nedenle, kısa ekstraksiyon süresinde yüksek verim ve düşük solvent miktarı, UDE tekniğinin dikkate değer avantajlarıdır (Gulzar ve Benjakul). Ayrıca, ultrasonik teknikler nispeten ucuz, enerji ve maliyet tasarrufu sağlayan yöntemlerdir. (Dedebaş ve ark., 2021) Ayrıca hızlı, verimli ve düşük sıcaklıklarda yapılabildiği için ekstraktların termal hasarını önleyen ve biyoaktif bileşiklerin moleküler özelliklerini koruyan gıda endüstrisinde ideal bir seçenektir. Mevcut çalışmada, etanol (%96) kullanılarak yapılan ultrasonik destekli ekstraksiyonda yüksek bir oranda yulaf ekstraktı elde edilmiştir. Ekstraksiyon verimi %7.31 ± 0.2 olarak hesaplanmıştır.

Yulaf tane ekstraktının yağ asidi komposizyonu

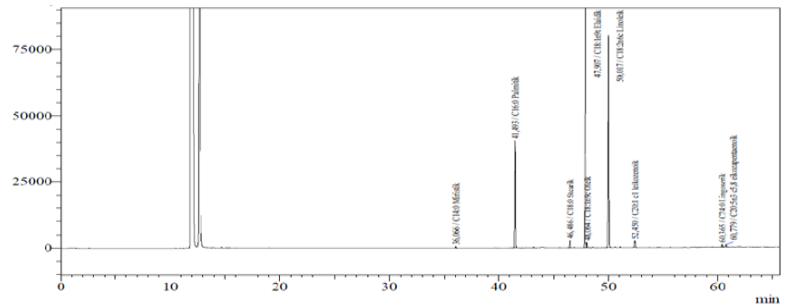
GC-FID analizine göre yulaf tanelerinin yağ asidi bileşimi Çizelge 1'de gösterilmektedir. Yulaf ekstraktının yağ asitleri GC-FID kromotogramı ise Şekil 1' de sunulmuştur. Şekil 1' de gösterildiği gibi yulaf ekstraktının yağ asitlerinin karbon zincir uzunluğuna bağlı olarak alınkonma zamanları farklılık göstermiştir. Yulaf ekstraktı, değişen miktarlarda yağ asitlerinin yanı sıra doymuş yağ asitleri (DYA), tekli doymamış yağ asitleri (TDYA) ve çoklu doymamış yağ asitlerinden (ÇDYA) oluşmaktadır. Yulaf ekstraktında en fazla bulunan major yağ asidinin oleik asit (% 43.62) olduğu tespit edilmiştir. Oleik asidi sırasıyla linoleik asit (% 34.02) ve palmitik asit (%17.38) takip etmiştir. Yulaf ekstraktının yağ asidi profilinin neredeyse % 80' i doymamış yağ asitlerinden oluşmaktadır.

Bu yağ asitlerinden linoleik asitin vücutta çok önemli fonksiyonları bulunmaktadır. Linolenik ve linoleik asit gibi doymamış yağ asitlerinin yanı sıra E vitamini içeriği bakımından da zengin olan sabit yağların kullanımı besin değeri özelliklerinden dolayı sıklıkla tercih edilmektedir (Raes ve ark., 2004). Dahası bu tip yağ asitleri bakımından zengin olan sabit yağların kozmetik ürünlerde de kullanımı yaygın hale gelmiştir (Vermaak ve ark., 2011). Çünkü linoleik asit (ω -6, omega 6), epidermiste en fazla bulunan yağ asidi olmakla beraber, stratum korneum' un işlevsel epidermal su geçirgenlik bariyerinin korunmasında da özel bir role sahiptir (McCuskerve ve Grant-Kels, 2010). Bu nedenle, deride yetersiz çoklu doymamış yağ asitleri varlığı transepidermal su kaybının artmasına neden olmaktadır (Meguro ve ark., 2000).

Çizelge 1. Yulaf tane ekstraktının yağ asidi komposizyonu
Table 1.Fatty acid composition of oat grain extract

Yağ asitleri *Fatty acid*	**İsim** *Name*	**Alınkonma zamanı** *Retention time*	**%**
C14:0	Miristik asit	36.066	0.24 ± 0.01
C16: 0	Palmitik asit	41.493	17.38 ± 0.015
C18: 0	Stearik asit	46.486	1.37 ± 0.013
C18: 1 cis-9 (n-9)	Oleik asit	48.064	43.62 ± 0.17
C18: 2 cis9,12	Linoleik asit	50.017	34.02 ± 0.14
C20: 1 cis11 (n-9)	Eikosenoik asit	52.450	1.51 ± 0.01
C24: 0	Lignoserik asit	60.365	0.6 ± 0.05
Σ DYA *(SFA)*			19.59 ± 0.09
Σ TDYA *(MUFA)*			45.13 ± 0.18
Σ ÇDYA *(PUFA)*			34.02 ± 0.14

GC-FID ile elde edilen veriler bağıl değerler (%) olarak ifade edilir: Σ DYA, doymuş yağ asitlerinin toplamı; Σ TDYA, tekli doymamış yağ asitlerinin toplamı; Σ ÇDYA, çoklu doymamış yağ asitlerinin toplamı.
Data obtained by GC-FID are expressed as relative values (%): mean % of total FAs ± SD; Σ SFA, sum of saturated fatty acids; Σ MUFA, sum of monounsaturated fatty acids; Σ PUFA, sum of polyunsaturated fatty acids

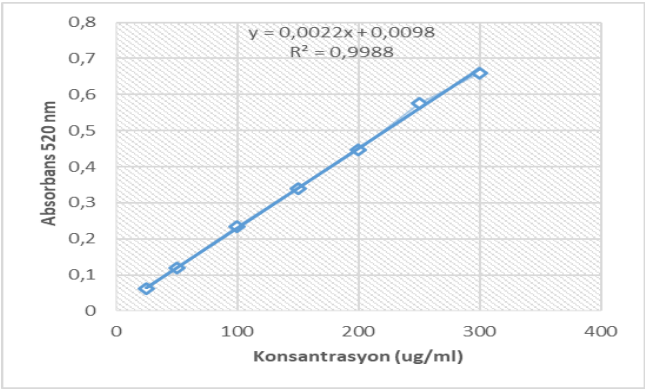


Şekil 1. Yulaf ekstraktının yağ asitleri GC-FID kromotogramı
Figure 1. *GC-FID chromatogram of fatty acids of oat extract*

Musa Özcan ve ark. (2006) tarafından bildirilen bir çalışmada dört farklı yulaf tanelerinin yağ asitleri bileşimini palmitik asit: %15.72; oleik asit: %33.97-51.26 ve linoleik asit: %22.80-35.90 olarak bulunmuştur. Mevcut çalışmada sunulan bulgular, daha önceden bildirilen yulaf taneleri yağı sonuçlarıyla karşılaştırıldığında benzer bulunmuştur. Yağlı tohum bitkilerinde de ultrason destekli ekstraksiyonların, genel olarak yağ verimini arttırdığı yağ asidi kompozisyonunu ise çok etkilemediği görülmüştür (Li ve ark., 2004).

Toplam tokoferol içeriği

Yulaf ekstraktlarının toplam tokoferol içeriği spektrofotometrik olarak tespit edilmiştir. Şekil 2' de gösterilen *α*– tokoferolün absorbansa karşı konsantrasyon kalibrasyon grafiği denklemi (y= 0.0022x+0.0098) kullanılarak yulaf ekstraktlarının toplam tokoferol içeriği 1376.3 ± 16 mg kg^{-1} yulaf ekstrakt olarak bulunmuştur (Çizelge 2).



Şekil 2. α-tokoferol kalibrasyonu için absorbans gradyanına karşı konsantrasyon grafiği
Figure 2. *Gradient of absorbance vs. concentration graph for α-tocopherol calibration*

Literatürde yulaf tanelerinden farklı çözücü ekstraksiyonlarından elde edilen yağlarda *α*–tokoferol içeriğinin 3-1724 µg g^{-1} arasında değiştiği rapor edilmiştir ve en fazla *α*–tokoferol içeriği ise etanol ekstraksiyonu (1724 µg g^{-1}) ile yapılanda bulunduğu bildirilmiştir (Chen ve ark., 2016). Analiz sonucu, yulaf ekstraktlarının toplam tokoferol içeriği bakımından oldukça zengin olduğunu ortaya çıkarmıştır.

Toplam antioksidan kapasitesi

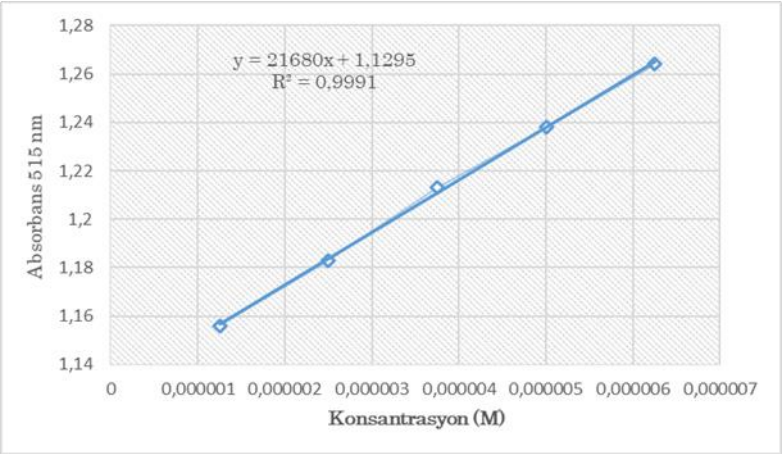
Bu çalışmada yulaf ekstraktının toplam antioksidan kapasitesi CUPRAC yöntemine göre hesaplanmıştır. Yulaf taneleri ekstraktının CUPRAC metoduna göre toplam antioksidan kapasitesi 1.5 ± 0.03 mmol TR $100g^{-1}$ yulaf ekstraktı olarak hesaplanmıştır (Çizelge 2). Yulaf ekstraktının bu kadar etkili antioksidan kapasite göstermesinin sebebi içerdiği yüksek miktarda E vitamini ile ilişkilendirilmiştir.

Serbest radikal süpürme aktivitesi

Troloks için DPPH•'nin molar absorbsiyon katsayısı (ε_{TR}) çözücüye bağlı olduğundan (Villaño ve ark., 2007), çalışma koşullarında ε_{TR} tahmin edilmiştir. Troloks bileşiğinin standart kalibrasyon eğrisi, absorbansa karşı konsantrasyon olarak bu şekilde oluşturulmuştur ve trolox için DPPH yönteminin molar absorptivitesi, ilgili kalibrasyon çizgisinin eğiminden bulunmuştur. Etanol ortamında DPPH testi ile elde edilen trolox kalibrasyon eğrisi için denklem ve regresyon katsayısı ($y = 2.17 \times 10^4 c + 1.130$; R^2: 0.9991) Şekil 3'te gösterilmiştir. Bu veriler eşitlik 5'te kullanılarak yulaf tanelerinin ekstraktlarının serbest radikal süpürme aktivetesi belirlenmiştir. Yulaf ekstraktının serbest radikal süpürme aktivitesi Çizelge 2' de sunulmuştur.

Çizelge 2. Yulaf ekstraktının toplam antioksidan kapasitesi, radikal süpürme aktivitesi ve toplam tokoferol içeriği
Table 2. Total antioxidant capacity, radical scavenging activity and total tocopherol content of oat extract

	CUPRAC-Toplam Antioksidan Kapasite (mmol TR $100g^{-1}$ ekstrakt) *CUPRAC-Total Antioxidant Capacity (mmol TR $100g^{-1}$ extract)*	DPPH süpürme aktivite (mmol TR $100g^{-1}$ ekstrakt) *DPPH scavenging activity (mmol TR $100g^{-1}$ extract)*	Toplam tokoferol içeriği (mg kg^{-1} ekstrakt) *Total tocopherol content (mg kg-1 extract)*
Yulaf ekstraktı Oat extract	1.5 ± 0.03	2.02 ± 0.01	1376.3 ± 16



Şekil 3. Standart Troloks bileşiğinin kalibrasyon eğrisi
Figure 3. *Calibration curve of standard Trolox compound*

Yulaf ekstraktının DPPH• radikali süpürme aktivitesi 2.02 ± 0.01 mmol TR $100g^{-1}$ yulaf ekstraktı olarak hesaplanmıştır. Literatürde, etanol ile elde edilen yulaf ekstraktının, en güçlü DPPH• radikal süpürme aktivitesini sergilediği rapor edilmiştir. Chen ve ark. (2016), yulaf tanelerinin DPPH• radikali süpürme kabiliyetindeki artan sırasını, petrol eteri<heksan< izopropanol<etil asetat<etanol ile elde edilen ekstraktlar için olduğunu bildirmişlerdir. Çözücü polaritesi arttığında yulaf ekstraktının DPPH• radikal süpürme yeteneğinin de arttığı görülmüştür.

SONUÇ ve ÖNERİLER

Mevcut çalışmada yulaf tanelerinin ultrasonik destekli etanolik ekstraktlarının, toplam antioksidan kapasitesi, serbest radikal süpürme aktivitesi, yağ asitleri komposizyonu ve toplam tokoferol içeriği dahil olmak üzere detaylı bir şekilde araştırıldı. Yulaf tanelerinden ultrasonik destekli ekstraksiyon yöntemiyle daha kısa sürede, daha az enerji ve daha az solvent kullanılarak ekstraktlar elde edilmiştir. *İn vitro* antioksidan yöntemler kullanılarak ultrasonik destekli yulaf ekstraktlarının güçlü radikal süpürme kapasitesine ve antioksidan özelliklere sahip olduğu belirlendi. Araştırma bulguları, yulaf ekstraktlarının oleik ve linoleik yağ asitleri bakımından zengin olmasının yanı sıra toplam tokoferol içeriği bakımından da zengin olduğunu gösterdi. Veriler, ultrasonik destekli ekstraksiyonun yağlı tohum bitkileri için uygun olduğunu göstermiştir.

Araştırmacıların Katkı Oranı Beyan Özeti

Yazarlar makaleye eşit oranda katkı sağlamış olduklarını beyan eder.

Çıkar Çatışması Beyanı

Makale yazarları aralarında herhangi bir çıkar çatışması olmadığını beyan ederler.

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Berberis crataegina Meyve Ekstraktının Antioksidan ve Antibakteriyel Etkisinin Araştırılması

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ÖZET

Bitkiler çok eski zamanlardan beri hem Türkiye'de hem de dünyada tıbbi amaçlı olarak kullanılmaktadır. Bu bitkilerin çoğu antimikrobiyal özellikleri nedeniyle pek çok hastalığın tedavi sürecine dahil edilmektedir. Benzer şekilde meyveler de geleneksel tedavi amaçlı halk arasında sıklıkla kullanılmaktadır. Bir yabani meyve türü olan *Berberis crataegina* (karamuk) meyveleri tanen, organik asitler, yüksek oranda C vitamini ve antosiyanin içermekte olup; ateş düşürücü, kaşıntı önleyici ve diüretik etkilerinden dolayı yaygın olarak tüketilmektedir. Bu çalışmada, *Berberis crataegina* meyvelerinin farklı çözücülerle (etanol, metanol, hekzan, diklorometan) hazırlanan ekstratlarının total antioksidan/oksidan etkileri ve *Stenotrophomonas maltophilia* bakterisine karşı antibakteriyel etkisinin belirlenmesi amaçlandı. Çalışmada, toplanıp kurutulan meyve tanelerinin farklı çözücülerle hazırlanan ekstraktlarında total antioksidan/oksidan seviyeleri ticari kitler ile spektrofotometrede ölçüldü. Berberis crataegina meyvelerinin *Stenotrophomonas maltophilia* bakterisine karşı antibakteriyel etkisinin olup olmadığı disk difüzyon yöntemiyle belirlendi. Çalışmaya göre *Berberis crataegina* meyvelerinin yüksek antioksidan seviyeye sahip olduğu, *Stenotrophomonas maltophilia* bakterisine karşı antibakteriyel etkisinin olmadığı tespit edildi.

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Anahtar Kelimeler
Berberis crataegina
Stenotrophomonas maltophilia
Total antioksidan seviye
Total oksidan seviye

Investigation of Antioxidant and Antibacterial Effect of *Berberis crataegina* Fruit Extract

ABSTRACT

Plants have been used for medicinal purposes both in Türkiye and in the world since ancient times. Many of these plants are included in the treatment process of many diseases due to their antimicrobial properties. Similarly, fruits are frequently used among the people for traditional treatment purposes. *Berberis crataegina* (barberry) fruits, a wild fruit species, contain tannins, organic acids, high levels of vitamin C and anthocyanins; it is widely consumed due to its antipyretic, antipruritic and diuretic effects. In this study, it was aimed to determine the total antioxidant/oxidant effects of *Berberis crataegina* fruit extracts prepared with different solvents (ethanol, methanol, hexane, dichloromethane) and antibacterial effect against *Stenotrophomonas maltophilia* bacteria. In the study, the total antioxidant/oxidant levels of the extracts of the collected and dried fruit grains prepared with different solvents were measured in spectrophotometer with commercial kits. The antibacterial effect of *Berberis crataegina* fruits against *Stenotrophomonas maltophilia* was determined by disk diffusion method. According to the study, it was determined that *Berberis crataegina* fruits had high antioxidant level and had no antibacterial effect against *Stenotrophomonas maltophilia* bacteria.

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GİRİŞ

İnsanlığın var oluşundan itibaren beslenmede önemli bir yer tutan bitkiler, yaşamın vazgeçilmez temel kaynaklarından biridir. İnsanlar bitkilerden sadece beslenme amaçlı değil geçmiş yıllardan beri sağlığın korunması ve çeşitli hastalıkların tedavisinde de faydalanmaktadırlar (Njume ve ark., 2009; Deveci ve ark., 2016).

Son zamanlarda tıbbi bitkilerden hazırlanan ilaç hammaddeleri üzerine yapılan araştırmalar önem kazanmıştır. Sentetik ilaçların tehlikeli yan etkileri mevcutken, birçok olumlu biyolojik aktiviteye sahip olan bitki özütlerinin yan etkisinin olmaması dikkat çekicidir. Bu nedenle sentetik ilaçlara göre tıbbi bitkiler ile yapılan ilaçlar daha fazla tercih edilmektedir (Diken, 2009).

Normal fizyolojik durumlarda, hücrelerde devamlı oluşan oksidanlar ve bunlarla etkileşim içinde olan antioksidanlar arasında mevcut bir denge söz konusudur. Bu dengenin oksidanlar lehinde bozulmasına yani antioksidan savunma sisteminin yetersiz olmasına oksidatif stres adı verilmektedir (Sies ve Cadenas, 1985; Halliwell, 2007; Deveci, 2017a). Bitkilerle yapılan bazı çalışmalarda bitkisel antioksidanların oksidatif stresi engellediği ve hücre ölümlerini azalttığı belirtilmektedir (Schoeter ve ark., 2000; Youim ve Joseph, 2001; Parihar ve Hemnani, 2004). Bitkilerin yapısında bulunan antioksidan maddeler, hücreleri deforme eden oksijene ve diğer zararlı maddelere karşı koruyucu bir kalkan görevi yapar. Bitkisel antioksidanlar, oksidasyonu önleyerek vücutta antibakteriyel ve antikanserojen etki de gösterirler (Meral ve ark, 2012; Deveci ve ark., 2016). Bu durum bitkisel doğal ürünlere olan ilgiyi daha da arttırmaktadır. *Berberis crataegina* (karamuk), Berberidaceae familyasının Berberis cinsine ait önemli bir tür olup, Türkiye ve İran bölgesinde geniş bir alanda yayılım göstermektedir. Türkiye'de yetişen Berberis cinsine ait türler; *B. crataegiana, B. cretica, B. integerrima* ve *B. vulgaris*'tir. *Berberis crataegina*, Anadolu'nun farklı yörelerinde karamuk, dikenli üzüm, amber ekmeği, ekşimen ve yılkı çalısı gibi değişik isimlerle anılmaktadır (Baytop, 1999; Gedikli, 2006). *Berberis crataegina* bileşiminde organik asitler, tanen, C vitamini ve yüksek oranda antosiyanin içermektedir (Gedikli, 2006). Bitkinin meyvesinin tadı hafif ekşi olup, eliptik ve üzümsü yapıda olan meyveler olgunlaştığında daha da siyahlaşır (Davis, 1982; Baytop, 1999). Şekil 1.'de *Berberis crataegina* meyvesinin şekli görülmektedir.

Şekil 1. *Berberis crataegina* meyvesinin bitki üzerindeki görünümü (0rijinal).
Figure 1. View of *Berberis crataegina* fruit on the plant.

Son zamanlarda farklı bitki ekstraktlarının antioksidan ve antimikrobiyal etkilerinin insan sağlığı açısından önemli görevleri olduğu

bilindiğinden, bu çalışmada da *Berberis crataegina* bitkisinin etanol metanol hekzan ve diklorometan ile hazırlanan meyve ekstraktının total antioksidan/oksidan seviyeleri (TAS/TOS) ve *Stenotrophomonas maltophilia* üzerine antibakteriyel etkisinin olup olmadığı araştırılmıştır.Yapılan geniş literatür çalışmaları sonucunda *Berberis crataegina* meyvesinin oksidan seviyesini ve *Stenotrophomonas maltophilia'* ya karşı antibakteriyel aktivitesini araştıran herhangi bir çalışmaya rastlanmamış olup, çalışma bu yönüyle orijinallik arz etmektedir.

MATERYAL ve METOD

Berberis crataegina Meyvelerinin Toplanması ve Teşhisi

Çalışma materyali olan *B. crataegina* meyveleri 2019 Eylül ayının ilk haftasında, Kayseri ili Sarız ilçesinin Fettahdere, Oğlakkaya ve Ayranlık köylerinin yüksek bölgelerinden toplandı. Çalışmaya uygun olacak parlak ve taze görünümlü olan meyveler tercih edildi. Gaziantep Üniversitesi Fen Edebiyat Fakültesi Biyoloji Bölümü'nün Botanik Anabilim Dalı'nda teşhis edildi.

Meyve Ekstraktlarının Elde Edilmesi

Arazi şartlarından ileri gelen toz, yabancı ot, diken, dal, yaprak vb oluşumlar manuel olarak temizlendi.Yıkama işlemi yapılmaksızın kurutma kağıdına serilerek gölgede kurutulan meyve taneleri, ekstraksiyon işlemi için mekanik öğütücü ile öğütüldü. Toz haline gelen meyveler 10 gram olarak tartılarak Soxtherm cihazının beherleri içine yerleştirildi. Özüt eldesi için Etanol (C_2H_6O), Metanol (CH_3OH), Hekzan (C_6H_{14}), Diklorometan (CH_2Cl_2) kullanıldı. Tüm çözücüler için ayrı ayrı olmak kaydıyla 5.1 bar basınçta, 2 saat boyunca 10 grama 150 ml çözücü konularak 130 ºC' de işleme tabi tutuldu. Elde edilen özütlerden, Rotary Evaporator'de 90ºC'de evaporasyon (buharlaştırma) yöntemiyle çözücüler uzaklaştırıldı. Hazırlanan ekstraktlar kullanılana kadar +4 ºC'de muhafaza edildi.

TAS, TOS ve Oksidatif Stres İndeksi (OSİ) Değerlerinin Belirlenmesi

Farklı çözücülerle elde edilen meyve ekstraktlarının total antioksidan seviyeleri (TAS), Erel (2004) tarafından geliştirilmiş Total Antioxidant Durum kiti (Rel Assay Kit Diagnostics, Türkiye) ile analiz edildi. TAS değeri mmol Trolox eşdeğeri/L olarak hesaplandı ve kalibratör olarak Trolox kullanıldı. Meyve ekstraktlarının total oksidan seviyeleri (TOS), Erel (2005) tarafından geliştirilmiş olan Total Oksidan Durum kiti (Rel Assay Kit Diagnostics, Türkiye) ile analiz edildi. TOS değeri µmol H_2O_2 eşdeğeri/L olarak hesaplandı ve kalibratör olarak hidrojen peroksit kullanıldı. Meyve ekstraktlarının OSİ değerleri ise, TOS değerlerinin TAS değerlerine oranının yüzde derecesi olarak ifade edildi. OSİ değerleri hesaplanırken, TAS sonuçların birimindeki mmol değeri TOS sonuçlarındaki gibi µmol birimine çevrildi. Sonuçlar aşağıda verilen formüle göre hesaplandı (Eren ve ark., 2015).

OSİ= [(TOS/TAS) x 100]

Stenotrophomonas maltophilia'ya Karşı Antibakteriyel Etkinliğin Belirlenmesi

B. crataegina ekstraktlarının, antimikrobiyal aktivitelerinin değerlendirilmesi amacıyla *Stenotrophomonas maltophilia* bakterisi kullanıldı. *S.maltophilia* suşları Gaziantep Üniversitesi Tıp Fakültesi Mikrobiyoloji Laboratuvarından temin edildi. Antibakteriyel etkinliğin blirlenmesi için disk difüzyon yöntemi uygulandı (Bauer ve ark 1966). Bu yönteme göre daha önceden hazırlanmış olan örneklerin 30µl'si 6mm çapındaki steril blank (boş) disklere emdirildi. Nutrient-Broth Agar (NBA) ortamında 37°C'de inkübe edilen test mikroorganizmalarından, 0.5 McFarland (son inokulum 1.5 x 108 CFU/mL) bulanıklık değerine göre süspansiyonlar hazırlandı. Bu süspansiyonlar daha sonra Mueller-Hinton besiyerlerine 100 µl inoküle edilerek steril eküvyon çubuk ile yayılıp disk difüzyon testinde kullanılmak amacıyla ekimi yapıldı ve kurutulmuş diskler steril bir penset yardımıyla besiyeri üzerine yerleştirildi. Hazırlanan besiyerleri 24 saat süreyle 37°C'de inkübe edildi. İnkübasyon süresi sonunda disklerin etrafında zon oluşup oluşmadığı incelendi.

BULGULAR

B. crataegina Meyvesinin TAS, TOS ve Oksidatif Stres İndeksi (OSİ) Değerleri

B. crataegina meyvelerinin farklı çözücülerdeki TAS, TOS ve OSİ değerleri Çizelge 1. 'de gösterildi.

Çizelge1. Farklı çözücülerdeki TAS, TOS ve OSİ değerleri
Table 1. TAS, TOS and OSI values in different solvents.

Parametreler / Çözücüler	TAS (mmol Trolox eşdeğeri./L)	TOS (µmol H_2O_2 eşdeğeri./L)	OSİ (Arbitrary unit)
Etanol	4.303 ± 0.020	41.580 ± 0.105	0.966 ± 0.013
Metanol	5.096 ± 0.019	46.403 ± 0.133	0.911 ± 0.012
Hekzan	0.891 ± 0.017	24.227 ± 0.095	2.719 ± 0.034
Diklorometan	1.235 ± 0.016	14.312 ± 0.108	1.159 ± 0.018

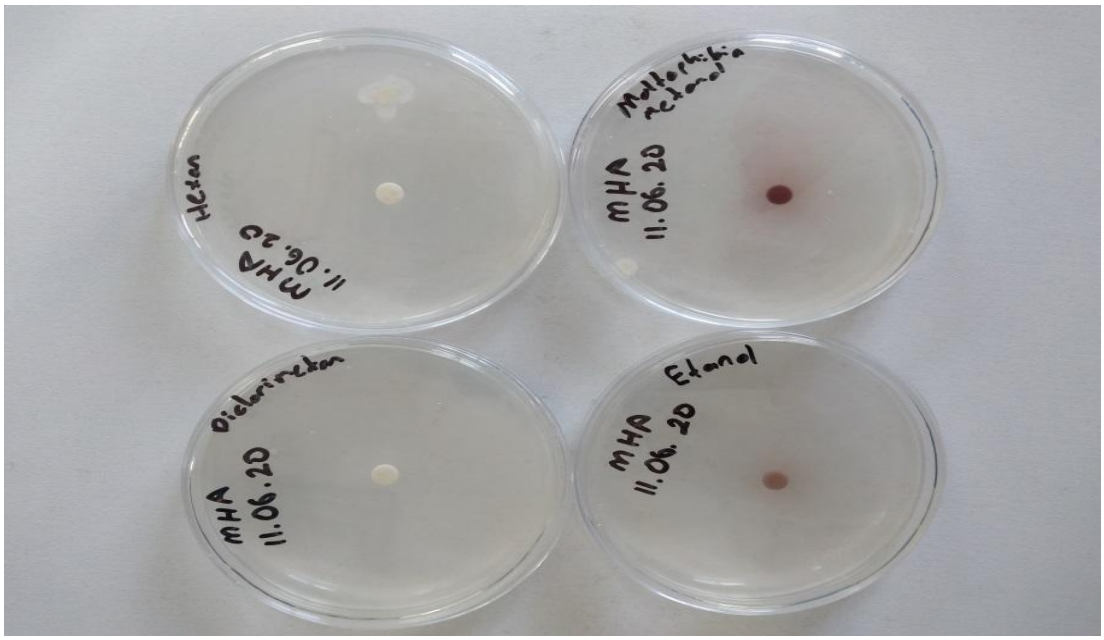
B. crataegina meyvesinin farklı çözücüler ile elde edilen ekstraktlarının TAS değerleri karşılaştırıldığında Çizelge 1.'de görüldüğü gibi etanol ve metanol çözücüleriyle elde edilen ekstraktın çok yüksek, diklorometan ile elde edilen ekstraktın normal seviyede, hekzan ile elde edilen ekstraktın ise daha az antioksidan özellik gösterdiği belirlendi.

B. crataegina meyvesinin farklı çözücüler ile elde edilen ekstraktlarının TOS değerleri karşılaştırıldığında ise en yüksek oksidan özelliği sırasıyla metanol, etanol, hekzan ve diklorometan ile elde edilen ekstraktların gösterdiği tespit edildi.

B. crataegina meyvesinin farklı çözücüler ile elde edilen ekstraktlarının OSİ değerleri incelendiğinde ise TAS değerleriyle doğru orantılı olarak sonuç verdiği görüldü. Buna göre OSİ değerleri sırasıyla en düşükten yükseğe doğru metanol, etanol, diklorometan ve hekzan ile elde edilen ekstraktların gösterdiği belirlendi.

B. crataegina Meyvesinin *Stenotrophomonas maltophilia*'ya Karşı Antibakteriyel Etkinliği

Antibakteriyel etkinliğin belirlenmesi amacıyla yapılan disk difüzyon testine göre 24 saatin sonunda ölçülen zon çapları aşağıda Şekil 2'de verildi. Buna göre *B. crataegina* meyvesinin farklı çözücülerle elde edilen ekstraktlarının, disk difüzyon testinde 24 saat sonunda herhangi bir zon oluşturmaması sonucu, *B. crataegina* meyvesinin *S. maltophilia*'ya karşı herhangi bir antibakteriyel aktivitesinin olmadığı tespit edildi.



Şekil 2. Disk difüzyon test sonucu
Figure 2. Disk diffusion test result.

TARTIŞMA ve SONUÇ

Türkiye; bulunduğu coğrafi konumu, uygun toprak yapısı, iklim şartları ve ekolojisiyle diğer ülkelere göre zengin bir bitki florasına sahiptir. Dolayısıyla Türkiye'deki tıbbi ve aromatik bitkilerin çeşitli araştırmalarda çalışılma potansiyelleri yüksektir (Kendir ve Güvenç, 2010). Günümüzde insanlar beslenmelerinde daha doğal ve organik olan gıdaları tercih etmektedir. Böylece hem dünyada hem de Türkiyede doğal ve organik gıdaya yönelim hızla artmaktadır. Ayrıca hem kırsal kesimde hem de şehirlerde insanlar, hastalıklardan korunmada ve hastalıkların tedavisinde tıbbi ve aromatik bitkilerden faydalanmaktadır (Mosihuzzaman, 2012; Özkol ve ark., 2017). Tıbbi ve aromatik bitkilerin meyve, çiçek, yaprak ve kök gibi bölümlerinden elde edilen ekstraktlar pek çok tıbbi ilacın ham maddesini oluşturmaktadır (Özyurt ve ark., 2021).

Sağlıklı bireylerde antioksidan ve oksidan moleküller hassas bir denge halindedir. Ancak bu dengenin oksidanların lehine bozulmasıyla hücre ve dokularda oksidatif stres meydana gelir. Oksidatif stres, hücresel ve moleküler doku hasarı oluşum mekanizmalarının bir parçasıdır (Tabakoğlu ve Durgut, 2013; Deveci, 2017b; Deveci ve ark., 2021). Yapılan birçok araştırmada, belli başlı bitkisel antioksidanların oksidatif stres sonucu oluşan hücre ölümlerini engellediği bildirilmektedir (Schoeter ve ark., 2000; Youim ve Joseph, 2001; Parihar ve Hemnani, 2004).

Bitkisel antioksidanların aktivitelerinin bilinmesi, hastalıkların çoğunun başlamasına öncülük eden

oksidatif stresin engellenmesi ve oksidan maddelerin zararlı etkilerinin ortadan kaldırılması açısından büyük önem arz eder. Son yıllarda doğal antioksidanların önemli bir bölümünü oluşturan tıbbi ve aromatik bitkiler üzerine bir çok araştırma yapılmış, hala da bu çalışmalar devam etmektedir (Diken, 2009; Deveci ve ark., 2016; Özyurt ve ark., 2021).

Berberis crataegina, Türkiyede Ankara, Antalya, Denizli, Erzincan, Karaman, Kahramanmaraş, Kayseri, Kastamonu, Konya, Kütahya, Malatya, Muğla, Niğde, Şanlıurfa, Tunceli ve Yozgat'ta doğal olarak yetişmektedir (Arslanoğlu ve Ayna, 2019). Türkiyede geleneksel kullanımda bitkinin meyveleri; kan yapıcı, tansiyon düşürücü, mide ve bağırsak rahatsızlıkları, hemoroit, sarılık, ishal, kabızlık ve soğuk algınlığı tedavisinde sıklıkla kullanılmaktadır (Baytop, 1999; Yeşilada ve Küpeli, 2002; Tuzlacı, 2016; Arslanoğlu ve Ayna, 2019).

Yapılan bu çalışmada, Anadolu'da birçok hastalık ve rahatsızlığın tedavisinde sıklıkla kullanılan *Berberis crataegina* meyvesinin total antioksidan /oksidan seviyesi ve *Stenotrophomonas maltophilia*'ya karşı antibakteriyel etkisinin olup olmadığı araştırıldı. Farklı çözücülerle elde edilen *B. crataegina* meyve ekstraktlarının total antioksidan seviyelerinin test kiti referans değerlerine göre yüksek olduğu belirlendi. Buna göre *B. crataegina* meyvesinin antioksidan seviyesinin belirlenmesinde metanol ve etanol çözücülerinin kullanılmasının daha uygun olabileceği kanaatine varıldı. Charehsaz ve ark. (2015), yaptıkları çalışmada *B. crataegina* meyvesinin serbest radikal süpürme yeteneğini; DPPH, süperoksit radikal süpürme ve TEAC testleri ile değerlendirilmiştir. Buna göre *B. crataegina* meyvesinin iyi bir antioksidan özelliğe sahip olduğunu bildirmiştir. Eroğlu ve ark. (2020), *B. crataegina* meyvelerinin antioksidan kapasitesi üzerine yaptıkları çalışmada, *ß*-karoten ağartma yönteminde Berberis meyvelerinin antioksidan düzeylerinin %62.83 ile %92.19 arasında olduğunu, DPPH radikal süpürücü aktivite testinde antioksidan seviyenin %11,92–40,44 aralığında olduğunu, ABTS yönteminde ise su ekstraktının, diğer ekstratlara kıyasla daha yüksek antioksidan seviyeye sahip olduğunu bildirmiştir. Gıdık (2021), *B. crataegina* ile yapmış olduğu bir çalışmada antioksidan kapasiteyi belirlemek için DPPH yöntemi kullanmış ve sonucun 0.36 ± 0.01 olduğunu bildirmiştir. Yapılan bu çalışmanın sonuçları farklı yöntemlerle ölçülen antioksidan aktivite belirlemeye yönelik sınırlı sayıdaki çalışmalarla uyumludur.

Yapılan bu çalışmada farklı çözücülerle elde edilen *B. crataegina* meyve ekstraktlarının, total oksidan seviyeleri de total antioksidan seviyelere benzer oranda yüksek çıkmıştır. B. crataegina meyve ekstraklarının total oksidan seviyeleri ile ilgili herhangi bir çalışmaya rastlanmamıştır, bu durum çalışmanın özgün olduğunu göstermektedir. Total oksidan seviyelerin yüksek olması bu çalışmanın en önemli bulgularından birisidir. Ancak hem antioksidan seviyenin hem de oksidan seviyenin birlikte değerlendirilmesine olanak sağlayan oksidatif stres indeksi baz alındığında, çalışma sonuçlarının antioksidan seviyeleri lehine olduğu görülmektedir. Bu da *B. crataegina meyve* ekstraktının güçlü antioksidan etkiye sahip olduğu ve oksidatif stresi önleyebileceğini göstermektedir.

Geniş literatür taramaları sonucu *Berberis crataegina* meyvesinin *Stenotrophomonas maltophilia*'ya karşı antibakteriyel aktivitesini araştıran herhangi bir çalışmaya rastlanmamış olup, çalışma bu yönüyle orijinallik arz etmektedir. *Stenotrophomonas maltophilia*, sıklıkla rastlanan fırsatçı patojen bir bakteridir (Dülger ve Berktaş, 2007).*Stenotrophomonas maltophilia;* aminoglikozid asetil transferaz, beta-laktamaz ve eritromisini inaktive eden enzimleri, eflüks pompaları kodlayan genleri sebebiyle çoğu antibiyotiğe dirençlidir. Günümüzde Karbapenemler dahil çoğu antibiyotiğe dirençli olduklarından bu bakterilerin neden olduğu hastalıkların tedavisi oldukça güçtür (Valdezate ve ark., 2001). Eroğlu ve ark. (2020), *Berberis crataegina* meyvesinin antimikrobiyal etkisini inceledikleri bir çalışmada meyve ekstraktının *Staphylococcus aureus, Salmonella typhimurium, Yersinia enterocolitica*'ya karşı etkili, *Escherichia coli ve Bacillus cereus'a* karşı ise etkisiz olduğunu bildirmişlerdir. Kyzy (2017), böğürtlen meyvesinin metanol ile hazırlanan ekstraktlarının farklı konsantrasyonlarının *S.maltophilia* üzerine antibakteriyel etkilerini araştırmıştır. Çalışma sonucunda böğürtlen meyvesinin *S.maltophilia* üzerine antibakteriyel etkilerinin; böğürtlen ekstraktının konsantrasyonu ile doğru orantılı olarak yüksek olduğunu bildirmiştir. Çiçek (2019), yaptığı bir çalışmada saf nar ekşisinin ve limon suyu ilaveli nar ekşisinin, sulandırılmadan kullanılmalarının *S. maltophilia* üzerine antimikrobiyal etkisinin olduğunu bildirmiştir. Bu çalışmada ise *Berberis crataegina* meyve ekstraktlarının disk difüzyon testinde 24 saat sonunda herhangi bir zon oluşturmaması, *S. maltophilia*'ya karşı herhangi bir antibakteriyel aktivitesininin olmadığını göstermektedir.

Bu çalışma ile *B. crataegina* meyvesinin, hem antioksidan hem de oksidan seviyelerinin yüksek olduğu, ancak oksidatif stres indeksi değerlendirildiğinde yüksek antioksidan aktiviteye sahip olduğu belirlenmiştir. Bitkinin meyvesinin antioksidan seviyelerinin yüksek olmasının yanında oksidan seviyenin de yüksek olmasının nedeni; meyvede fazla sayıdaki antioksidan bileşiklerin prooksidan etki göstermesi olabilir. Bu durum aşırı miktarda antioksidan içeren bitkilerin fazla

tüketilmemesi gerektiğini göstermektedir. Ayrıca *B. crataegina* meyvesinin *S. maltophilia* bakterisine karşı antibakteriyel bir etkisinin olmadığı tespit edilmiştir. Çalışmanın daha sonra yapılacak araştırmalara ışık tutacağı kanısındayız.

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Lokal Endemik Tür *Alchemilla orduensis* B. Pawl Üzerine Eko-Biyolojik Bir Çalışma

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ÖZET

Bu araştırmada menderes coğrafi oluşumları ile karakterize edilen Ordu İli Aybastı İlçesi Perşembe yaylasından toplanan lokal endemik *Alchemilla orduensis* ile çalışılmıştır. Bitkinin vejetatif ve generetif dönemlerinde toplanan örneklerinin toprak üstü ve toprak altı kısımlarında N, C, H ve S elementlerinin miktarları tespit edilmiştir. S elementine çiçekli dönemdeki bitki örneklerinde az miktarda rastlanılmıştır. N miktarının toprak altı kısmında generatif dönemde, toprak üstü kısmında ise vejetatif dönemde yüksek olduğu görülmektedir. C ve H miktarları ise hem toprak altı hem de toprak üstü kısmında vejetatif dönemde yüksek bulunmuştur. Çalışma alanı düşük sıcaklık ve bol yağış alan iklim özelliklerine sahiptir. Bitkinin bulunduğu bölge killi-tınlı karakterde hafif alkali, az kireçli ve az tuzlu toprak özelliklerine sahiptir. Bununla beraber, toprağın organik madde, pH, yarayışlı potasyum, fosfor, kalsiyum ve magnezyum miktarları açısında da yeterli olduğu belirlenmiştir. Türün neslinin devamında önemli rolü olan çiçek ve meyve kısımları da morfolojik ve mikromorfolojik olarak incelenmiştir. Türün çiçeğinde periant epikaliks ve kaliksten oluşmaktadır. İnfloresens çok çiçekli ve çiçekler sarı renklidir. Epikaliksin iç yüzeyindeki hücreler dalgalı çeperlidir. Epikaliks iç yüzeyi retikulat-rugose, sepal iç yüzeyi ise retikulat yüzey şekillidir. Meyve aken tipindedir ve yüzeyi retikulat yapıdadır.

Biyoloji

Araştırma Makalesi

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An Eco-Biological Study on the Localy Endemic Species *Alchemilla orduensis* B. Pawl

ABSTRACT

In this study, the local endemic *A. orduensis* collected from Perşembe plateau of Aybastı district of Ordu provinve, which is characterized by meander geographical formations, was studied. The amounts of N, C, H and S elements were determined in the aboveground and underground parts of samples collected from vegetative and generative periods of plants. S element was found in small amounts in plant samples belonging to the flowering period. It is seen that the amount of N is high in the generative period in the underground part and in the vegetative period in the above ground part. The amounts of C and H were found to be high in both the underground and aboveground parts during the vegetative period. The study area has low temperature and high precipitation climate characteristics. The region where the plant is located has clayey-loamy, slightly alkaline, little calcareous and salty soil properties. However, it was determined that the soil was sufficient in terms of organic matter, pH, available potassium, calcium and magnesium amounts. The flower and fruit, which have an important role in the continuation of the species' extinction, have also been examined morphologically and micromorphologically. In the flower of the species, the perianth consists of epicalyx and calyx. The inflorescence is multi-flowered and the flowers are yellow. Cells on the inner surface of the epicalix have wavy walls. Epicalyx inner surface is reticulate-rugose, sepal

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inner surface is reticulate surface shaped. The fruit is achene type and its surface is reticulate.

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GİRİŞ

Alchemilla L., başta kuzey yarımkürede olmak üzere tüm dünyada yayılış gösteren yaklaşık 100 cins ve 3500 tür içeren Rosaceae familyasına ait bir cinstir. Tek veya çok yıllık otsu, çalı veya nadiren ağaç formunda bitkilerdir (Heywood ve ark., 2007; Aymerich ve Saez, 2015). Türkiyede bu familyaya ait 36 cins ve 250 kadar tür bulunmaktadır. *Alchemilla* 1000 türe sahip büyük bir cins olup, Potentileae tribusunda, Alchemilinae alt tribusunda sınıflandırılmaktadır (Fröhner ve Faghir, 2022). Cins Türkiye'de 82 tür ile temsil edilmekte olup bu türlerden 36 tanesi endemiktir. Cinsin endemizm oranı %33,8' dir (Davis, 1972; Özhatay ve Kültür, 2006; Özhatay ve ark., 2011). Tıbbi, gıda ve süs bitkisi olarak kullanılan birçok türü vardır. *Alchemilla* türlerinin "fındık otu, aslan pençesi, keltat, şebnemli gibi yöresel isimleri bulunmaktadır (Baytop, 1994).

Yaprak ve çiçek bitkinin önemli vejetatif ve generetif organlarıdır. Yapraklar bitkinin beslenme durumunu en iyi yansıtan, fotosentez, solunum ve transpirasyon gibi önemli fizyolojik olayların gerçekleştiği primer organlardır (Bilgin ve ark., 2016; Yalçın, 2018). Bu nedenle bitkinin tümündeki besin maddesi konsantrasyonunun değerlendirilmesinde yapraklardaki besin maddesi konsantrasyonu oldukça önemlidir (Kutbay ve ark., 2003; Kılıç ve ark., 2012). Azot (N), Karbon (C), Hidrojen (H) ve Kükürt (S) bitkilerin büyüme ve gelişmesi için bulundukları ortamdan büyük miktarda alması gereken makro elementlerdir. Bununla beraber, topraktan inorganik olarak absorbe edilen besin elementlerinin alınımı, ortamın pH'sına, sıcaklığına, ışık miktarına, toprağın yapısına, bitkinin türüne ve diğer bitkilerle olan rekabetine bağlı olarak değişmektedir (Comerford, 2005; Bolat ve Kara, 2017). Bitkinin önemli bir generatif organı olan çiçek ise eşeyli üreme yoluyla bitkinin neslini devam ettirmeye yarayan sürgün veya sürgün kısımları (Özçağıran, 2002) olup önemli morfolojik karakterlerdendir.

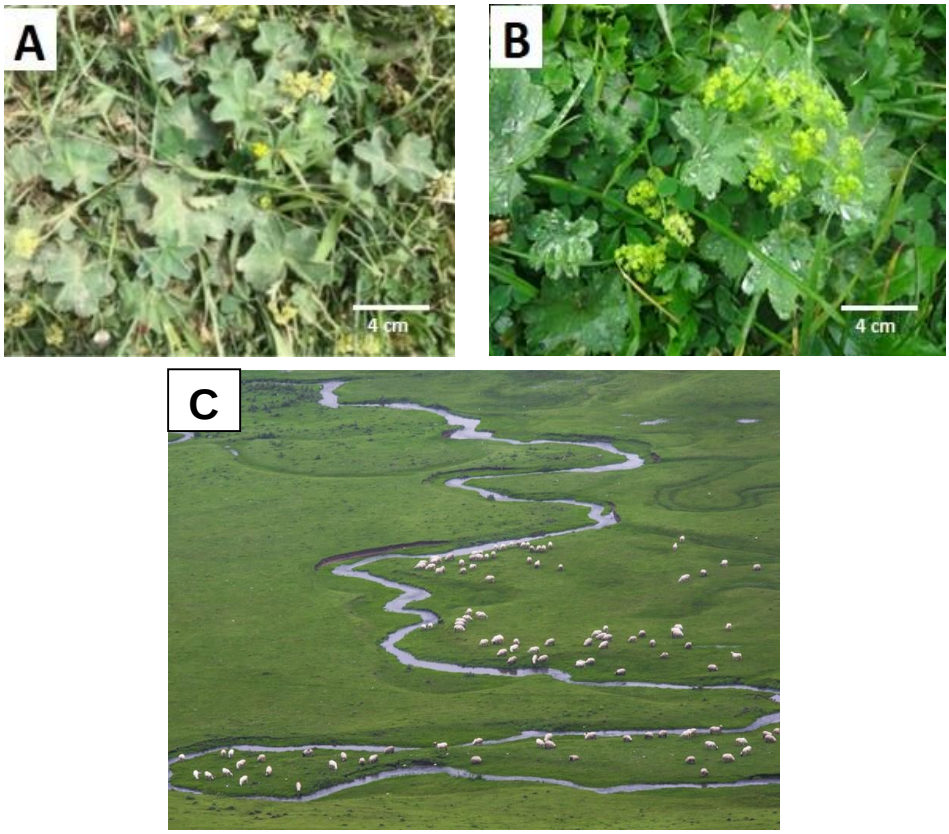
Alchemilla cinsine ait *Alchemilla orduensis* B. Pawl türü 30-40 cm boylanabilen çok yıllık bir bitkidir. Türkiyede lokal endemik olarak yayılış gösteren tür, Doğu Karadeniz Bölgesinde Ordu, Giresun ve Trabzon illerinde dar bir alanda bulunmaktadır. Türün yöresel adı "Ordu keltatı" olarak bilinmektedir (Güner ve ark., 2012). Avrupa- Sibirya fitocoğrafik elementi ve hemikriptofit hayat formuna sahip olan tür, IUCN kriterlerine göre, EN (Endangered- doğada yok olma riski çok yüksek) kategorisindedir (Deveci, 2012). Belirtilen yayılış alanlarının çok dar olması nedeniyle yeni lokalitelerin var olup olmadığının araştırılması, soyu tehlike altında olan bu türün neslinin devamının sağlanabilmesi için popülasyon çalışmalarının yapılması gerekmektedir. Bu çalışmada doğal popülasyonlarda lokal endemik bir tür olan *A. orduensis*'e ait özelliklerin gözlenmesi ve detaylandırılması amacıyla tür popülasyonunun yayılış gösterdiği lokalitelerin ekolojik özelliklerinin ve bitkinin neslinin devamında önemli rolü olan çiçek kısımlarının morfolojik ve mikromorfolojik özellikleri belirlenmeye çalışılacaktır. Türkiye için oldukça önemli olan endemik bitkilerin incelenmesi, araştırılması ve özelliklerinin belirlenmesi biyoçeşitliliğin sürdürülebilir şekilde korunması adına oldukça önemlidir.

MATERYAL ve METOD

Bu çalışmada Ordu İli Aybastı İlçesi Perşembe yaylasından toplanan *A. orduensis*'e ait örnekler ile çalışılmıştır. Türün toplanması için hedeflenen arazi çalışmalarının çoğunda türe ait örneklere rastlanmamıştır. Çalışma materyalleri 1500m (40° 41' 6.5832" kuzey paralelleri ile 37° 23' 39.1992" doğu meridyenleri arasında) yükseklikte bitkinin yayılış gösterdiği populasyonlardan vejetatif ve generatif periyotlarda (Haziran-Eylül) toplanmış ve fotoğrafları çekilmiştir (Şekil 1). Örnekler Flora of Turkey (Davis, 1972)'e göre teşhis edilerek Ordu Üniversitesi Fen-Edebiyat Fakültesi Botanik laboratuvarında koruma altına alınmıştır.

Toprak altı ve toprak üstü kısımları alınan bitki örneklerinde Elemantar/Vario Mikro Cube Elementel Analiz Cihazı kullanılarak N, C, H ve S elementlerinin miktar tayinleri yapılmıştır. Elementel analizler için toprak üstü kısmına generatif dönemde çiçek kısımları da katılmıştır. Toprak altı kısmı olarak rizom ve kök kısımları kullanılmıştır. Bitki örneklerinin toplandığı popülasyonlardan alınan toprak örneklerinde fiziksel, kimyasal ve elementel analiz tayinleri yapılmıştır. Toprak örneklerinin fiziksel ve kimyasal analizlerinde organik madde (%) Walkkey – Black metodu ile, N (%) mikro-Kjeldhal metodu ile, P (%) amonyum-molibdat- Stannus klorid metodu ile K (%),

Ca (%) ve Mg (%) ise atomik absorbsiyon spektrofotometre ile belirlenmiştir. Topraktaki % nem miktarı ise toprağın yaş ve kuru ağırlık farkının belirlenmesi ile ortaya konulmuştur. Toprak tekstür analizi Bouyoucus hidrometre metodu ile toprak pH'sı pH metre ile ölçülmüştür (Kaçar, 1984).



Şekil 1. A,B- *A. orduensis* bitkisinin genel görünüşü. C- Çalışma alanının genel görüntüsü ve otlatma baskısı (ordu.gov.tr.)

Figure 1. A-B. General view of A. orduensis . C- A general view of field and grazing pressure.

Çalışılan bölgenin iklim özelliklerinin belirlenmesi için, Ordu İli Meteoroloji İstasyon Müdürlüğü'nden alınan 1961-2019 yılları arasındaki iklim verileri ile Walter iklim diyagramı çizilmiştir. Çalışılan bölgede meteoroloji istasyonu bulunmadığı için ile ait veriler enterpolasyon yapılarak bölgenin iklim verileri hesaplanmış ve grafik çizilmiştir (Şekil 2). İklim grafikleri çalışılan bölgenin önemli iklim elemanları olan sıcaklık ve yağış özelliklerindeki değişimi ortaya koyması adına önemlidir. İklim diyagramına bakıldığında 1848.4 mm. lik yüksek yağış miktarı, 5.8 °C'lik düşük sıcaklık ile nispeten düzenli yağış eğrisi ve kurak devrenin olmayışı göze çarpan özelliklerdir.

Ayırt edici karakter açısından çiçek morfolojisi büyük önem taşıdığından bu kısımlara ait özellikler ışık mikroskobu ve taramalı elektron mikroskobu (SEM) kullanılarak belirlenmiştir. Mikromorfolojik incelemeler için herbaryum materyalleri kullanılmıştır. Elektron mikroskobunda çekim yapabilmek için öncelikle kurutulmuş çiçek örnekleri çift taraflı karbon bant üzerine yapıştırılarak sabitlenmiştir. Sabitlenen örnekler 12.5-15 nanometre (nm) altın ile kaplanmış ve 10-15 kilovolt'luk (kV) voltajla elekton mikroskobu çekimleri yapılmıştır. Yüzey şekilleri Stearn'e (1985) göre belirlenmiştir.

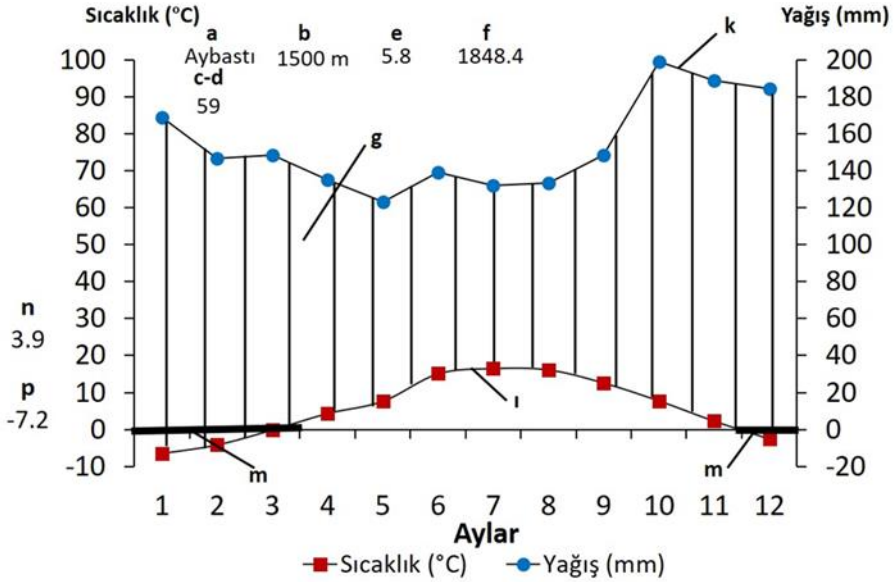
BULGULAR ve TARTIŞMA

Yapılan bu çalışmada Ordu ili Aybastı ilçesi sınırlarında bulunan Türkiyein ve ilin önemli coğrafik alanlarından birisi olan Perşembe yaylasından toplanan, lokal bir endemik olan ve ile özgü spesifik epithet taşıyan *A. orduensis* türünün bazı ekolojik ve morfolojik özellikleri çalışılmıştır. *A. orduensis*'in bölgeye özgü ve dar yayılışa sahip olması nedeniyle üzerinde çok fazla bilimsel çalışma yapılmadığı tespit edilmiştir. Bu noktadan değerlendirildiğinde hem çalışma alanı hem de çalışılan bitkinin ayrı bir öneme sahip olduğu görülmektedir.

Perşembe yaylası Aybastı ilçesine 17 km. uzakta bulunmaktadır. Çalışma alanında menderesler olarak bilinen S ve U harfi şeklindeki coğrafi oluşumlar dikkat çekmektedir (Şekil 1C). Yaylanın sahip olduğu menderesleri ile dünya miras listesine aday başvurusunda bulunulmuştur (Bahtiyar Karadeniz ve Sarı, 2018). Alan, Bakanlar Kurulu'nun 20.09.1991 tarih ve 2997 sayılı kararıyla Turizm Merkezi,

Başbakanlık Özel Çevre Koruma Kurumu Başkanlığı'nca da Özel Koruma Alanı olarak ilan edilmiştir. Alanda orman, çayır, sucul ve bozkır gibi farklı habitatlar bulunmaktadır. Yapılan arazi gözlemlerde çalışma alanında turistik tesislerin etrafına dikilen ağaçlar dışında ağaca rastlanmamıştır. Bitki çeşitliliği bakımından özellikle Asteraceae ve Poaceae familyalarına ait türlerin homojen ve geniş bir yayılışa sahip olduğu görülmektedir. Sahip olduğu çayır-mera özelliği nedeniyle alanda kontrolsüz ve aşırı otlatma baskısı olduğu dikkatimizi çekmiştir (Şekil 1C). Ayrıca arazi çalışmalar sırasında *A. orduensis* örneklerine otlama baskısından uzak menderesin çukur taraflarında rastlamasıda bu durumun bir kanıtıdır. Otlatma baskısı dışında yerli ve yabancı turistlerin oldukça ilgisini çeken alanda her yıl temmuz ayında şenlikler düzenlenmektedir. Bu da alandaki antropojenik kökenli kirlilik ve tahribat nedenlerinden bir tanesi olarak ifade edilebilir. Güler (2021) tarafından Perşembe yaylasının ekoturizm açısından önemli bir potansiyele sahip olduğu belirtilmiştir. Bu potansiyelin kullanılmasında yapılacak planlı ve denetimli uygulamaların doğada ekoturizme bağlı oluşabilecek tahribatı önleyebileceği bildirilmiştir.

Bitki örneklerinin generatif ve vejetatif dönemlerinde toprak üstü ve toprak altı kısımlarının elementel analiz sonuçları Çizelge 1'de verilmiştir.



Şekil 2. Çalışma alanına ait iklim diyagramı (a: Meteoroloji istasyonunun yeri, b: İstasyonun deniz seviyesinden yüksekliği, c: Sıcaklık için ölçüm süresi (yıl), d: Yağış için ölçüm süresi (yıl), e: Yıllık ortalama sıcaklık (°C), f: Yıllık toplam yağış (mm), g: Yağışlı devre, h: Kurak devre, ı: Sıcaklık eğrisi, k: Yağış eğrisi, m: Muhtemel donlu aylar, n: En soğuk ayın ortalama minimum sıcaklığı, p: Mutlak minimum sıcaklık)

Figure 2. Climatic diagram of study area (a: Location of meteorological station, b: Altitude of the station above sea level, c-d: Length of observation period of temperature and precipitation, e: Average annual temperature (°C), f: Average annual precipitation (mm), g: Wet period, h: Dry period, ı:Temperature curve, k: Precipitation curve, m: Frosty months, n: Mean Daily minimum temperature of the coldest month, p: Minimum temperature)

Çizelge 1. *A. orduensis* türünün toprak altı ve toprak üstü kısımlarının elementel analiz sonuçları.
Table 1. Elemental analysis results of underground and above ground parts of A. orduensis

Bitki Elementel Analiz		Azot (%) Ort ± SD	Karbon (%) Ort ± SD	Hidrojen (%) Ort ±SD	Kükürt (%) Ort ± SD
Toprak Altı	Çiçekli	1.150 ± 0.057	39.007 ± 0.063	5.407 ± 0.005	0.000 ± 0.000
	Çiçeksiz	0.969 ± 0.039	40.354 ± 0.756	5.556 ± 0.100	0.000 ± 0.000
Toprak Üstü	Çiçekli	2.089 ± 0.130	39.504 ± 0.074	5.433 ± 0.019	0.058 ± 0.009
	Çiçeksiz	2.148 ± 0.059	40.868 ± 0.109	5.567 ± 0.021	0.067 ± 0.004

Ort; Ortalama, SD;Standart sapma

Her iki döneme ait örneklerin toprak altı ve üstü kısımlarında S elementine rastlanmazken, toprak üstü kısımlarında cok az miktarda S elementine rastlanmıştır. Bitkiler kükürdü topraktan sülfat iyonları veya havadan stomalar vasıtasıyla kükürt dioksit şeklinde alabilirler. Fosil yakıtların kullanımına bağlı olarak havaya kükürt dioksit salınımı gerçekleşmektedir (Tonguç ve ark., 2017). Mobil bir element olan S'ün taşınımı genel olarak yukarıdan aşağıya doğru olmaktadır. Aşağıdan

yukarıya taşınım çok azdır. Bu durum bitkinin toprak üstü kısımlarında az miktarda tespit edilen S elementinin nedeni olabilir.

N miktarının toprak altı kısmında generatif dönemde, toprak üstü kısmında ise vejetatif dönemde yüksek olduğu görülmektedir. N miktarı generatif dönemde toprak üstü kısımlarda daha yüksektir. C ve H miktarları ise toprak altı ve üstünde birbirine yakın değerler göstermekle beraber hem toprak altı hem de toprak üstü kısmında vejetatif dönemde yüksek bulunmuşlardır. Geofit bitkilerde makro element konsantrasyonlarının toprak üstü bitki kısımlarında vejetatif dönemde toprak altı bitki kısımlarından, generatif dönemde ise toprak altı kısımlarında toprak üstü kısımlarından daha yüksek bulunduğunu gösteren top senesens denilen bir strateji bulunmaktadır (Kutbay ve Kılınç, 2002). Bu çalışmada bunun aksine, generatif dönemdeki bitki örneklerinin toprak üstü kısımlarındaki N, C ve H miktarlarının toprak altı kısımlardaki azot miktarından daha yüksek olduğu belirlenmiştir. N elementi sonucu literatürlerle benzerlik göstermektedir (Kutbay ve Kılınç, 2002; Özbucak ve ark., 2017 a, b). Bununla beraber, yüksek rakımlarda bulunan bitkiler çalışma alanında olduğu gibi düşük sıcaklıklar, rüzgâr hızı gibi daha zor iklim şartlarında yaşamak zorundadırlar (Lemke ve ark., 2012). Shi ve ark. (2012), düşük sıcaklıklarda azot bakımından zengin enzimlerin verimliliğindeki düşüşün neden olduğu biyokimyasal reaksiyonlardaki azalmanın yüksek yaprak azot içeriği ile telafi edilebileceğini bildirmiştir. Özen (2006) tarafından yapılan bir çalışmada da benzer sonuçlar bulunmuş olup elementlerin generatif gelişme döneminde vejetatif döneme göre daha düşük olmasının bu dönemdeki fizyolojik faaliyetlerin yoğun olması nedeniyle bitkiye taşınmasından kaynaklanabileceği bildirilmiştir. Ayrıca N, P, K gibi metabolizma ile ilişkili olan elementlerin konsantrasyonları yaprak ya da diğer organlar ilk oluştuğunda hücre duvarı materyalinin artması ile en yüksek değerdedir (Lambers ve ark., 2008). Toprak üstü ve toprak altı kısımları kendi arasında değerlendirildiğinde toprak üstü kısımlarının N, C, H ve S element miktarlarının toprak altı kısımlara göre daha yüksek olduğu görülmektedir. Özellikle çiçekli yani generatif dönemde toprak üstü kısımlarında bu elementlerin vejetatif döneme göre yüksek olması beklenen bir sonuçtur. Rong ve ark. (2015) çalışmalarında N, C ve P değerlerinin önemli derecede mevsimsel varyasyonlar gösterdiğini ifade etmişlerdir. Mevsimler boyunca bitkilerin değişen büyüme hızı nedeniyle, bitki organları içinde yapısal, fonksiyonel ve depolanan bileşenlerin dağılım oranları değişen büyüme hızı altında besin ihtiyaçlarını karşılamak için mevsimlere göre önemli ölçüde değişmektedir (Sterner ve Elser, 2002; Kerkhoff ve ark., 2006).

Araştırma alanında bulunan toprak örneklerinde yapılan fiziksel ve kimyasal analizler sonucunda bitkinin bulunduğu bölgelerin killi-tınlı karakterde hafif alkali, az kireçli ve az tuzlu özellikte olduğu görülmektedir. Bununla beraber, toprağın organik madde, pH, yarayışlı potasyum, fosfor, kalsiyum ve magnezyum miktarları açısında da yeterli olduğu görülmektedir (Çizelge 2). Çizelge 3'te ise toprak element analiz sonuçları görülmektedir. Bu sonuçlara bakıldığında toprakta en fazla miktarda C elementinin bulunduğu görülmektedir. Onu H ve N elementleri izlemektedir. Bu da araştırma alanında bulunan toprağın organik madde açısından zengin olduğunu göstermektedir.

Çizelge 2. Toprak analiz sonuçları
Table 2. Soil analysis results

Toprak Analizleri	Ph	% Kireç	% Tuz	% Organik madde	Yarayışlı Potasyum (K2O) (kg/da)	Yarayışlı Fosfor (P2O5)	% Suya doygunluk	Bünye sınıfı	% N	Yarayışlı Kalsiyum (mg/kg)	Yarayışlı Potasyum (mg/kg)
Analiz sonucu	7.15	1.56	0.07	6.86	51.25	12.78	57.3		0.11	4725	512.25
Değerlendirme	H. alkali	Az kireçli	Az tuzlu	İyi	Yeterli	Yeterli		Killi-Tınlı	Yetersiz	Yüksek	Yüksek
Analiz sonucu	7.17	1.56	0.07	6.40	56.32	13.83	5606		0.10	4625	502.25
Değerlendirme	H. alkali	Az kireçli	Az tuzlu	İyi	Yeterli	Yeterli		Killi-Tınlı	Yetersiz	Yüksek	Yüksek
Analiz sonucu	7.23	1.56	0.06	6.53	56.21	11.77	58.7		0.11	4685	511.05
Değerlendirme	H. alkali	Az kireçli	Az tuzlu	İyi	Yeterli	Yeterli		Killi-Tınlı	Yetersiz	Yüksek	Yüksek

Çizelge 3. Toprak elementel analiz sonuçları
Table 3. Soil elemental analysis results

Elementel analiz	Azot (%) Ort ± SD	Karbon (%) Ort ± SD	Hidrojen (%) Ort ± SD	Kükürt (%) Ort ± SD
	0.763±0.075	4.639 ± 3.943	2.549 ± 1.197	0.000 ± 0.000

Ort; Ortalama, SD;Standart sapma

Araştırma alanı yaklaşık 1500m rakımda olup toprak özellikleri bölgenin topografik yapısına, ana materyale, yıkanma zonuna, mikroiklim farklılıklarına, vejetasyona ve abiyotik faktörlerin değişkenliğine bağlı olarak farklılık gösterebilmektedir (Bilgin ve ark., 2016). Tekstür veya toprağın mineral içeriği gibi bazı durağan toprak özellikleri, oluşum süreçleri tarafından belirlenmesine karşın su içeriği, elektriksel iletkenlik, sıkışma, organik madde miktarı gibi bazı dinamik toprak özellikleri arazi yönetim uygulamalarına bağlı olarak değişkenlikler gösterebilmektedir (Jabro ve ark., 2006). Çalışmanın sonuçlarına bakıldığında toprağın özellikle organik madde ve K, P, Ca, Mg elementler bakımından yeterli düzeyde olduğu görülmektedir. Toprakta N içeriğinin düşük olmasının nedeni ortamda azotun bitki tarafından kullanılabilir formlarının bulunması olabilir. Ayrıca bölgesel ve yıllık iklim farklılıkları azot alım etkinliğini değiştirmektedir. Azot alımı iklim şartlarından büyük ölçüde etkilenmektedir. Farklı ekolojik koşulların, hava sıcaklığının ve neminin, bitkinin azot alımını ve kullanımını etkilediği varsayılmaktadır (Karaşahin, 2014).

A.orduensis türüne ait çiçek kısımlarının morfolojik incelemesi sonucunda çiçeğin epikaliks ve kaliksten oluştuğu, koralla olmadığı, epikaliksin 4 parçalı, kaliksin 4 sepalli, kaliks ve epikaliks parçalarının almaşlı sıralandığı ve infloresensin çok çiçekli olduğu belirlenmiştir. Epikaliks loplarının uç kısımlarında belirgin tüyler bulunmaktadır. Türün çiçekleri sarı renklidir. Hipantium bulunmaktadır. Pedisel tüylüdür. Sepaller 0.6-0.7mm X 0.8-1mm ebatlarında, epikaliks loblarıda 0.5-1mm X 1-1.5 mm boyutlarındadır. Sepaller epikaliks loplarından daha dardır (Şekil 3A-D). Davis (1972) benzer şekilde türün epikalikslerinin daha geniş olduğunu belirtmiştir. Meyve özellikleri ile ilgili Flora of Turkey'de (1972) veriye rastlanılmamıştır. Yapılan çalışmada türün aken tipi meyveye sahip olduğu ve akenlerin yaklaşık 0.5-0.7mm X 1-1.2 mm boyutlarında olduğu belirlenmiştir. Faghir ve ark. (2017) 24 *Alchemilla* türünün çiçek morfolojisini incelemişler ve kaliks şekli ve uzunluğu, kaliks ve pediseldeki tüylerin durumunun türlerin ayırt edilmesinde çok önemli karakterler olduğunu belirtmişlerdir. Ayrıca cinsin tür tayin anahtarlarında çiçek özellikleri ayırt edici özellik olarak kullanılmaktadır (Davis, 1972).

Taramalı elektron mikroskobunun (SEM) kullanımı, bitkilerin vejetatif ve generatif organlarının yüzey özelliklerinin belirlenmesinde önemli katkılar sağlamıştır (Barthlott, 1984; Stace, 1984). Petal ve sepal morfolojisi ve mikromorfolojisi taksonların ayırt edilmesinde ayırtedeci özelliklerdir (Metcalfe ve Chalk, 1979; Özcan, 2002). Song ve ark., (2020) Rosaceae familyasında petal ve epikaliks gibi yapıların mikromorfolojik ve morfolojik karakterlerinin evolusyonuna farklı bir bakış açısı getirdiğini belirtmişlerdir. *A. orduensis* türünün epikaliks, sepal ve meyve yüzeylerinin taramalı elektron mikroskobu yardımı ile yapılan mikromorfolojik incelenme sonuçları Şekil 3 ve Şekil 4'te görülmektedir. Epikaliksin alt tüpsü yapısında belirgin tüyler bulunmaktadır (Şekil 3B). Epikaliks dış yüzeyi dikdörtgenimsi hücrelerden oluşmaktadır. Hücrelerin çeperleri içe gömülü durumdadır. Hücrelerin antiklinal çeperleri genellikle düz bazende kavislidir (Şekil 3 F-G). Epikaliksin iç yüzeyindeki hücreler dalgalı çeperlidir. Hücrelerin çeperleri belirgin şekilde içe gömülüdür. Epikaliks iç yüzeyi retikulat-rugose yüzey şekillidir (Şekil 3 H-I). *Onosma* L. türlerinin petalleri ile yapılan çalışmada petal dorsal yüzeylerinin mikromorfolojik yapılarının diğer morfolojik ve mikromorfolojik özellikler ile birlikte türlerin ayırt edilmesinde kullanılacağı belirtilmiştir (Akçin, 2009).

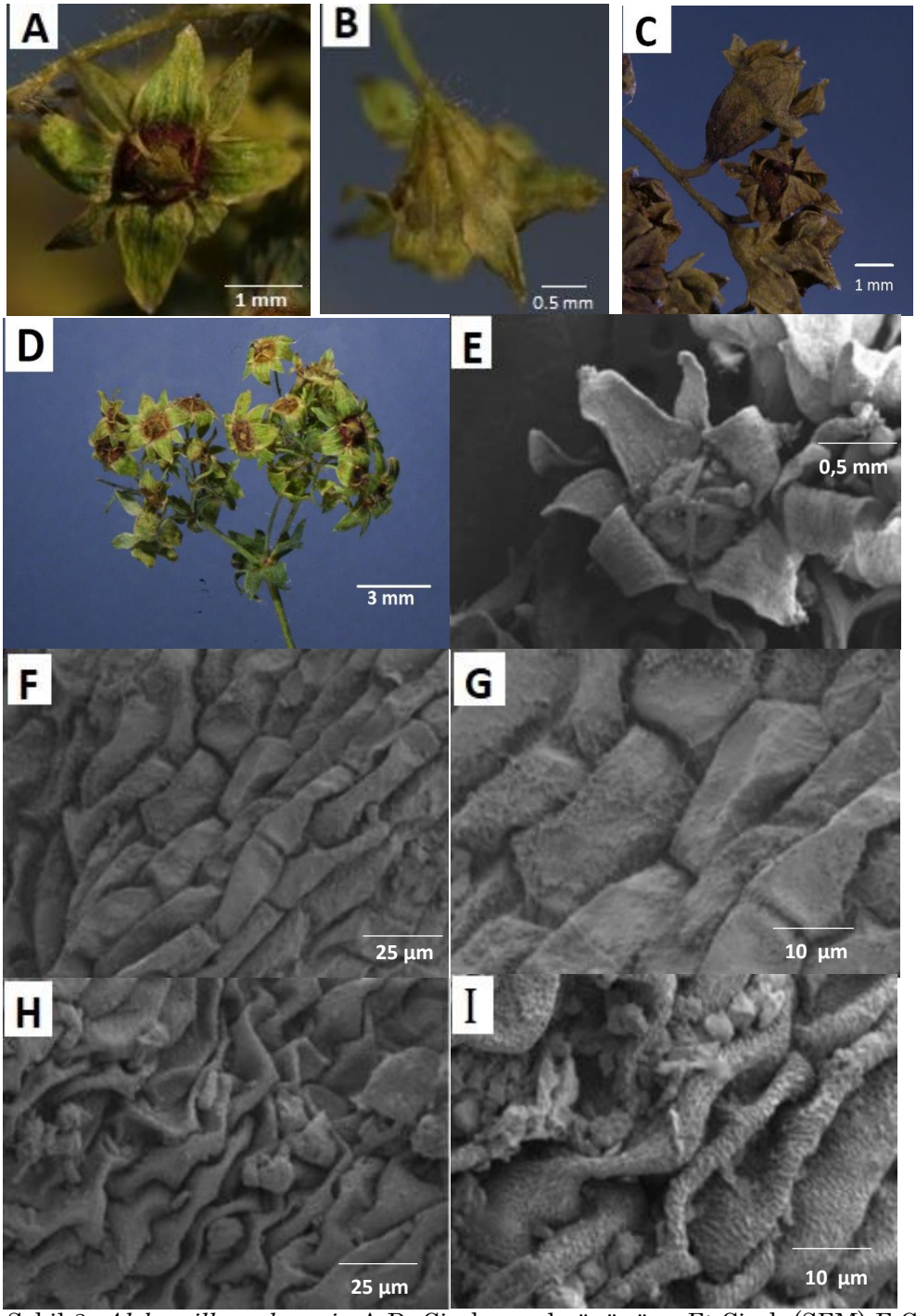
Çiçeklerin sepallerinin dış yüzeyinde epidermis hücreleri az-çok dikdörtgenimsi bir yapıdadır. Yüzey üzerinde kütikular katlanmalar ve vaks yapısı bulunmaktadır. Antiklinal çeperler genellikle kavislidir. Hücre çeperleri içe gömülü vaziyettedir (Şekil 4 A-B). Sepallerin iç yüzeyleri dalgalı çeperlidir. Hücreler girintili çıkıntılı yapıdadır. Hücrelerin üzerinde yoğun retikulat yüzey şekilleri bulunmaktadır (Şekil 4 C-D).

A. orduensis türünün meyvesi aken tipindedir. Yüzey üzerinde süslemeler bulunmaktadır. Meyve yüzey tipi retikulat tiptedir. Epidermis hücreleri az çok dikdörtgenimsi ve antiklinal çeperleri düz veya kavislidir (Şekil 4 E-F). Birçok bitkinin meyve ve tohum mikromorfolojileri taramalı elektron mikroskobu ile incelenmiş ve taksonomik önemleri ortaya konulmuştur (Juan ve ark., 2000; Akçin, 2007). *Potentilla* L. (Rosaceae) türlerinin aken mikromorfolojilerinin cinsin türlerini ayırt etmek için güvenilir kriterler olarak kullanılabileceğini gösterilmiştir (Sadeghi ve ark., 2021).

SONUÇ ve ÖNERİLER

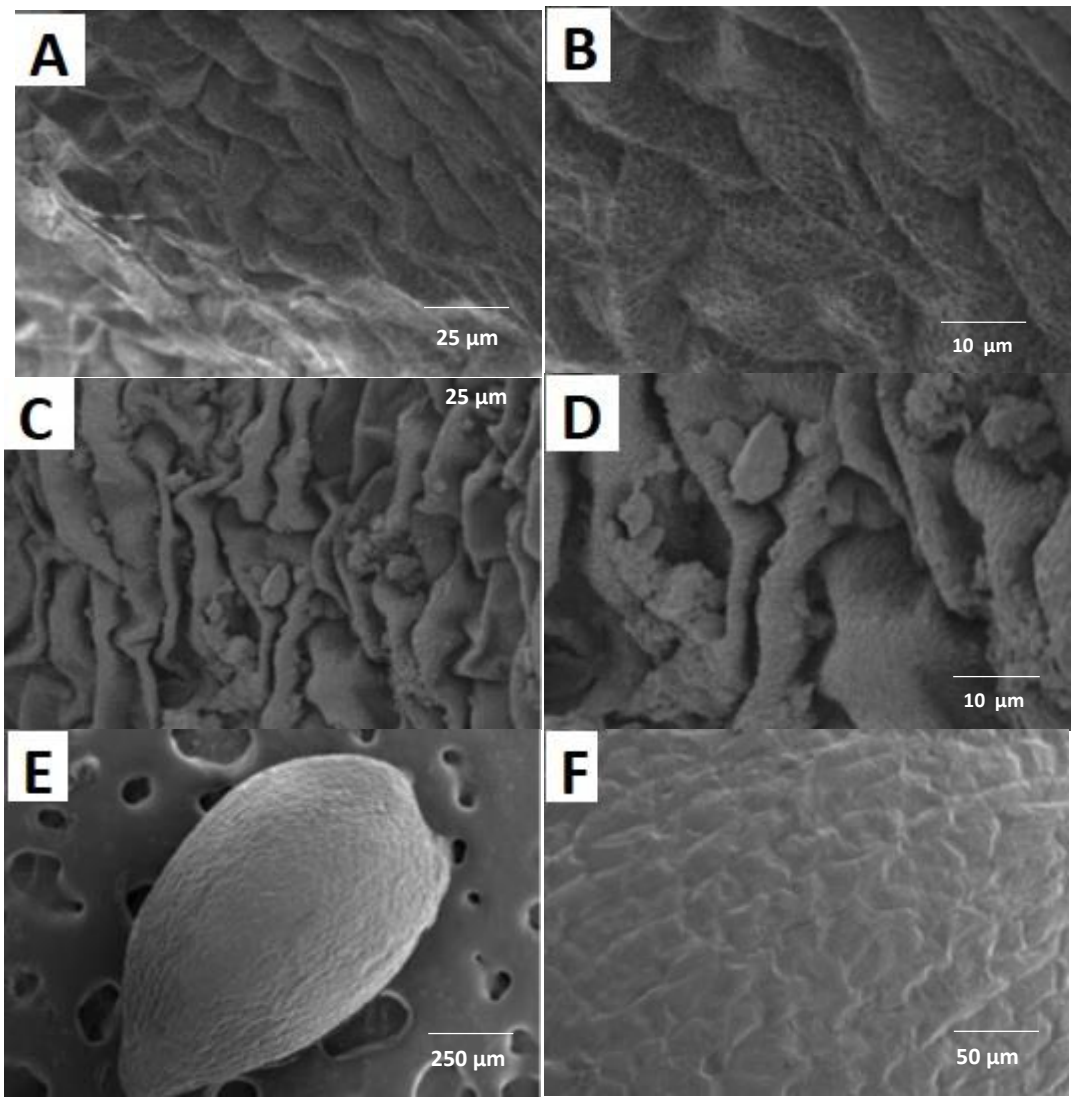
Bu çalışmada Ordu İli Perşembe yaylasında sınırlı bir alanda çok az bir popülasyon ile temsil edilen lokal endemik tür olan *A. orduensis*'in çiçek ve meyve morfolojisi ile bazı ekolojik özellikleri tespit edilmeye çalışılmıştır. Hem generatif hem de vejetatif dönemde N, C, H ve S element miktarlarının toprak üstü kısımlarda fazla olduğu belirlenmiştir. Bitkinin çiçek özellikleri ayrıntılı şekilde incelenmiştir. Cinsin tür tayin anahtarında bu özelliklerinden faydalanılmaktadır. Lokal endemik olan türün daha iyi tanınması için diğer özelliklerinin de ayrıntılı şekilde incelenmesi önerilmektedir. Farklı coğrafi ve

iklimsel özelliklere sahip alanın otlama baskısı altında olduğu gözlenmiştir. Çalışılan bitki örneklerinin otlatma baskısından uzak lokalitelerde bulunması tahribatın bir göstergesi olabilir. O nedenle alanda bulunan endemik ve tehdit altındaki türler ile yapılacak çalışmaların hem bitki çeşitliliğinin hem de mendereslerin korunması ve sürdürülebilirliğine katkı sağlayacağını düşünmekteyiz.



Şekil 3. *Alchemilla orduensis.* A-D. Çiçek genel gönünüm, E; Çiçek (SEM) F-G; Epikaliks dış yüzey (SEM), H-I; Epikaliks iç yüzey (SEM).

Figure 3. Alchemilla orduensis.A-D.Flower general appearance, E; Flower (SEM) F-G; Epicalyx outer surface (SEM), H-I; Epicalyx inner surface (SEM).



Şekil 4. *A. orduensis* SEM çekimleri. A-B; Sepal dış yüzey, C-D; Sepal iç yüzey, E; Meyve genel görünüş, F; Meyve yüzeyi.

Figure 4. A. orduensis SEM photos. A-B; Sepal outer surface, C-D; Sepal inner surface, E; Fruit general appearance, F; Fruit surface.

TEŞEKKÜR

Bu çalışma Ordu Üniversitesi Bilimsel Araştırma Projeleri Koordinasyon Birimi tarafından desteklenen A-1832 no'lu projenin bir kısmıdır. Katkılarından dolayı teşekkür ederiz.

Araştırmacıların Katkı Oranı Beyan Özeti

Yazarlar makaleye eşit oranda katkı sağlamış olduklarını beyan eder.

Çıkar Çatışması Beyanı

Makale yazarları aralarında herhangi bir çıkar çatışması olmadığını beyan ederler.

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Inocybe tarda Kühner: Türkiye Mikotası İçin Yeni Bir Kayıt

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ÖZET

Bu çalışmada, *Inocybe tarda*'nın bazidiyokarpları Türkiye'den ilk kez toplanmış, makroskobik ve mikroskobik yapıları, ilgili fotoğraflar ve kısa bir tartışma ile birlikte sunulmuştur. Türkiye örneği soluk kırmızımsı kahverengi veya kestane rengi şapka, badem biçiminde, 8–12 × 5–6 µm bazidiyosporlar ve iğ veya şişkin silindir biçimindeki, 50–80 × 12–21 µm sistityumlar ile akraba türlerden ayrılır.

Biyoloji

Araştırma Makalesi

Makale Tarihçesi
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Anahtar Kelimeler
Bazidiyomikota
Taksonomi
Trabzon
Bazidiyospor

Inocybe tarda Kühner: A New Record for the Turkish Mycota

ABSTRACT

In this study, basidiocarps of *Inocybe tarda* were collected for the first time from Turkey and are presented together with macroscopic and microscopic structures, related photos and a brief discussion. The Turkish specimen is distinguished from related species by its pale reddish brown or chestnut color cap, almond-shaped, 8–12 × 5–6 µm basidiospores and spindle-shaped or swollen cylindrical, 50–80 × 12–21 µm cystidia.

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GİRİŞ

Inocybe (Fr.) Fr. çeşitli renklerde, küçük ve orta büyüklükte, yüzeyi pullu veya lifli ve az çok yapışkan şapkası, sapa az veya çok birleşik, açık veya koyu renkli lamelleri, silindirik veya tabanda genişlemiş, tozlu, lifli veya pullu, az çok kortinalı sapı, sperm veya farklı kokulu ve büyük çoğunluğunda zehirli eti, açık sarımsı kahverengi spor izi, fasulye şeklinde, badem biçiminde, düğümlü-köşeli, düz yüzeyli ve açık kahverengimsi bazidiyosporları, iğ biçiminde, kalın çeperli ve tepesi kristalli sistityumları, az çok paralel veya bazen yüzeye doğru büyüyen ve kancalı şapka derisi hifleri ile diğer gruplardan ayırt edilen büyük bir cins olup son yıllardaki moleküler çalışmalar sonucunda birkaç alt cinse bölünmüştür (Kuyper, 1986; Vauras, 1992; Knudsen ve Vesterholt, 2008). Cins Türkiye'nin her yöresine yayılmış olup yüze yakın türle temsil edilmektedir. Yakın zamanda bilim dünyası için yeni ve Türkiye için yeni kayıt *Inocybe* türlerinin yayımlanması yeni çalışmaların gerekliliğini ortaya koymaktadır (Akata ve ark., 2014; Keleş ve ark., 2014; Altuntaş ve ark., 2019; Sadullahoğlu ve ark., 2019; Sesli, 2019; Bandini ve ark., 2020; Sesli, 2020; Sesli ve ark., 2020; Doğan ve ark., 2021; Uzun ve Kaya, 2022).

Bu çalışmanın amacı Türkiye'de yayılış gösterdiği ilk kez saptanan bazidiyokarpları inceleyip tanıtarak *Inocybe tarda* türünü Türkiye mikotası için yeni olarak bildirmektir.

MATERYAL ve METOD

Bu araştırmanın materyalini 06.12.2017 tarihinde Trabzon ili Ortahisar ilçesinde bulunan Karadeniz Teknik Üniversitesi yerleşkesinden toplanan bazidiyokarplar oluşturmaktadır. Toplama sahasında yayılış gösteren ağaçların türü, bazidiyokarpların genel özellikleri, renklerinin değişip değişmediği ve olası mikorizal ilişkileri kaydedildikten sonra birkaç tanesi toplanarak kese kağıtlarına konup laboratuvara getirildi. Spor izleri elde edildikten

sonra geriye kalan numuneler radyatör üzerinde birkaç saatte kurutuldu ve kataloglanarak fungaryuma yerleştirildi. Daha sonra kuru materyalin lamel, şapka derisi ve sap yüzeyinden mikroskop altında ince kesitler alındı. Mikroskopik incelemeler için kesitler %5'lik amonyak çözeltisi içerisine konuldu, birkaç dakika bekletildikten sonra bazidiyum, sistityum ve şapka derisi hifleri görüntüleme sistemi sayesinde ölçüldü ve mikroskopik fotoğrafları elde edildi. Bazidiyosporların toplu halde görüntülenebilmesi için bazidiyokarptan yaklaşık bir santimetre karelik bir parça kesildi, %10'luk amonyak çözeltisi içerisinde 5 dakika bekledildikten sonra lam üzerinde pens yardımı ile birkaç defa sıkılıp bırakıldı. Fotomikrografi sistemi sayesinde yaklaşık 30 tanesinin büyüklükleri ölçüldü, ortalaması alınarak en yakın sayıya yuvarlandı ve fotoğrafları çekildi. Numunelerin teşhisi ilgili kaynaklara göre yapıldı (Kuyper, 1986; Vauras, 1992; Knudsen ve Vesterholt, 2008; Sesli ve Bandini, 2019, 2020).

BULGULAR

Basidiomycota / Topuzlu mantaralar

Inocybaceae / Kümbetmantarıgiller

Inocybe tarda Kühner / Cücekümbet (Şekil 1)

Şapka konik veya düz, 25–55 mm, tepe çıkıntılı, pullu, lifli veya tüylü, soluk kırmızımsı veya turuncumsu kahverengi ve kenarlara doğru daha soluk renklidir. Lameller sınırlı bir bölgeden veya daralarak sapa bağlı, geniş, beyazımsı gri veya pembemsi kahverengi ve kenarları sillidir. Eti ince, spermatik kokulu, hafif tatlı, beyazımsı, grimsi veya soluk pembemsidir. Sap silindirik, kırılgan, bazen tabanda daha geniş, 35–65 × 3–8 mm, yüzeyi pembemsi veya turuncumsu kahverengi, tabana doğru daha açık renkli, beyazımsı ve lifli, yukarıda beyazımsı tozlu görünümdedir. Bazidiyumlar çomakçık biçiminde, 4 sporlu, kancalı ve 25–38 × 8–12 µm'dir. Bazidiyosporlar eliptik veya badem biçiminde, düz yüzeyli, kalın çeperli ve 8–12 × 5–6 µm'dir. Sistityumlar iğ biçiminde veya ortadan şişkin silindir şeklinde ve 50–82 × 12–21 µm'dir. Şapka derisi az çok düzgün ve paralel, 5–12 µm kalınlığında silindirik hiflerden oluşmuştur. Sonbaharda park ve bahçelerde, geniş yapraklı ağaçlardan oluşan orman kenarlarında, ağaçlar altında, genellikle gruplar halinde bazen de tek tek yayılış gösterir.

İncelenen örnekler: Türkiye, Trabzon, Karadeniz Teknik Üniversitesi Yerleşkesi, 40°59'39.07" K ve 39°46'17.22" D, 108 m, 06.12.2017, gruplar halinde, E. Sesli 3875.

TARTIŞMA

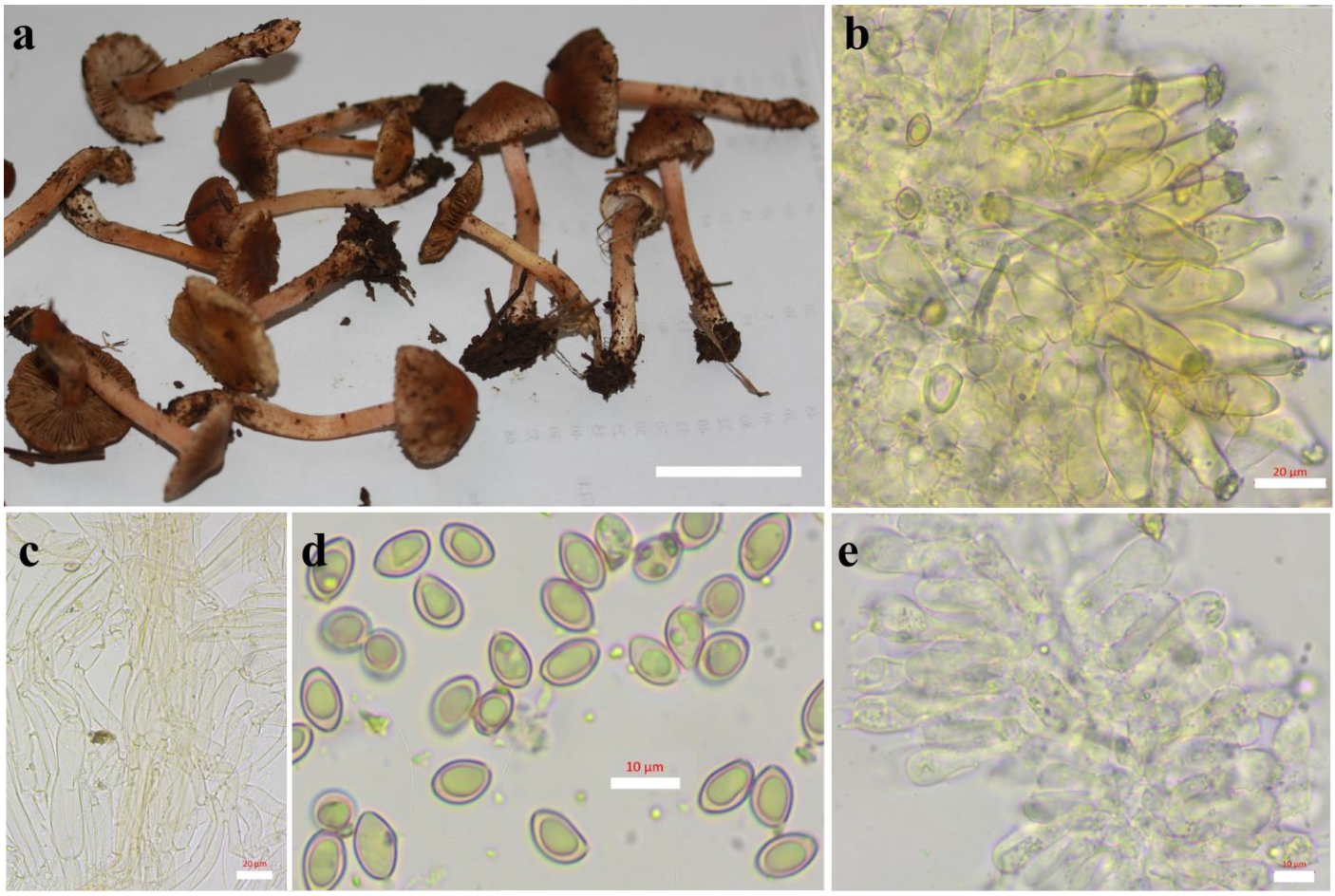
Inocybe tarda'nın ait olduğu alt cins (sect. *Tardae* Bon) üyelerinde şapka beyaz, sarı, grimsi beyaz, turuncumsu kahverengi, toprak, cilt, leylak veya menekşe renginde olabilmektedir. Bu alt cins içerisinde yeni kayda yakın bireylerde şapka nispeten zarif ve küçüktür. En yakın tür olan *Inocybe nitidiuscula* (Britzelm.) Sacc., kozalaklı ağaçlar ve özellikle ladinle mikorizal yaşaması ve daha küçük şapkalı (10–40 mm) olması ile ayırt edilir. Diğer yakın bir tür olan *I. involuta* Kuyper genellikle çatlak şapkası, daha küçük boyutlu olması (şapka: 20–40, sap 30–50 × 4–7 mm), daha geniş bazidiyosporları (6–7.5 µm) ve iğne yapraklı ağaçlar ile mikorizal olması ile yeni kayıttan fark eder. *Inocybe pusio* P. Karst oldukça küçük (10–35 mm) şapkaya, az veya çok menekşe renginde sap ve lamellere ve daha küçük bazidiyosporlara (8–11 × 4.5–6 µm) sahiptir. *Inocybe aeruginascens* Babos beyaz ve zedelenince mavimsi yeşil sapa, 8–10 × 4–5.5 µm bazidiyosporlara sahiptir ve daha çok kumlu toprakları tercih eder. *Inocybe nespiakii* Bon oldukça küçük şapka ve sapı (8–13 mm, 20–30 × 1.5–3 mm) ve polimorfik sistityumları ile kolayca ayırt edilir. *Inocybe obscurobadia* ladinle mikorizal yaşam sürmesi, daha kısa bazidiyosporları (7–10.5 µm), şişe ve/veya armut biçimindeki sistityumları ile farklılık gösterir. Diğer bir nispeten yakın fakat farklı bir tür, *Inocybe ochroalba* Bruylants 10–30 mm, konik, yarım küre veya çan biçiminde şapkaya, 7.6–10 × 4.5–5.5 µm bazidiyosporlara ve daha küçük sistityumlara sahiptir. *Inocybe luteipes* J. Favre koyu grimsi ve oldukça küçük şapkaya, sarımsı veya turuncumsu kahverengi sapa, eliptik ve 8.5–9.5 × 6–6.5 µm bazidiyosporlara sahiptir. *Inocybe rupestris* J. Favre kahverengimsi sapı, 10–15 mm şapkası, silindirik ve 45–65 × 10–16 µm sistityumları ile *Inocybe tarda*'dan ayrılır. *Inocybe posterula* (Britzelm.) Sacc. eliptik ve daha küçük bazidiyospor (7.5–9 × 4.5–6 µm) ve şişe şeklinde, 45–60 × 12–20 µm sistityumları ile farklılık gösterir. *Inocybe subporospora* Kuyper 9–36 mm şapkalı, kırmızımsı kahverengi saplı ve daha kısa (8.5–10 µm) bazidiyosporludur (Kuyper, 1986; Vauras, 1992; Breitenbach ve Kränzlin, 2000; Knudsen ve Vesterholt, 2008).

SONUÇ ve ÖNERİLER

Bu çalışma sonucunda *Inocybe tarda* Türkiye için yeni kayıt olarak betimlenmiş ve Türkiye mikotasına katkıda bulunulmuştur. *Inocybe* grubu mantarların dışarıdan bakıldığında teşhisi zor görünmesine rağmen, renkleri, mikorizal ilişkileri, oldukça ilginç sistityumları, eliptik veya nodüllü olabilen bazidiyosporları, ezildiğinde renk değiştirebilme özellikleri, şapka derisinin mikroskopik yapısı, kokusu ve benzeri çok değişken ve kullanılabilir karakterleri sayesinde dikkatli çalışıldığında kolay bir grup olduğu söylenebilir.

Genellikle küçük boyutlu olduklarından ve çeşitli habitatlarda kendilerini kamufle edebildiklerinden

arazide dikkatli aranmaları gerekir. Fakat bu zorluğun iyi yönü olarak her zaman yeni kayıt veya yeni tür *Inocybe* bulma olasılığının yüksek olduğunu söyleyebiliriz. Diğer yönden bazı yıllar yaz ve sonbahar aylarında beklenen yağmurların yağmaması mantar araştırmacılarının arazi çalışmalarını olumsuz etkilediği bir gerçektir. Fakat birçok *Inocybe* türünü geç sonbaharda ve hemen hemen kışa kadar toplayabilme şansınız mevcuttur. Küçük boyutlu olduklarından kurutulmaları da kolaydır. Bu nedenlerle mantar araştırmacılarına bu grup mantarları çalışmalarını öneririz.



Şekil 1. Inocybe tarda: a- bazidiyokarplar, b- sistityumlar, c- şapka derisi kesiti, d- bazidiyosporlar, e- bazidiyumlar (Ölçek çubukları: a: 50 mm, b ve c: 20 µm, d ve e: 10 µm)

Figure 1. Inocybe tarda: a-basidiocarps, b-cystidia, c-section from the pileipellis, d-basidiospores, e-basidia (Scale bars: a: 50 mm, b and c: 20 µm, d and e: 10 µm)

TEŞEKKÜR

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Example of Sexual Dimorphism and Structural Defects in Some Aquatic Insects (Coleoptera)

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ABSTRACT

Freshwater is a very important part of the ecosystem. Aquatic insects are at the center of importance of fresh waters due to their remarkable number and diversity.. The purpose of this study was to compare the morphological differences in male and female individuals with the arithmetic mean of some body measurements and to give a summary of rare unexpected structural disorders. On observing sexual dimorphism, *Helophorus aquaticus* Linnaeus, 1758 (Coleoptera) specimens were selected from the samples collected from Erzurum Wetlands (Turkey) between April and October (2021), as it has a large body and their number in the collection was sufficient. Measurements were performed under a stereo microscope. In this context, some differences in measurements were evaluated by considering sexual dimorphism, some structural disorders and gender-developmental stages in our study. Along with some ecological data, like sexual difference of approximately 100 samples, measurement differences were identified with body length 0.1-0.4 mm, body width 0.1-0.3 mm and leg length 0.1-0.3 mm. There are differences in measurements between the two groups, and in some species belonging to some families of the other Coleptera order, individuals with structural disorders have been identified.. It is believed that the developmental stages and sexual dimorphism size differences are important data for future ecological and evolutionary studies.

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Bazı Sucul Böceklerde (Coleoptera) Eşeysel Dimorfizm ve Yapısal Bozukluklar Örneği

ÖZET

Tatlı sular ekosistemin çok önemli bir tamamlayıcıdır. Sucul böcekler sayı ve çeşitlilik açısından dikkat çekmeleri nedeniyle tatlı suların önem bakımından merkezindedir. Bu çalışmanın amacı; erkek ve dişi bireylerde morfolojik farklılıkların bazı vücut ölçülerindeki aritmetik ortalamasıyla karşılaştırılmasını sağlamak ve nadir karşılaşılan beklenmedik yapısal bozukluklara önek vermektir. Eşeysel dimorfizmi görebilmek için; Erzurum Sulak alanlarından (Turkey) nisan ve ekim ayları arasında toplanan örneklerden büyük vücuda sahip olması ve toplanma sayısı yeterli olduğu için *Helophorus aqauticus* Linneus, 1758 (Coleoptera) türü seçilmiştir. Bir stereo mikroskopta ölçümleri yapılmıştır. Bu bağlamda; ölçümlerdeki bazı farklılıklar çalışmamızda eşeysel dimorfizm, bazı yapısal bozukluklar ve cinsiyet-gelişim evreleri dikkate alınarak değerlendirilmiştir. Bazı ekolojik verilerle birlikte yaklaşık 100 örneğin eşesel farklılığı olarak; vücut uzunluğunda 0,1mm.-0,4 mm., vücut genişliğinde 0,1 mm.-0,3 mm. arası ve bacak uzunluğunda 0,1 mm.-0,3 mm. arası ölçüm farklılıklarının olduğu, diğer Coleptera takımının bazı familyalarına ait bazı türlerde ise yapısal bozukluk örneği görüldüğü bireyler tespit edilmiştir. Sonuçların, gelişim evreleri ve eşeysel dimorfizm boyut farklılıklarının gelecekteki ekolojik ve evrimsel çalışmalar için önemli veriler olacağına inanılmaktadır.

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INTRODUCTION

Freshwater ecosystems provide several functions for both nature and society. Freshwater affects biogeochemical processes, ecological dynamics of biodiversity, ecosystem productivity, and human health and welfare at local, regional and global scales (Albert et al., 2021). Freshwater insect abundance needs to be evaluated cautiously in the context of distinct (Jähnig et al., 2020) and morphometric characters.

Dey (2007) suggested that morphometrics can play a very important role in understanding some of the unique features and additional information about insects. Geometric morphometrics is a relatively new technique that has generated valuable results in the study of insects and ecology. The advantage of a geometric framework is comprehensive use of information about shape (Vijayakumar and Jayaraj, 2013). Because insects have morphometric variations, geometric morphometry has an important role in studying aquatic ecosystems. Study of morphometrics is a simple and quick technique, with low cost and very good discriminatory power. Also, Zelditch et al. (2004) mentioned that this new information from geometric morphometrics for biologists is divided into three parts topically: basics of shape data, analysing shape variables, and applications of morphometric methods.

Sexually dimorphic organisms provide elegant comparative systems in which to study adaptation (Dawson and Geber, 1999). The comparison of anatomical features of organisms, and understanding how variation in these features is associated with variation in other traits, has long been of interest to ecologists and evolutionary biologists (Adams and Castillo, 2013). Additionally, geometric morphometrics can be used in a phylogenetic context (Monteiro, 2013).

Finally, invasive species are well known to impact many aspects of ecosystems, including biodiversity (Li et al., 2021). Helophorus spp. insects are abundant in freshwater (Bektas, 2015). For morphometric aims, some measurements of geometrical features of aquatic insects, shape variation and descriptions of numerous functions are obtained as both two-dimensional and three-dimensional data (Adams and Castillo, 2013).

MATERIAL and METHODS

Collection of samples

One species of *Helophorus aquaticus* Linnaeus, 1758 (Coleoptera: Helophoridae) was used as a model (Fig. 1). Adult individuals of *Helophorus* spp. species and other aquatic coleoptera were collected from a randomly selected area of Erzurum Wetland (Turkey) from April-October 2021, using aerial nets in the late afternoon (Table 1). After the collected individuals were sorted and examined in using a stereo microscope (Zeiss Stemi 305) in the Entomology laboratory (Agricultural Faculty of Ataturk University) and East Anatolia High Technology Application and Research Center (DAYTAM) laboratories (Ataturk University), they were separated to two sections according to use in research. Then they were scanned, measured and parameters were calculated under light microscopy. The materials (hexapod) were stored in small bottles. The hexapod was cleaned with a brush before identification, and then the adeagophore of the hexapod was dissected under a stereo microscope in the laboratory. Bektaş (2015) was used for morphological identifications. The sampling time was not a special time, but a rain-free day when the insect could be in the adult phase. Also, the coordinates of localities (different sampling-points of Erzurum wetland) where the insect samples were taken were obtained by using a GPS device (Garmin Gpsmap 66S Handheld).

Figure1. Sexual dimorphism at *Helohrus aqauticus* Linneus, 1758 (Coleoptera: Hydrophilidae).
Şekil 1.*Helohrus aqauticus* Linneus, 1758 (Coleoptera: Hydrophilidae) türünde eşeysel dimorfizm.

Methodology

Every metric has the possibility to clearly contribute

to understanding (Daan, 2005). Aquatic ecosystem analysis was considered the aim of this study. The differences between males and females in morphometric measurements were tested using statistical analysis under stereo microscopy (Zeiss Stemi 305). To visualize the shape (Fig. 2 and Table 1), maximum and minimum values and variation coefficients were calculated using mean values and standard deviations. Measurements used the technological device which was located at DAYTAM. Additionally, the difference between measurements of morphometrics for male and female structures and structural defects may be an important focus in evolutionary research. Approximately 100 aquatic insect specimens (43 males; 54 females) of *Helophorus aquaticus*, were caught. This species was chosen because it is found in abundance by chance. Moreover, sexual dimorphism (in terms of size) is evident and body size is easy to measure. Other aquatic insects with structural defects were found by chance and the insects were photographed to make use of this opportunity (Fig. 3).

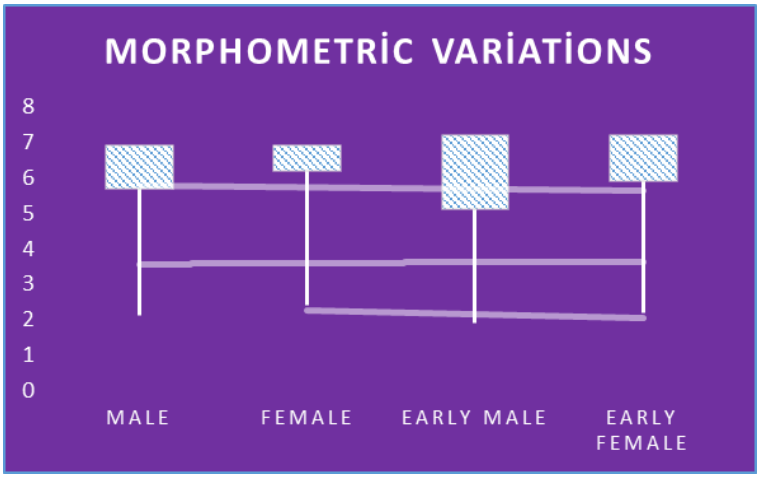

Figure 2. Morphometric variaons on stage and sexual of collected *Helophorus* spp. species.
Şekil 2. Helophorus spp. türlerinde yaşam evreleri ve cinsiyetlerine göre morfometrik varyasyonlar.

Table 1. Morphometric scales on *Helophorus aqauticus* Linneus, 1758 (Coleoptera: Hydrophilidae) species.
Çizelge 1. Helophorus aqauticus Linneus, 1758 (Coleoptera: Hydrophilidae) türünde morfometrik ölçümler.

Gender	Collected Information	Body Length (mm)[a]	Body Width (mm)[a]	Leg length (mm)[a]	Freshwater temperature (°C)[a]	Freshwater pH*
Male ♂	Coordinates: 39°58'02.0"N/41°17'52.5"E Location: Vegetation Regions, Local Conserved Wetlands. Erzurum Wetlands, Turkey Collected time: April-October	5,7±0,1	2,1±0,1	3,5±0,1	25±1	6.9±0,1
Female ♀	Coordinates: 39°58'02.0"N/41°17'52.5"E Location: Vegetation Regions, Local Conserved Wetlands. Erzurum Wetlands, Turkey Collected time: April-October [b]	6,2±0,1	2,4±0,1	3,8±0,1	25±1	6.9±0,1

[a]Average measurements
[b]There are twin sercus at last sternit of abdomen

Figure 3. Structural defects at some aquatic insects (Coleoptera).
Şekil 3. Bazı sucul böceklerde (Coleoptera) yapısal bozukluklar.

RESULTS and DISCUSSION

The most conspicuous secondary sexual traits in animals are morphological features. This is exemplified by body size in insects (Ariza-Marín and Luna, 2022). In past research, populations of insects confirmed that morphological traces were not more structured than genetic patterns and did not always correlate (Zubrii et al., 2022). Also, teratology (structural defect) studies the causes that produce structural malformations, defects or abnormalities in organisms (Burke et al., 2018). Before genetic support, insect studies are needed to reveal morphological differences and obtain preliminary data. These analyses were conducted for both structural defects and morphometric differences. Here we attempt to demonstrate that utility of morphometric and structural analysis as a new methodology for research. Also, there are some groups that are:

For animals, some anatomical structure appears to be good markers of quality of diet, with feedback mediated by sex (Gutiérrez-Cabrera et al., 2022). Insects are among the most successful animals among species in the world. Their success is partly attributed to their modified, sclerotized forewings, known as elytra, that protect their body against physical damage, desiccation, predation and thermal stress, enabling them to occupy a wide range of ecological habitats (Katlav et al., 2021). An increase in insect abundance may reflect improved water quality (Jähnig et al., 2020), as distribution and behaviour of insect populations are based on their powerful structures and adaptation to the environment. Wings, legs and genital organs are excellent structures for studying morphological variations. Comparisons of results among these studies are difficult because of the different morphometric methods and concepts of modularity and integration (Joji´c et al., 2012). Morphometrics is defined as the quantitative description, analysis and interpretation of shape and variation of structures in biology (Mondal et al., 2015). As in other applications of geometric morphometrics, some adjustments may be needed (Klingenberg et al., 2003). Plus, these studies contributed to resolving taxonomic problems in aquatic insects and other species (Petrarca et al., 1998). Dawson and Geber (1999) indicated that females are more often limited in their reproductive success by resources, whereas males are limited by opportunities for mating. Consistent with this view is the very strong evidence of larger resource (biomass) investments in reproduction in females compared to males. This approach may be supported for aquatic insects. Morphological integration also manifests at a macroevolutionary level (Klingenberg, 2008). It is important to study teratomorphs because they can last a long time and manifest in the phylogeny of a certain lineage; also these morphological abnormalities pinpoint the underlying genetic determinism of the morphology. Morphological anomalies are not uncommon in insects, and Coleoptera is one of the orders with the highest number of described teratological cases (Popa et al., 2021). Therefore, it is very important to find these teratological images in our morphometric and ecological studies of aquatic insects.

Some differences in animal body shape arise even when there are minimal variations in diet (Huie et al., 2020). A similar study (Almeida et al., 2021) found females were larger than males in body length, but males had thicker arms and forearms. The diet of males and females is similar for *Hylodes perere* Silva

& Benmaman, 2008 (Anura, Hylodidae), comprising insects, arachnids and crustaceans, suggesting a similar foraging strategy for both sexes. Francoy et al. (2009) examined the forewing venation patterns of males and females of five stingless bee species and reported that the patterns of males and females from the same species were more similar than the patterns of individuals of the same sex from different species. They suggested that the features extracted from the wings of males and females were very informative in discriminating the five species. Metrics, however, measure something specific, while indicators are supposed to tell us something different from what they actually measure (Daan, 2005).

Above all, the analyses showed that there was strong integration of geometric morphometric analysis, and that this approach might help future studies not only by contributing to solving problems in systematics but also in understanding the flight mechanism of bumblebees (Aytekin et al., 2007). Klann et al. (2021) performed on study of sensory mechanisms that cause severe structural defects on sensilla pattern in *Tribolium castaneum* larva (Tenebrionidae). Moreover; a study of an insect species belonging to the Pentatomidae family found correlation between sexual dimorphism and epicuticular hydrocarbon. Differences were found in the epicuticular hydrocarbon pattern among nymphs and adults, as well as sexual dimorphism in adult stink bugs (Sessa et al., 2021).

The most common hypothesis is that females are larger than males and this is prevalent in many animals (Kelly et al., 2008). Instruments of geometric morphometrics will be able to answer many specific questions about evolutionary diversification of shape. A foundation of other multivariate methods can also be used in a comparative context to understand geometric morphometrics and structural defects (Revell and Harmon, 2008). Results show:

Since it was invented, the microscope has become very important to investigate microinvertebrates and macroinvertebrates, especially insects. With the development of technology, microscopes have been developed for scientific research. This search has progressed with the ability of the researcher to observe tiny organisms.

So we undertook a comparative study of correlations of morphologies for male and female body structures, such as body length in approximately 100 aquatic insects (43 males; 54 females) in the genera *Helophorus*. *Helophorus* spp. includes aquatic insects, distributed in a wide habitat, that were collected from Erzurum province in Turkey. In this genus, morphometric characteristics are vital for understanding how organism shapes evolve. Additionally, comparative studies are necessary due to the identification of specific data about structure. Studies that emphasise the variability in body measurements are very useful for identification and ecological dynamics.

The results showed that both male and female insects have different measurements. Additionally, sexual dimorphism may be an important force with ecological dimensions based on measurements of morphometrics of male and female organs. There is no inherent limitation in phylogenetic comparative approaches that restricts them to regression. On the contrary, all the usual tools of geometric morphometrics can be used in a phylogenetic comparative context (Monteiro, 2013). Similarly, it is unsurprising that insect structural defects are explained by their necessary generation as a function of local area variation in related tissue and cells (Kim et al., 2016). When viewed in this light, the approach described here provides a general conceptual framework for understanding patterns of change in morphometrics. Our results may indicate that differences in sexual dimorphism between these *Helophorus* insects could be due to differentiation of developmental methods and possible distinctions of feeding activity between individuals. It is believed that further investigations are needed.

CONCLUSION

These methods are mathematical applications of geometric morphometric. Geometric morphometry is a simple and quick technique with low costs, and revealed itself to be a valuable tool in this research. Distribution and behaviour of insect populations are based on their powerful structures and adaptation to the environment. Wings, legs and genital organs are excellent structures for studying morphological variations. This research is the first to demonstrate that a wide range of morphometric methods can be combined with morphological defects, which is concerned with structural changes. It is believed that these data will support the improvement of these new methods.

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Author's Contributions

The contribution of the authors is equal.

Statement of Conflict of Interest

Author has declared no conflict of interest.

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Diyarbakır'da Bulunan *Pipistrellus kuhlii* (Kuhl, 1817) (Chiroptera: Vespertilionidae) Türünün Morfolojik ve Karyolojik Özellikleri

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ÖZET

Diyarbakır'ın Eğil yöresinden farklı iki lokaliteden toplanan 11 (10♀♀, 1♂♂) *Pipistrellus kuhlii* örneğinin morfolojik ve karyolojik özellikleri incelenmiştir. Morfolojik karakterler, ölçüler ve karyolojik özellikler daha önce yayınlanmış çalışmalarla karşılaştırılmıştır. Diploid kromozom sayısı 2n = 44, kromozom kol sayısı NF = 54 ve otozomal kromozom kol sayısı NFa =50'dir. X kromozomu orta büyüklükte metasentrik, Y kromozomu nokta şeklinde olup, son akrosentrik kromozom çiftlerinden daha küçüktür. Bu karşılaştırmalı çalışma sonucunda, tragus ve üst diş özellikleri dışında diğer morfolojik ve karyolojik özellikler Türkiye'deki *Pipistrellus kuhlii* özelliklerine sahiptir.

Zooloji

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Anahtar Kelimeler
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Pipistrellus kuhlii
Morfoloji
Karyoloji
Türkiye.

Morphology and Karyology of *Pipistrellus kuhlii* (Kuhl, 1817) (Chiroptera: Vespertilionidae) in Diyarbakır Province/Turkey

ABSTRACT

In this study, the morphological and karyological peculiarities of eleven (10♀♀, 1♂♂) specimens of *Pipistrellus kuhlii* collected from Diyarbakir province were examined. The external and cranial measurements and weights of the specimens were taken and also the field notes were recorded. The diploid chromosome number of the specimens was 2n = 44, fundamental chromosome number was NF = 54 and autosomal chromosome number was NFa = 50. The X chromosome was middle sized metacentric and Y chromosome was small acrocentric. The external and cranial measurements and karyological and morphological peculiarities were compared with previously published accounts. The results revealed that the species of *P. kuhlii* distributed in this region.

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GİRİŞ

Türkiye yarasalarıyla ilgili yapılmış çok sayıda önemli morfolojik, moleküler ve taksonomik çalışmalar bulunmaktadır (Çağlar, 1969; Felten ve ark., 1977; Kumerloeve, 1978; Helversen, 1989; Benda ve Horáček 1998; Albayrak ve Aşan, 1999; Horáček ve ark., 2000; Kryštufek ve Vohralik, 2001, Yağcı ve Aşan, 2019). Türkiye'de farklı sayıda yarasa türü yaşadığı ifade edilmekle beraber toplamda yaklaşık 39 tür ve Vespertilionidae familyasına ait 30 tür bulunmaktadır (Benda ve Horáček, 1998; Kryštufek ve Vohralik, 2001; Grimmberger ve Rudloff, 2009). Vespertilionidae familyası içerisinde yer alan *Pipistrellus* cinsinin Türkiye'de *Pipistrellus, Pipistrellus kuhlii, Pipistrellus nathusii, Pipistrellus pygmaeus* olmak üzere toplam 4 türü bulunduğu ifade edilmektedir (Albayrak ve Aşan, 1999; Karataş, 2005).

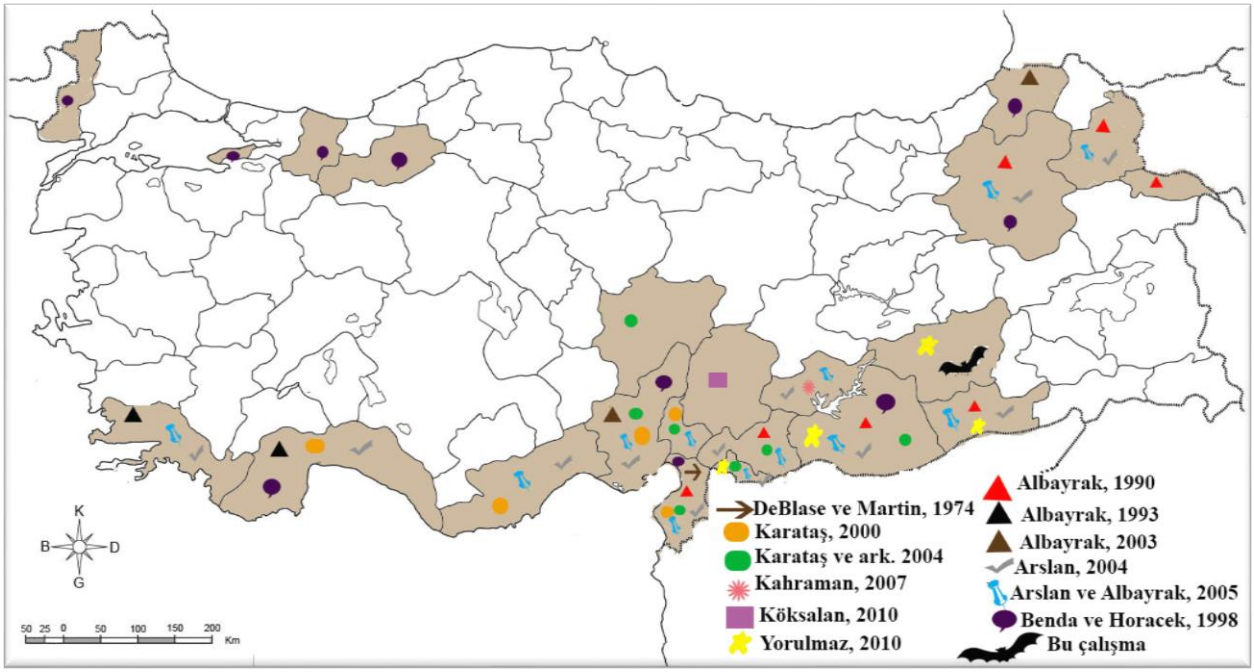
Pipistrellus kuhlii türü Palearktik bölge memelilerinden olup dünyada Akdeniz çevresindeki ülkelerde, Kuzey Afrika, Ortadoğu, Kafkaslar ve Avrupa'nın büyük bir kısmında dağılış göstermektedir (Benda ve Horáček, 1998; Wilson ve Reeder, 2005; Grimmberger ve Rudloff, 2009).

Pipistrellus kuhlii türünün Türkiye'nin güney kesimi ve Kuzeydoğu Anadolu'da kayıtları bulunmasına karşılık Türkiye'nin Kuzey bölgesi için durum belirsizdir (Albayrak ve Aşan, 1999). Türkiye'nin 21 ilinde *Pipistrellus kuhlii* türü kayıt edilmiştir (DeBlase ve Martin, 1974; Benda ve Horacek, 1998; Albayrak, 1990; 1993; 2003; Karataş, 2000; Karataş ve ark., 2004; Kahraman, 2007; Köksalan, 2010; Yorulmaz, 2010).

Diyarbakır'ın yakın çevresinde, Adıyaman, Şanlıurfa ve Mardin illerinde *Pipistrellus kuhlii* türüne ait kayıtlar bulunmaktadır (Arslan, 2004a; Arslan ve Albayrak, 2005). Ancak, Diyarbakır'a komşu olan Elazığ, Bingöl, Malatya, Batman ve Muş gibi kuzey, batı ve doğusunda bulunan illerde kayıt bulunmayışı türün karyolojik ve morfolojik özellikleri, taksonomik durumu, dağılış alanları açısından boşluk oluşturmaktadır. Bu çalışmada Diyarbakır'da dağılış gösteren *Pipistrellus kuhlii* türünün morfolojik ve karyolojik özellikleri hakkında bilgi eksikliğini gidermek ve dağılış alanlarını belirlemekle birlikte, Güneydoğu Anadolu bölgesi biyoçeşitliliğinin tespitine katkıda bulunmak amaçlanmıştır.

MATERYAL ve METOD

Diyarbakır'ın Eğil ilçesinde bulunan evlerin ve Yatılı Bölge Okulunun duvarlarındaki çatlaklar içerisinde, çengel şekline getirilen tel çubuklar yardımıyla, toplamda 11 (10♀♀, 1♂♂) *Pipistrellus kuhlii* türü örnekleri 09-16 Aralık 2016 tarihleri arasında yakalanmıştır (Şekil 1). Çağlar, 1969; DeBlase, 1980; Palmeirim, 1990; Albayrak, 1993; Dietz ve Helversen, 2004 tarafından oluşturulan tayin anahtarlarları ile karşılaştırma yapılarak toplanan örneklerin tür tespiti yapılmıştır. Örneklerin cinsiyetleri belirlenip, dış vücut ve baş iskeleti ölçüleri Karataş, 2000; Arslan, 2004b ve Yorulmaz, 2010'a göre 0,1 mm duyarlı dijital kumpas ile (mm) ve ağırlıkları (g) olarak ölçülmüştür. Ford ve Hamerton (1956) yöntemine göre kromozom preparasyonları yapılmıştır. Sentromer pozisyonlarına göre diploid kromozom sayısı (2n), kromozom kol sayısı (NF) ve otozomal kromozom kol sayısı (NFa) belirlenmiştir. Örneklerin derileri ve baş iskeletleri Dicle Üniversitesi Fen Fakültesi Biyoloji Bölümünde muhafaza edilmektedir.

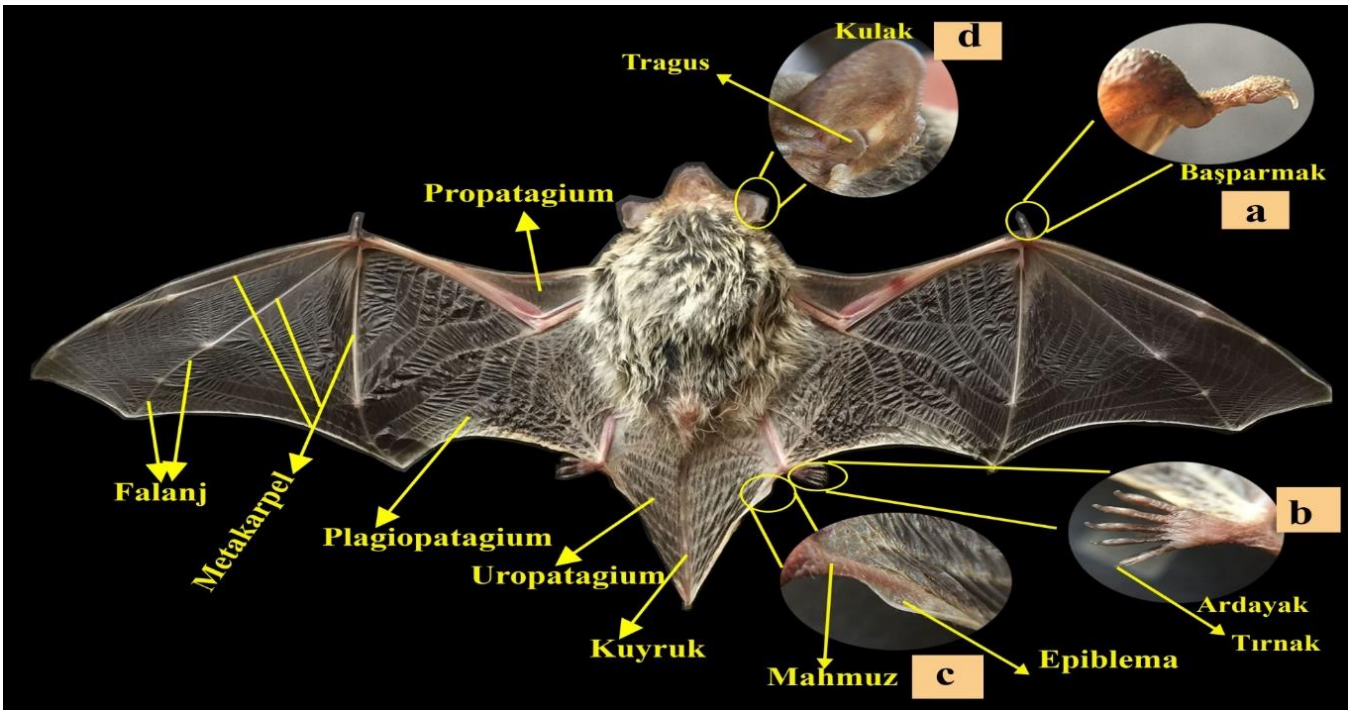


Şekil 1. Materyalin toplandığı lokaliteler: Diyarbakır-Eğil-Döşemeler Köyü ile Bölge Yatılı Okulu ve *Pipistrellus kuhlii*'nin Türkiye'de kayıt edildiği iller.

Figure 1. Collection localities: Diyarbakır-Eğil-Döşemeler köyü, Bölge Yatılı Okulu and previously recorded province of Pipistrellus kuhlii in Turkey.

BULGULAR

Pipistrellus kuhlii türü boyut olarak oldukça küçük yarasadır. Sırt ve karın kürkü yoğun bir şekilde ince ve yumuşak kıllarla örtülüdür. Türün kürkünü oluşturan kıllar farklı iki renk tonuna sahiptir. Sırtta bulunan kılların taban kısımları siyah, uç kısımları daha açık sarımsı-kahverengidir (Şekil 2). Karında bulunan kılların ise taban kısımları siyah, uç kısımları sarımsı-beyaz renktedir. Kanat zarı geniş, uzun ve yüzeyi çıplaktır. Zar grimsi-kahverengi tonlarda, yarı saydam, ince bir yapıya sahip ve damarlanmalar net bir şekilde görülmektedir. Kanat zarında, her iki yönde üçüncü parmaktan itibaren başlayan ve uropatagiumun serbest kenarı boyunca devam eden simetrik, şerit şeklinde olan dar beyaz alan görülmektedir (Şekil 2).



Şekil 2. *Pipistrellus kuhlii*'nin dış morfolojik özellikleri. a. Baş parmak tırnağı, b. Arka ayak tırnağı, c. Mahmuz ve epiblema, d. Kulak ve tragus.

Figure 2. Morphological characters of Pipistrellus kuhlii. a. Claw of thumb, b. Hind foot claw c. Spur and epiblema d. Ear and tragus.

Bacaklar kısa ve ayakları küçük. Ayak parmaklarındaki tırnaklar çengel şeklinde ve keskindir (Şekil 2b). Epiblema ve mahmuz bulunmaktadır (Şekil 2c). Bacaklardan kuyruğa doğru her iki yönden simetrik şekilde uzayan "V" şeklinde daha açık alanlar bulunmaktadır. Bu açık alanlar uropatagiumun yüzeyindebeyaz şeritler varmış gibi görünmesine neden olmaktadır (Şekil 2).

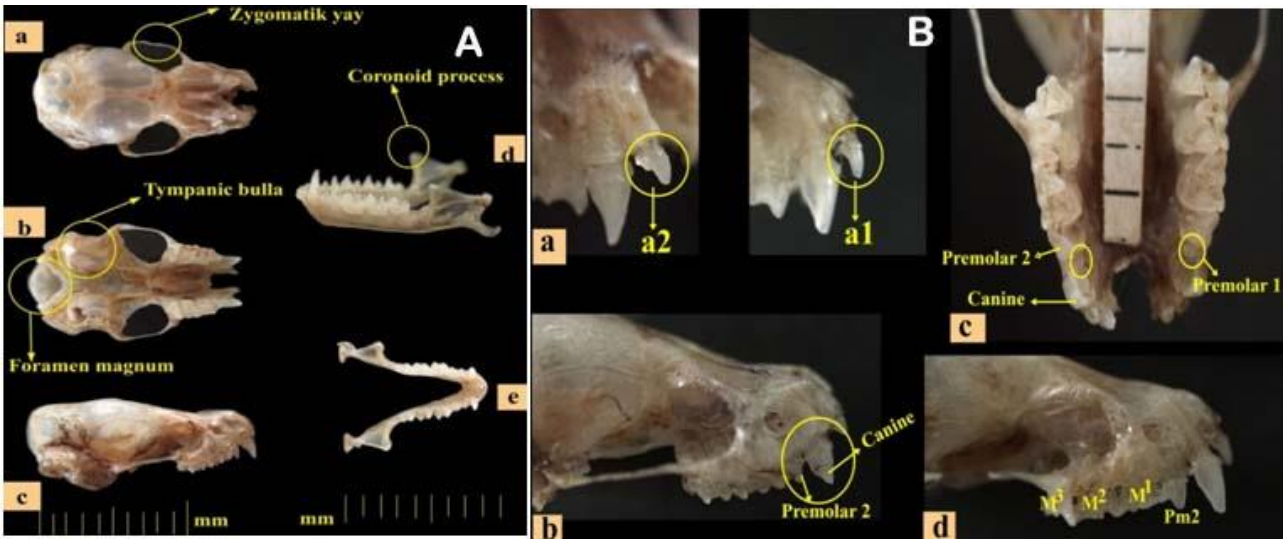
Kulaklar küçük ve uca doğru daralarak oval bir şekil almıştır. Kulakların kenar kısmında oldukça belirgin dışa doğru bir çıkıntı ve iç kısmında 5 tane yatay çizgi şeklinde deri kıvrımları bulunmaktadır (Şekil 2d). Kulaklar ve tragus kahverengi tonlarındadır. Tragus, kulağın yarı uzunluğuna ulaşmamakta,uca doğru daralmış ve oval bir şekil almıştır. Tragusun kenar kısmında bir çıkıntı mevcuttur ve tragusun iç kısmının kenarında içe doğru belirgin bir girinti vardır. Bu da uç kısmın içe doğru bükülmesine ve kama şeklini almasına neden olmuştur. Antitragus belirgin, kulağın dış kenar tabanından çıkıntı yapmış ve tepe kısmı oval bir şekil almıştır (Şekil 2d).

Beyin kapsülü rostrumdan geniştir. Rostrum öne doğru daralan ve az da olsa çıkıntılı bir yüzeye sahiptir. Zygomatik yayın orta kısmında hafif içe doğru bir çöküntü vardır (Şekil 3Aa-c). Tympanic bullae iyi gelişmiştir. Foramen magnum geniştir (Şekil 3Ab). Mandibula oldukça sağlam ve coronoid process belirgindir (Şekil 3A, d ve e).

Üst çenedeki birinci kesici diş tek zirvelidir (Şekil 3 Ba-a1 ve a2); İkinci kesici diş, birinci kesici diş ile temas halinde ve aralarında boşluk yoktur. Birinci kesici diş belirgin şekilde ikinci kesiciden uzundur. Örneklerde üstçenede bulunan canine ile ikinci premolar dişleri arasında çok az ya da oldukça belirgin bir boşluğun bulunduğu ve tamamıyla temas halinde olmadığı görülmüştür (Şekil 3 Bb ve c). Çok küçük olan üst birinci premolar diş dizi sırası içerisinde yer almaz, ağız içine doğru yerleşmiş ve yandan bakıldığında çok az ya da belirgin bir şekilde görülebilmektedir (Şekil 3 Bc ve d). Molar dişler yandan bakıldığında uç kısımları birbirine eşit üç tane zirve şeklinde görülmektedir. Üstçene üçüncü molar diğer molar dişlerden genişlik açısından daha dardır. Altçenedeki kesici dişlerin uç kısımları üç zirvelidir. Bu dişler sıkışık bir şekilde dizilmiş ve aralarında boşluk bulunmamaktadır. Alt çenedeki dişlerin tamamı diş sırası hizasında ve yandan bakıldığında görülmektedir. Üstçene diş dizisi uzunluğu 4.69-5.14 mm ve altçene diş dizisi uzunluğu 5.05-5.34 mm'dir. Diyarbakır örneklerinin baş iskeleti ölçüleri Çizelge 1'de verilmiştir.

Diyarbakır *Pipistrellus kuhlii*'nin tüm örneklerinde diploid kromozom sayısı 2n=44, otozomal kromozom kol sayısı NFa=50 ve kromozom kol sayısı NF=54 olarak bulunmuştur (Şekil 4). Otozom kromozomların 3 çifti kromozom setinin en büyük metasentrik kromozomudur. Bir çifti küçük submetasentrik, 17

çifti ise orta büyüklükten küçüğe doğru sıralanan akrosentriktir. X kromozomu metasentrik olup, küçük submetasentrik ile en büyük metasentrik kromozom çifti arasında bir büyüklüğe sahiptir. Y kromozomu ise oldukça küçük olup, nokta şeklindedir. Diyarbakır *Pipistrellus kuhlii* karyotipinin sonuçları önceki çalışmalarla karşılaştırılması Çizelge 2'de gösterilmiştir.



Şekil 3. *Pipistrellus kuhlii*'nin A. Baş iskeleti özellikleri; a. Dorsalden, b. Ventralden, c. Lateralden, d. Mandibula lateral, e. Mandibula. B. Üstçene diş yapısı a. Kesici dişlerin yapısı, b. Canine ve Premolar 2 (pm^2) dişin durumu , c. Premolar 1 (pm^1) 'in durumu, d. Molar dişlerin lateral'den görünümü.

Figure 3. Skull of Pipistrellus kuhlii, A. Skull peculiarities; a. Dorsal view, b. Ventral view, c. Lateral view, d. Mandibula lateral, e. Mandibula. B. Upper tooth row, a. Incisive, b. Canine and Premolar 2 (pm^2), teeth location, c. Upper Premolar 1 (pm^1), d. Lateral view of upper molars.

Table 1. Body and skull measurements of *Pipistrellus kuhlii* samples (mm) and weight (g); sample size (n), ekstrems (min./max.), avarage (x), standart deviation (±Sd).

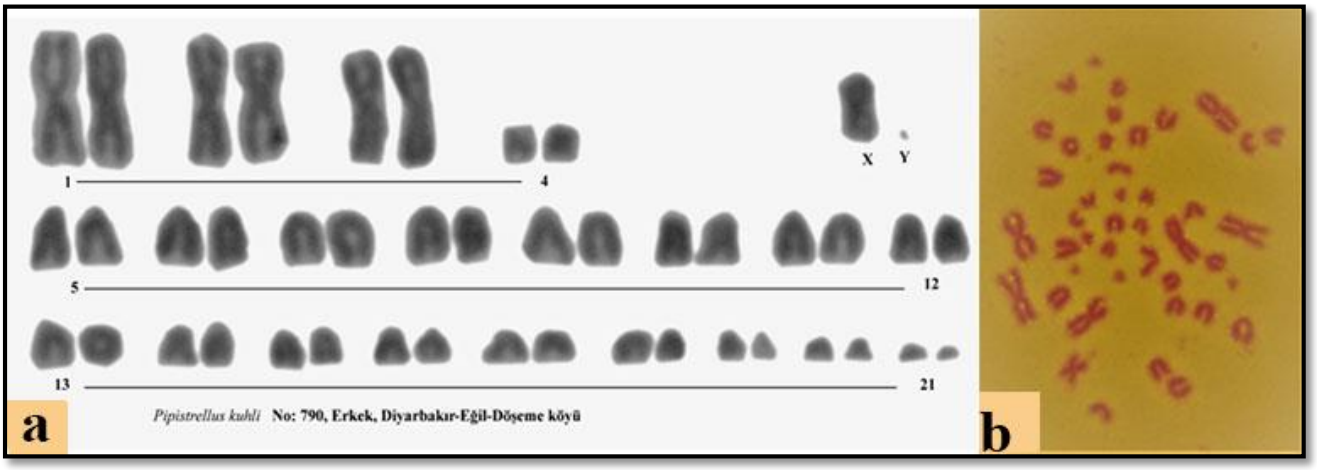
Çizelge 1. Pipistrellus kuhlii örneklerinin dış vücut ve baş iskeleti ölçüleri (mm) ve ağırlık (g); örnek sayısı (n), varyasyon alt ve üst sınırı (min./max.), ortalama (x), standart sapma (±Sd) değerleri.

Özellikler	n	min./max.	X	±Sd		
Tüm boy uzunluğu			11	84-95	90.7	3.23
Baş-beden uzunluğu			11	44-59	53.2	3.87
Kuyruk uzunluğu			11	34-40	37.5	2.21
Ağırlık (g)			11	4.9-7.1	6.1	0.56
Önkol uzunluğu			11	34-39	35.4	1.44
Kulak uzunluğu			11	10-12	10.9	0.54
Ardayak uzunluğu			11	6-10	8.0	1.18
Kanat açıklığı			11	240-270	247.9	8.81
Tüm kafatası uzunluğu			11	12.94-13.61	13.2	0.24
Condylobasal uzunluk			11	12.3-12.98	12.6	0.19
Zygomatik genişlik			11	8.45-8.98	8.6	0.18
İnterorbital genişlik			11	3.35-3.54	3.4	0.06
Mastoid genişlik			11	7.39-7.89	7.7	0.14
Beyin kapsülü genişliği			11	6.42-7.03	6.7	0.16
Rostral genişlik			11	4.17-5.44	4.5	0.39
Kafatası yüksekliği			11	5.82-6.36	6.1	0.15
Altçene uzunluğu			11	9.45-10.04	9.7	0.18
Üstçene diş dizisi uzunluğu			11	4.69-5.14	4.9	0.13
Altçene diş dizisi uzunluğu			11	5.05-5.34	5.2	0.09

TARTIŞMA ve SONUÇ

Pipistrellus kuhlii için ayırıcı karakter olarak belirtilen kanat membranında beyaz şeridin varlığı ve bu şeridin kanat sistemi içerisinde bulunması özelliği bakımından Diyarbakır *Pipistrellus kuhlii* örneklerinde beyaz şerit üçüncü parmaktan başlayıp, kuyruk ucuna kadar kesintisiz bir şekilde devam etmektedir. Bu özellik yalnızca Çağlar (1969)

tarafından ifade edilen durumla benzerlik gösterir. Ognev (1962), Albayrak (1990, 1993), Karataş (2000), Arslan (2004a), Arslan ve Albayrak (2005) ve Yorulmaz (2010) 'ın çalışmalarında ise genellikle beyaz şerit beşinci parmaktan başlayıp devam ettiği için farklılık göstermektedir.



Şekil 4. Diyarbakır erkek *Pipistrellus kuhlii* örneğinin; a. Karyotipi (No: 790 erkek, Eğil), b. Metafaz plağı.
Figure 4. A male sample of Pipistrellus kuhlii from Diyarbakır province; a. Karyotipi (No: 790 male, Eğil), b. Metaphase plate.

Table 2. Karyoype comparation of Pipistrellus kuhlii with previously published accounts; 2n; diploid chromosome number, NF; chromosomal arm number, NFa; autosomal arm number, A; acrocentrik, M+SM; metacentric+submetasentrics.

Çizelge 2. Diyarbakır Pipistrellus kuhlii karyotipinin önceki araştırmalarla karşılaştırılması; 2n; diploid kromozom sayısı, NF; kromozom kol sayısı, NFa; otozomal kromozom kol sayısı, A; akrosentrik, M+SM; metasentrik+submetasentrik.

	2n	NF	NFa	M+SM	A	X	Y	Referanslar	Ülkeler
Pipistrellus kuhlii	44	-	50	4	17	M	-	Capanna ve Civitelli (1970)	İtalya
	44	-	50	-	-	-	-	Volleth ve ark. (2001)	Yunanistan
	44	54	50	4	17	M	A	Arslan (2004)	Antalya ve Şanlıurfa
	44	-	50	4	17	M	A	Karataş ve ark. (2004)	Türkiye
	44	54	50	4	17	M	A	Gharkheloo ve ark. (2008)	İran
	44	-	-	4	17	M	A	Yorulmaz (2010)	Türkiye
	44	54	50	4	17	M	-	**Bu çalışma**	**Diyarbakır**

Pipistrellus kuhlii örneklerinde tragusun uca doğru daralıp, oval bir şekil alması; Ognev (1962), Harrison ve Bates (1991) ve Arslan (2004b)'ın belirttiği özelliklerle aynıdır. Tragusun dış kenarında belirgin bir çıkıntı ve iç kısmında içe doğru bir girinti bulunması durumu ise Arslan (2004b) ile benzerlik gösterirken, Harrison ve Bates (1991) ve Dietz ve Helversen (2004)'den faklılık göstermektedir. Harrison ve Bates (1991) *Pipistrellus kuhlii*'de antitragusun iyi geliştiğini ve kulağın dış kısmının tabanından ayrı bir çentik şeklinde sınırlandırıldığını ifade etmişlerdir. Diyarbakır *Pipistrellus kuhlii* örnekleri de benzer özelliklere sahiptir.

Harrison ve Bates (1991) *Pipistrellus kuhlii*'de altçene kesici dişlerin uç kısımlarının üç eşit parçalı, birinci premoların ikincisinin yarı uzunluğunda olduğunu ve Palmeirim (1990) alt kesici dişlerin oldukça sıkışık bir şekilde dizilmiş olduğunu ifade etmişlerdir. Bu özellikler Diyarbakır *Pipistrellus kuhlii* örneklerinin tamamında benzerlik göstermektedir.

Karyolojik özellikleri, diploid kromozom sayısı 2n=44, otozomal kromozom kol sayısı NFa=50 ve kromozom kol sayısının NF=54 olup literatür çalışmalarıyla karşılaştırılması sonucunda herhangi bir farklılığın olmadığı anlaşılmıştır (Capanna ve Civitelli 1970, Volleth ve ark. 2001, Arslan 2004a, Karataş ve ark. 2004, Gharkheloo ve ark. 2008, Yorulmaz 2010).

Dış vücut ve baş iskeleti ölçüleri ile Diyarbakır *Pipistrellus kuhlii* örneklerinin hem Türkiye hem de Ortadoğu ülkelerinde verilen ölçülerin sınırları içerisinde olduğu ve önemli farklılık göstermediği görülmüştür. Yalnızca interorbital genişliğin Arslan (2004b)'ın çalışmasında belirttiğinden önemli derecede farklılık göstermektedir.

Pipistrellus kuhlii türünün yaşa bağlı olmaksızın karakteristik özelliklerinden olduğu belirtilen canine ve ikinci premolar dişleri arasında boşluğun bulunmaması ve birinci premoların yandan bakıldığında görülmemesi durumu, Diyarbakır

Pipistrellus kuhlii örnekleri için geçerli olmadığı; örneklerin beş tanesinde canine ve ikinci premolar dişi arasında az bir boşluk bulunduğu ve birinci premolar yandan bakıldığında görülebilmektedir. Altı örnekte ise canine dişi ve ikinci premolar arasında belirgin bir boşluk bulunmakta ve birinci premolar yandan bakıldığında net bir şekilde görülmektedir. Tür için karakteristik olarak kabul edilen canine ve ikinci premolar dişleri arasında boşluğun bulunmaması ve birinci premoların yandan görülmemesi özelliğinin farklılık gösterebileceği ortaya çıkmıştır.

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Yazarlar olarak makaleye eşit oranda katkı sağlamış olduğumuzu beyan ederiz.

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Makale yazarları aralarında herhangi bir çıkar çatışması olmadığını beyan ederler.

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New Data on The Soil Mite Fauna (Acari: Oribatida) from Soğucak Plateau (Turkey)

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ABSTRACT
During the investigations of soil mites of the Soğucak Plateau, three species belonging to families Oribotritiidae, Neoliodidae, and Collohmanniidae (Acari: Oribatida) were found. *Oribotritia* (*O.*) *krivolutskyi* Liu, Niedbała, and Starý, 2011 is secondly recorded throughout the world. SEM images of the species are also provided.

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Soğucak Yaylası'ndan (Türkiye) Toprak Akarı Faunası (Acari: Oribatida) Üzerine Yeni Veriler

ÖZET
Soğucak Yaylası toprak akarları üzerinde yapılan araştırmalarda Oribotritiidae, Neoliodidae ve Collohmanniidae (Acari:Oribatida) familyalarına ait üç tür tespit edilmiştir. *Oribotritia* (*O.*) *krivolutskyi* Liu, Niedbała ve Starý, 2011 dünyada ikinci kez kaydedilmiştir. Ayrıca türlerin SEM görüntüleri de verilmiştir.

Zooloji

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INTRODUCTION

Oribatid mites are one of the major microarthropod groups in soil organic horizons, and they perform vital roles during litter decomposition (Norton, 1990; Scheu *et al.*, 2005). While this taxa includes more than 11,000 species in 162 families (Subias, 2004, updated 2021), the fauna of Turkey is little known with around 250 species (Baran *et al.*, 2018).

The genus *Oribotritia* Jacot, 1924 has 95 known species and only four of them (*O. hermanni*, *O. schusteri*, *O. turcica* and *O. berlesei*) previously recorded from Turkey (Gümüş, 2002; Niedbala, 2006, 2008). The genus *Neoliodes* Berlese, 1888 has 39 known species and only two of them (*N. theleproctus* and *N. ionicus*) previously recorded from Turkey (Per *et al.*, 2015; Sevimli and Baran, 2016). The genus *Collohmannia* Sellnick, 1922 has 3 known species and one of them (*C. gigantea*) previously recorded from Turkey (Baran and Bezci, 2017).

During the investigations of soil mites of the Soğucak Plateau in Turkey, three species belonging to the families Oribotritiidae, Neoliodidae, and Collohmanniidae were found. These species are *Oribotritia (O.) krivolutskyi* Liu, Niedbała and Starý, 2011, *Neoliodes theleproctus* (Hermann, 1804), and *Collohmannia gigantea* Sellnick, 1922. The first one has been recorded only from Caucasus up to date and secondly recorded throughout the world from Turkey. The species *N. theleproctus* has a semicosmopolitan and *C. gigantea* has a Southern Palearctic distribution and both of them previously recorded from Turkey. The SEM images of *O. (O.) krivolutskyi*

firstly given in this paper.

The Marmara Region constitutes a transition area between the two continents, namely Europe and Asia. Soğucak plateau is well represented by moist forests. Beech (*Fagus* sp.) is the most common tree among the moist forests in these regions and it has a wide distribution on the Soğucak plateau. Sessile oak (*Quercus petraea*) and chestnut (*Castanea sativa*) are also occasionally mixed into these forests. The latest supplementary checklist of oribatid mite species in Turkey has already been published by Baran *et al.* (2018). When compared with the number of species known from the world, it is seen that the number of species known from Turkey is quite low. We estimate that with sufficient taxonomic research, the number of oribatid mites in Turkey, located at the junction of the Asian and European continents, will be much higher. The main goal of the present paper is to make a contribution to oribatid fauna and facilitate the further study.

MATERIAL and METHOD

Samples taken from Soğucak Plateau (1100 msl altitude) in Sakarya province (Fig 1) in May and June 2020 and mites were extracted using a Berlese funnel apparatus.

They were fixed and stored in 70% ethanol. Specimens were cleaned by soaking in Tergazyme® solution for 6–12 h. They were mounted on aluminum stubs with conductive double-sided adhesive carbon tape then critical point dried and gold-coated in a Hummer sputter apparatus before SEM investigation. All measurements are given in micrometers (µm).

Terminology and taxa identification were according to Balogh and Balogh, (1992) and Weigmann, (2006).

Microscopic investigation: Mites were selected under Olympus SZX51 stereomicroscope and investigated under Leica DM1000LED microscope. SEM images were taken by JEOL JSM 6060 LV and Vega Tescan II.

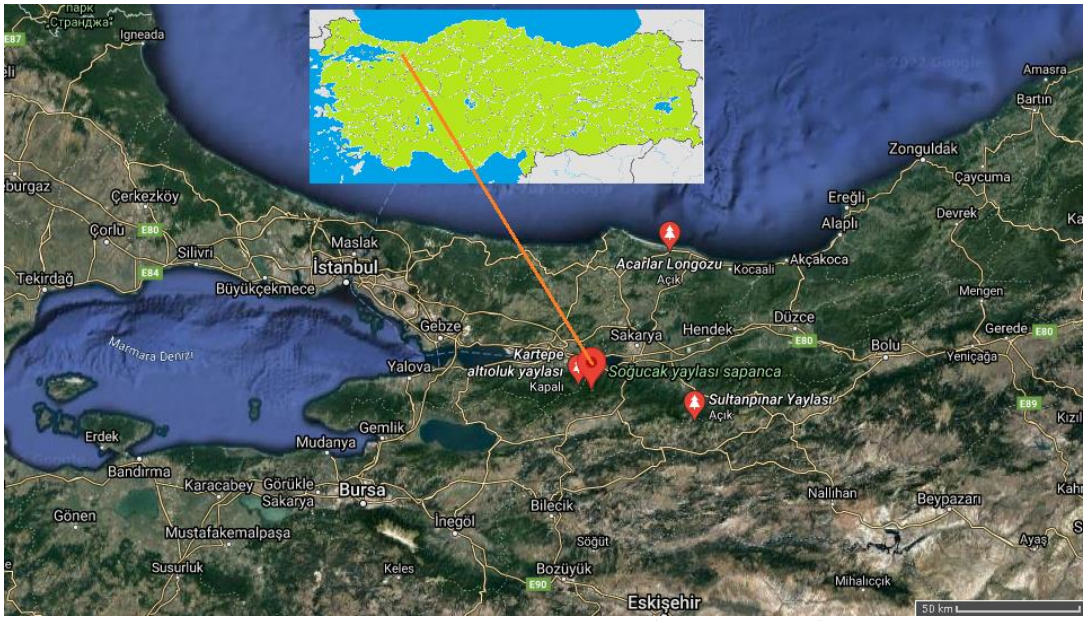

Figure1. Map of the study area (Google Earth)
Şekil 1. Çalışma alanının haritası (Google Earth)

RESULTS

Family: Collohmanniidae

Collohmannia gigantea Sellnick, 1922

Measurements and colour: Body length: 1571, body width: 867 µm (n=1). Colour dark brown.

Diagnosis (Figure 2): Prodorsum subtriangular and rostrum broadly rounded. Sensillus long rod shaped, distally thickened. The notogaster convex dorsally with an imbricate cuticle pattern. Notogaster with three flagellate setae; d_1, h_2 and p_1; seta other notogastral setae relatively short. *gla* present. Eight pairs of genital, two pairs of aggenital, three pairs of anal and three pairs of adanal setae present. Genital plate not divided transversely. Legs are tridactylous.

Material examined: Turkey, Sakarya, Soğucak platue, 40°36´36.6´N, 30°10´32.3´E, 27.05.2020, grassy soil under *Pinus* sp., 1 specimen.

Distribution: Soğucak platue. The second locality record for the species in Turkey (previously recorden from Amasya province Baran and Bezci, 2017). Holoarctic (Weigmann, 2006; Subias, 2004, updated 2021).

Family: Oribotritiidae

Oribotritia (O.) krivolutskyi Liu, Niedbała and Starý, 2011

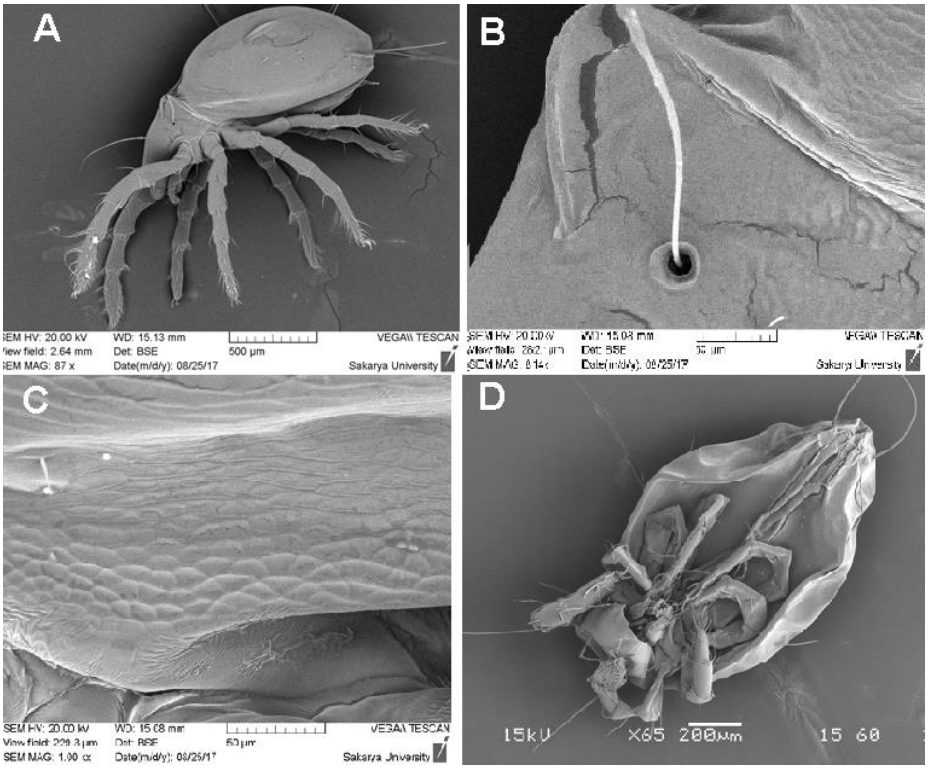

Figure 2. *Collohmannia gigantea* Sellnick, 1922. A - latera view; B - bothridium; C – notogastral cuticle pattern; D- ventral view

Şekil 2. Collohmannia gigantea Sellnick, 1922. A – yandan görünüş; B - bothridium; C – notogaster kütükula deseni; D- karından görünüş

Measurements and colour: Prodorsal length: 558-600 width:474-487 height:170-188 µm. Notogastral length 1210-1403, width 980-1010, height 895-903 µm (n=3). Colour brown.

Diagnosis (Figure 3): Body surface finely punctate. Rostral, interlamellar, lamellar and exobothridial setae smooth, fine and setiform. Sensillus short and robust 120-125 µm in length. One pair of long and strong lateral carina. Notogastral setae thin and setiform except thick and rigid setae ps_2 and ps_3. Eight pairs of genital setae, g_1-g_3 longer than the others. Two pairs of thin and long aggenital setae present. One pair of minute anal setae, three pairs of thin adanal setae present. Genito-aggenital plate 270-300, ano-adanal plate 565-576 µm in length. Mutual distance between ad_1 and ad_2 smaller than that between ad_2 and ad_3; setae ad_3 the shortest, setae ad_1 the longest; lyrifissures *iad* located between setae ad_3 and anal setae. Legs are heterotridactylous.

Material examined: Turkey, Sakarya, Soğucak plateau, 40°36´37.0´N, 30°10´33´E, 27.05.2020, soil and litter from the mixed forest, 3 specimens.

Distribution: Soğucak plateau. The second locality record for the species throughout the world and the first in Turkey (previously only known from Azerbaijan Lui *et al.* 2011). Palearctic.

Family: Neoliodidae

Neoliodes theleproctus (Hermann, 1804)

Measurements and colour: Body length: 1064- 1085, body width: 754-760 µm (n=3). Colour brown.

Diagnosis (Figure 4): Rostrum broadly rounded. Bothridia dorso-laterally opened, Sensillus short-stalked, thick club-shaped. Notogaster oval, with longitudional carina in front of notogaster. Anterior border of notogaster convex. The surface of notogaster with nymphal scalps and with laterally arranged lines. Six pairs of notogastral setae present. Genital and anal plates large and close to each other. Genital plate transversely divided into two parts. Seven pairs of thin genital setae (5+2) present. Adanal plate with three pairs of minute setae.

Material examined: Turkey, Sakarya, Soğucak plateau, 40° 36'37.6'' N, 30° 10'32.3'' E, 27.05.2020, soil and litter under *Pinus* sp., 3 specimens.

Distribution: Soğucak plateau. The second locality record for the species in Turkey (previously recorded from Yozgat province Per *et al.*, 2015). Semicosmopolitan (Subias, 2004, updated 2021; Weigmann, 2006).

DISCUSSION

Oribotritia (O.) krivolutskyi, has been recorded only from the Caucasus (Azerbaijan) in 2011 by Liu et al. and secondly recorded throughout the world from

Turkey by this study. This species can be distinguished from congeners by the presence of thick and rigid notogastral setae *ps2* and *ps3*, one pair long and strong prodorsal lateral carina, shape of sensillus, number and position of adanal setae (Liu *et al.* 2011). The notogastral length and width of the Turkish specimens (1210-1403x 980-1010µm) are in accordance with the Azerbaijan nominal species. Length of strong notogastral setae *ps2* and *ps3* in the Turkish specimens are shorter than the length of the type specimens. The other morphological feature of the Turkish specimens are appropriate with the original description (Liu *et al.* 2011).

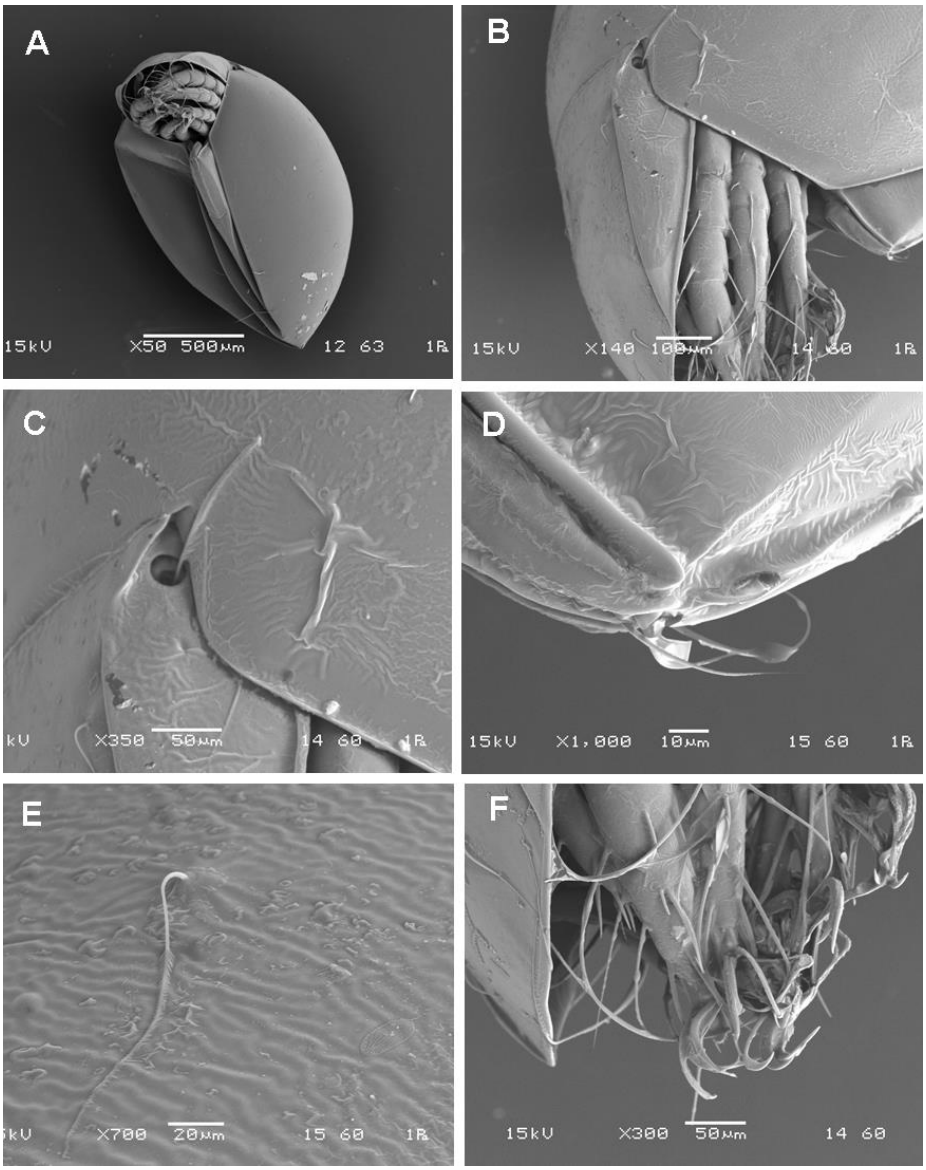

Figure 3. *Oribotritia (O.) krivolutskyi* Liu, Niedbała *and* Starý, 2011. A - ventral view; B - prodorsum; C - sensillus; D - genital setae (g_1-g_3); E - notogastral setae; F – legs

Şekil 3. Oribotritia (O.) krivolutskyi Liu, Niedbała ve Starý, 2011. A – karından görünüş; B - prodorsum; C - sensillus; D - genital kıllar (g1-g3); E - notogaster kılı; F – bacaklar

Phthiracaroid genera are especially diverse in areas with well developed organic debries layer (Woas, 2002). *Oribotritia (O.) krivolutskyi* was found in moss and litter sample under beech forest in the Talysh Mountains of Azerbaijan (Liu *et al.* 2011). We found in litter and soil under mixed forest in Soğucak plateau. Beech (*Fagus* sp.) is the most common and widely distributed tree among the moist forests in the Soğucak plateau. According to available data it can be considered as a typical inhabitant of litter and soil under beech forests in mountains.

Neoliodes theleproctus has a semicosmopolitan distribution (Palearctic, Oriental, Australia and Neotropic) (Subias, 2004, updated 2021). In Turkey previously recorded from Yozgat province by Per *et al.* (2015). The body length of *Neoliodes theleproctus* was

previously given between 1100–1375 µm (Weigmann, 2006; Per *et al.*, 2015). Body lengths of the specimens (1064- 1085µm) are smaller than the previously given. The other morphological features are in agreement with the features given by Weigmann (2006) and Per *et al.* (2015) for this species.

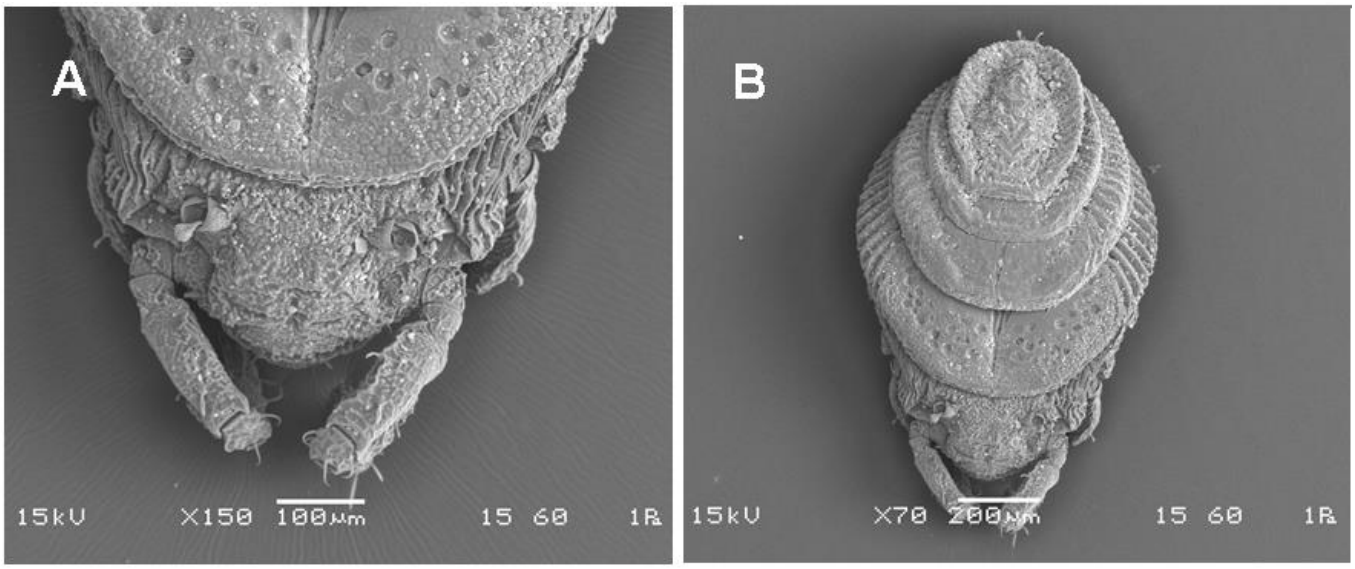

Figure 4. *Neoliodes theleproctus* (Hermann, 1804). A - prodorsum; B - dorsal view
Şekil 4. Neoliodes theleproctus (Hermann, 1804). A - prodorsum; B – sırttan görünüş

In temperate regions mites of Liodidae family tend to prefer warmer and dryer places and their distribution restricted to woodlands (Woas, 2002). This species was detected in bark, rotten wood (Weigmann, 2006), tree debris, moss and lichen (Per et al. 2015). We found in litter and soil under *Pinus* sp..

The species *Collohmannia gigantea* is show distribution in the Holarctic region. In Turkey it was recorded from Amasya province (Baran and Bezci, 2017). *Collohmannia gigantea* is similar to *C. chusteri* and *C. asiatica* but differs from it by bigger body dimensions and the length of setae *d2*, *p1* and *h2*. The body length of the species was previously given between 1450-2025 µm (Weigmann, 2006) and the body length of the specimen shows conformity with this. The other morphological features of the specimens closely resemble given by Weigmann (2006) for this species. This is the second record of species from Turkey.

Family Collohmanniidae shows Holoactic distribution (Woas 2002; Weigmann, 2006; Subias, 2004, updated 2021). The species *Collohmannia gigantea* has been reported from deciduous and mixed forests such as sweet chestnut forests and thermophilic (Woas 2002). We also found it in grassy soil from mixed forest under *Pinus* sp..

This study presents newly recorded and known species to the oribatid fauna of Turkey. For *Oribotritia (O.) krivolutskyi* Turkey is the first locality record outside its type locality Azerbaijan. The species *N. theleproctus* has a semicosmopolitan and *C. gigantea* has a Southern Palearctic distribution and they are previously recorded in Tukey from Yozgat and Amasya provinces respectively.

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Researchers Contribution Rate Declaration Summary

The authors declare that they have contributed equally to the article.

Conflicts of Interest Statement

None of the authors had any financial or personal relationships with other individuals or organizations that might inappropriately influence their work during the submission process.

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Anthelmintic Activities of Methanol Extracts of Five Different Geophyte Plant Species

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ABSTRACT

The purpose of this study was to look into the *in vitro* anthelmintic activities of methanol extracts of *Hyacinthella lineata* (Steud. ex Schult. & Schult.f.) Chouard, *Ornithogalum umbellatum* L., *Allium reuterianum* Boiss., *Cyclamen coum* Mill., and *Sternbergia clusiana* (Ker Gawl.) Ker Gawl. ex Spreng.. The paralysis and death times of the Indian earthworm, Cosmocerca ornata, were used to assess anthelmintic activity. Three different concentrations, (5,10 and 20 mg mL^{-1}) of methanol extracts were used to determine the time required to paralyze and to induce death in the earthworms. Albendazole (15 mg mL^{-1}) was used as reference standard drug. Plant extracts at higher concentrations (20 mg mL^{-1}) produced faster paralytic effects and a shorter time to death. The results revealed that *S. clusiana* killed all of the test worms within 20 ± 0.6 min of exposure and was 100% effective. Among all five plants extracts tested, *S. clusiana* was found to be highly effective and significant against *C. ornata*. The findings will aid in understanding the significance and application of these medicinal plant species in the food industry, traditional medicine, and pharmaceutical applications. This anthelmintic study of the plant extracts revealed that traditional medicine may be as effective as modern medicine in combating pathogenic micro-organisms.

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Beş Farklı Geofit Bitki Türünden Elde Edilen Metanolik Ekstraktların *ın vitro* Antihelmint Aktivitesinin Araştırılması

ÖZET

Bu çalışma *Hyacinthella lineata, Ornithogalum umbellatum, Allium reuterianum, Cyclamen coum, Sternbergia clusiana* türleri metanol ekstraktlarının in vitro antihelmintik aktivitelerini araştırmak için tasarlanmıştır. Hint toprak solucanı *Cosmocerca ornata*'nın paraliz ve ölüm zamanının hesaplanması ile antihelmint aktivite değerlendirilmesi yapılmıştır. Paraliz ve ölüme yol açan süreyi belirlemek için üç farklı konsantrasyon (5,10 ve 20 mg mL^{-1}) kullanılmıştır. Referans standart olarak Albendazol (15 mg mL^{-1}) kullanılmıştır. Bitki özütleri yüksek konsantrasyonlarda (20 mg mL^{-1}) daha hızlı paralitik etkiler ve daha kısa zamanda ölüm göstermiştir. Sonuçlar göstermiştir ki, *Sternbergia clusiana* türüne maruz kalan test solucanlarının tümü 20±0.6 dakika içinde ölüm göstererek %100 etkili olmuştur. Beş bitki türü arasında *S. clusiana*'nın solucanlara (*Cosmocerca ornata*) karşı oldukça etkili olduğu ve önemli sonuçlar verdiği bulunmuştur. Sonuçlar, bu tıbbi türlerin gıda endüstrisindeki, geleneksel tıptaki ve farmasötik uygulamalardaki önemini ve kullanımını anlamamıza yardımcı olacaktır. Bitki özleri üzerinde yapılan bu antelmintik çalışma, geleneksel tıbbın patojenik mikroorganizmaların üstesinden gelmek için modern tıp kadar etkili olabileceğini ortaya koydu.

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INTRODUCTION

Helminthes infections are among the most common infections in humans. Human intestinal parasitic worms are vectored through air, food, and water, causing disease state, secreting toxins, and stealing vital nutrients from host bodies. The current treatment regimens for these diseases have limitations because the anthelmintic drugs used are primarily microfilaricidal, with little effect on the adult worms; thus new drugs are urgently needed. In this regard, natural products have made and continue to make important contributions to this therapeutic area. The drugs currently used for helminthes infections include combinations of DEC (diethylcarbamazine) and albendazole, ivermectin and albendazole or the use of DEC fortified salt, which has also been described in (Murugamani et al., 2012).

The plant mediated drugs may be a potential alternative to synthetic anthelmintic drugs. The resistance against the synthetic anthelmintic results in the search of alternative natural resources to overcome the drug resistance (Thooyavan et al., 2018).

The plants are known to provide a rich source of botanical anthelmintics. A number of medicinal plants have been used to treat parasitic infections in man and animals. In traditional system of medicine the practitioners use various indigenous plants for the treatment of anthelmintic (Vidyadhar et al., 2010). Secondary metabolites isolated from plants by different isolation and purification techniques play major role as alternative to synthetic drugs.

To eliminate the harmful side effects of the synthetic anthelmintic drugs and to provide newer and cheaper alternative, it is important for us to promote the studies of traditionally used anthelmintic plants, which will lead to the development of new anthelmintic substances with ease of availability and lesser side-effects.

Hyacinthella Schur (Liliaceae) is a genus of 17 species distributed in mainly Mediterranean regions.*Hyacinthella* genus is constantly changing place between families (Liliaceae, Hyacinthaceae, recently Asparagaceae). Genus represented 12 species, in which 10 of them are endemic, in Turkey. Recently, several scientific studies on the floristic, morphology, and anatomy of this genus have been performed (Aydin and Mammadov, 2017).

The genus *Ornithogalum* (family: Liliaceae) comprises 150 species, distributed in temperate Europe, Asia, and Africa. Some *Ornithogalum* plants are known to be poisonous; of these, several cardenolide glycosides have been isolated and identified (Ghannamy *et al.*, 1987). Phytochemical studies revealed that the bulbs of some species contain a variety of steroidal compounds and steroidal glycosides such as cholestane glycosides, acylated cholestane bisdesmosides, saponins, and spirostanol glycosides, some of which exhibit significant cytotoxic activities against cultured tumor cells and have anticancer potential (Delazar et al., 2009). Some *Ornithogalum* species (sp.) have been reported to exhibit a wide range of biological activities such as anticancer, anti-inflammatory, antimicrobial, antioxidant and antitumor (Plančić et al., 2014).

The species of *A. reuterianum* are members of the *Alliaceae* family. *Allium* L., which is a genus, is important because it creates a group of natural antioxidants. Since ancient times, many *Allium* species, such as onion, garlic, leek, and chives, have been used as foods, spices, and herbal remedies in widespread areas of the world, especially in the northern hemisphere. The *Allium* genus is one of the major sources of polyphenolic compounds and the antioxidative activity of some *Allium* species has been reported and has been mainly attributed to a variety of organo-sulfurous compounds as well as their precursors.

The genus *Cyclamen*, which possesses geofit plant species, belongs to the family of Primulaceae (Davis,1978). In Turkey, this genus is represented by 12 taxa, 5 of which are endemic (Guner et al., 2000). *Cyclamen* plants are used as medicinal plants and also as ornamental plants (Mathew et al., 2001). Pharmacological investigations into the extracts or isolated saponins of *Cyclamen* spp. tubers exhibited in vitro cytotoxic, antimicrobial, analgesic, and anti-inflammatory activities. Also, analgesic, anti-inflammatory, and antimicrobial activities of some *Cyclamen* species such as *C. repandum* and *C. mirabile* have been reported (Dall'acqua et al., 2010). *C. coum* is used in traditional medicine for the treatment of hemorrhages, ecchymosis, inflammations, hemorrhoids, and cancer (Jaradat et al., 2017). The tubers were used in Turkish folkloric medicine for infertility treatment in women. Recent studies have shown that *C.coum* can be used as a perspective medicinal plant in clinical practice (Bokov et al., 2020).

Sternbergia Waldst & Kit. (winter daffodil) is a genus of bulbous monocotyledons belonging to the family Amaryllidaceae (Mathew and Davis, 1999). The species of *Sternbergia* always takes the interest of scientists; therefore, several studies have been done on this species (Mammadov et al., 2011). Plants of the family Amaryllidace are well known not only for their ornamental value but also for the alkaloids they produce. Studies on *Sternbergia* species yielded compounds belonging to the basically different groups of Amaryllidaceae alkaloids (Kıvcak and Gözler, 1993).

Amaryllidaceae is a family of monocotyledonous plants with significant economic and medical value. Amaryllidaceae alkaloids have been shown to possess important biological activities, including antitumor, antiviral, and acetylcholinesterase inhibitory activity (Kaya et al., 2010; Berkov et al., 2009). Also, analgesic and antimicrobial activities have been reported for extracts and alkaloids from S*. clusiana, S. sicula,* and *S. lutea* (Unver et al., 2005).

The purpose of this study is to evaluate the anthelmintic activity of methanol extracts of bulbs and leaves of *H. lineata*, *O. umbellatum, A. reuterianum, C. coum*, and *S. clusiana.*

MATERIALS and METHODS

Preparation of plant extracts

H. lineata species were collected in the spring of 2020 from the Honaz locality, near Denizli province, in Turkey and identified from the book of Flora of Turkey. The fresh bulbs and leaves of the plant samples were cleaned and dried in the shadow for extraction. The voucher specimen was deposited at the herbarium of Pamukkale University the Laboratory of Botanics, under the "PAU2020-2023" number.

O.umbellatum species were collected in April 2020 from the Honaz locality, near Denizli province in Turkey and identified from the book of Flora of Turkey. The fresh bulbs and leaves of the plant samples were cleaned and dried in the shadow for extraction. Dried plant parts (bulbs and leaves) were pulverized. Each ground sample was transferred into a beaker. The voucher specimen was deposited at the herbarium of Pamukkale University the Laboratory of Botanics, under the "PAU2020-2024" number.

S.clusiana species were collected in the spring of 2019 from the Kavaklıdere locality, near Muğla province, in Turkey and identified from the book of Flora of Turkey. The fresh bulbs and leaves of the plant samples were cleaned and dried in the shadow for extraction. The voucher specimen was deposited at the herbarium of Pamukkale University the Laboratory of Botanics under the "PAU219-2021" number.

A. reuterianum species were collected in the spring of 2019 from the Kötekli locality, near Muğla province, in Turkey and identified from the book of Flora of Turkey. The fresh bulbs and leaves of the plant samples were cleaned and dried in the shadow for extraction. The voucher specimen was deposited at the herbarium of Pamukkale University the Laboratory of Botanics, under the "PAU219-2022" number.

C. coum species were collected in the spring 2019 from Kötekli locality, near Muğla province, in Turkey and identified from the Flora of Turkey book. Each part (tubers and leaves) was dried in the shadow at room temperature and low humidity. The voucher specimen was cataloged as "PAU219-2023" in the Pamukkale University the Laboratory of Botanics.

Each material (Table1) was thoroughly cleaned with water and dried (bulbs and leaves) under shade for seven days. All samples were kept at 25˚C until extraction. In our laboratory, the dried materials were ground and powdered before being stored separately for future use. Dried plant parts are kept in tightly-sealed dark containers until they are needed. About 200 g of each plant material was used for extraction. Methanol (100mL) was added in the ratio of 1:10 and each plant material was put in a water bath at 55 ºC for 6 h . The extraction mixture was separated from the residue by filtration through filter paper (Whatman No. 1). The plant residue was re-extracted twice with methanol. After the filtration, the two extracts were combined. The residual solvent of methanol extracts of samples was removed under reduced pressure at 48 - 49°C using a rotary evaporator (IKA RV10D, Staufen, Germany). Extracts were produced in duplicates and used to assay the biological activity (Aydin and Mammadov, 2017).

Table 1. *Plant materials evaluated for their anthelmintic activity*
Çizelge 1. Antihelmintik aktiviteleri açısından değerlendirilen bitki materyalleri

Botanical name	***Family***	***Parts used***
H. lineata	*Asparagaceae*	Bulb, leaf
O. umbellatum	*Asparagaceae*	Bulb, leaf
A. reuterianum	*Amaryllidaceae*	Bulb, leaf
C. coum	*Primulaceae*	Bulb, leaf
S. clusiana	*Amaryllidaceae*	Bulb, leaf

Evaluation of the in vitro anthelmintic activities

The anthelmintic activities were performed according to the method mentioned in Ghosh et al. (2005). The adult *Cosmocerca ornata* (of the *Cosmocercidae* family) resembles intestinal round worm parasites in terms of anatomy and physiology. *C. ornata* was placed in a petridish containing three different concentrations (5, 10, and 20 mg mL^{-1}) each of plant methanol extracts. Each petridish was placed with six worms and observed for paralysis (or) death. The mean time for paralysis was noted when no movement of any sort could be observed except when the worm was shaken vigorously; the time of death of the worm (min) was recorded after ascertaining that worms neither moved

when shaken nor when given external stimuli. In the same manner, albendazole was included as a reference compound. The test results were compared to samples that had been treated with the reference substance albendazole (15 mg mL-1).

RESULTS and DISCUSSION

The results in Table 2 show the anthelmintic activities of the extracts obtained from each plant species. The present study also shows that there was an increase in the anthelmintic activities as the concentrations increased. The extracts were effective in causing the death of the worms as well as promoting paralysis. All the doses of the extracts of each plant showed better anthelmintic activity than the standard. *S. clusiana* was the most potent, requiring less time for paralysis and death of the worms. It showed a concentration-dependent anthelmintic property (Table 2). *S. clusiana* bulb extract was the most active against *C. ornata* with paralysis time of 12 ± 0.6 and death time of 20 ± 0.6 in minutes at 20 mg mL^{-1},while *O. umbellatum* leaves extract was the least active with paralysis time of 98 ± 0.3 and death time of 105 ± 0.5 in minutes at 20 mg mL^{-1} (Table 2). The strong anthelmintic activity of the extract of *S. clusiana* may be due to the presence of rich polyphenolic compounds in its essential oil, which are monoterpenoid phenols and alkaloids (Berkov et al., 2009; Kaya et al., 2010).

Table 2. *Anthelmintic activity of plant materials*
Çizelge 2. Bitki materyallerinin antihelmintik aktivitesi

Plant species		*Concentration (mg mL^{-1})*	*Time taken for paralysis (min)*	*Time taken for death (min)*
dH_2O control			-	-
Albendazole (Reference)		15	39±0.5	58±0.4
A. reuterianum	*Bulb*	5	46±0.3	57±0.4
		10	26±0.2	38±0.7
		20	19±0.4	30±0.4
	Leaves	5	52±0.6	66±0.8
		10	37±0.7	45±0.5
		20	26±0.3	35±0.3
H.lineata	*Bulb*	5	115±0.3	120±0.7
		10	89±0.2	100±0.7
		20	73±0.6	80±0.2
	Leaves	5	86±0.7	98±0.3
		10	83±0.2	95±0.8
		20	79±0.4	90±0.4
O. umbellatum	*Bulb*	5	100±0.3	115±0.7
		10	95±0.4	105±0.5
		20	91±0.2	100±0.6
	Leaves	5	116±0.5	130±0.2
		10	108±0.4	120±0.7
		20	98±0.3	105±0.5
S. clusiana	*Bulb*	5	33±0.6	48±0.6
		10	22±0.7	35±0.4
		20	12±0.6	20±0.6
	Leaves	5	38±0.4	55±0.4
		10	27±0.6	40±0.4
		20	24±0.5	30±0.3
C.coum	*Bulb*	5	65±0.7	78±0.2
		10	48±0.6	60±0.7
		20	36±0.6	47±0.5
	Leaves	5	50±0.5	65±0.7
		10	47±0.4	58±0.5
		20	35±0.3	46±0.4

The anthelmintic activities of the species investigated in this study wereilluminated for the first time. Studies have shown that phenolic and alkaloids possess plants to produce anthelmintic activities

because alkaloids chemically belong to polyphenolic compounds. Phenolics and alkaloids are known to interfere with the generation of energy in helminth parasites by uncoupling the oxidative phosphorylation and also bind to free proteins in the GIT of the host animal or glycoprotein on the parasite cuticle, leading to death (Mali and Wadekar, 2008).

It is possible that phenolic compounds and alkaloids also present in *S. clusiana* extracts may be responsible for the anthelmintic activity. *S. clusiana* shows moderate anthelmintic activity comparable to other plants.

All values are present as mean+SD; n=6 in each group. Comparisons made between standard versus treated groups.

Results obtained from the anthelmintic assay indicated that higher concentrations of plant extracts produced faster paralytic effects and showed a shorter time of death. Methanol extracts of plant species demonstrated paralysis as well as death of worms, especially at a higher concentration of 20 mg mL^{-1}, while a 5 mg concentration also showed activity but at a longer time. An *in vitro* assay was used in this present investigation in agreement with the findings. This provides a cheaper, more economical and rapid turnover in contrast to *in vivo* assays as far as anti-parasitic properties of plants and plant extracts are concerned (Blessing et al., 2019).

Iqbal *et al.* (2001), has been reported that the alcoholic extract of the bulb of *A. sativum* shows in vitro anthelmintic activity against human *Ascaris lumbricoldes*. *A. sativum*'s alcoholic extract has demonstrated anthelmintic activity in vitro against *Heterakis gallinae* and *Ascaridia galli*, *Haemonchus contortus*, a free-living worm of *Rhabditis sp.*, larvae of *Nippostrongylus brasiliensis*, and eggs of *Ascaris summ* (Chybowski, 1997; Nagaich, 2000).Singh et al. (2008) reported the alcoholic extract of *A. sativum* causes a paralytic effect on liver amphistomes *Gigantocotyle explanatum*.

It has been shown that the ethanolic extracts of the tuber and leaf parts of the *Cyclamen alpinum* have anthelmintic activity. After 6 minutes, it was seen that the tuber part (LC_{50}: 0.52 mg mL^{-1}) is more toxic than the leaf part (LC_{50}: 1.32 mg mL^{-1}) with an LC_{50} value (Turan and Mammadov, 2018).

The phytochemical constituents showing anthelmintic effects include alkaloids, saponins, polyphenols, tannins, etc. Alkaloids suppress the transfer of sucrose from the stomach to the small intestine, diminish the support of glucose to the helminths, and act on the CNS(Central Nervous System) causing paralysis. Synthetic anthelmintic drugs are usually associated with various side effects. More attention is attracted by the increasing problems of resistance development in helminthes against synthetic anthelmintics. However, plants are the richest source of bioactive compounds. The best alternative to modern synthetic drugs is plant-derived medicine. Many investigators have worked on similar aspects, and their reports support this investigation, revealing that plants are potent anthelmintic agents.

CONCLUSION

It is concluded that all possess a varying degree of anthelmintic activity. Ultimately, it is possible that methanol extracts of *Sternbergia clusiana* bulb possess significant anthelmintic activity.The results suggest the presence of biologically active components in *S. Clusiana,* which may be worth further investigation. These findings of this geophyte species will be the basis for future work on the purification and identification of the active compounds, which may be useful for medicinal purposes, and on the potential use of this plant for nutrition. This is the first comprehensive study on the anthelmintic activities of species. These results indicated that extracts have strong anthelmintic activities, and that these extracts can be used as a food additive in food processing and pharmaceutical industries.

Author's Contributions

The contribution of the authors is equal.

Statement of Conflict of Interest

Authors have declared no conflict of interest.

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Mardin İlindeki Su Örneklerinde *Legionella* ve Bazı Kimyasalların Analizi

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ÖZET

Bu çalışmada, Mardin İlinde bir kamu kurumuna ait iki su kuyusu, bir su deposu ve binaların musluklarından alınan su ve sürüntü numunelerinde mikrobiyolojik ve kimyasal analiz yapılmıştır. Bu örneklerde *Legionella* ve serogruplarının varlığı ile klor, nitrat, nitrit, pH ve elektriksel iletkenlik gibi parametreler analiz edilmiştir. Alınan 25 su örneğinden *Legionella* üremesi gözlenmezken, aynı yerlerden alınan 25 sürüntü kültür örneğinin 4 tanesinde *L. pneumophila* serogrup 1 saptanmıştır. Su örneklerinin pH değerlik aralıkları 7.53-8.02 olarak tespit edilirken en düşük pH değeri 7.53 ile bir nolu kuyuda gözlenirken en yükseği ise 8.02 ile iki nolu kuyuda gözlenmiştir. En düşük elektrik iletkenlik değeri 376.44 µS/cm ile iki nolu kuyuda, en yükseği ise 446.57 µS cm^{-1} ile bir nolu kuyuda ölçülmüştür. Araştırmada yapılan analizler neticesinde hiçbir su numunesinde nitrit tespit edilmezken, nitrat miktarı en düşük bir nolu kuyuda 4.30 ppm ile en yüksek ise 10.85 ppm ile iki nolu kuyuda tespit edilmiştir. Su numunesi alınan bir nolu ve iki nolu kuyu ile ana depodaki klor değerleri sırasıyla 2.21, 2.05 ve 2.43 ppm olarak ölçülürken, bina musluklarından alınan örneklerde ise bu değerler 1.68-1.73 ppm aralığında olduğu tespit edilmiştir.

Mikrobiyoloji

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Anahtar Kelimeler
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Legionella
Su analizi
Mikrobiyolojik
Kimyasal

Analysis of *Legionella* and Some Chemicals in Water Samples in Mardin Province

ABSTRACT

In this study microbiological and chemical analyzes were carried out on water and swab samples taken from two water wells, a water tank and taps of buildings belonging to a public institution in Mardin Province. In these specimens the presence of *Legionella* and its serogroups as well as the parameters such as chlorine, nitrate, nitrite concentration, pH level and electrical conductivity were analyzed. While *Legionella* growth was not observed in 25 water samples, *L. pneumophila* serogroup 1 was detected in 4 of 25 swab culture samples taken from the same places. The pH values of water samples ranged from 7.53 to 8.02 the lowest pH value was observed in well no 1 as 7.53 while the highest was observed in well no 2 as 8.02. The lowest electrical conductivity value was measured at well 2 as 376.44 µS cm^{-1} and the highest was measured as 446.57 µS/cm from well 1. As a result of the analyzes made in our research nitrite was not detected in any of the water samples. Whilst the nitrate amount was found to be the lowest with 4.30 ppm in the well , the highest with 10.85 ppm in the well 2. The chlorine values in the well 1 and 2 from which water samples were taken and the main tank were measured as 2.21, 2.05 and 2.43 ppm respectively. These values were found to be in the range of 1.68-1.73 ppm in the samples taken from the building taps.

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GİRİŞ

Amerika Birleşik Devletleri'nin, Philadelphia şehrindeki bir otelde 1975 yılı Haziran ayında 58. yıllık Amerikan Lejyonu Kongresine katılan yaklaşık 4400 emekli Amerikalı asker arasında daha önce benzeri görülmemiş bir zatürre salgını meydana gelmiş ve *Legionella pneumophila* salgını ilk defa bu askeri toplantıda tespit edilmiştir. Toplantıya katılanların 189'unda pnömoni salgını tanımlanmış ve bunların 29'u bu salgından ötürü hayatını kaybetmiştir (Alary ve Joly, 1992; Yu, 1995). ABD Hastalık Kontrol ve Önleme Merkezi (CDC) ve Pensilvanya'daki sağlık çalışanları tarafından yürütülen epidemiyolojik araştırmalar netice vermemiş ve salgının nedeni ve etkeni teşhis edilememiştir. Salgının üzerinden altı ay geçtikten sonra, CDC'deki bilim insanları salgının etkeninin zor üreyen Gram negatif basil olduğunu duyurmuşlardır (McDade ve ark., 1977; Newton. ve ark., 2018). Keşfedilen bu yeni hastalık etkeni sınıflandırmada Legionellaceae familyasının, *Legionella* cinsinin *pneumophila* türüne dâhil edilmiştir (Qiu ve Luo, 2017; Mondino ve ark., 2020).

Legionellaceae familyası tek cins *Legionella'* dan oluşur. *Legionella* 60'dan fazla tür 70 serogrup içermektedir. Hastalardan yaklaşık 30 tür izole edilmiş ve insanlar için patojen olduğu ispatlanmıştır. Lejyoner hastalığının oluşmasındaki en önemli etken *L. pneumophila*'dır. *L. pneumophila* ise 16 farklı serogruptan meydana gelen bir bakteri türüdür (Fields ve ark., 2002). İnsanlarda ortaya çıkan enfeksiyonun çoğunlukla *L. pneumophila* serogrup 1, serogrup 4 ve serogrup 6 tarafından meydana getirildiği tespit edilmiştir. (Mahon, 2007).

L. pneumophila'nın akışkan su kaynaklarında, koloni oluşturma olasılığı oldukça düşüktür. Fakat bu patojenler, evsel su sistemlerine bulaştıklarında koşullar daha uygun olduğundan üreyip gelişebilmekte ve insanlar için önemli bir sağlık riski oluşturmaktadır (Cramer, 2003; Brady ve Sundareshan, 2019). *Legionella* spp. türlerinin en çok tespit edildiği ve onlar için elverişli yaşam koşullarını içeren alanlar; "örn., merkezi klima ve havalandırma sistemleri, sıcak su tankları, soğutma kuleleri, su sertliğini kırma tankları, duş başlıkları ve sıcak su muslukları, termal banyolar, çamurlar ve kaplıcalar, hastanelerde bulunan solunum cihazları, süs havuzu ve bahçe fıskiyeleri, yangın söndürme işleminde kullanılan springler, evaporatörler ve nebulizatörler" olarak sıralanabilir. Soğutma kuleleri ve merkezi klima sistemlerinden salınan *Legionella* spp. içeren aerosoller özellikle bağışıklık sistemi zayıf kişiler ve risk grupları için tehlike oluşturmaktadır (Hornei ve ark., 2007; Garrison ve ark., 2016). Lejyoner hastalığının benzeri olan Pontiac ateşi sendromunda da kontaminasyon kaynağının merkezi klima sistemleri, soğutma kuleleri, klimalar ve jakuziler olduğu tahmin edilmektedir (Yu, 1995). Moleküler parmak izi (fingerprinting) çalışmalarında *L. pneumophila*'nın alt türlerinin genotipleri belirlenerek, musluk suyu kaynaklarının da bu patojenin yaşam alanı olduğu belirtilmiştir (Yu, 1995; Hamilton ve ark., 2019). Yukarıda sayılan muhtemel rezervuarlarının yanısıra belkide en çok risk oluşturanı hastanelerdeki su şebeke sistemlerinin *Legionella* spp. ile kontamine olmasıdır. *Legionella* spp.' lerin nozokomiyal enfeksiyon etkenleri arasında önemli bir yerinin olduğu belirtilmiştir (Hornei ve ark., 2007; Garrison ve ark., 2016). Bu bakterilerinin insanlara bulaşması genellikle; kontamine aerosoller, aspirasyon veya entübasyon esnasında kontamine olmuş ortamın solunmasıyla oluşmakta ve direkt olarak pulmoner sistemi etkilemektedir (Gürler ve İğnak, 2012). Bilimsel raporlarda, soğutma kuleleri ve klimalar tarafından ortama yayılan aerosollerin hava akımıyla 1.6 km'den daha uzak mesafeye taşındığı vurgulanmıştır. *Legionella* kontaminasyonunda aerosolizasyon partikülleri ortalama 1-5 µm boyutlarında olup bu partiküllerdeki enfeksiyon etkeni iki saat kadar canlılığını koruyabilmektedirler (Baskerville ve ark., 1983; Memish ve ark., 1992; Hlady ve ark., 1993).

Özellikle hastanelerdeki solunum sistemi araç gereçleri *L. pneumophila* ile kontamine olmuş musluk suları ile temizlenip daha sonra bu ekipmanların hastalarda tekrar kullanılması direkt aspirasyon aracılığıyla nozokomiyal hastalığa yakalanma olasılığını arttırmakta, bu da risk grupları için büyük bir tehlike arz etmektedir (Park ve ark., 2020). *L. pneumophila* ile kontamine suyun deriye temas etmesi deri lezyonlarına ve dolayısıyla da enfeksiyon oluşmasına sebebiyet vermektedir (Baskerville ve ark., 1983; Memish ve ark., 1992). Nazogastrik tüplerin de kontamine suyla mikroaspirasyonu sonucu nozokomiyal Lejyoner hastalığına neden olduğu bazı çalışmalarda belirtilmiştir (Brabender ve ark., 1983). Ayrıca yapılan bazı çalışmalarda toprağın kazılması sebebiyle oluşabilecek aerosolizasyondan dahi bulaşma riski olasılığının olabileceği iddia edilmiştir (Yu, 1995; Khodr ve ark., 2016).

Legionella spp. su sistemlerinin her tarafında bulunabilir ve bazı protozalar ve biyofilm tabakalarıyla yakın ilişki içindedir. En az 20 amip ve iki silialı protozoonların *Legionella* spp. için konak olduğu gösterilmiştir (Erdoğan, 2018). Sudaki biyofilm tabakaları, amip ve silialı protozalar hem besin kaynağı hem de olumsuz şartlarda korunak görevi görürler ve *Legionella* spp. için yaşam alanlarıdır (McBurnett ve ark., 2018). Özellikle otel, hastane, okul, yurt gibi kalabalık yaşam alanlarındaki su tesisat sistemlerinin eski olması ve su sıcaklığının yüksek olması bakterinin üreme ve çoğalmasını arttırarak salgınlara neden

olabilmektedirler (Garrison ve ark., 2016). Lejyoner hastalığı tanısı konmuş kişinin veya kişilerin konakladığı binadaki su deposundan, su tanklarından, klima sistemi içinde dolaşan sudan, binanın tüm katlarının en az birer odasının duş başlıklarından, musluklardan ve termal havuzlardan su örnekleri alınmalıdır. Şüphelenilen alan hastane binası ise örnekler yalnızca katları temsilen alınmaz. Yoğun bakım ve ameliyathanelerden, kliniklerde yatan hastaların solunum ekipmanlarının su haznelerinden de örnek alınmalıdır (Huang ve ark., 2011).

Bu hastalık, Sağlık Bakanlığı, Halk Sağlığı Genel Müdürlüğü, Bulaşıcı Hastalıklar Daire Başkanlığı'nın kontrolünde takip edilmekte ve bununla ilgili Lejyoner Hastalığı Kontrol Programı Rehberi bulunmaktadır (Anonim, 2018). Ayrıca 13 Mayıs 2015 tarihli resmi gazetede Sağlık Bakanlığı Türkiye Halk Sağlığı Kurumu tarafından "Lejyoner Hastalığı Kontrol Usul ve Esaslari Hakkında Yönetmelik" yayımlanmıştır (Anonim, 2015). Bu yönetmeliğe göre; insanların topluluk halinde bir arada bulunabildiği hastane, okul, otel, fabrika, yurt ve restoran gibi yerlerde *Legionella* ya da vaka tespitinin bildiriminin zorunlu ve derhal yapılması gerekmektedir. Bunu takiben iligili konaklama biriminde etkenin belirlenmesi için çevre sürveyansının yapılması elzem bir husustur.

Yapılan bu çalışmada, Mardin ilinde bir kamu kurumuna ait iki su kuyusu, bir su deposu ve binaların musluklarından toplamda 25 su ve 25 sürüntü numunesinin mikrobiyolojik olarak *Legionella* cinsi bakteri ve serogruplarının varlığı ile klor, nitrat, nitrit, pH ve elektriksel iletkenlik gibi kimyasal özellikleri analiz edilmiştir. Ayrıca bu çalışma *Legionella* analizi açısından Mardin ili için ilk bilimsel rapor olma özellğini de taşımaktadır.

MATERYAL ve METOD

Su numuneleri

Mardin ilindeki bir kamu kurumuna ait iki su kuyusu, bir su deposu ve binaların musluklarından 25'er adet su ve sürüntü numuneleri alınmıştır.

Araç ve Gereçler

Sınıf II biyogüvenlik kabini (Biosafety, BlLF2000), kabinli UV (C-MAG), buffered charcoal yeast extract (BCYE), GVPC ve kanlı agar besiyerleri (Liofilchem, İtalya), vorteks (VelpScientifica), bunzen beki, -20°C buzdolabı (Arçelik), vakum pompası (Sartorius), kronometre, etüv (Sanyo, MR262), +4 °C buzdolabı (Arçelik), binoküler koloni mikroskobu (Hund Wetzlar), membran filtrasyon cihazı (Sartorius), *Legionella* spesifik lateks aglütinasyon kiti (Migrogen M45, İngiltere), membran filtre (0.45 µm), hidroklorik asit (HCl, Merck) potasyum klorür (KCl, Merck), cam L baget, N95 maske (3M), tek kullanımlık plastik pastör pipetler (1mL, 10 mL), 500 ml steril burgu kapaklı plastik kap, 15x120 mm vidalı steril plastik tüp, 15x160 mm kapaklı vidalı steril plastik tüp, 25x160 mm vidalı kapaklı pyrex tüp, tek kullanımlık steril plastik öze, tek kullanımlık drigalski spatülü ve sarf malzemeler kullanılmıştır. Su örneklerinde klor, nitrat ve nitrit anyonları analizi içinde iyon kromatografisi cihazı (ICS 5000+ DİONEX), pH ve elketriksel iletkenlik ölçümleri Multi Mettler Toledo marka cihazı ile ölçülmüştür.

Mikrobiyolojik analizler

Alınan su ve sürüntü örneklerinin *Legionella* spp. analizleri Diyarbakır İl Sağlık Müdürlüğü, Halk Sağlığı Laboratuvarlarında yapılmıştır. Su örnekleri toplandıkları yerlere göre membran filtrastyon, direkt ekim ve filtre asit gibi farklı metodlarla deriştirilir. Su örneklerinde *Legionella* cinsi dışında farklı bakterilerin de olduğu ve bu bakterilerin üreme hızlarının *Legionella* bakterisine göre daha yüksek olduğundan *Legionella* bakterilerini baskıladığı bilinmektedir. Bunu önlemek amacıyla toplanan su numunelerine asit ile muamele işlemi uygulanmıştır. İşlem görmüş ve görmemiş su numuneleri, daha sonra *Legionella* için seçici agar ortamı içeren plaklara ekilir ve inkübe edilir. İnkübasyon işleminden sonra seçici ortamda gelişen morfolojik olarak karakteristik koloniler, olası *Legionella* türleri olarak kabul edilir ve doğrulama için kanlı agar ve BCYE agara parelel ekimler yapılır (Erdoğan ve Arslan, 2007).

Eküvyon ile depo ve musluklardan alınan sürüntü örnekleri filtre edilmeden HCl-KCl (pH 2.2) asit çözeltisi içeren tüpe daldırılıp, pamuklu uç kuvvetlice tüp içindeki asitle karıştırıldı. Eküvyon çubukları tüp kenarından süzdürülerek çıkartılıp atık kutusuna atıldı. Tüp 3 dakika arayla iki defa vortekslendikten sonra tüpten 0.1'ler ml örnek BCYE ve GVPC besiyerlerine paralel ekim yapılmış ve L öze ile yayma işlemi gerçekleştirilmiştir (Erdoğan ve Arslan, 2007).

Depo ve musluklardan şişeye doldurularak alınan örnekler filtre edildikten sonra asit ile muameleye tabi tutulmuştur. Öncelikle filtrasyon işlemi için su örneğinin 50 ml'si filtrasyon cihazında filtre edilmiş, filtre ucu alevden geçirilen penset ile dikkatlice tutulduktan sonra 5 ml steril su içeren tüpe (25 x 160 mm) konmuş ve tüp 30 saniye boyunca vortekslenmiştir (Erdoğan ve Arslan, 2007).

Asitle muamele işlemi için ise vortekslenmiş filtre içeren tüpten 2 ml örnek, 2 ml HCl-KCl (pH 2.2) asit çözeltisi içeren tüpe aktarılmıştır. Tüp vortekslenip 3 dakikaya ayarlanmış saate basıp 3 dakika dolduğunda tüp tekrar vortekslenmiştir. Tüpten 0.1'er ml örnek BCYE ve GVPC besiyerilerine konulmuş ve L öze ile yayma ekim yapılmıştır

(Erdoğan ve Arslan, 2007).

Legionella ekimi yapılan besiyeri plakları, kapakları alta gelecek şekilde etüv tepsisine dizilmiştir. Plaklar oda ısısında, yüzeyleri kuruyuncaya kadar bekletildikten sonra plaklar ters çevrilerek tepsi 36.5±0.5 °C etüvde aerob koşullarda 5-10 gün süresince inkübe edilmiştir. Plaklar 3. günden itibaren okunmaya ve değerlendirilmeye başlanmıştır. Ayrıca bazı su sistemlerinde *Legionella* türü bakterilerin bulunacağından kuvvetle kuşkulanılıyorsa; özellikle mavi-beyaz floresan veren koloniler en erken bir haftada üreyebildikleri göz önüne alınarak üreme süreleri 10. güne kadar uzatılabilir. Bazı *Legionella* türleri (*L. bozemanii*, *L. gormanii*, *L. dumoffii* ve *L. anisa*) 362 nm dalga boyunda UV lamba ışığı altında mavi-beyaz floresan verirler. Bu özellik bu türlerin tanısında bir ön basamak olarak kullanılır (Erdoğan ve Arslan, 2007).

İnkübasyon sonunda plaklar koloni mikroskopu altında incelenir; *Legionella* spp. ile uyumlu, yüzeyleri düzgün, hafif bombeli, gri-beyaz, 1-3 mm çaplı, mikroskop altında kenarları pembe, mor, yeşil veya mavi buzlu cam görünümü veren koloniler muhtemel *Legionella* kolonileri olarak ayırt edilir (Erdoğan ve Arslan, 2007).

Sonraki tanımlama basamağında *Legionella* kuşkulu kolonilerin görüldüğü her plaktan bir veya daha fazla koloni seçilir. Bir plaktaki tüm koloniler aynı görünümde ise bir koloni seçilir, farklı iseler her birini temsilen birer koloni seçilir. Seçilen her koloni 1'den başlayarak sırasıyla numaranladırılır. Tanımlama çalışmasının ilk basamağı, *Legionella*'ların %5 kanlı agarda üreyemediğinin gösterilmesi prensibine dayanır. Bu nedenle kanlı agara ve BCYE besiyerinden seçilen her koloniden paralel pasaj yapılır. Seçilen bütün kolonilere bu işlem uygulanır. Bu şekilde pasajlanan paralel ekim plakları etüve kaldırılıp, 24-48 saat sonra değerlendirilir. Genellikle üremeler için 24 saat yeterlidir. Kanlı plaklarda üreme olmamış iken, BCYE'de üremiş olan kolonilerin yüksek olasılıkla *Legionella* olduğu kabul edilir. Kültür sonunda tespit edilen *Legionella* cinsi bakterilerin tür ve serogrup düzeyinde tanımlanması için lateks aglütinasyon testi yapılmıştır (Fields, 1996).

Su numunelerinin kimyasal analizleri

Alınan su ve sürüntü örneklerinin kimyasal analizleri Diyarbakır İl Sağlık Müdürlüğü, Halk Sağlığı Laboratuvarlarında yapılmıştır. Su numunelerinin pH ölçümü Türk Standardları Enstitüsü'nün (TSE), TS 9748 EN 27888 nolu (Anonim, 2010), su kalitesi elektrik iletkenliği ölçümü TS 3263 ISO 10523 nolu (Anonim, 2010) ve sudaki klor nitrat ve nitrit analizleri ise TS EN ISO 10304-1 nolu (Anonim, 2010), metodlarına göre yapılmıştır. Her bir analiz üçer tekrarlı olarak yapılıp, ortalamaları ve standart sapmaları hesaplanmıştır.

BULGULAR ve TARTIŞMA

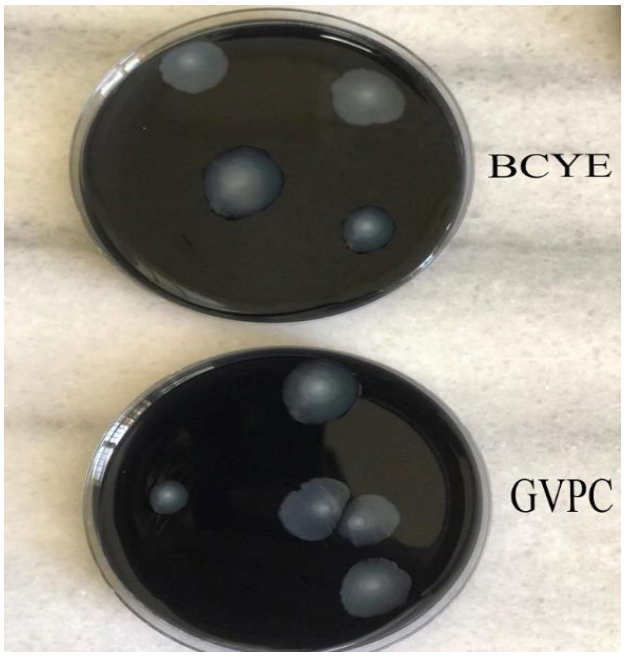
Alınan su örneklerinin hiçbirinde *Legionella* spp. üremesi gerçekleşmezken, sürüntü örneklerinin dördünde ise *L.* pneumophila serogrup 1'e rastlanılmıştır (Şekil 1.1., 1.2. ve 1.3.). 6 nolu muslukta 20 (ml/koloni), 4 nolu da 40, 8 nolu da 60 ve 9 nolu da ise 100 adet koloniye rastlanılmıştır. Hedef (2019), yaptığı çalışmada, Halk Sağlığı Genel Müdürlüğü Ulusal Solunum Yolu Patojenleri Referansları Laboratuvarına, konut, fabrika ve hastanelerden gelen 64 adet su numunesinin 23 tanesinde *Legionella* varlığına rastlandığını belirtmiştir. Lateks aglütinasyon kiti ile yapılan tanımlamada 3 numunede *L. pneumophila* serogrup 1, 8 numunede *L. pneumophila* serogrup 2, 12 numunede *Legionella* spp. tespit edilmiştir. *Legionella* tespit edilen numunelerin ml'de 2 koloniden 2176 koloniye kadar geniş bir aralıkta olduğu belirtilmiştir. Bir başka çalışmada 131'i su ve 84'ü sürüntü örneği olmak üzere toplam 215 örnekte (cami, okul, bina su depoları, doğal kaynak suyu) *Legionella* spp. analizi yapılmış, bunların 18'inde *Legionella* spp. üremesi tespit edilmiştir (Ayhan, 2020). Akkaya ve Özbal'ın (2011) yapmış olduğu *Legionella* spp. analzinde Kayseri'de 16 binanın depo sularından alınan örneklerin analizi neticesinde 3 örneğin *L. pneumophila* serogrup 1, 64 musluk ve duş başlığı suyundan üretilen 2 *Legionella* türünün 1'i *L. pneumophila* serogrup 1 ve 40 musluk ve duş başlığı sürüntüsünden üretilen 3 *Legionella* türünün 2'sinin *L. pneumophila* serogrup 1 olduğu belirtilmiştir. İspanya'nın Huesca bölgesinde boyunca şehire su taşıyan boru hatlarından 6 ay boyunca değişik aralıklarla alınan 21 su örneğinin *Legionella* analizinde, örneklerin 11 tanesinde serogrup 2, gözlenirken 10 tanesinde ise *Legionella* tespit edilmediği raporlanmıştır (Gruas ve ark., 2013).

Suların pH yani asitlik ve alkalilik derecesi suda bulunan tuzlara bağlı olarak değişmektedir. Kalsiyum bikarbonat ve alkali karakterde tuzlar mevcutsa alkali. aşırı miktarda karbondioksit bulunduğu durumlarda ise asidik karakter sergiler. Suyun aşırı miktarda alkali olması suyun kirlilik nedeniyle kokuştuğunun göstergesidir. Suyun asiditesinin kaynağı karbondioksit dışındaki etkenler ise suyu korozif karaktere dönüştürür. İdeal su pH'nın ise nötre yakın yada hafif alkali olması gerekmektedir (Demirer, 1995).

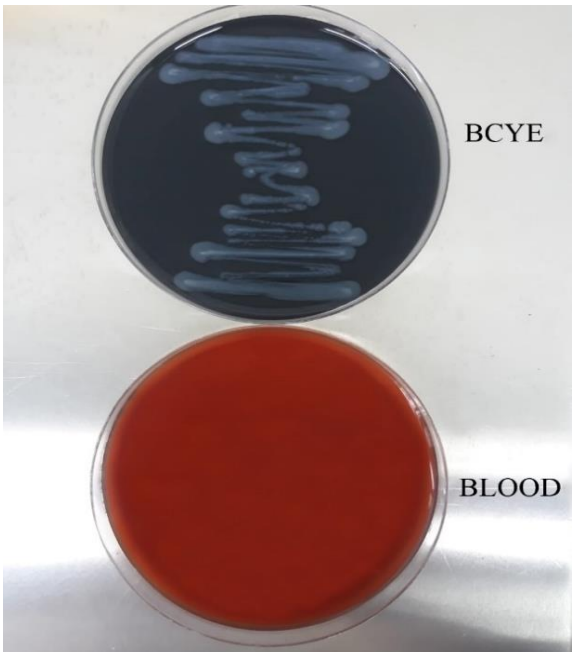
Yapılan çalışmada su numunelerinin pH değerlik aralığı 7.53±0.76-8.02±0.87 olarak tespit edilmiştir (Çizelge 1.). En düşük pH değeri 7.53±0.76 ile bir nolu kuyuda gözlenirken, en yükseği ise 8.02±0.87 ile iki nolu kuyuda gözlenmiştir.

Zonguldak'ta yapılan bir çalışmada (Bora, 2016), merkez ilçeye bağlı köylerden alınan su numunelerin

hepsinde pH değerleri içilebilir seviyede çıkmıştır. pH değerlerinden en yükseği Kaleoğlu köyünden alınan numunede 8.79 olarak ölçülürken en düşük pH değeri ise Köroğlu köyünden alınan numunede 6.50 olarak ölçülmüştür. Köylerden alınan tüm numunelerin pH değeri ortalama 7.80 olarak belirlenmiştir Bu çalışmanın en düşük ve en yüksek değerleri bu çalışmadaki değerlerden sırasıyla daha düşük ve daha yüksek çıkmıştır. Van'ın Erciş İlçesinde su depolarından alınan örneklerin pH değerlerinin 7.60, musluk sularında ise 7.27 olduğu (Ağaoğlu ve ark., 2007a), içme sularında ise bu değerin 6.95 olduğu belirtilmiştir (Atıcı ve ark., 2016). Muğla'nın Kavaklıdere ile Aydın'ın Bozdoğan ilçelerinin yer altı ve kaynak sularının pH analizlerinin yapıldığı bir çalışmada değer aralığının 6.34-7.33 olduğu tespit edilmiştir (Karaoğlu, 2001).



Şekil 1. Besiyerinde *Legionella* spp. Üremesi
Figure 1. Growth of Legionella spp. *in the medium*



Şekil 2. Besiyerinde doğrulama ekimi
Figure 2.Conformation cultivation in the medium

Şekil 1. *Legionella* serotiplendirme aglutinasyon testi
Figure 3. Agglutination test for Legionella serotyping

Değişik çalışmalardan elde edilen bulguların farklı olması biyotik ve abiyotik faktörler nedeniyle gerçekleşebilmektedir. Biyotik faktörler için üreticilerin, ayrıştırıcıların ve tüketicilerin faaliyetleri sayılırken abiyotik faktörlerin ise fiziksel fakörler (sıcaklık, nem, ışık ve atmosfer basıncı), inorganik maddeler ve organik maddeler olduğunu söyleyebiliriz. Farklı çalışmalarda elde edilen ölçüm değerlerinin farklı olması, su kaynaklarının bulunduğu yerin kayaç ve toprak yapısı, su kaynağına yakın bölgelerde yapılan tarım ve hayvan çiftliği faaliyetlerinin atık ve artıklarının su kaynaklarına karışması ile diğer çevre kirleticilerinin su kaynağına bulaşması gibi nedenlerden kaynaklandığını düşünülmektedir.

TSE standartlarına göre içilebilecek suyun pH değeri 6.5-9.5 aralığında olması istenirken (Anonim, 2010), bu değer aralığı Dünya Sağlık Örgütüne göre ise 6.5-8.5 'tur (WHO, 1996). Bu değerler ışığında alınan ölçümler neticesinde analiz edilen suyun pH açısından içilebilir olduğu görülmektedir.

Araştırmada su numunelerin elektrik iletkenlik değerlerinin en düşüğü 376.44±10.87 µS cm^{-1} ile iki nolu kuyudan, en yükseği ise 446.57±15.76 µS cm^{-1} ile

bir nolu kuyudan alınan numunede ölçülmüştür. Binaların musluklarından alınan örneklerde ise bu aralığın 382.40 µS cm^{-1} ile 418.06 µS cm^{-1} olduğu görülmüştür (Çizelge 1.).

Çizelge 1. Su numunelerinin kimyasal analiz sonuçları
Table 1. Chemical analysis results of water samples

	Parametreler				
Su numuneleri	Klor (ppm)	Nitrat(ppm)	Nitrit (ppm)	İletkenlik(µS cm^{-1})	pH
Bir nolu kuyu	2.21±0.15	4.30±0.45	*	446.57±15.76	7.53±0.76
İki nolu kuyu	2.05±0.08	10.85±1.12	*	376.44±10.87	8.02±0.87
Ana depo	2.43±0.07	7.00±1.06	*	390.98±10.85	7.90±0.64
1 nolu musluk	1.70±0.09	7.00±0.87	*	382.40±12.78	7.84±0.45
2 nolu musluk	1.70±0.11	7.00±0.98	*	394.57±13.43	7.82±0.82
3 nolu musluk	1.69±0.10	6.98±0.97	*	402.63±12.36	7.79±0.76
4 nolu musluk	1.71±0.05	7.02±1.08	*	401.17±10.53	7.80±0.61
5 nolu musluk	1.70±0.05	7.02±1.10	*	406.41±12.90	7.81±0.92
6 nolu musluk	1.70±0.05	7.03±1.15	*	407.55±14.63	7.77±0.37
7 nolu musluk	1.73±0.12	7.01±0.87	*	415.67±12.92	7.76±0.74
8 nolu musluk	1.68±0.02	6.97±0.85	*	406.58±11.24	7.76±0.75
9 nolu musluk	1.69±0.10	6.97±0.76	*	401.02±12.73	7.76±0.73
10 nolu musluk	1.68±0.05	6.95±0.68	*	404.22±12.97	7.78±0.21
11 nolu musluk	1.68±0.04	6.97±0.87	*	407.36±13.64	7.78±0.56
12 nolu musluk	1.69±0.07	7.03±0.98	*	415.77±12.79	7.73±0.65
13 nolu musluk	1.69±0.06	7.04±0.85	*	409.89±12.35	7.81±0.52
14 nolu musluk	1.69±0.07	7.03±0.65	*	411.33±11.09	7.75±0.74
15 nolu musluk	1.69±0.09	7.02±0.87	*	410.12±10.32	7.75±0.45
16 nolu musluk	1.70±0.04	7.03±0.64	*	408.49±10.43	7.88±0.44
17 nolu musluk	1.70±0.07	7.04±1.52	*	418.06±12.62	7.75±0.27
18 nolu musluk	1.69±0.08	6.97±0.97	*	412.25±11.23	7.74±0.62
19 nolu musluk	1.69±0.06	6.96±0.76	*	404.59±12.48	7.74±0.67
20 nolu musluk	1.72±0.08	6.97±0.95	*	404.34±14.23	7.76±0.73
21 nolu musluk	1.69±0.09	7.01±0.75	*	415.40±11.87	7.76±0.76
22 nolu musluk	1.69±0.06	6.92±0.63	*	409.90±10.65	7.81±0.52

*: Tespit edilmedi, Her bir analiz üçer tekrarlı olarak yapılmıştır.

Farklı bir araştırmada (Bora, 2016), Zonguldak'a bağlı merkez köylerden alınan numunelere ait en yüksek elektriksel iletkenlik değeri Çukurören köyünden alınan numunede 1020 µS cm^{-1} olarak en düşük iletkenlik değeri ise Olukyanı köyünden alınan numunede 21 µS cm^{-1} olarak ölçülmüştür. Çalışmadaki en yüksek veri Bora'nın (2016) bulgularından oldukça düşük, en düşük veri ise oldukça yüksektir. Tunceli'de sekiz farklı istasyondaki su kaynaklarının iletkenlik değerleri. 165.8 ile 760 µS cm^{-1} arasında değiştiği belirtilmektedir (Çetin, 2017). Karaoğlu ve ark., (2008) yaptıkları çalışmada suların iletkenlik değerlerini en yüksek ve en düşük sırasıyla 42 ve 1000 µS cm^{-1} olarak ölçmüşlerdir. Erciş'te ölçülen değerler musluk sularında 301.9 µS cm^{-1}, depo sularında 294.5 µs/cm şeklindedir (Ağaoğlu ve ark., 2007b). Erciş içme sularında ise bu değer 313.0 µS cm^{-1} (Atıcı ve ark., 2016) olduğu tespit edilmiştir.

Suda çözünen organik ya da inorganik madde miktarı arttıkça suyun elektrik iletkenliği de artmaktadır. Yani iletkenlik derecesi yüksek olan sularda çözünmüş madde miktarının da yüksek olduğu kabul edilmektedir.

İçme sularındaki elektrik iletkenliğinin farklı standartlara göre maksimum 2500 µS cm^{-1} seviyesinde olması istenmektedir (WHO, 1996; Anonim, 2010). Saha çalışmasındaki kuyu, depo ve musluk su numunelerinin hepsinin bu değerin altında olduğu görülmüş ve dolayısıyla iletkenlik açısından da içilebilir bir su olduğu tespit edilmiştir.

Araştırmada yapılan ölçümler neticesinde hiçbir su numunesinde nitrit tespit edilmezken, nitrat miktarı en düşük bir nolu kuyuda 4.30±0.45 ppm (mg L^{-1}) ile en yüksek ise 10.85±1.12 ppm ile iki nolu kuyuda ölçülmüştür (Çizelge 1.).

Çetin'in (2017), Tunceli'de yaptığı çalışmada sekiz farklı su kaynağından alınan örneklerin nitrit değerleri. <0.001 ile 0.6734 mg L^{-1} arasında. nitrat değerleri ise 0.0162 ile 10.010 mg L^{-1} arasında ölçülmüştür. Karaoğlu ve ark., (2008) tarafından belirlenen nitrit miktarının 0.0003 mg L^{-1} nitrat miktarının ise 0.0035 ile 1.88 mg L^{-1} dir. Koçak ve Güner (2010), Erzurum'un içme ve kullanma sularında yaptıkları nitrit miktar tayininde sonucun 0.03 mg L^{-1} olduğu belirtilmiştir. Ağaoglu ve ark. (2007a) yaptığı bir çalışmada göre nitrit düzeyi ortalamasının 0.1 mg/L'in altında olduğu

bildirilmiştir. Çavuş ve ark., (2017) Van şehir merkezi ve ilçelerinde bulunan dere sularının tamamında bulunan nitrit miktarını 0.060-0.691 mg L^{-1} aralığında tespit etmiş, Erciş'teki içme sularında ise nitrit miktarı 0.012 mg L^{-1} olarak ölçülmüştür (Atıcı ve ark., 2016). Karaoğlu, (2001) yılında yaptığı çalışmada Kavaklıdere (Muğla). Bozdoğan (Aydın) yerleşim yerlerindeki kaynak ve yer altı sularınının nitrit değerlerini 0.003-0.073 mg L^{-1} aralığında ölçmüştür. Erzurum İl merkezindeki içme ve kullanma sularının nitrat değerleri üzerine yapılan bir çalışmada en düşük değer 4.08 mg L^{-1}, en yüksek değer ise 128.39 mg L^{-1} olarak bildirilmiştir (Koçak, 2007).

Doğada ayrıştırıcıların faaliyetleri sonucu oluşan amonyak (NH_3) nitrit bakterileri tarafından nitrite (NO_2^-), nitritten de nitrat bakterileri tarafınadan nitrata (NO_3^-) dönüşür (Sipahi ve ark., 2016). İçme ve kullanma sularında nitrit bulunması istenen bir madde olmadığından kalitatif analiz yapılır.

İçme sularındaki nitrit seviyesinin TSE standartlarına göre en fazla 0.5 mg L^{-1}, Dünya Sağlık Örgütüne ise 3 mg L^{-1} olması istenirken, nitrat için üst sınır değeri ise 50 mg L^{-1}'dir (WHO, 1996; Anonim, 2010). Su numunelerinin nitrit ve nitrat değerleri açısından standartlara uygun ve dolayısıyla içilebilir olduğunu belirtebiliriz.

Yapılan çalışmada su numunesi alınan iki nolu kuyu. bir nolu kuyu ve ana depodaki klor değerleri 2.05±0.08-2.21±0.15 ve 2.43±0.07 ppm olarak ölçülmüştür. Bina musluklarından alınan örneklerde ise 1.68±0.02-1.73±0.12 ppm aralığında olduğu tespit edilmiştir (Çizelge 1).

Tunceli'nin doğal su kaynaklarının klor değerleri 0.9997 ile 10.996 mg L^{-1} arasında olduğu belirtilmiştir (Çetin, 2017). Çavuş ve ark. (2017) Van şehir sularının ortalama serbest klor değerinin 0.05 mg L^{-1} olarak ölçmüştür. Erciş'te yapılan analiz çalışmasında musluk suyunun klor değeri 8.99 mg L^{-1} iken, depo suyunun klor değeri ise 9.07 mg L^{-1} olarak tespit edilmiştir (Ağaoğlu ve ark., 2007b). Çalışma bulguları Ağaoğlu ve ark. (2007a) bulgularından daha düşük seviyededir. Bitlis ve ilçelerindeki içme suyu klor ölçümleri neticesinde ortalama değerin 7.69 mg L^{-1}, en yüksek değerin Ahlat ilçesinde 12.21 mg L^{-1}, en düşük değer ise 2.14 mg L^{-1} olarak Hizan ilçesinde belirlenmiştir (Alemdar ve ark., 2009). Erciş içme sularında yapılan ölçümlerde serbest klor miktarının 0.02 mg L^{-1} olarak bulunduğu rapor edilmiştir (Atıcı ve ark., 2016).

Klorün, TSE standartlarına (Anonim, 2010) göre içme sularındaki üst değerinin 0.5 mg L^{-1} olduğu bunun üzerindeki klor değerindeki suların içilemez olduğu belirtilmiştir.

SONUÇ ve ÖNERİLER

Su örneklerinde yapılan mikrobiyolojik analiz neticesinde 25 su örneğinde *Legionella* spp. bakterileri tespit edilememişken, 25 sürüntü örneğinin dördünde *L. pneumophila* serogrup-1 patojen bakterisi tespit edilmiştir. Su örneklerinin kimyasal açıdan değerlendirilmesi pH, iletkenlik, klor, nitrit ve nitrat değerleri üzerinden yapılmıştır. Analiz edilen su numuneleri klor haricindeki parametreler göz önüne alındığında TSE ve Dünya Sağlık Örgütü standartlarını karşılar niteliktedir.

L. pneumophila'nın tespiti ve vaka bildirimleri Sağlık Bakanlığı, Halk Sağlığı Genel Müdürlüğü, Bulaşıcı Hastalıklar Daire Başkanlığı'nın kontrolünde takip edilmektedir. Başkanlığın bununla ilgili Lejyoner Hastalığı Kontrol Programı Rehberi bulunmaktadır. İnsanların topluluk halinde bir arada bulunabildiği hastane, okul, otel, fabrika, yurt ve restoran gibi yerlerle ilişkili vakaların bildiriminin zorunlu ve derhal yapılması gerekmektedir. Bunu takiben iligili konaklama biriminde etkenin belirlenmesi için çevre sürveyansının yapılması elzem bir husustur. İlgili yönetmelik uyarınca elde edilen veriler ve bunun gereği olarak alınması gereken önlemler ilgili kuruma iletilmiştir.

Yapılan bu çalışmadan elde edilen veriler ışığında önerileri şu şekilde sıralayabiliriz.

- Birçok hastane, okul, yurt, otel, restoran ve konutlar gibi insan sirkülasyonunun youğun olduğu yerleşkelerden ilgili ve yetkili birimlerce hem su hemde sürüntü örnekleri alınıp *Legionella* spp. varlığı açısından incelenmelidir.
- Özellikle hastane, kreş, yaşlı evi, huzur evi ve okul gibi bağışıklık sistemi nispeten zayıf bireylerin bulunabildiği alanlarda su sistemlerinin periyodik olarak incelenmesi önem arz etmektedir.
- Lejyoner hastalığı insandan insana solunum yoluyla bulaşabilen bir hastalıktır. Üniversiteler mobilite açısından daha aktif bireylerin bir araya geldiği mekanlar olduğundan, buradan kaynaklanan bir hastalığın yayılım hızının diğer yerleşkelere nazaran daha hızlı olabileceği unutulmamalı gereken önlemler alınmalıdır.
- Yukarıda belirtilen yapıların depo, kuyu ve musluk gibi su sistemlerinde yapılan analiz neticesinde *Legioenella* spp. tespit edildiğinde bir an önce su sistemlerinin ilgili protokoller rehberliğinde dezenfeksiyon ve dekontamisyonu sağlanmalıdır.
- Ayrıca yapılan analiz neticesinde *Legionella* spp. varlığı tespit edilen su sistemlerine bağlı iklimlendirme cihazları *Legionella* spp'nin hızlı bir şekilde yayılmasına yol açacağından cihazların bakımı ve dezenfeksiyonu bir an evvel yapılmalıdır.
- Bakır-gümüş iyonizasyon dezenfeksiyon yönteminin hem en etkili yöntem ve hem de insan

sağlığına olumsuz etkilerinin diğer yöntemlere nazaran daha düşük olduğundan dezenfeksiyon yöntemi olarak tercih edilmelidir.

 Legionella spp.'nin sebep olduğu hastalıklardan *Legionella*'dan şüphelenilmemesi bu hastalığın insidansını düşük göstermektedir. Bundan dolayı bu hastalığın belirtileri daha iyi bilinmelidir.

 Su numuneleri içerisinde yaşamını sürdüren *Legionella spp.* bakterilerini daha yoğun bir şekilde kültürde üretmek için filtrasyonla konsantrasyon işlemi uygulanmalıdır. Kültür yönteminde *Legionella* spp. üremesini artırmanın diğer bir yolu ise çevre biyotayı baskılayan asitle muamele işleminin uygulanması olduğu sonucuna varılmıştır.

 Lejyoner hastalığının da diğer hastalıklar gibi sigara, alkol, stres, dengesiz beslenme, hareketsiz yaşam vb. nedenlerle immün sistemi baskılanmış ya da zayıflamış bireylerde daha yıkıcı olduğu düşünüldüğünde bu tür olumsuz etkenlerden uzak durmak önem arz etmektedir. Bu riski taşıyan bireylerin etkenin kaynağından mümkün oldukça uzak durmaları ve bu etkene karşı dikkatli davranmaları önerilmektedir.

 Hızlı ve doğru tanı sonucunda hastalığın teşhisi ile doğru antibiyotik kullanımı hastalığın seyrini olumlu yönde etkileyen bir unsur olmaktadır. Bu amaçla hastalığın tedavi süreci doğru yönetilmelidir.

 Legionella bakterilerinin sürüntü örneğinde bulunma olasılığı normal su örneğinden daha yüksektir.

TEŞEKKÜR

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Yazarlar makaleye eşit oranda katkı sağlamış olduklarını beyan eder.

Çıkar Çatışması Beyanı

Makale yazarları aralarında herhangi bir çıkar çatışması olmadığını beyan ederler.

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Anticandidal Activity and Anticandidal Mechanism of Essential Oil of *Cuminum cyminum* L. and *Myrtus communis* L. Mixture

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ABSTRACT

C. cyminum (cumin) and *M. communis* (myrtle) comprise many biologically active molecules. In this study, cumin seed and myrtle leaves were mixed then hydrodistilled and analyzed by GC-MS. The main components of the essential oil were cuminal (50.71%), 1,8 cineole (8.30%), O-cymene (7.88%), ß-pinene (7.62%), α-pinene (7.16%), γ-terpinene (6.09%) and α-terpinolene (2.19%). The antifungal activity of the essential oil against *C. albicans*, *C. parapsilosis* and *C. tropicalis* was investigated using spectrophotometric broth microdilution, colorimetric broth microdilution, and agar well diffusion tests. Antimicrobial mechanism of the EO was researched by TTC-dehydrogenase relative activity, protein and DNA leakage analysis. While the MIC values of the oil were 3.29 mg mL^{-1} for *C. albicans*, 3.57 mg mL^{-1} for *C. parapsilosis* and 3.65 mg mL^{-1} for *C. tropicalis*, the 50% cytotoxic concentration values were between 0.17 mg mL^{-1} and 2.61 mg mL^{-1} for *Candida* species. The inhibition values against yeasts in dark and light conditions were found as 42.1 mm on *C. parapsilosis* and 39 mm on *C. tropicalis* exposed dark and light, respectively. The inhibition zones of oil in water, glucose, glycerol and salt environment. As a result, the highest IZ was found as 26.6 mm on *C. albicans* and 26 mm on *C. tropicalis* in the presence of a 3% glycerol environment. The DNA and protein levels were increased when yeast was exposed to the EO. As a result, the oil of mixed *C. cyminum* and *M. communis* preserved its antimicrobial stability in different environmental conditions and should contribute to new antifungal research.

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Cuminum cyminum L. ve *Myrtus communis* L. Karışım Uçucu Yağının Antikandidal Aktivitesi ve Antikandidal Mekanizmasının Belirlenmesi

ÖZET

C. cyminum (kimyon) ve *M. communis* (murt) pek çok aktif bileşeni olan özellikle antimikrobiyal, antioksidan ve antidiyabetik özellikleriyle bilinmektedirler. Bu çalışmada, ilk kez olarak, kimyon tohumları ve murt bitkisinin yaprakları birlikte su distilasyonuna tab tutulmuş ve elde edilen uçucu yağın bileşenleri GC-MS cihazıyla belirlenmiştir. Uçucu yağın temel bileşenleri cuminal (%50.71), 1,8 sineol (%8.30), osimen (%7.88), ß-pinen (%7.62), α-pinen (%7.16), γ-terpinen (6.09%) ve α-terpinolen (%2.19) olarak belirlenmiştir. Antifungal aktiviteyi belirlemek amacıyla uçucu yağın *C. albicans*, *C. parapsilosis* and *C. tropicalis* mantarlarına karşı Spektrofotometrik Broth Mikrodilüsyon, Kolorimetrik Broth Mikrodilüsyon ve Agar Well Difğzyon metodlarıyla etkileri belirlenmiştir. Uçucu yağın animikrobiyal etkisini belirlemek amacıyla TTC-dehidrogenaz aktivite, protein kaçağı ve DNA aktiviteleri incelenmiştir. Yağlarin MIC değerleri *C. albicans* için 3.29 mg mL^{-1}, *C. parapsilosis* için 3.57 mg mL^{-1} ve *C. tropicalis* için 3.65 mg mL^{-1}, CC50 (%50 sitotoksisite konsantrasyonu) değerleri ise

Mikrobiyoloji

Araştırma Makalesi

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Anahtar Kelimeler
Kimyon
Murt
Uçucu yağ
Antikandidal
Antifungal

çalışılan kandidalar için 0.17 mg mL^{-1} ve 2.61 mg mL^{-1} olarak belirlenmiştir. Yağların mayalara karşı karanlık ve ışık (standart LED ışık) koşullarında etkilerinin de belirlendiği çalışmada en yüksek inhibisyon 42.1 mm ile *C. parapsilosis* ve 39 mm ile *C. tropicalis*'de sırasıyla karanlık ve ışıkta bulunmuştur. Çalışmada ayrıca yağların su, glikoz, gliserol ve tuz koşullarında inhibisyon bölgeleri (IZ) 26.6 mm ile *C. albicans* ve 26 mm ile *C. tropicalis*'de %3 gliserol koşullarında bulunmuştur. Mayaların DNA ve protein seviyelerinde yağa maruz kalma durumlarına göre artış tespit edilmiştir. Sonuç olarak, kimyon ve murt birlikte distile uçucu yağının antimikrobiyal stabilite gösterdiği ileri çalışmalar yapılması gerektiği tespit edilmiştir.

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INTRODUCTION

Candidiasis is a common infection found in the gastrointestinal tract, oral cavity, and esophagus, skin, vagina, and vascular system. The most important species isolated is *Candida albicans* (Calderone et al., 2001). However, in the last two decades, infections caused by non-albicans *Candida* species have been observed to increase (Miguel et al., 2005). Candida species for instance *C. albicans*, *C. parapsilosis* and *C. tropicalis* could cause multiple nosocomial bloodstream infections with high morbidity and mortality rates (Wisplinghoff et al., 2004; Pappas et al., 2016). *C. parapsilosis* could cause invasive candida related disease (Trofa et al., 2008). The other emerging human pathogen is *C. tropicalis*, identified with more detailed screening methods and it causes serious infections with other Candida pathogens (Silva et al., 2012).

Many antifungal drugs are used in the treatment of candidal infections, and the side effects of these drugs occur especially in immunocompromised patients. In addition, candidal resistance emerges as another important problem even in healthy people (Whaley et al., 2017). For this reason, plants are being constantly being researched to discover new antifungal agents. Essential oils, (EOs), which have particularly strong effects against microbes, are extracted from a wide variety of plants and are widely used in antimicrobial research (Bakkali et al., 2008). Some of those are the essential oils of cumin, fennel, manuka (Elisa et al., 2019), thyme, pennyroyal, and lemon (Mahdavi et al., 2010), which have strong antifungal performance. Among medicinal plants, *C. cyminum* (cumin) and *M. communis* (myrtle) are known as two important aromatic plants with very high biological activity. *Myrtus communis* L., belongs to Myrtaceae family, is an evergreen aromatic tree in bush form, and widely grows throughout the Mediterranean region (Aleksic and Knezevic, 2014). Especially leaves of myrtle have been used in medicine for years as an antidiabetic, antiseptic, analgesic, emmenagogue, astringent, haemostatic, and cardiotonic agent, and in lithotripsy applications (Sumbul et al., 2011). Furthermore, myrtle leaves essential oil have been used perfume and cosmetic industry and, owing to high antimicrobial activity even in post-harvest applications (Zomorodian et al., 2013; Bahadirli et al., 2020). *M. communis* plants cultivated in the Mediterranean is for its fruits, but for leaves, demands consume mostly by collecting from nature. *C. cyminum* L., from the Apiaceae family, is an annual herbaceous plant, that has been used as a medicinal herb in Asia, Europe, and Africa (Johri, 2011; Al-Rubaye et al., 2017). Cumin is an important export crop of Turkey after poppy, oregano and bay laurel (Kırıcı et al., 2020). Cumin is cultivated in ca. 361.761 da area, especially in Central Anatolia (Kırıcı et al., 2020; Baydar, 2020). Dried seeds of *C. cyminum* are commonly used for culinary and medicinal purposes (VanderJagt et al., 2002). Many cumin types are used in traditional medicine and veterinary applications as antispasmodic, due to their appetizing, carminative, stimulant, and astringent effects and as a remedy against flatulence, diarrhea, and indigestion (Morton, 1976).

Aromatic plants are very active against microorganisms due to their bioactive molecules. Research conducted so far on the essential oil contents of these plants has made important contributions to microbiology. In recent years, studies have been investigating the antimicrobial effects of combinations of essential oils with different herbs and antibiotics to control pathogenic microorganisms and to reveal resistance mechanisms and multiple biochemical processes (Bassolé and Juliani, 2012; Bajpai and Dash, 2012). Also, essential oils used for pharmaceutical, food, or cosmetic purposes are generally not used alone. They are mostly found in

mixtures in the drugs or foods (Başer, 2009), and exposed to environmental factors such as pH, food additives (Gutierrez et al., 2008), and light (Erdoğan Eliuz, 2017). *C. cyminum* as a stimulant, carminative, therapeutic in respiratory diseases (Thippeswamy and Naidu, 2005) and *M. communis* as a reliever, therapeutic in lung diseases are indispensable (Asgarpanah and Ariamanesh, 2015) food and medicine sources used by the people.

This study, best of our knowledge is the first study to make essential oil extraction from two plants (*M. communis* and *C. cyminum*) mixed in equal proportions. The antifungal effect and antifungal mechanism of the essential oil obtained from the mixture was investigated against *C. albicans*, *C. parapsilosis*, and *C. tropicalis*. In addition, it was researched whether there was any change in the antifungal performance of this mixture oil in NaCl, glucose, glycerol, and LED light environments.

MATERIAL and METHOD

The Extraction Method and GC-MS Analysis

Cumin seeds were purchased from an herbalist in Hatay and myrtle leaves were collected from nature in Hatay/Turkey, 2020. Cumin seeds was grounded and myrtle leaf dried and cut into small pieces. The seeds of *C. cyminum* and *M. communis* leaves were mixed (1:1) and hydro-distilled with Clevenger-type apparatus for 4 hours. The mixture of essential oil was stored at 4 °C until the time of analysis. The essential oil composition was determined with GC-MS (Agilent 7890A-5975C) equipped with an Agilent 19091S-433 model column (30m X 250 µm film X 0.25 µm). Helium was a carrier gas. The detector transfer line temperature was 260 °C and the detector ionization temperature was 250 °C. The analysis program was as follows: starting with 60 °C increased to 150 °C with 5 °C min^{-1}, from 150 to 200 °C with 1.5°C min^{-1}, from 200 °C to 240°C with 4 °C min^{-1}. The essential oil components were defined using Wiley and NIST database. Retention indices were calculated against *n*-alkanes (C8–C40).

Antifungal Screening:

Antifungal activity of the essential oil of the plant mixture against *Candida albicans* (ATCC 90028), *C. tropicalis* (ATCC 750) and *C. parapsilosis* (ATCC 22019) were tested by Spectrophotometric Broth Microdilution and Agar Well Diffusion methods. Yeasts' inoculums were prepared in 4 mL of Sabouraud Dextrose Broth media and incubated at 35°C, overnight followed the directions of document M27-A of the NCCLS. After 24 hours the yeast cultures were adjusted to 0.5 McFarland Standard (NCCLS, 1997).

Spectrophotometric Broth Microdilution Method

This method was performed to determine MIC (Minimum Inhibitory Concentration) of *C. cyminum* and *M. communis* EO mixture on *C. albicans*, *C. tropicalis* and *C. parapsilosis* using a 96-well microplate. Firstly, a 50 µL portion of the Mueller Hinton Broth (MHB) was poured in all wells and then a 50 µL portion of the essential oil mixture (3.024 mg mL^{-1}) in %10 DMSO (Dimethyl sulfoxide) was poured into the first order wells. Columns 11 (media and yeast) was the negative control and 12 (fluconazole; FLC:128 µg mL^{-1}) was a positive control. Lastly, 5 µL cultures of yeast were inoculated on all the wells except negative control. Then, the two-fold serial dilution of the EO mixture was made and the final diluted was 0.05 mg mL^{-1}. The same procedure was applied for the antibiotic. The plates were incubated at 35°C for 24 hours and the optical density of growth was measured at 415 nm after incubation by spectrophotometer (Thermo Scientific, MULTISKAN GO). The MIC of the EO mixture, which results in 99.9% inhibition of growth, was calculated using the regression curve obtained from Eq. 1 and Eq. 2 (Erdoğan Eliuz, 2020).

$$\text{Growth (\%)} = \left(\frac{OD_{test}}{OD_{control}}\right) \times 100 \quad \text{Eq. 1}$$

$$\text{Inhibition (\%)} = \left[1 - \frac{OD_{test\ well}}{OD_{corresponding\ control\ well}}\right] \times 100 \quad \text{Eq. 2}$$

Cytotoxicity Assay for Candida Species

Colorimetric (MTT: 4,5 dimethyl-2-thiazole-2,5-diphenyl-2H-tetrazolium bromide) broth microdilution method was performed to determine the 50% cytotoxic concentration (CC50) of the oil based on the method previously applied for microorganisms (Wang et al., 2010; Cruz et al., 2018). Briefly, MTT (Sigma) solution was prepared to obtain the concentration of 5.0 g/L and stored -20 °C at until the experiment. MHB medium was loaded and then two-fold serial diluted of the essential oil was added to all wells. No essential oil was added to the negative control well alone. Then, 5 µl of the yeast prepared according to Mcfarland 0.5 was added to all wells. Subsequently, the plates were left to incubate for 24-48 hours and then 25 µl of MTT (1/10 diluted from stock solution) was added to all wells and the incubation was continued for 1 more hours at 37 °C. The metabolically active fungal cells disrupted the tetrazolium chain that gave MTT its yellow color and turned into purple-colored MTT-formazan crystals. By ensuring that these crystals were dissolved in 200 µl of DMSO added to each well, the resulting color density was measured in the spectrophotometer at 575 nm wavelength, and the % viability was determined by proportioning the optical densities of

the control and test wells. The CC50 reflects the concentration of the oil required to reduce the yeast cell viability by 50% and was calculated using the equation below (Eq 3 and Eq 4). All experiments were repeated three times in the study.

$$\text{Viability (\%)} = \left(\frac{OD_{test}}{OD_{control}}\right) \times 100 \quad \text{Eq. 3}$$

Cytotoxicity (%) = 100- Viability (%) Eq. 4

Agar Well Diffusion Method

The inhibition zones of the *C. cyminum* and *M. communis* mixture on *C. albicans*, *C. parapsilosis* and *C. tropicalis* were studied with AWD method, the analysis was replicated 3 times (Erdoğan Eliuz et al., 2017). The experiment was carried out under both light and dark incubation conditions. The 50 µL of the EO mixture add to the wells and incubated at 35°C for 24 hours (dark treatment). The same procedure was repeated for the light incubation experiments and a standard white LED source (470 Lumens / 40 watts) was used as the light (light treatment). After one day-incubation, caliper and sterile distilled water were measured as negative controls.

To determine the inhibition area of the EO against the yeast in different stress conditions, the test was performed in the presence of NaCl, Glucose, Glycerol. For this, 1 mL of 1%, 3% and 9% of NaCl, glucose, glycerol solutions and 100% distilled water were prepared in aseptic conditions. The pure essential oil (100 µL) of *C. cyminum* and *M. communis* mixture was added to each solution and mixed for 2 minutes at 100 rpm. The 50 µL of the samples were added to the wells in the MHA petri dishes which were prepared and where the *C. albicans*, *C. parapsilosis* and *C. tropicalis* were spread as in the first agar well diffusion experiment. All tests were performed three times and after 24-hours of incubation, the inhibition zones were measured. Solutions with no added oil were used as a negative control.

Antifungal Mechanism of the EO of *C. cyminum* and *M. communis* Mixture

The inhibition mechanism of the essential oil of the EO of *C. cyminum and M. communis* mixture on the yeast was also determined. The yeast exposed to the essential oils then TTC (Triphenyl tetrazolium chloride dye)-DRA (Dehydrogenase relative activity) of the yeasts were analyzed according to the Ding et al., (2016) and Ersoy et al., (2019) after some modifications. TTC-DR produces insoluble reddish colored TF (2,3,5-triphenyl formazan) which is reduced by the H^+ acceptor TTC dye in the living cells. Regarding this absorbance of the TF was evaluated at 485 nm to obtain dehydrogenase enzyme activity in the cells. A 0.2 mL of yeast (McFarland 0.5) was incubated with 0.2 mL of the mixture and glucose solution (0.1 mL) with TTC (0.1 mol/L glucose and 0.4% TTC solution) for 35°C during 2.5 hours. The mixture was centrifuged at 14,000×g for 3 min and the supernatant (50 µL) was transferred to the well (96-plate) and finally, 10 µL ethanol was added. The plates were kept for 5 min in a shaking incubator to develop red. The measurement of absorbance at 485 nm was taken every 10 minutes using the UV-Vis spectrophotometer. The experiments were repeated 3 times and DRA was formulated as follows:

$$\% = \left[\frac{ODx}{ODc}\right] * 100$$

where OD_x is the absorbance of treated and OD_c is the control samples.

Intracellular protein and DNA leakage in the yeast was studied according to Qi and Hung, (2019). The 0.2 mL of yeast culture (McFarland 0.5) was incubated with the 0.2 mL of the EO mixture for 37 ° C, for 2 hours. The sample was centrifuged at 14,000×g for 3 min and the supernatant (50 µL) was withdrawn, then stained with Bradford dye reagent (50 µL) for 15 minutes to determine leakage of protein. The absorbance of the culture was measured at 595 nm.

For DNA leakage, all procedures were the same as the protein leak test. No dye was used in the process and the absorbance was determined at 260 nm (Qi and Hung, 2019).

The increase of leakage of protein and DNA was formulated as follows:

$$\% = \left[\frac{ODx - ODc}{ODc}\right] * 100$$

where OD_x is the absorbance of treated and OD_c is the control samples

Statistical Analyses

The MICs, CC50, and IZ of the EO were measured by One-way ANOVA with posthoc Tukey HSD Test ($p<0.01$ and $p<0.05$). To compare IZs of dark treatment with light treatment was used T-Test for 2 Independent Means (<0.05).

RESULTS and DISCUSSION

The Components of the EO Mixed *C. cyminum* and *M. communis*

The compounds of the essential oil of mixed *C. cyminum* and *M. communis* were determined by GC-MS analysis and a total of 19 compounds were identified. The quality values of the compounds were higher than 83% (Table 1). The main compounds were found cuminal, which was determined at a peak ratio of 50.71% and with a peak quality of 98%. Cuminal was followed by 1,8 cineole (8.30%), O-cymene (7.88%), ß-pinene (7.62%), α-pinene (7.16%), γ-terpinene (6.09%) and α-terpinolene (2.19%). The

other compounds were camphene, 1-Terpinen-4-ol, nerol, ß-myrcene, p-cymen-7-ol, phellandral, α-thujene, isobutyl isobutyrate, terpinolene, α-terpinene, O-cymene, and 2,4-dimethyl-3-pentanone.

Table 1. Chemical composition of *C. cyminum* and *M. communis* mixture essential oil
Çizelge 1. C. cyminum ve *M. communis* karışımının uçucu yağ bileşenleri

No	[a]RT	[b]RRI	Compound	% [c]RA	MW	%Quality
1	3.133	616	2,4-Dimethyl-3-pentanone	0.03	$C7H_{14}O$	90
2	6.012	736	Isobutyl isobutyrate	0.12	$C_8H_{16}O_2$	90
3	6.469	937	α-Thujene	0.17	$C_{10}H_{16}$	87
4	6.754	943	α-Pinene	7.16	$C_{10}H_{16}$	96
5	8.469	979	ß-Pinene	7.62	$C_{10}H_{16}$	94
6	9.104	992	ß-Myrcene	0.28	$C_{10}H_{16}$	92
7	9.881	1008	P-Cymene	0.05	$C_{10}H_{16}$	93
8	10.226	1015	α-Terpinene	0.05	$C_{10}H_{16}$	97
9	10.736	1026	O-Cymene	7.88	$C_{10}H_{14}$	97
10	10.944	1030	1,8-Cineole	8.30	$C_{10}H_{18}O$	99
11	12.309	1059	γ-Terpinene	6.09	$C_{10}H_{16}$	97
12	13.680	1087	Terpinolene	0.08	$C_{10}H_{16}$	98
13	14.618	1107	α-terpinolene	2.19	$C_{10}H_{16}$	95
14	18.375	1185	1-Terpinen-4-ol	0.35	$C_{10}H_{18}O$	98
15	19.230	1202	Camphene	0.98	$C_{10}H_{16}$	91
16	22.007	1260	Cuminal	50.71	$C_{10}H_{12}O$	98
17	23.266	1286	Phellandral	0.21	$C_{10}H_{16}O$	91
18	27.486	1374	p-Cymen-7-ol	0.28	C10H14O	87
19	28.500	1395	Nerol	0.33	$C_{10}H_{18}O$	83
			Total-identified compounds	92.88		
			Non-identified compounds	7.12		

[a]Retention Time relative, [b]Calculated Kováts index, [c]Relative area (peak area relative to the total peak area).
MW: Molecular Weight (g mol^{-1})

In this study, cuminal was the most detected compound in the mixed oil, and it has been shown in previous studies that it is the basic component of cumin (Li and Jiang, 2004; Sahana et al., 2011; Chaudhary et al., 2014). The 1,8 cineole, which was detected at a high rate after cumin, is one of the most important components detected in both myrtus and cumin essential oil (Chalchat et al., 1998; Özek et al., 2000; Yadegarinia et al., 2006; Gachkar et al., 2007; Mahboubi and Bidgoli, 2010; Bahadirli et al., 2021). The other molecules such as O-cymene (Chalchat et al., 1998; Gachkar et al., 2007), ß-pinene (Li and Jiang, 2004; Chaudhary et al., 2014; Curini et al., 2003), α-pinene (Chalchat et al., 1998; Allaq et al., 2020; Yadegarinia et al., 2006), γ-terpinene (Li and Jiang, 2004; Gardeli et al., 2008; Sahana et al., 2011), and α-terpinolene (Chalchat et al., 1998; Curini et al., 2003, Li and Jiang, 2004) were previously detected by several studies in both *C. cyminum* and *M. communis* EO. In literature, the compound of 2,4-Dimethyl-3-pentanone was found only in *M. communis* (Mahboubi et al., 2010), but no record was found with *C. cyminum*.

The EOs of *C. cyminum* (Allaq et al., 2020) and *M. communis* (Aleksic et al., 2014; Cannas et al., 2013) have antifungal properties, about which a great amount of data has already been published. A broad-spectrum antifungal performance of the EO obtained from both plants is due to the high percentage of cumin molecule that cumin (Naeini et al., 2014; Petretto et al., 2018) contains and α-pinene and 1,8-cineole that myrtle has (Jamoussi et al., 2005; Bouzabata et al., 2013; Bahadirli et al., 2021). There is a great lack of literature on the combination of essential oils. This study is original in this respect. The only study, that combined cumin with another herb was Minooeianhaghighi's study. Minooeianhaghighi et al. (2017), found that *C. cyminum* with *Lavandula binaludensis* mixed oils were inhibited many *C. albicans* isolates than cumin alone. In that study, only essential oils were mixed. However, in this study, it was left to hydrodistillation with cumin and myrtle, together.

The evaluation of antifungal activity and cytotoxicity of the EO on Candida species

The mean MICs, CC50 and IZs were calculated and, the results showed that the essential oil of *C. cyminum* and *M. communis* mixture were effective against *C. albicans*, *C. parapsilosis* and *C. tropicalis* by spectrophotometric broth microdilution and agar well diffusion method (Table 2). The MICs of the EO against the yeasts were between 3.29 and 3.65 mg mL^{-1} and they were 3.29 for *C. albicans*, 3.57 mg mL^{-1} for *C. parapsilosis* and 3.65 mg mL^{-1} C. tropicalis. ($p<0.05$).

Table 2. MICs and IZs of the EO of *C. cyminum* and *M. communis* mixture against *C. albicans, C. parapsilosis* and *C. tropicalis.*

Çizelge 2. C. cyminum ve M. communis karşımının uçucu yağının C. albicans, C. parapsilosis ve C. tropicalis'e karşı MIC ve IZ değerleri

Species	EO-MIC (mg mL^{-1})	FLC-MIC (µg mL^{-1})	EO-CC$_{50}$ (mg mL^{-1})	IZ (mm) in dark environment	EO-IZ (mm) in LED environment
C. albicans	3.29^{a} ±2.07	17.9^{a} ± 1.8	2.61^{a} ±1.4	19.3^{abcdT}±1.5	23^{efgT}±0.8
C. parapsilosis	3.57^{b} ±1.43	102.5^{ac} ±3.8	0.58^{b} ±0.5	42.1^{aeT}±0.8	33.01^{cT} ±6.6
C. tropicalis	3.65^{c} ±3.94	25.7^{bc} ± 2.8	0.17^{c} ±0.16	41.01^{bft} ±2.1	39^{dgt}±3.6

(ANOVA, p<0.05, Tukey HSD). Values on the same column with same superscript letters differ statistically (a,b,c,de,f,g). IZs in the same line (IZs in dark environment and IZs in LED environment) with "T" superscript letters: statistically significant, with "t" superscript letters: no statistically significant (T-Test for 2 Independent Means p<0.05). FLC: antibiotic

The cytotoxicity of different concentrations of the essential oils (0.38-3 mg mL^{1}) was studied on *C. albicans*, *C parapsilosis* and *C. tropicalis* (Table 2). The EO significantly decreased the viability of the yeasts and IC50 of the essential oil was 2.61 mg mL^{-1} for *C. albicans*, 0.58 mg mL^{-1} for *C. parapsilosis* and 0.17 mg mL^{-1} for *C. tropicalis* (p<0.05). When the MTT dye was reduced by the viable yeast cells, the yeast cells and was in the form of clusters of beads-like cells and completely covered with dye (Figure 1).

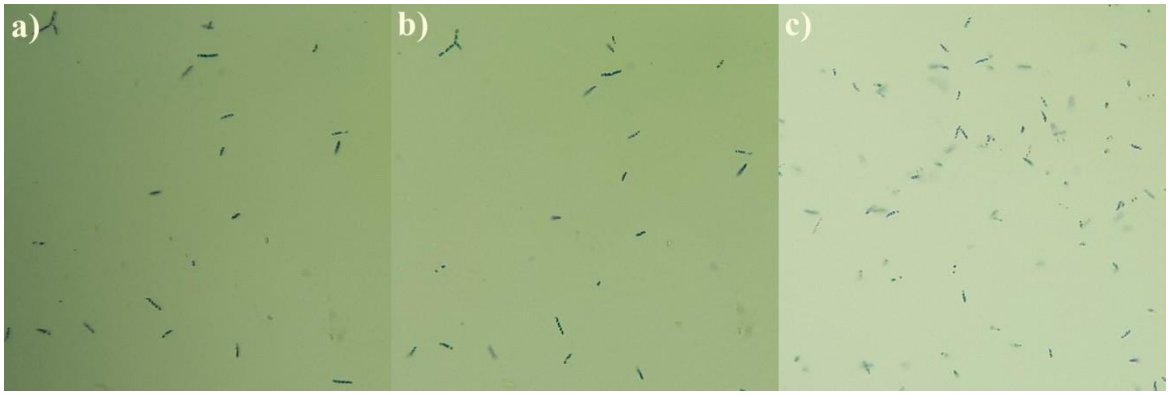

Figure 1. The micrograph in the light microscope (40X) of yeast cell-formazan crystal complexes formed in the MTT reduction solution

Şekil 1. MTT indirgeme solüsyonunda oluşan maya hücresi-formazan kristal komplekslerinin ışık mikroskobundaki (40X) mikrografı

In the present study, strong antifungal activity of the EO of cumin and myrtle part mixture was determined in standard condition (in dark incubation) against *C. albicans*, *C. parapsilosis*, and *C. tropicalis* (Figure 2). The essential oil exhibited different levels of antifungal activities depending on the dark and light treatments applied. The inhibition zones of the EO were 19.3 mm against *C. albicans*, 42.1 mm against *C. parapsilosis* and 41.01 mm against *C. tropicalis* in dark treatment, while IZs were 23 mm on *C. albicans*, 33.01 mm on *C. parapsilosis* and 39 mm on *C. tropicalis* in light treatment (p<0.05).

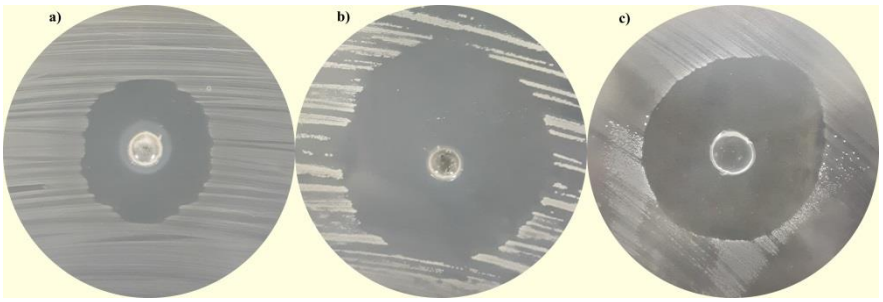

Figure 2. The images of inhibition zone of the EO of the plant mixture on C. albicans (a), C. parapsilosis (b) and C. tropicalis (c)

Şekil 2. Uçucu yağ karışımının *C. albicans* (a), *C. parapsilosis* (b) ve *C. tropicalis*'e karşı inhibisyon bölgeleri

There were any MTT study found on the effects of cumin and myrtle against Candida species, however, the CC50 values of the oil obtained from both plants can be expressed in the following order: *C. tropicalis*

(0.17 mg mL^{-1}) < *C. parapsilosis* (0.58 mg mL^{-1}) < *C. albicans* (2.61 mg mL^{-1}). When compared with the data in the literature, it is stated that cumin and myrtle alone were active against Candida-type fungi. The MICs of cumin alone were 4.375 mg mL^{-1} for many *C. albicans* strains and 2.188 mg mL^{-1} for *C. parapsilosis* because of cuminaldehyde, γ-terpinene, and ß-pinene components of cumin (Vieira et al., 2019). Considering the MIC value (3.29 mg mL^{-1}) of the mixed plants against *C. albicans* in this study, it can be said that the MIC in *C. albicans* decreased due to a synergistic effect between plants. For *Candida parapsillosis*, (3.57 mg mL^{-1}), an antagonistic effect can be mentioned because the MIC value increased with mixing the cumin and myrtle. In another article, the MICs of *M. communis* EO, the main components of which were 1,8-cineole, a-pinene, and limonene, were 2.5 mg mL^{-1} for Candida albicans ATCC 10231, *C. krusei*, C. tropicalis ATCC 13803, and *C. parapsilosis* ATCC 90018 (Bouzabata et al., 2015). In this study, the MICs of the mixture were between 3.65 and 3.29 mg mL^{-1} for Candida species. In this case, it was determined that when the cumin was mixed into the myrtle plant, the MICs increased. Similarly, the MICs of *C. cyminum* oil was 289 mg L-1 against *C. albicans* and *C. dubliniensis* (Naeini et al., 2014). Compared to Naeini's study, the enrichment of cumin with myrtle in this study may have enhanced its antimicrobial effect against *C. albicans*. In this case, an antagonistic effect can be mentioned among the oils.

The compounds of essential oil are highly affected by environmental alterations. In this study, changes in IZs were controlled by adding the EO into different solutions. As a result, it was observed that the effects of the essential oil on yeast continued in different concentrations of the solutions, but differences occurred between inhibition zones. This may be due to the interactions of the essential oil with glycerol, glucose, and NaCl. or, IZs for each fungal strain may have changed depending on their resistance status.

Antifungal activity in the stress conditions

The inhibitory effect of the EO of *C. cyminum* and *M. communis* mixture, at different NaCl, glucose, glycerol concentrations and distilled water added the EO on the yeasts is shown in Table 3. No inhibition was observed against *C. albicans*, *C. parapsilosis* and *C. tropicalis* in the presence of pure water, 1%, 3%, 9% NaCl, glucose, glycerol without EO as negative control. The maximal IZs of the EO tested in different stress conditions can be presented as: 3% glycerol (26.6 mm)> 3% glucose (24 mm)> 3% NaCl (20 mm)> Water (19 mm) for *C. albicans*; 9% glucose (20 mm)> 3% NaCl (19.6 mm)> 9% NaCl (18 mm)> 9% glycerol (17.6 mm)> water (14.3 mm) for *C. parapsilosis*; 3% glycerol (26 mm)> 1% NaCl (21.3 mm)> water (21.1 mm)> 1% glucose (17 mm) for *C. tropicalis*. The lowest IZs were 13.3 mm and 10 mm for *C. albicans* and *C. parapsilosis*, respectively, at 1% NaCl concentration, while the lowest IZ was 7.6 mm at 9% glucose for *C. tropicalis*.

Table 3. Anticandidal effect of the essential oil (10%) in the presence of NaCl, glucose, glycerol and water environments

Çizelge 3. Uçucu yağların (%10) NaCl, glikoz, gliserol ve su ortamlarında antikandidal aktiviteleri

		C. albicans			*C. parapsilosis*			*C. tropicalis*		
	C %	With EO		NG	With EO		NG	With EO		NG
NaCl	1	13.3^{abc}	±1.07	*	10^{ab}	±0.9	*	21.3^{a}	±0.08	*
	3	20^{f}	±2.0	*	19.6^{a}	±1.07	*	19.3^{b}	±0.09	*
	9	16^{d}	±2.8	*	18^{c}	±2.3	*	11.6^{e}	±1.09	*
Glucose	1	22.6^{a}	±3.0	*	16^{d}	±2.2	*	17^{f}	±1.27	*
	3	24^{b}	±3.0	*	16^{e}	±2.1	*	16.6^{g}	±2.31	*
	9	20^{g}	±1.08	*	20^{f}	±0.09	*	7.6^{abc}	±0.07	*
Glycerol	1	19.6^{h}	±1.08	*	14^{g}	±2.02	*	17.6^{h}	±1.3	*
	3	26.6^{cde}	±1.9	*	13.3^{h}	±1.11	*	26^{cd}	±1.07	*
	9	16^{e}	±1.011	*	17.6^{i}	±1.08	*	12.3^{d}	±2.04	*
Water	100	19^{i}	±2.13	*	14.3^{b}	±2.03	*	21.1^{i}	±2.07	*

C: The concentration of NaCl, Glucose, Glycerol, and water. NG: negative control, *: no inhibition zone. The mean IZs were expressed with the standard deviation (±) and significance level (ANOVA, $p<0.05$, Tukey HSD). Values on the same column with same superscript letters differ statistically at the level of 0.05.

Antifungal mechanism of the EO of *C. cyminum* and *M. communis* mixture

When a microorganism is exposed to an antimicrobial agent, the cell structure damaged and therefore many intracelluler macromolecules are left out. In this study, it was investigated the effect of the EO of *C. cyminum* and *M. communis* mixture on yeast cells (Table 4). All the treatments showed the DRA dropped from 100% to near 75% within 15 min. In yeast treated with the EO, DRA dropped to 75.01%, 81.9%, 80.1% in supernatant of *C. albicans*, *C. parapsilosis* and *C. tropicalis*, respectively, within 20 min.

Table 4. The dehydrogenase relative activity (DRA%) and the percentage increase (%) of intracellular DNA and protein leakage of *C. albicans*, *C. parapsilosis* and *C. tropicalis* after the EO of *C. cyminum and M. communis* mixture treatment

Çizelge 4. *C. cyminum* ve *M. communis* karışım uçucu yağına maruz bırakılan *C. albicans*, *C. parapsilosis* ve *C. tropicalis*'in dehidrogenaz bağıl aktivite (DRA%) ve Hücre içi DNA ve protein kaçışının ortalama artış (%) değerleri

	Time	DRA (%)	Protein leakage (%)	DNA leakage (%)
C. albicans	5. min.	90.18	6.76	1.006
	10. min	80.12	7.78	2.03
	15.min.	75.01	8.01	3.01
C. parapsilosis	5. min.	93.7	0.08	3.78
	10. min.	82.6	1.25	3.04
	15.min.	81.9	5.25	3.04
C. tropicalis	5. min.	90.1	2.46	3.83
	10. min.	80.2	3.25	4.1
	15.min.	80.1	4.01	5.2

All experiments were done in triplicate (P≤0.05). For EO activation, Standard deviation of all 3 replicate experiments was between 0.1 and 0.001. Min: minute.

After treatment by the EO at MIC, the fastest increase in protein and DNA leakage in supernatant of yeast was found in the first 20 minutes compared to the control. Percent increase in the water-soluble proteins of yeast exposed to the EO was 6.76%-8.01% for *C. albicans*, 0.08%-5.25% for *C. parapsilosis*, and 2.46%-4.01% for *C. tropicalis* in 15 min. Percent increase in DNA leakage of the yeast exposed to the EO was 1.006%-3.01% for *C. albicans*, 3.78%-3.04% for *C. parapsilosis*, and 3.83%-5.2% for *C. tropicalis* in 15 min. These results implied the cell structures of the yeast were destroyed after treatment by the EO, causing protein and DNA leakage.

The mechanism of action of most antibiotics used recently destroys the microorganism by targeting specific regions such as the cell wall of microorganisms, nucleic acids, and proteins. However, after a while, yeast gain resistance against these new antibiotics by changing the structures targeted by the antibiotic (Yao and Moellering, 2011). Therefore, it is necessary to understand the antimicrobial mechanism correctly and to reveal the effects of new antimicrobial compounds. In this study, when the yeasts were exposed to EO, there was cell destruction due to the increase in protein and DNA in the environment.

CONCLUSION

Essential oils are widely used in various fields such as the cosmetics, pharmaceutical, and food industries, and antimicrobial activity of them are their antimicrobial effect is the most powerful biological activity known. The stability of these oils alone or in mixtures is a powerful alternative in the fight against resistant strains. Therefore, we have to develop various methods against antibiotic resistance, which will be the most important problem of the future. Antimicrobial agent combinations are one of these methods. Essential oils are often not found alone in foods and medicines. With this study, it was shown that there may be changes in the antimicrobial activity of the oil, depending on the amount of salt, water, and light. When aromatic oils are hydro distilled alone or with another plant, it has been shown that there may be changes in their active ingredients. This situation can be turned into an advantage for biological activity studies. Cumin and myrtle are among the most significant herbs of traditional medicine and are the two recommended plants especially for diabetes. In diabetic studies, new drug formations can be created using these plants. These two herbs can be used in skin surface infections. Finally, environmental factors such as heat, light, and water should be included and investigated in more detail in future studies.

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Author's Contributions

The contribution of the authors is equal.

Statement of Conflict of Interest

The authors have declared no conflict of interest.

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Antibacterial Effects of *Phlomoides molucelloides* (Bunge) Salmaki

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ABSTRACT

The insufficiency of existing antibiotics in the combat against antibiotics-resistant bacteria has necessitated the discovery of new and effective antibacterial drugs. The discovery that bacteria synthesize various virulence factors by the quorum sensing system has suggested that quorum sensing inhibitors may be used in the fight against infectious diseases. This study aimed to determine the antibacterial and anti-quorum sensing activities of methanol and water extracts of aerial and root parts of *Phlomoides molucelloides* (Bunge) Salmaki. The minimal inhibitory concentration (MIC) values of the extracts were investigated against reference bacterial strains using the broth microdilution method. Anti-quorum sensing activities were examined by violacein and pyocyanin pigments inhibition and swarming motility inhibition assays against *Chromobacterium violaceum* ATCC 12472 and *Pseudomonas aeruginosa* PAO1 bioreporter strains. The antibiofilm activities of the extracts were tested against *P. aeruginosa* PAO1 using the crystal violet staining method. The MIC value (> 2000 µg/mL) of all extracts against the tested bacteria could not be determined at the concentrations studied. All extracts partially inhibited the swarming motility of *P. aeruginosa*. Methanol extract of the aerial part inhibited pyocyanin production by 81.7% without interfering with *P. aeruginosa* growth. The extracts had no significant inhibitory activity on biofilm formation and violacein pigment production. These results showed that the extracts of *P. molucelloides* may be good anti-quorum sensing agents. Further research can be done to elucidate the mechanisms underlying these biological activities.

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Phlomoides molucelloides (Bunge) Salmaki'nin Antibakteriyel Etkileri

ÖZET

Antibiyotiklere dirençli bakterilerle mücadelede mevcut antibiyotiklerin yetersizliği, yeni ve etkili antibakteriyel ilaçların keşfini zorunlu kılmıştır. Bakterilerin quorum sensing mekanizması ile çeşitli virülans faktörlerini sentezlediğinin keşfedilmesi, quorum sensing inhibitörlerinin enfeksiyon hastalıkları ile mücadelede kullanılabileceğini düşündürmüştür. Bu çalışmada, *Phlomoides molucelloides* (Bunge) Salmaki türünün toprak üstü ve kök kısımlarının metanol ve su özütlerinin antibakteriyel ve anti-quorum sensing aktivitelerinin araştırılması amaçlanmıştır. Özütlerin referans bakteri suşlarına karşı minimal inhibitör konsantrasyon (MİK) değerleri sıvı mikrodilüsyon yöntemi ile araştırılmıştır. Anti-quorum sensing aktivite, *Chromobacterium violaceum* ATCC 12472 ve *Pseudomonas aeruginosa* PAO1 biyoraportör suşlarda viyolasin ve piyosiyanin pigment üretiminin ve

Mikrobiyoloji

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swarming (yayılma) hareketinin inhibisyonu üzerine belirlenmiştir. Antibiyofilm aktivite testi *P. aeruginosa* PAO1 suşuna karşı kristal viyole yöntemi ile değerlendirilmiştir. Tüm özütlerin test edilen bakterilere karşı çalışılan konsantrasyonlarda MİK değeri (> 2000 µg/mL) tespit edilmemiştir. Buna karşılık tüm özütlerin *P. aeruginosa*'nın swarming hareketini baskıladığı saptanmıştır. Ayrıca, bitkinin toprak üstü kısımlarının metanol özütlerinin bakteri üremesini baskılamadan piyosiyanin üretimini kontrole göre %81.7 oranında inhibe ettiği belirlenmiştir. Tüm özütlerin biyofilm oluşumu ve viyolasin pigment üretimi üzerine önemli bir inhibitör etkinliği saptanmamıştır. Sonuçlar *P. molucelloides* özütlerinin iyi bir anti-quorum sensing ajanı olabileceğini göstermiştir. Bu biyolojik aktivitelerin altında yatan mekanizmaları aydınlatmak amacıyla ileri çalışmaların yapılması gerekmektedir.

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INTRODUCTION

Antibiotic resistance, which has become widespread today, has necessitated the development of new antibacterials or alternative treatment methods in the combat against infectious diseases (Uddin et al., 2021). The fact that bacteria modulate the synthesis of virulence factors using the Quorum Sensing (QS) system suggested that QS inhibition could be an alternative antibacterial treatment method. QS is the regulation of some phenotypic and biochemical activities of bacteria reaching a certain population density with signal molecules called autoinducers (Jiang et al., 2019).

Some autoinducers as QS signal molecules have been identified in bacteria. These are autoinducing peptides (AIP) used by Gram-positive bacteria, N-acyl homoserine lactone (AHL) used by Gram-negative bacteria, and autoinducer-2 (AI-2) used by both Gram-positive and Gram-negative bacteria (Prazdnova et al., 2022). These signaling molecules regulate the expression of virulence-related genes such as biofilm formation, swarming motility, pigment, enzyme and toxin production in many bacteria strains (Eberl et al., 1996; McClean et al., 1997; Ohtani et al., 2002; Marketon et al., 2003; Rice et al. 2005).

QS inhibition in bacteria can occur such as i) by inhibiting autoinducer synthesis, ii) by cleaving autoinducers, iii) by preventing autoinducers from binding to the specific receptor through competition for the binding site, iv) by blocking the binding of the signal-receptor complex to the gene promoter and inhibiting gene expression (Prazdnova et al., 2022). There are many compounds known to have QS inhibitory activity in the literature. However, most of them are not suitable for human use (Nain et al., 2020). Therefore, it is significant to discover natural and reliable compounds.

Plants have the potential to develop new and effective antibacterial with their secondary metabolites (Gorlenko et al., 2020). *Phlomoides molucelloides* (Bunge) Salmaki (also known as *Eremostachys molucelloides* Bunge) is a plant species belonging to the Lamiaceae family. It is a perennial herbaceous plant. Limestone hills, schist and volcanic slopes are its natural habitats (Babaç, 2004). It is stated that *Eremostachys* species have ethnopharmacological activities and have essentials oils and secondary metabolites such as flavonoids, monoterpenes, irioid, and chrysoeriol glycosides (Mohammadhosseini et al., 2019). Previous studies have demonstrated the potential of *Eremostachys* genus to have various biological effects. For example, the iridoid glycosides of *Eremostachys laciniata* (L) Bunge have been reported to have antibacterial effects (Modaressi et al., 2009). It was shown that essential oils obtained from the root, stem, and flower parts of *Eremostachys laevigata* Bunge had high antibacterial effects in another study (Esmaeilli et al., 2012). It was emphasized that *Eremostachys labiosa* Bunge has a high potential in terms of anti-leishmanial, anticancer, and anti-inflammatory activity in a study (Taghizadeh Rabe et al., 2014). Dichloromethane, n-hexane and methanol extracts of rhizomes of *Eremostachys azerbaijanica* Rech.f. have been reported to have antibacterial and antioxidant activity, respectively. Moreover, it has been reported that all extracts exhibit cytotoxic activity against the A549 cell line. The presence of fatty acids and steroids as the main compounds in the extracts has been held responsible for those bioactivities (Asnaashari et al., 2017).

To the best of our knowledge, there is no previous

report about the antibacterial effects of *P. molucelloides* in the literature. Therefore, this study aimed to investigate the antibacterial and anti-QS activities of methanol and water extracts of aerial and root parts of *P. molucelloides*.

MATERIALS and METHODS

Plant material and preparation of extracts

P. molucelloides were collected from Konya province (Akbaş Village, Bozdağ Natural park around, 1020 m, 38° 02' 51" N, 32° 56' 20" E), Türkiye in June 2019. The plant picture from natural location is given in Figure 1. The plants were confirmed by a botanist at Selcuk University (Konya, Türkiye) and one voucher specimen has been deposited in Selcuk University (EY-3004). The aerial and root parts of the plant were kept in a shaded environment and room temperature for 10 days and dried until weighing constant. The samples were then pulverized (particle size about 2 mm) by using a laboratory mill (Retsch SM-200, Germany). The pulverized samples were stored in a dark environment and at room temperature. In the present work, it was used methanol and water as solvents. Maceration was used as an extraction method. In addition, infusion was prepared with water. In the maceration, the plant materials (10 g) were macerated with 200 mL methanol at room temperature for 24 h. Then, after the extracts were filtered, the methanol was removed by using a rotary evaporator. Regarding infusion, the plant materials (10 g) were kept with 200 mL boiled water for 15 min and then filtered (2 µm, Whatman 589/3). Water extracts were lyophilized and all extracts were held at 4°C until study.

Figure 1. *Phlomoides molucelloides*
Şekil 1. Phlomoides molucelloides

Bacterial strains and growth conditions

Antibacterial activities of the extracts were investigated against three Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Bacillus subtilis* ATCC 6633) and nine Gram-negative bacteria (*Escherichia coli* ATCC 25922, *E. coli* NCTC 13846, *Klebsiella pneumoniae* ATCC 13883, *K. pneumonia* ATCC 700603, *K. pneumonia* NCTC 13440, *Salmonella* Typhimurium ATCC 14028, *Pseudomonas aeruginosa* ATCC 27853, *Enterobacter aerogenes* ATCC 13048, *Acinetobacter haemolyticus* ATCC 19002).

P. aeruginosa PAO1 and *C. violaceum* ATCC 12472 strains were used as bioreporters for the anti-QS activities of the extracts. These strains regulate the synthesis of various phenotypic behaviors using the QS mechanism, so they are widely used to investigate QS inhibition. For example, *C. violaceum* ATCC 12472 produces violacein pigment using *cvi* system via long-chain (C10-C16) AHL molecules (Morohoshi et al., 2008). *P. aeruginosa* PAO1 regulates the synthesis of pyocyanin pigment, swarming motility and biofilm formation using *rhI* system via short-chain C4-AHL molecules and *las*, *rhI*, *pqs*, and integrated QS systems via various signal molecules, respectively (Tapia-Rodriguez et al., 2019; Vetriel et al., 2021).

All bacteria were National Collection of Type Cultures (NCTC) and American Type Culture Collection (ATCC) reference strains. They were

obtained from the culture collection of the Department of Medical Microbiology, Faculty of Medicine, Karadeniz Technical University.

C. violaceum was grown in Luria-Bertani (LB) medium at 30ºC, and the others were grown in Mueller-Hinton medium at 37ºC aerobically.

Determination of minimal inhibitory concentration

The minimal inhibitory concentration (MIC) values of the extracts were determined by the broth microdilution method as previously described (Wiegand et al. 2008). Briefly, the test was carried out in 96-well plates every well containing 100 µL of Mueller Hinton broth (MHB; Merck, Darmstadt, Germany). The tested concentration range of the extracts was from 2000 to 62.5 µg mL^{-1}. Levofloxacin (Chemical Industry, Tokyo, Japan) was used as a standard antibiotic in the concentration range of 0.015-128 µg mL^{-1}. The inoculums of bacteria prepared from the fresh cultures were set to 0.5 McFarland (~1.0×10^8 CFU mL^{-1}) turbidity standards using a turbidimeter (DEN-1B, Biosan, Latvia) and then diluted 10-fold. Five µL of prepared inoculums were put into the wells. One well without the extracts was used as a growth control and one well without the bacteria was used as a sterility control. The plate was covered with a lid and incubated at 37ºC for 24 h. The minimal extract concentration without visible bacterial growth was determined as the MIC value.

Anti-quorum sensing inhibition assays

Violacein inhibition

The abilities of the extracts to inhibit violacein pigment production was investigated by the soft agar method on *C. violaceum* ATCC 12472 (McClean et al. 1997). Briefly, 50 µL of an overnight culture of *C. violaceum* was transferred into a 5 mL volume of molten soft LB agar (0.5% w v^{-1}) (NZYTech, Lisbon, Portugal). The agar-culture mixture was vortexed and directly spilled over the surface of prewarmed LB agar plates. After solidifying the agar, a set of blank discs in 6 mm diameter were on the seeded agar media and then impregnated with 20 µL from each extract (20 mg mL^{-1}) to obtain 400 µg extract per disc. The supernatant (20 µL) of an overnight culture of *P. aeruginosa* PAO1 strain was used as a positive control. The cultures were incubated at 30°C for 18 h. Violacein inhibition was determined by a colorless, opaque, but viable halo around the discs.

Pyocyanin inhibition

The MIC value of the extracts against *P. aeruginosa* PAO1 has been determined using the broth microdilution assay. For avoiding any antibacterial effect, the sub-MIC values (500 µg mL^{-1}) of the extract have been used for this assay. The pyocyanin production inhibitory activities of the extracts were performed using the pyocyanin extraction method described by Tüfekci et al. (2020). Briefly, the supernatants from 16 h cultures of *P. aeruginosa* PAO1 grown in broth with and without the extracts were obtained. The pigment in the supernatant was extracted by chloroform, then by 0.2 N HCl. The absorbance was determined at 520 nm against 0.2 N HCl using a spectrophotometer (Multiskan Go, Thermo Fisher Scientific). Whether bacterial growth was interfered or not was determined by viable cell count. For this purpose, serial dilutions were prepared from the cultures and spread on MHA medium. After overnight incubation, colonies were counted on the media and compared with the control.

Swarming inhibition

Swarming inhibition was done based on a previously described assay (Saliha et al., 2020). Briefly, a small piece of the colony was taken from a fresh culture of *P. aeruginosa* PAO1 on MHA with a sterile toothpick and inoculated onto swarm agar plates. Swarm agar contained 1% glucose, 0.5% peptone, 0.5% bacteriological agar, 0.2% yeast extract and the test material (500 µg mL^{-1} of the extract). Sterile distilled water was added to the control plate instead of the extract. The plates were incubated in the upright position at 37°C for 24 hr. At the end of the incubation, the swarming motility was determined from the point of inoculation to the environment.

Biofilm inhibition

This experiment was done in 96-well flat-bottom polystyrene plates as described previously (O'Toole, 2011). In brief, a 16 h broth culture of *P. aeruginosa* PAO1 was diluted 100-fold in fresh LB broth and 180 µL was inoculated into each well. Then, 20 µL of the extract at a concentration of 5 mg mL^{-1} was added to provide a final concentration in the wells was 500 µg mL^{-1}. For control wells, 20 µL sterile distilled water was put to the wells as the solvent of the extracts. Typically, three replicate wells were used for each treatment. The plate was incubated at 37ºC without agitation for 24 h after the plate was covered with a lid. After incubation, it was determined whether the bacterial growth in the wells was suppressed by measuring the plate at 600 nm using the spectrophotometer. Then, the media from the wells was discarded and biofilm was washed three times by gently dipping into a container including sterile distilled water. The biofilm layer was stained with 200 µL 0.1% (w v^{-1}) crystal violet (Merck) solution for 5 min, and then the unbound stain was discarded. The washing process was repeated as described above. The crystal violet retained by the biofilm layer was dissolved with 100 µL absolute ethanol. The absorbance was determined at 570 nm against absolute ethanol using the spectrophotometer.

Statistical analysis

The tests were repeated three times and the data were given as mean ± standard deviation (SD) values. Wilks-Shapiro test (Shapiro and Wilk, 1965) has been used for normality of the data analysis. Mann-Whitney *U* test and independent samples t-test were done using IBM-SPSS statistics version 23.0 (IBM Inc., Armonk, NY, USA) for the analysis of nonparametric data and parametric data, respectively. A consider of $P < 0.05$ was statistically significant.

RESULTS and DISCUSSION

Anti-quorum sensing activity results

Pyocyanin is a secondary metabolite produced by *P. aeruginosa* strains and exhibits cytotoxicity by inducing oxidative stress in host cells (Gonçalves and Vasconcelos, 2021). Methanol extract of the aerial part inhibited pyocyanin production by 81.7 ± 0.2% ($P < 0.05$ versus control) without interfering with bacterial growth. However, other extracts could not inhibit pyocyanin production at the concentration tested (Figure 2). This means that there are compounds that inhibit the production of pyocyanin pigment in the aerial part of *P. molucelloides*. Since no inhibition was detected in water extracts, the compounds in question were probably extracted with methanol.

Swarming motility is a virulence factor that enables *P. aeruginosa* strains to attach to biotic or abiotic surfaces and form a biofilm. Also, bacteria adapt to the environment they are in with their swarming motility (Khan et al., 2020). As with pyocyanin, *P. aeruginosa* PAO1 swarming motility is regulated by the system of *rhI* using C4-AHL signal molecules (Tapia-Rodriguez et al., 2019). In the presented study, it was observed that all extracts suppressed the swarming motility of the *P. aeruginosa* PAO1 strain (Figure 3). Considering that pyocyanin pigment production is also regulated by the *rhI* system, it can be said that *P. molucelloides* interferes with the *rhI* system.

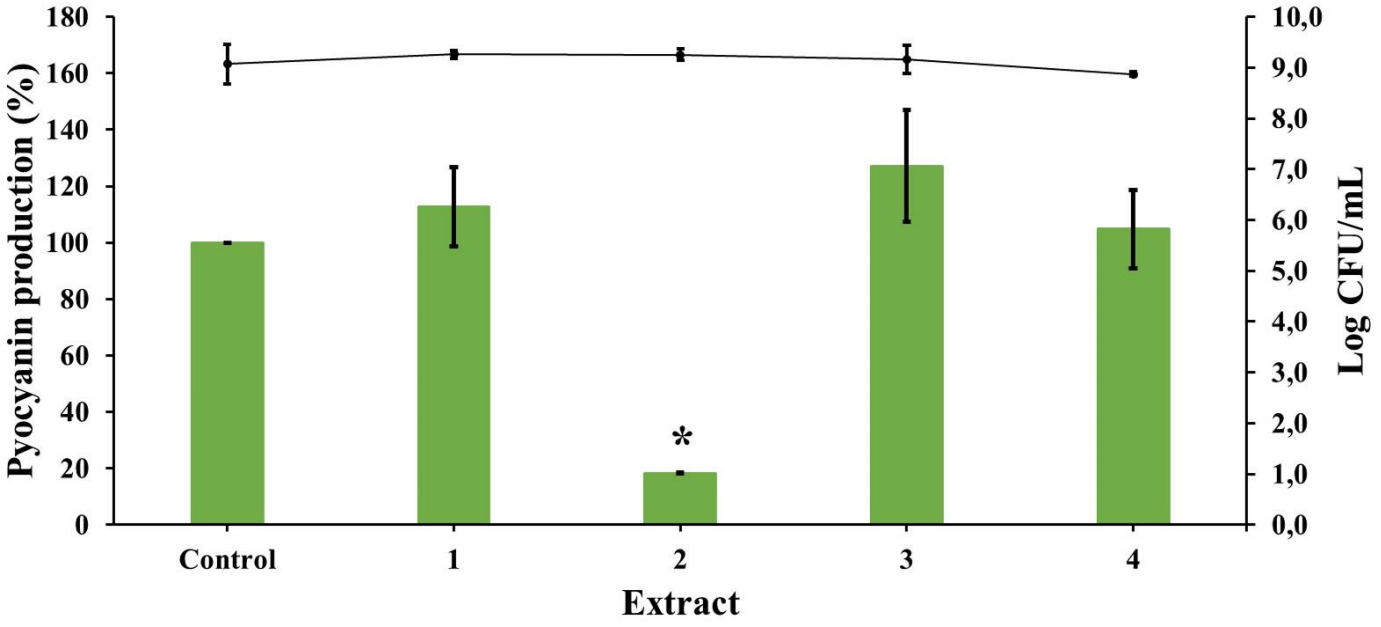

Figure 2. The result of the pyocyanin inhibition assay (1; water extract of aerial part, 2; methanol extract of aerial part, 3; water extract of root part, 4; methanol extracts of root part). Data are presented as the mean ± SD. *P < 0.05.

*Şekil 2. Piyosiyanin inhibisyon deneyinin sonucu (C; kontrol, 1; toprak üstü kısmın su özütü, 2; toprak üstü kısmın metanol özütü, 3; kök kısmın su özütü, 4; kök kısmın metanol özütü). Veriler ortalama ± standart sapma olarak sunulmuştur. *P < 0.05.*

Biofilms are cell communities formed by bacteria on biotic and abiotic surfaces. The biofilm layer protects bacterial cells against various environmental stresses such as disinfectants, antibiotics, and the host's immune system (Maurice et al., 2018). However, the extracts did not have any inhibitory activity on biofilm formation at the concentrations studied (Figure 4). This may be due to the inability of the extracts to inhibit the QS pathways for biofilm synthesis in *P. aeruginosa*. Moreover, none of the extracts could inhibit violacein pigment production in this study (Figure 5). This result showed that the extracts could not have any inhibitory effect on the *cvi* system.

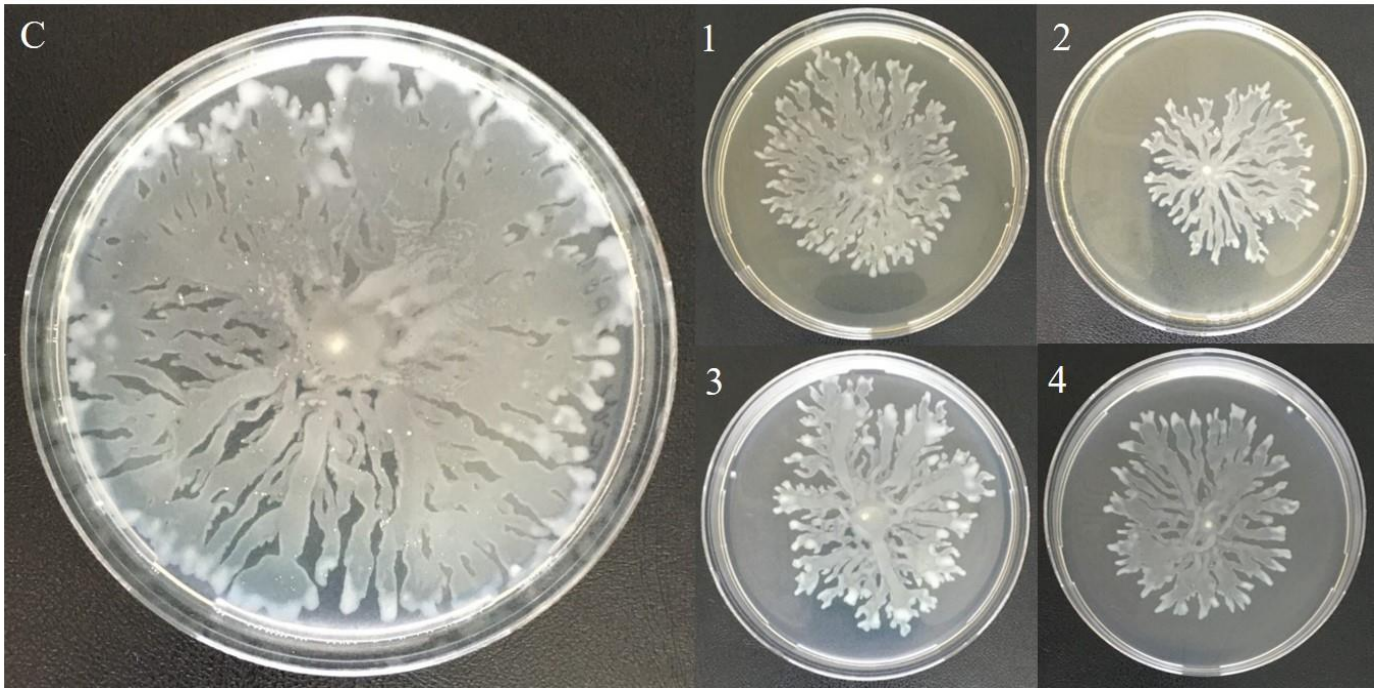

Figure 3. Swarming inhibition assay (C; control, 1; water extract of aerial part, 2; methanol extract of aerial part, 3; water extract of root part, 4; methanol extract of root part).

Şekil 3. Swarming inhibisyon deneyi (C; kontrol, 1; toprak üstü kısmın su özütü, 2; toprak üstü kısmın metanol özütü, 3; kök kısmın su özütü, 4; kök kısmın metanol özütü).

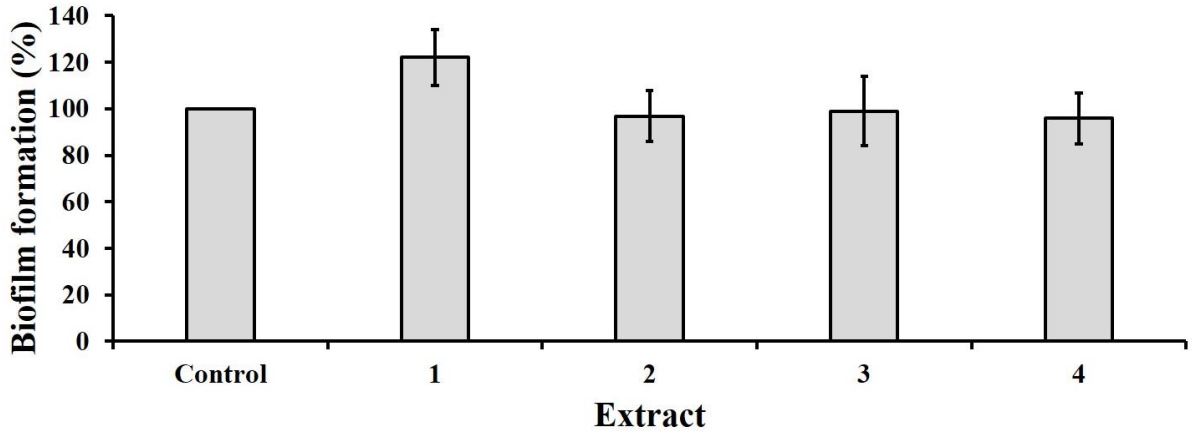

Figure 4. The result of antibiofilm activity (1; water extract of aerial part, 2; methanol extract of aerial part, 3; water extract of root part, 4; methanol extract of root part). Data are presented as the mean ± SD. $P > 0.05$.

Şekil 4. Biyofilm inhibisyon deneyinin sonucu (C; kontrol, 1; toprak üstü kısmın su özütü, 2; toprak üstü kısmın metanol özütü, 3; kök kısmın su özütü, 4; kök kısmın metanol özütü). Veriler ortalama ± standart sapma olarak sunulmuştur. $P > 0.05$.

Antibacterial activity results

The antibacterial activity of the extracts of aerial and root parts of *P. molucelloides* was investigated against some reference bacterial strains representing pathogens in the presented study. The broth microdilution method was done to test the antibacterial activity of the extracts at high concentrations in this study. However, MIC values of the extracts against all tested bacteria could not be determined (MIC > 2000 µg/mL). There are studies in the literature researching the antibacterial activity of various *Eremostachys* species. For example, it has been reported that n-hexane, dichloromethane, and methanol extracts of the aerial parts of *Eremostachys macrophylla* Montbret & Aucher ex Benth. and methanol extracts of the rhizomes of *E. azerbaijanica* had no antibacterial activity as in the current study (Asgharian et al., 2017; Asnaashari et al., 2017).

Figure 5. The result of violacein inhibition assay (1; positive control, 2 and 4; methanol extracts of aerial and root parts, respectively, 3 and 5; water extracts of aerial and root parts).

Şekil 5. Viyolasin inhibisyon deneyi sonucu (1; pozitif kontrol, 2 ve 4; sırasıyla toprak üstü ve kök kısımlarının metanol özütleri, 3 ve 5; sırasıyla toprak üstü ve kök kısımlarının su özütleri).

However, dichloromethane and n-hexane extracts of the rhizomes of *E. azerbaijanica* have been shown to exhibit antibacterial activity against *S. aureus* and *Staphylococcus epidermidis* (Gram-positive bacteria) (Asnaashari et al., 2017). Moreover, it has been stated that essential oils obtained from the root, stem, and flower parts of *E. laevigata* also exhibit high antibacterial activity (Esmaeilli et al., 2012). In addition, it was reported that iridoid glycosides purified from *E. laciniata* have a remarkable antibacterial activity especially against *Bacillus cereus*, *S. aureus* (Gram-positive bacteria) and *E. coli*, *Proteus mirabilis* (Gram-negative bacteria) (Modaressi et al., 2009). In the current study, it was determined that methanol and water extracts of the aerial and root parts of *P. molucellides* did not exhibit any antibacterial activity against the Gram-positive (*S. aureus*, *E. faecalis* and *B. subtilis*) and Gram-negative (*E. coli*, *K. pneumoniae*, *S.* Typhimurium, *P. aeruginosa*, *E. aerogenes*, *A. haemolyticus*) bacteria tested, even at high concentrations. This result may be due to the chemical content of the plant, the geographical region where it grows, the harvesting, or the extraction method.

CONCLUSION

The lack of determination of the chemical content of the plant and the lack of investigation of other biological activities such as cytotoxic and antioxidant activities limit this study. This research was reported as preliminary study based on the *in-vitro* antibacterial and anti-QS effects of methanol and water extracts of aerial and root parts of *P. moluccellides.* Based on the results, *P. molucelloides* had not been found antibacterial and antibiofilm activities. However, its inhibitory activities on swarming motility were noteworthy. In particular, methanol extract of the aerial part of *P. molucelloides* had strong inhibitory activity on pyocyanin production. These results showed that *P. molucelloides* is a good candidate for the development of anti-QS agents. Further research can be done to elucidate the mechanisms underlying these biological activities.

Author's Contributions

Authors declare the contribution of the authors is equal.

Statement of Conflict of Interest

The authors have declared no conflict of interest.

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Hasat Sonrası Benzo-Thiadiazol Karbotioik Uygulamalarının Kiraz Meyvesinde Muhafaza Performansı ve Fitokimyasal Maddeler Üzerine Etkisi

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ÖZET

Çalışmada, kiraz meyvelerine depolama öncesinde uygulanan BTH'nın depolama sürecindeki kalite değişimi ile toplam fenolik madde, antosiyanin ve antioksidan kapasitesi üzerine etkileri incelenmiştir. Çalışmada, BTH'nın iki farklı dozu (100 ve 200 ppm) uygulanmış ve 200 ppm daha etkili olacak şekilde her iki dozun da depolama sürecinde oluşan ağırlık kaybını azaltmada etkili olduğu tespit edilmiştir. Yirmi bir günlük depolama sonunda, 200 ppm BTH'nın asit içeriğinde önemli bir artışa SÇKM içerinde ise azalmaya neden olduğu belirlenmiştir. Depolamanın yedinci gününde yapılan ölçümlerde kontrol meyvelerinin toplam fenolik madde miktarı, antosiyanin içeriği ile antioksidan kapasitesinde belirgin bir artış gözlenmiştir. Bu artış BTH uygulanan meyvelerde görülmemiştir. Depolamanın 14 ve 21. gününde belirlenen toplam fenolik madde miktarı ve antosiyanin içeriği ile antioksidan kapasitesi açısından kontrol ve BTH uygulamaları arasında önemli bir farklılık ortaya çıkmamıştır.

Bahçe Bitkileri

Araştırma Makalesi

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Anahtar Kelimeler
Antosiyanin
Antioksidant
Benzo-Thiadiazol karbotioik
Depolama
Fenolik madde

Effect of Post-Harvest Benzo-Thiadiazole Carbotioic Applications on Storage Performance and Phytochemicals in Sweet Cherry Fruit

ABSTRACT

In the study, the effects of BTH applied to cherry fruits before storage on changes of the quality, total phenolic, anthocyanin and antioxidant capacity during the storage period were investigated. In the study, two different doses of BTH (100 and 200 ppm) were applied and it was determined that both doses were effective in reducing weight loss during storage, with 200 ppm being more effective. At the end of 21 days of storage, it was determined that 200 ppm BTH significantly increased acid content and decreased total soluble solid content. On the 7th day of storage, a significant increase was observed in total phenolic compounds, anthocyanin content and antioxidant capacity of control fruits. This increase was not observed in fruits treated with BTH. There were no significant differences between control and BTH applications in terms of total phenolic compounds and anthocyanin content and antioxidant capacity determined on the 14th and 21st days of storage.

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GİRİŞ

Sağlık konusunda bilinç düzeyinin artması, bütün dünyada tüketici tercihlerini etkileyen kalite faktörlerinde önemli değişmelere yol açmıştır. Özellikle gelişmiş ülkelerde meyve kalitesi, sadece tat ve dış görünüşle değil, aynı zamanda meyvenin içermiş olduğu sağlık açısından ilave faydalar sağlayan bioaktif madde miktarı ile ölçülmektedir (Wang, 2006). Bu anlamda kiraz gerek tat ve çekici rengi gerekse de yüksek antioksidan içeriği ile tüketici talebinin her geçen gün artış gösterdiği bir üründür (Wani ve ark., 2014; Öztürk ve ark., 2019).

Yüksek oranda antioksidan içeriğinden dolayı, kiraz meyvesinin sağlıklı bir yaşam sürdürme ve bazı hastalıklara karşı korunmada etkili olduğunu bildiren araştırma sonuçları bulunmaktadır (Yılmaz ve ark., 2009; Yoo ve ark., 2010). Kirazın önemli bir fenolik madde kaynağı olduğu, fenolik madde içeriğinin çilek, kırmızı kuş üzümü ve kırmızı ahududu meyveleri ile benzer düzeyde, maviyemiş gibi koyu renkli meyvelerinkinden ise daha düşük düzeyde olduğu araştırmacılar tarafından bildirilmiştir (Jakobek ve ark., 2007; Jakobek ve ark., 2009).

Daha iyi beslenme ve sağlıkla ilgili kaygılar, biyoaktif madde içeriği yüksek yeni tür ve çeşit arayışı yanında, hâlihazırda, tat ve aroması ile tüketicilerin beğenisini kazanmış meyvelerin biyoaktif madde içeriğini artırmaya yönelik ya da hasattan sonra besin değerinin en iyi şekilde korunmasına yönelik depolama çalışmalarına olan ilgiyi artırmıştır.

Bitkilerin patojenlere karşı dayanıklılığını artırmak amacıyla yapılan bazı uygulamaların bitkide polifenol sentezini artırdığının tespit edilmesinden sonra, yüksek fenolik madde içeriğine sahip bitki elde etmek için alternatif bir yol gündeme gelmiştir. Yüksek veya düşük sıcaklık, ultraviole veya gama ışınları gibi fiziksel uyarıcılar ile çitosan (chitosan), benzo-thiadiazole, harpin, ve 1-metilsiklopropane gibi kimyasal uyarıcıların polifenol sentezini uyardığına dair bulgular bulunmaktadır (Cantos ve ark., 2003; Obradovic ve ark., 2004; Liu ve ark., 2005; Ağlar ve ark., 2016). Bunların dışında jasmonik asit, metil jasmonat ve benzo-thiadiazole karbotik asidin (BTH) farklı meyve türlerinde toplam fenolik madde içeriğini etkilediği bildirilmiştir (Hukkanen ve ark., 2007; Belhadj ve ark., 2008; Cao ve ark., 2010; 2011; Öztürk ve ark., 2013; Saraçoğlu ve ark., 2017).

BTH depolama sürecinde taze meyve ve sebzelerde önemli bir sorun olan çürümelere karşı fungusitlere alternatif bir uygulama olarak gündeme gelmiştir (Buonaurio ve ark., 2002). Bu gelişme düzenleyicinin sistemik kazanılmış dayanıklılık mekanizmasını teşvik ederek meyvelerin dayanıklılığını artırdığı ileri sürülmektedir. (Benhamou ve Belanger, 1998). BTH'nin bitkide dayanıklılık mekanizmasında önemli bir yere sahip olan fenolik madde sentezinde de etkili olabileceği düşünülerek bu konuda çalışmalar yapılmaktadır. Nitekim bazı çilek çeşitleri ile yapılan çalışmalarda hasat sonrası BTH uygulamasının fenolik madde birikimini ve meyvelerin antioksidan aktivitesini artırdığı kaydedilmiştir (Hukkanen ve ark., 2007; Cao ve ark., 2010; 2011).

Yukarıda verilen bilgileri ışığında, bu çalışmada gerek iç pazar gerekse dış pazar için önemli bir ürünümüz olan 0900 Ziraat kiraz çeşidine ait meyvelere uygulanan BTH'nin depolama sürecinde meyve kalitesine etkisini incelemek, bunun yanında uygulamanın fenolik madde içeriği ve antioksidan aktivite üzerine etkisinin belirlenmesi amaçlanmıştır.

MATERYAL ve METOD

Örnekleme Yöntemi

Araştırmada Tokat Gaziosmanpaşa Üniversitesi Tarımsal Uygulama ve Araştırma Merkezi bünyesinde bulunan, kurulumu 2008 yılında '0900 Ziraat'/Gisela 5 çeşit-anaç kombinasyonu üzerinde 2018 büyüme döneminde elde edilen meyveler kullanılmıştır. Hasat edilen meyvelerden homojen renklenmiş, yeknesak büyüklükte, herhangi bir hasar görmemiş, sağlıklı ve kusursuz olanlar seçilerek, plastik ambalajlara istiflenmiş ve meyveler derhal -5 ile +10 C' ye kadar muhafaza olanağı sunan, aynı zamanda nem kontrolü yapılabilen, 25 m3 hacme sahip soğuk hava deposuna transfer edilmiştir. Soğuk hava deposunda bir gün süreyle ön soğutmaya tabi tutulmuş, bu süre sonunda laboratuvara getirilen meyveler 1000 gramlık polipropilen plastik kutulara yerleştirilmiştir. Her bir grupta 12 adet kutu olacak şekilde meyveler üç gruba ayrılmıştır. Birinci gruba 100 ppm, ikinci gruba 200 ppm BTH uygulaması daldırma yöntemi ile yapılmıştır. Üçüncü grup meyveler kontrol olarak bırakılmış ve sadece su ile muamele edilmiştir. Uygulama sonrası meyveler kurumaları için 20 dakika bekletilmiş, daha sonra meyveler 1 °C ve % 90±5 oransal nem koşullarında 21 gün boyunca depolanmıştır. Depolamanın 0, 7, 14 ve 21. gününde örnekler alınarak gerekli analizler yapılmıştır. Deneme tam şansa bağlı tesadüf parselleri deneme desenine göre üç tekerrürlü olarak kurulmuştur. Buna göre her bir örnekleme tarihinde, her bir uygulama için, tekerrür başına 1 paket olacak şekilde 3 paket alınarak analizler yapılmıştır. Denemede her bir örnekleme dönemi için aşağıdaki ölçümler yapılmıştır.

Laboratuvar analizleri

Ağırlık Kaybı, Meyve Kabuk Rengi, Suda Çözünebilir Kuru Madde, Titrasyon Asitliği ve pH

Farklı örnekleme tarihlerinde ağırlık kayıpları belirlenmiştir. Kümülatif ağırlık kayıpları her bir muhafaza döneminde örnek kapları üç tekerrürlü olarak tartılarak, güncel ağırlıklar orijinal ağırlığa oranlanarak yüzde olarak ifade edilmiştir. Meyve kabuk rengi CIE L*, a* ve b* cinsinden bir renk ölçer (Minolta, model CR–400, Tokyo, Japonya) kullanılarak her bir analiz döneminde, her bir tekerrürde 20 meyvenin ekvatoral kısmından bir ölçüm yapılarak belirlenmiştir. Belirlenen a ve b değerlerinden kroma değeri (C= (a2+b2)1/2) ve hue açısı değerleri (h^o= tan-1 x b/a) hesaplanmıştır (McGuire, 1992). Her tekerrürde 10 meyve elle sıkılarak meyve suları çıkarılmış daha sona dijital refraktometre (PAL-1, McCormick Fruit Tech. Yakima, ABD) ile SÇKM değerleri % briks olarak ölçülmüştür. Titrasyon asitliği değerini belirlemek

için elde edilen meyve püresi örneğinden alınan 10 mL'lik örnek 10 mL saf su ile seyreltildikten sonra pH 8.1 değerine ulaşana kadar 0.1 mol L-1sodyum hidroksit (NaOH) ile titre edilmiş ve titrasyonda harcanan NaOH miktarı esas alınarak malik asit cinsinden (g malik asit 100 mL-1) ifade edilmiştir. Homojenizatörde püre hale getirilen meyvelere pH-metre ile doğrudan cam elektrot daldırılarak pH ölçülmüştür.

Toplam Fenolik Madde, Toplam Antosiyanin İçeriği ve Toplam Antioksidan Kapasitesi

Toplam fenolik madde miktarı Folin-Ciocalteu (Singleton ve Rossi,1965) yöntemine göre yapılmıştır. Bu amaçla, homojenize edilen püre aseton, su ve asetik asit (70:29.5:0.5) çözeltisi kullanılarak bir saat boyunca tüpler içerisinde ekstraksiyon işlemine tabi tutulmuştur. Folin-Ciocalteu's kimyasalı ve saf su karıştırılarak 8 dakika bekletilmiştir. Sonra %7'lik sodyum karbonat ilave edilmiştir. İki saat inkübasyondan sonra mavimsi bir renk alan çözeltinin absorbansı spektrafotometrede 750 nm dalga boyunda ölçülmüştür. Sonuçlar gallik asit cinsinden µg gallik asit eşdeğer/g taze meyve olarak hesaplanmıştır.

Meyvedeki toplam antosiyanin tayini, pH farkı metodu kullanılarak yapılmıştır (Giusti and Wrolstad 2005). Ekstraktlar pH 1.0. ve 4.5 çözeltiler hazırlanarak 520 ve 700 nm dalga boylarında ölçülmüştür. Toplam antosiyanin miktarı (molar extinction coefficient of 26900 siyanidin 3-glikozit) absorbanslar [(A520–A700) pH 1.0 - (A520–A700) pH 4.5] µg antosiyanin /g kuru madde olarak hesaplanmıştır.

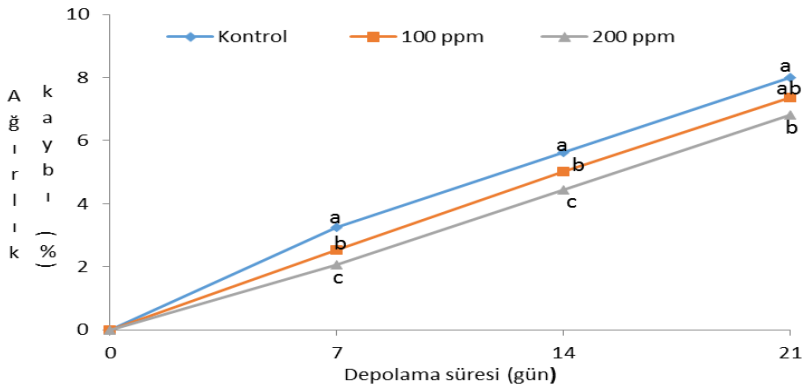
Meyvelerin anitioksidan kapasiteleri için (Saracoglu, 2018) 7 nm ABTS (2,2'-Azino-bis 3-ethylbenzothiazoline-6-sulfonic acid) 2,45 mM potasyum bisülfat ile karıştırılarak karanlık ortamda 12-16 saat bekletilmiştir. Daha sonra bu solüsyon 20 mM sodium asetat (pH4.5) bafırı ile spectrofotometrede 734 nm dalga boyunda 0,700±0,01 absorbans olacak şekilde sadeleştirilmiştir. Nihayetinde 30 µL ekstrakt 2.97 mL hazırlanan bakır karıştırılarak absorbance 10 dakika sonra spektrafotometrede 734 nm dalga boyunda ölçülmüştür. Elde edilen absorbans değerleri Trolox (10–100 µmol/L) standart eğim çizelgesi ile hesaplanarak µmol Troloks eşdeğeri/g yaş ağırlık olarak sunulmuştur.

İstatistik Analizler

Deneme tam şansa bağlı tesadüf parselleri deneme desenine göre üç tekerrürlü olarak kurulmuştur. Buna göre her bir örnekleme tarihinde, her bir uygulama için, tekerrür başına 1 paket olacak şekilde 3 paket alınarak analizler yapılmıştır. Veriler varyans analizi ile analiz edildikten sonra uygulama ortalamaları arasındaki farkların önem düzeyi Tukey testi ile belirlenmiştir. Bütün istatistik analizler SAS paket proğramı kullanılarak yapılmıştır.

BULGULAR

Kiraz meyvelerinin ağırlık kaybı bütün uygulamalarda depolanma sürecinde düzenli bir artış göstermiştir. İlk ölçümün yapıldığı 7. günde kontrol meyvelerindeki ağırlık kaybı %3.25±0.09 iken, on dördüncü günde %5.62±0.18, yirmi birinci günde ise %8.01±0.19'a kadar çıkmıştır. BTH'nın her iki dozu da ağırlık kaybını azaltmıştır. Depolamanın son günü olan 21. günde yapılan ölçümlerde 100 ppm BTH uygulanan meyvelerin ağırlık kaybı %7.37±0.15 iken, 200 ppm BTH uygulamasında bu değer %6.82±0.14 olarak ölçülmüştür (Şekil 1).

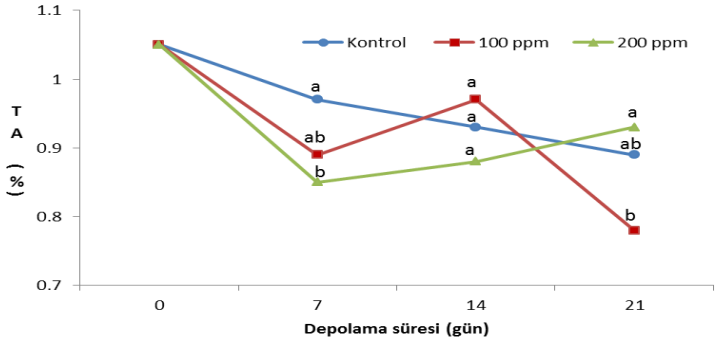


Şekil 1. 0900 kiraz çeşidinde, depolama sürecinde oluşan ağırlık kayıpları üzerine BTH uygulamalarının etkisi.
Figure 1. The effect of BTH applications on weight loss during storage in 0900 cherry cultivar.

Depolama sürecinde kontrol meyvelerinin titre edilebilir asit içeriğinde düzenli bir azalma gözlenmiştir. Depo başlangıcında % 1.05±0.03 olarak ölçülen asitlik değeri 21 günlük depolama sonunda %0.89±0.03'e düşmüştür. BTH uygulamalarında ise TA'nın değişimi depolama sürecinde dalgalı bir seyir izlemiştir. Depolamanın 7. günüde 100 ve 200 ppm

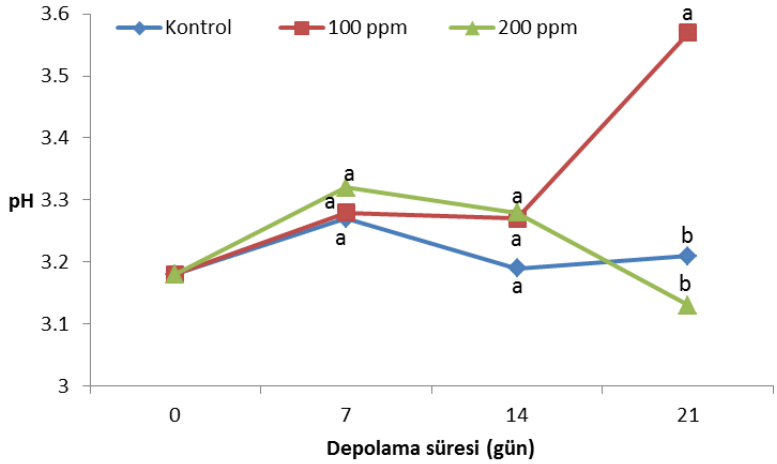
BTH uygulanan meyvelerin asit içeriğinde depolama başlangıcına kıyasla düşüş gözlenmiştir. 100 ppm BTH uygulamasında 14 günde artan asitlik 21. günde tekrar azalmıştır. 21 günlük depolama sonunda 100 ppm BTH uygulanan meyvelerin asit içeriği (%0.78±0.05) kontrol meyvelerine kıyasla daha düşük bulunurken 200 ppm uygulaması (%0.93±0.02) ile kontrol arasındaki fark önemsiz çıkmıştır (Şekil 2).

Depolama sürecinde 14. güne kadar uygulamalar arasında pH değeri açısından belirgin bir farklılık ortaya çıkmamıştır. Depolamanın son günü olan 21. günde ise 100 ppm BTH uygulanan meyvelerin pH değerinde, diğer uygulamalara kıyasla, belirgin bir artışın olduğu tespit edilmiştir. (Şekil 3). Bu dönemde yapılan ölçümlerde, kontrol ve 200 ppm uygulamasında sırasıyla 3.21±0.01 ve 3.13±0.02 olan ph değeri 100 ppm de 3.6±0.17 olarak tespit edilmiştir. Kontrol meyveleri ile BTH uygulamaları arasındaki fark, istatistiki olarak önemli bulunmamıştır.



Şekil 2. BTH uygulamalarının depolama sürecinde,0900 Ziraat çeşidine ait meyvelerin titre edilebilir asit içeriğindeki değişim üzerine etkisi.

Figure 2. The effect of BTH applications on the change in titratable acid content of 0900 Ziraat fruits during the storage period.



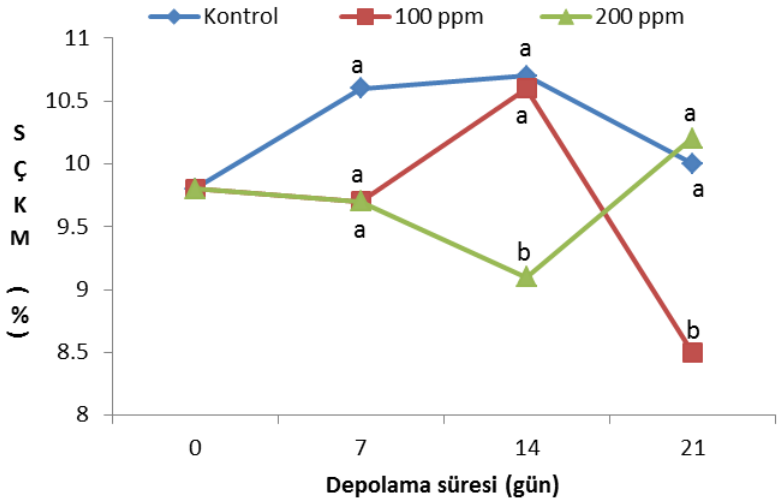
Şekil 3. Farklı dozlarda BTH uygulanmış 0900 Ziraat meyvelerinin depolama sürecindeki pH değişimi.

Figure 3. The pH change of 0900 Ziraat fruits applied at different doses during the storage period.

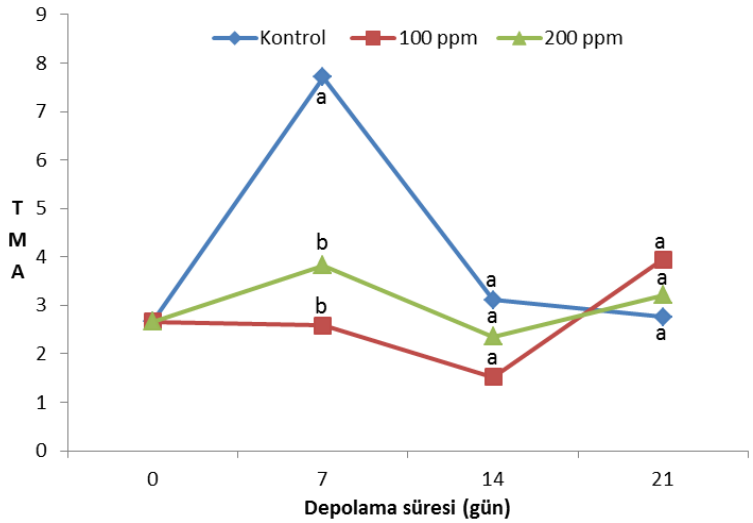
Hiçbir uygulama yapılmadan depolanan kontrol meyvelerinin SÇKM değerleri depolama sürecinin 7 ve 14. gününde bir miktar artmış, 21. günde ise tekrar azalarak başlangıçtaki seviyesine düşmüştür. BTH uygulamalarında ise dalgalı bir seyir izlemiştir. 14. günde 100 ppm BTH uygulanmış meyvelerin SÇKM içeriği ile kontrol meyvelerinin SÇKM içeriği benzer seviyede iken 200 ppm BTH uygulamasının SÇKM değerinde belirgin bir azalmaya neden olduğu görülmüştür. 21. günde ise SÇKM içeriği üzerine 200 ppm BTH uygulamasının etkisi önemsiz bulunurken, 100 ppm BTH uygulamasından kaynaklanan önemli bir azalma tespit edilmiştir. 21 günlük depolama sonunda kontrol ve 200 ppm BTH uygulanan meyvelerin SÇKM içeriği sırasıyla %9.97±0.63 ve % 10.2±0.65 iken 100 ppm BTH uygulanmış meyvelerde bu değer % 8.5±0.68'e düşmüştür (Şekil 4.).

Depolama öncesi 2.66±0.49 µg siy-3-gli gta^{-1} olarak ölçülen toplam monomerik antosiyanin miktarı, depolamanın 7. gününde kontrol meyvelerinde 7.71±0.44 µg siy-3-gli gta^{-1} kadar çıkarken, 100 ve 200 ppm BTH uygulanan meyvelerde sırasıyla 2.58±0.46 ve 3.82±0.64 µg siy-3-gli gta^{-1} olarak belirlenmiştir. Daha sonraki günlerde ölçülen antosiyanin miktarı

açısından kontrol ile BTH uygulamaları arasında belirgin bir farlılığın ortaya çıkmadığı belirlenmiştir. Depolamanın son günü olan 21. günde kontrolde 2.76±0.68 µg siy-3-gli gta^{-1}, 100 ve 200 ppm BTH uygulamalarında sırasıyla 2.95±1.71 ve 3.21±0.47 µg siy-3-gli gta^{-1} antosiyanin bulunmuştur.



Şekil 4. Farklı dozlarda BTH uygulanmış 0900 Ziraat meyvelerinin depolama sürecindeki SÇKM değişimi.
Figure 4. The change in SSC of 0900 Ziraat fruits applied at different doses during the storage period.

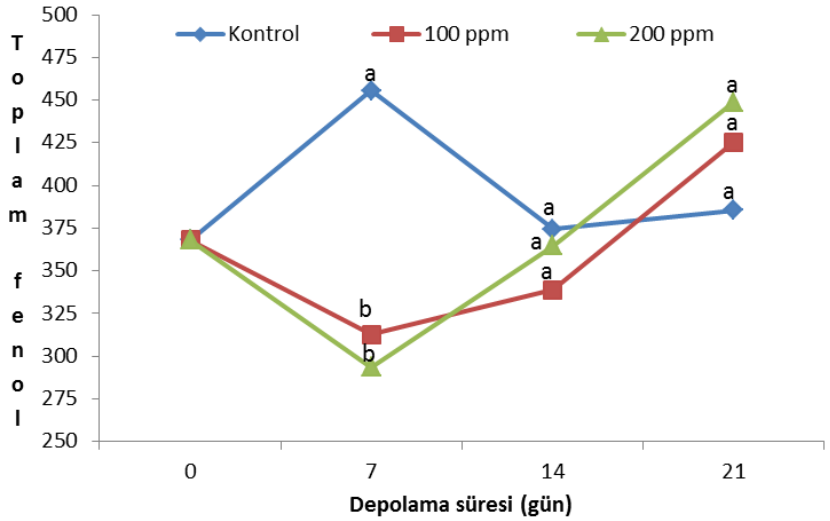


Şekil 5. Farklı dozlarda BTH uygulanmış 0900 Ziraat meyvelerinin depolama sürecindeki toplam antosiyanin (µg siy-3-gli gta^{-1}) içeriğindeki değişimler.
Figure 5. Changes in the total anthocyanin (µg cy-3-glu/g ta) content of 0900 Ziraat fruits applied at different doses during the storage period.

Toplam fenolik madde içeriğinde, antosiyanin içeriğindeki değişime benzer bir seyir gözlenmiştir. Depolamanın 7. günde kontrol meyvelerinin fenolik madde içeriği artarken, BTH uygulanan meyvelerin fenolik madde içeriğinde azalma görüşmüştür. Bu değişim sonucunda 7. günde kontrol meyvelerinde ölçülen toplam fenolik madde miktarı BTH uygulamasında ölçülenden önemli derecede daha yüksek çıkmıştır. Meyvelerin toplam fenolik madde içeriği depo başlangıcında 368.15±7.4 µg GAE/g ta iken bir haftalık depolama sonunda yapılan ölçümlerde kontrol meyvelerinde 455.41±48.9 µg GAE/g ta, 100 ve 200 ppm BTH uygulanmış meyvelerde sırasıyla 312. 57±5.9 ve 293.67±16.4 µg GAE/g ta olarak ölçülmüştür. Hem 14. hem de 21. günde yapılan ölçümlerde kontrol ve BTH uygulamalarında toplam fenolik madde içeriği benzer seviyelerde bulunmuş ve aradaki farklar istatistiki olarak önemsiz çıkmıştır.

TEAC cinsinden ölçülen antioksidan kapasiteleri incelenmiş ve sonuçlar Şekil 7'de verilmiştir. Şekilden de görüldüğü gibi bir hafta depolama sonunda kontrol meyvelerinin antioksidan kapasitesinde belirgin bir artış görülmüştür. Depolama başlangıcında 0.78±0.09 µmol TE/g ta olarak belirlenen antioksidan kapasitesi 7 günlük depolama sonunda 1.5±0.04 µmol TE/g ta değerine ulaşmıştır. 14. günde ise tekrar azalarak BTH uygulanan meyvelerle benzer

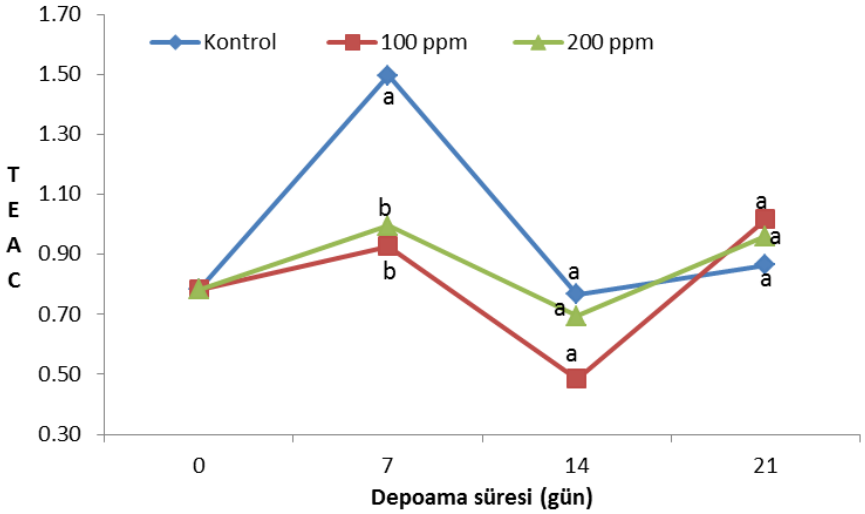
seviyelere (0.77±0.11) inmiştir. 21 gün depoda bekletilen meyvelerin antosiyanin kapasiteleri kontrol, 100 ve 200 ppm BTH uygulamalarında sırasıyla 0.86±0.11, 1.02±0.13 ve 0.96±0.07 µmol TE/g ta olarak ölçülmüştür (Şekil 7). 21 günde belirlenen bu değerler arasındaki farklar istatistiki olarak önemsiz bulunmuştur.



Şekil 6. Farklı dozlarda BTH uygulanmış 0900 Ziraat meyvelerinin depolama sürecindeki toplam fenolik madde (µg GAE/g ta) içeriğindeki değişimler.

Figure 6. Changes in total phenol (µg GAE/g ta) content of 0900 Ziraat fruits applied at different doses during storage.

Yapılan ölçümlerde renk parametrelerinde depolama sürecinde bazı önemli değişimler gözlenmekle birlikte hem L hem hue açı değeri hem de kroma değerinde uygulamalardan kaynaklanan önemli bir farklılık tespit edilememiştir. L değeri kontrol meyvelerinde 14. güne kadar yükseliş göstermiş 21. günde ise tekrar azalmıştır. Her iki BTH uygulamasında da 14 ve 21. günde belirlenen L değeri depolama başlangıcında belirlenen L değerine göre önemli derecede yüksek bulunmuştur. Depolamanın sonunda kontrol, 100 ve 200 ppm BTH uygulamalarında sırasıyla 25.2±0.8, 25.8±1.0 ve 24.8±0.7 olacak şekilde birbirine oldukça yakın değerler elde edilmiştir.



Şekil 7. Farklı dozlarda BTH uygulanmış 0900 Ziraat meyvelerinin depolama sürecindeki antioksidan kapasiteleri (TEAC: µmol TE/g ta).

Figure 7. Antioxidant capacities (TEAC: µmol TE/g ta) of 0900 Ziraat fruits applied at different doses during storage.

Hem kontrol hem de BTH uygulanmış meyvelerin kroma değerlerinde depolama süreci uzadıkça azalma eğilimi göstermiştir (Çizelge 1). Kontrol meyvelerinde depolama başlangıcında 33.9±0.2 olarak tespit edilen kroma değeri 21 günlük depolama sonunda 26.6±0.6'ya düşmüştür. 100 ppm BTH uygulandıktan

sonra depolanan meyvelerin kabuk rengine ait kroma değerinde de depolama sürecinde azalma yönünde bir eğilim belirlenmekle birlikte bu değişim istatistiki olarak önemsiz bulunmuştur. 200 ppm uygulamasında 14. güne kadar azalan kroma değerinde 21. günde bir miktar artış gözlenmiş görülmüştür.

Hue değerlerinde de depolama süresine bağlı olarak her üç uygulamada da azalma eğilimi görülmüştür. Bu azalma kontrol meyvelerinde önemli bulunurken 100 ve 200 ppm BTH uygulamalarındaki değişimler önemsiz çıkmıştır (Çizelge 1). Depolama başlangıcında 14.7±0.5 olarak ölçülen hue değeri 21 günlük depolama sonunda kontrol meyvelerinde 12.5± 0.5'e, 100 ppm BTH uygulanan meyvelerde 13.7±1.6 ve 200 ppm BTH uygulanan meyvelerde ise 13.1±0.9 olarak tespit edilmiştir.

Çizelge 1. BTH uygulamalarına bağlı olarak depolama sürecinde 0900 Ziraat meyvelerinin kabuk rengine ait L, kroma ve hue değerlerindeki değişimler

Table 1. Changes in the L, chroma and hue values of the skin color of 0900 Ziraat fruits during the storage process depending on BTH applications

Renk parametreleri	Uygulama	Depolama süresi			
		0	7	14	21
L	Kontrol	21.1±1.1 b	23.5±0.3 b	29.0±1.7 a	25.2±0.8 ab
	100 ppm BTH	21.1±1.1 b	23.1±1.1 ab	25.6±0.6 a	25.8±1.0 a
	200 ppm BTH	21.1±1.1 b	23.8±0.8 ab	27.2±0.4 a	24.8±0.7 a
Kroma	Kontrol	33.9±0.2 a	30.2±1.1 ab	26.3±1.1 c	26.6±0.6 bc
	100 ppm BTH	33.9±0.2 a	34.0±1.6 a	30.1±1.1 a	28.9±2.0 a
	200 ppm BTH	33.9±0.2 a	33.2±0.4 a	25.2±2.3 b	28.5±1.2 ab
Hue	Kontrol	14.7±0.5 a	13.3±0.3 ab	12.3±0.2 b	12.5± 0.5 b
	100 ppm BTH	14.7±0.5 a	14.7±0.3 a	13.4±0.6 a	13.7±1.6 a
	200 ppm BTH	14.7±0.5 a	14.9±0.9 a	14.4±1.7 a	13.1±0.9 a

*Aynı satırda aynı harfle gösterilen ortalamalar arasındaki farklar Tukey çoklu karşılaştırma testine göre önemli değildir($p<0.05$).
** The difference between the means indicated with the same letter on the same row is not significant according to the Tukey test ($p< 0.05$).*

TARTIŞMA

Meyve kabuğunun difüzyona karşı direncinin düşük olması nedeni ile kirazda ağırlık kaybının diğer ürünlerden daha yüksek olduğu bildirilmektedir (Crisosto, 1992). Benzer durum bu çalışmada da gözlenmiş olup, herhangi bir uygulama yapılmadan 1 C° sıcaklık % 90 oransal nem koşullarında depolanan kontrol meyvelerinde 21. günün sonunda % 8 civarında ağırlık kayıpları ortaya çıkmıştır. Kiraz üzerine yapılan önceki çalışmalarda, depo koşullarına ve uygulanan tekniklere bağlı olarak farklı oranlarda ağırlık kayıpları rapor edilmekle birlikte genel olarak yapılan çalışmada elde edilen sonuca benzer düzeylerde ağırlık kayıplarının olduğu belirlenmiştir (Çavuşoğlu ve ark., 2018; Koyuncu ve ark., 2018). Yaşar (2017) 0900 Ziraat çeşidinde salisilik asit uygulamasının ağırlık kaybını azalttığını bildirmiştir. Benzer şekilde, bu çalışmada da bir salisilik asit analoğu olduğu bildirilen BTH uygulaması ile ağırlık kaybının azaltılabileceği belirlenmiştir.

Meyvelerin asit içeriği, pH değeri ve toplam suda çözünür (SÇKM) kuru madde içerikleri birer olgunluk parametresi olması yanında tat ve aromayı belirleyen özellikler olduğundan depolama sürecinde bu özelliklerin değişimi her zaman bilinmek istenir. Yapılan BTH uygulamaları, asit içeriğinde ölçüm tarihine bağlı olarak bazı önemli farklılıklar ortaya çıkarmasına rağmen, çalışmadan elde edilen bulgularla BTH'nın kiraz meyvelerinin depolama sürecindeki titrasyon asitliği ve SÇKM içeriği üzerine kararlı bir etkisinin olmadığını ortaya koymuştur. Huan ve ark (2021), erik meyveleri üzerine yaptıkları çalışmada muhafaza süresi boyunca titrasyon asitliği ve SÇKM değerlerinde net bir trend tespit edilemediğini ifade etmiş, benzer sonuçlar Li ve ark (2015) tarafından kavun üzerine yapılan çalışmada da belirlenmiştir.

Çilekte yapılan bir çalışmada, 0.2 g/L BTH çözeltisine batırıldıktan sonra depolanan meyvelerin antosiyanin içeriğinde depolamanın ikinci gününde belirgin bir artışın olduğu belirtilmiştir. Aynı çalışmada, antosiyanin sentezinde görev alan bazı enzim aktiviteleri de incelenmiş ve depolamanın erken dönemlerinde (0-6 gün) BTH bu enzimlerin aktivitesini teşvik ettiği belirlenmiştir. Araştırıcılar BTH'nın antosiyanin içeriğini artırıcı yöndeki etkisinin bu enzimlerin aktivitesindeki artıştan kaynaklanabileceği ileri sürmüşlerdir (Cao ve ark., 2010). Kiraz meyveleri ile yapılan bu çalışmada ise 100 ve 200 ppm BTH kullanılmış ve depolamanın 7, 14 ve 21. gününde yapılan ölçümlerde antosiyanin içeriğinde BTH uygulamasından kaynaklanan önemli bir artış belirlenememiş tam tersine 7 günde kontrole kıyasla azalma olduğu saptanmıştır. Bu durum çilek ve kiraz meyvelerinin BTH uygulamalarına tepkilerinin farklı olmasından kaynaklanabileceği

gibi ölçüm tarihlerinin farklı olmasından da kaynaklanmış olabilir. Çilekte yapılan çalışmada depolamanın 2 gününden başlanarak 2 gün aralıklarla 10 gün boyunca ölçümler yapılmasına karşın bu çalışmada ilk örnekler depolamanın 7. gününde alınmış ve ölçümler 1 hafta aralıklarla yapılmıştır.

Hormon benzeri etkileri olan salisilik asidin bir analoğu olan BTH'nın, bitkilerde sistemik kazanılmış dayanıklılık (SAR) yolunu başlatarak viral, bakteriyel ve fungal hastalıklara karşı bitki direncini artırdığı belirlenmiştir (Benhamou ve Belanger, 1998). Savunma sisteminin başlatılması bazı sinyal molekülleri ve bileşiklerin teşvik edilmesi ile ilişkili olup bunlardan birisi de fenolik maddelerdir. Hukkanen ve ark (2007), çilekte BTH uygulamasının, serbest ve hücre duvar materyallerine bağlı fenolik madde birikimini artırarak küllemeye karşı meyvelerin direncini artırdığını tespit etmiştir. Bu çalışmada ise depolama öncesi uygulanan BTH'nın 21. günlük depolama sürecinde kiraz meyvelerinin toplam fenolik madde içerinde beklendiği şekilde bir artışa neden olmamıştır. Literatür bulgularının aksine depolamanın 7. gününde kontrol meyvelerinin toplam fenolik madde içeriği BTH uygulanan meyvelerin toplam fenolik madde içeriğinden daha yüksek bulunmuştur. Bu farklı sonuç uygulama zamanında meyvenin olgunluk safhasının yada uygulamadan sonraki örnekleme sürelerinin farklı olmasından kaynaklanmış olabileceği düşünülmektedir.

Meyvelerde BTH uygulamalarının meyve kalitesi üzerine etkileri ile ilgili sınırlı sayıda çalışma vardır. Çilekte yapılan birkaç çalışmada hasattan sonra uygulanan BTH uygulamasının meyvelerin toplam fenolik madde ve antosiyanin sentezini teşvik ettiği buna bağlı olarak da antioksidan kapasitelerinde artışa neden olduğu bildirilmiştir (Hukkanen ve ark., 2007; Coa ve ark., 2010; 2011). Bu çalışmada ise hem toplam fenolik madde miktarı hem antosiyanin içeriğinde hem de meyvelerin antioksidan kapasitesinde BTH uygulamasından kaynaklanan önemli bir artış görülmemiştir. Çalışma ile benzer şekilde bazı meyve ve sebzelerde fitokimyasal maddelerde net bir değişim gözlenememiş olup muhfaza süresince değişkenlikler meydana gelmiştir (Hafez, 2010; Lin ve ark., 2011). Bazı araştırıcılar elde edilen sonuçların farklı türlerde meyve kabuk farklılıklarından kaynaklanabileceğini öne sürülmüşlerdir (Huan ve ark., 2021).

SONUÇ ve ÖNERİLER

İnsan sağlığı açısından biyoaktif maddelerin öneminin anlaşılmasında sonra, biyoaktif madde içeriği açısından zengin, antioksidan kapasitesi yüksek ürünlere ve ürünlerin biyoaktif madde içeriğini artırmaya yönelik çalışmalara olan ilgiyi artırmıştır. Bu amaçla bitki fizyolojisine yön veren gelişimi düzenleyici kullanımı ilk akla gelen yöntemlerden biri olmaktadır. Son yıllarda, BTH ile yapılan bazı çalışmalarda, bu gelişme düzenleyicinin fenolik madde sentezini teşvik ederek bitkide savunma sistemini başlattığı ileri sürülmüştür. Bu bilgiler ışığında, bu çalışmada gerek tat ve aroması gerekse içermiş olduğu besin değeri ile sevilerek tüketilen kiraz meyvesinde BTH uygulaması ile antosiyanin ve fenolik madde içeriğinin artırılıp artırılamayacağının belirlenmesi amaçlanmıştır. Bu amaç doğrultusunda 100 ve 200 ppm olarak iki doz uygulanmış ve depolama boyunca hem biyoaktif madde içeriğinde hem de antioksidan kapasitesinde uygulamadan kaynaklanan herhangi bir iyileşme tespit edilememiştir. Bu çalışmada uygulamalar tek zamanda yapılmış olup, uygun olgunluk aşaması yakalanamamış olabilir. Farklı olgunluk aşamaları ve farklı dozlar kullanılması ile ayrıntılı çalışmalar yapılarak daha faydalı sonuçlara ulaşılabilir.

BTH uygulamalarının biyoaktif madde içeriğinde iyileştirici yönde bir etkisi tespit edilmemesine rağmen depolama sürecinde oluşan ağırlık kaybında azalmaya neden oldukları görülmüştür. Bu sonuç söz konusu gelişme düzenleyicinin kiraz meyvesinde depo performansını artırmaya yönelik olarak kullanılabilecek alternatif bir yöntem olabileceğini ortaya koymuştur.

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Araştırmacıların Katkı Oranı Beyan Özeti

Yazarlar makaleye eşit oranda katkı sağlamıştır.

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Makale yazarları aralarında herhangi bir çıkar çatışması yoktur.

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Comparison of Morphometric Traits of Pecan Cultivars

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ABSTRACT
Pecan is a major horticultural nut tree that originated in North America and, in Turkey, it is generally cultivated in the Aegean and Mediterranean regions. In this study, morphometric traits of twenty-one cultivars grown under equal ecological conditions in the Southeastern region of Turkey were determined. In addition, a multivariate analysis was performed on morphometric traits for determining suitable cultivars that show the best performance in these ecological conditions. The nut weight ranged from 3.66 g for 'Cherokee' to 10.35 g for the 'Shoshoni' cultivar. The 'Choctaw' had the highest kernel weight and kernel ratio ranging from 5.63 g to 58.60%. While the 'Cherokee' had the lowest ranging from 0.91 g to 22.84 g, respectively. A significant positive correlation between nut weight and kernel ratio was determined. The kernel weight was also highly correlated with nut weight and height. The use of clustering analysis according to Ward's method allowed the establishment of relationships between pecan cultivars by separating them into four main clusters. Also, these results were supported by principal component analysis, and 'Big Z' and 'Ideal' cultivars were determined to be the closest cultivars. Results showed that there are quantitative differences regarding the genetic property of pecan cultivars and more than half of the examined cultivars showed a good performance in terms of nut quality criteria that are crucial in marketing. Furthermore, the multivariate analysis was effective in the differentiation of pecan cultivars and it has been evaluated that it can be used successfully in future studies.

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Pikan Çeşitlerinin Morfometrik Özellikler Bakımından Karşılaştırılması

ÖZET
Pikan, aslen Kuzey Amerika orjinli olan ve Türkiye'de genellikle Ege ve Akdeniz bölgelerinde yetiştirilen önemli bir sert kabuklu meyve türüdür. Çalışmada, Türkiye'nin Güneydoğu bölgesinden aynı ekolojik koşullar altında yetiştirilen yirmi bir pikan çeşidinin morfometrik özellikleri belirlenmiştir. Ayrıca, bu ekolojik koşullarda en uygun çeşitlerin belirlenmesi için morfometrik özellikler üzerine çok değişkenli istatiksel analiz uygulanmıştır. Çeşitlerin meyve ağırlığı 3.66 g 'Cherokee' ile 10.35 g 'Shoshoni' arasında değişmiştir. En yüksek iç ağırlığı ve iç oranı 'Choctaw' çeşidinde (5.63 g ve %58.60) en düşük ise "Cherokee" çeşidinde (0.91 g ile 22.84 %) belirlenmiştir. Meyve ağırlığı ile iç oranı arasında anlamlı pozitif bir ilişki bulunmuştur. Ayrıca, iç ağırlığının meyve ağırlığı ve yüksekliği ile yüksek önemli ilişkili olduğu belirlenmiştir. Ward yöntemine göre yapılan kümeleme analizi, pikan çeşitleri arasında ilişkilerin kurulmasına izin vermiş ve çeşitleri dört ana kümeye ayırmıştır. Elde edilen sonuçlar temel bileşen analizi ile desteklenmiş ve çeşitlerden en yakın ilişkisi 'Big Z' ile 'Ideal' arasında belirlenmiştir. Sonuçlar pikan çeşitlerinin genetik özellikleri ile ilgili niceliksel farklılıkların olduğu ve incelenen çeşitlerin yarısından fazlasının

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Pican
Caryaillinoensis
Morfometrik özellikler
Çok değişkenli analiz

pazar tarafından arzu edilen meyve kalitesi açısından iyi bir performans sergilediğini göstermiştir. Ayrıca çok değişkenli analiz pikan çeşitlerinin ayrımında oldukça etkili olmuş ve ileriki çalışmalarda başarıyla kullanılabileceği değerlendirilmiştir

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INTRODUCTION

Pecan is a hard-shelled valuable fruit tree species that have a high economic value nut belonging to the *Juglandaceae* family, which also includes other tree and Carya varieties (Rosengarten, 1984). The pecan is a deciduous tree native to the North American continent, especially the USA and Mexico. Today, the USA and Mexico are the world's largest commercial pecan producers with about 75%, and 20% of world production, respectively (Hadigeorgalis et al., 2005; Venkatachalam et al., 2007; Thompson and Conner, 2012). Pecan is a popular fruit with a pleasant taste, and medicinal and nutritive properties, so the demand for it is increasing worldwide. Plantations are being established in many countries outside of North America such as; Australia (Wakeling et al., 2000), Argentina (Giuffré et al., 2011), and China (Zhang et al., 2015). The other important pecan-producing countries are Israel, South Africa, and Turkey).

Pecan is a healthy food that has always found an important place in food production and human nutrition the pecan is consumed fresh or in various food products such as pies and cakes as well as in salads and topping of desserts (Thompson and Conner, 2012). In addition to being edible, pecan oil is also used in cosmetics, medicine, paints, and essential oil production (Prasad, 1993; Duke, 2001). Pecan consists of 9-18% protein, which is lower compared to other nuts, and it consists of 14% carbohydrate depending on cultivars. Pecans are a fine source of dietary fiber, and they are also rich in terms of minerals including manganese, phosphorus, magnesium, zinc, iron, and naturally low in sodium (Prasad, 1993; Gebhardt and Thomas, 2002; USDA, 2004; Curiel-Maciel et al., 2021). Furthermore, pecans have high oil contents, about 65-70%, depending on cultivars, and the oil profile is mostly comprised of monounsaturated (commonly oleic acid) and polyunsaturated fatty acids (linoleic acid). Pecan consists of high antioxidants including vitamin B complex, beta-carotene, ellagic acid, lutein, and vitamin E (Rudrappa, 2016; Binici Akkuş et al., 2020; Özdemir et al., 2021; Curiel-Maciel et al., 2012).

Research shows that pecan has major beneficial effects on cholesterol and triglyceride levels. Moreover, regular pecan consumption affects the increase of HDL cholesterol and the reduction of triglycerides and apolipoprotein B levels. Pecan is considered cholesterol-free heart-healthy friendly food and can improve human blood fat profiles and low-density lipoprotein levels because of the high monounsaturated fatty acid contents (Ros, 2010).

The pecan was introduced from North America to Turkey and has been generally spread in Antalya and İzmir regions that have subtropical and temperate climates. Therefore, pecan nut production has been intensive throughout the coastal regions of the Mediterranean and Aegean in Turkey. Some important cultivars were planted in the Southeastern Anatolia Agricultural Research Institute collection orchard in Sanlıurfa province in 1992 to determine the performance of pecan cultivars in the South Eastern Anatolia region. The pecan is commercially cultivated in temperate, tropical, and subtropical regions of the world. Since pecan growing is limited in Turkey due to the restriction of climatic conditions, which is more suitable for walnut (Juglans regia) growing, there is no data available on pecan production in Turkey.

There are studies on the chemical compositions of pecan cultivars. But, to the best of our knowledge, there have not been studies on the morphometric properties of pecan cultivars grown under equal ecological conditions. With the present study, we aimed to compare the morphometric traits of twenty-one pecan cultivars that were grown in the same cultural practices and climatic conditions by performing multivariate analysis on the main nut characteristics preferred by the market. The results of this study will make a positive contribution to the producers in the region regarding the cultivation of pecan.

MATERIAL and METHOD

Plant material

In the study, morphometric traits of 21 pecan cultivars (Big Z, Harris Super, Hastings, Ideal, Kiowa, Mahan, MahanxStuart, Mohawk, Pawnee, Royal, Schley, Shawnee, Shoshoni, Texhan, Western, and Wichita) from Southeastern Anatolia Agricultural Research Institute collection orchard at 477 m altitude in Şanlıurfa province were evaluated

through two sequential years. The trees were twenty-three years old and planted out 12x12 m within and between row spacings. Plants were regularly fertigated and weed, pest, and disease control were performed properly. No nutritional deficiency was observed on trees and all cultural practices were done properly during the study.

Method

Twenty nuts from each cultivar were measured over two years. Morphometric characters including nut width (NW), nut length (NL), nut height (NH), and shell thickness (SC) were measured using a caliper with an accuracy of 0.1 mm. Nut weight (NWT) and kernel weight (KWT) were measured with an electronic scale with a 0.01 g precision. Frequency distribution of used characters such as shell color, kernel color, kernel ratio, shell thickness, and harvest date were determined according to UPOV criteria, Pecan Descriptors Bulletin (available at: https://www.upov.int/meetings/en/doc.details.jsp?meeting_id=35045 &doc_id=297728).

Ecological data of research area

Climatic information, monthly maximum temperature (°C), monthly average temperature (°C), monthly precipitation (kg/m^2), monthly average humidity (%), and monthly average wind speed (m/s) were received from the Turkish State Meteorological Service (MGM) and shown from 1st January to 31st December for two consecutive years (2015 and 2016).

Statistical Analysis

Descriptive statistics in the examined characters as the minimum (min), maximum (max), mean, standard error (SE), Duncan's multiple range test, and coefficient of variations (CV%) were determined by using the statistical package program of SPSS. One-way ANOVA and Duncan test were used to compare the cultivars in terms of characteristics and the statistical significance level was considered as 5% in the calculations. Moreover, the relationship among characteristics was determined by Pearson's correlation analysis utilized by the R Studio software and the 'corrplot' package (Wei et al., 2017). The principal component analysis (PCA) was conducted to clarify the relationships of features with each other being utilized by R Studio software and the package of 'ggplot2' (Wickham, 2016). Clustering analysis (CA) was utilized to create a dendrogram showing the hierarchical distribution of cultivars in terms of studied characteristics.

RESULTS and DISCUSSION

Ecological data of research area

The monthly average temperature showed a regular rise from January to July and a slight decrease was observed in August. The highest monthly temperature was monitored in July at 44.1 °C. The monthly average relative humidity and monthly precipitation occurred mostly in the winter months, and a decline was observed in June and July. Also, parallelism was determined between monthly precipitation and relative humidity. The monthly average wind speed was slightly higher in the spring months (March, April, May, and June) than thin e others. While no significant difference was observed in temperature data between years, the precipitation amount and distribution were dissimilar compared to the previous year (Figure 1).

Fruit yield and quality are closely related to environmental conditions. Very low and high temperatures, light quantity and quality, soil temperature, altitude, relative humidity, and wind speed have detrimental effects on fruit trees. Unsuitable environmental conditions cause problems in flowering and fruit sets. The areas with below 55% humidity and up to 150-200m altitudes, which have long hot summers, short and not very hard winters, and rare early or late frosts, are suitable for pecan growing (Dolgun et al., 2020). In the research area, summers are hot and dry, winters are warm and rainy, and humidity is lower than 55% during the vegetation period that is suitable for pecan growing. On the other hand, the altitude is a little high, which may cause appearances of late and early frosts.

Morphometric traits of cultivars

A high variation and significant ($P<0.05$) differences were observed between the morphometric traits of cultivars. These differences were due to genetics because cultural practices and ecological conditions were equal for all cultivars. The morphometric traits that are important for consumers were determined. Shell color, nut dimensions (width, length, and height), nut weight, kernel weight, and color traits in twenty-one pecan cultivars are presented in Tables 1 and 2. Shell color and kernel weight showed the highest CVs (43.27 and 40.48%, respectively) while nut length (10.68%) and nut width (12.92%) had the lowest CVs. NWT, KWT, KC, KR, ST, and HD had higher than 20.00% CVs (Table 1). The NW, NL, and NH had low CVs indicating that these may be considered more stable traits. Similar CVs in terms of morphometric traits were observed on other nuts such as walnut (Khadivi et al., 2015; Başak et al., 2022), chestnuts (Corona et al., 2021), hazelnut (Mohammad et al., 2014), and other fruits such as hawthorn (Muradoglu et al., 2021).

Nut size is more important for marketing that prefers large nuts for the succinct quality kernel. Generally, nut weight above 9.5 g is desirable for pecan (Wells and Corner, 2012), while smaller nuts than 8.0 g are

not preferred by the market. In the investigated cultivars, nut traits are shown in Table 2. The nut width varied between 19.43 and 27.30 mm. the largest nut width was determined in the 'Wichita' cultivar, while the 'Shoshoni' cultivar was the smallest one. The largest nut length was determined 50.84 mm by the 'Mahan' cultivar, and the smallest one 35.86 mm by the 'Cape Fear' cultivar. The nut height varied from 22.98 to 29.45 mm, 'Choctaw' cultivar was found the largest in section, and the smallest section was found on the 'Cherokee' cultivar.

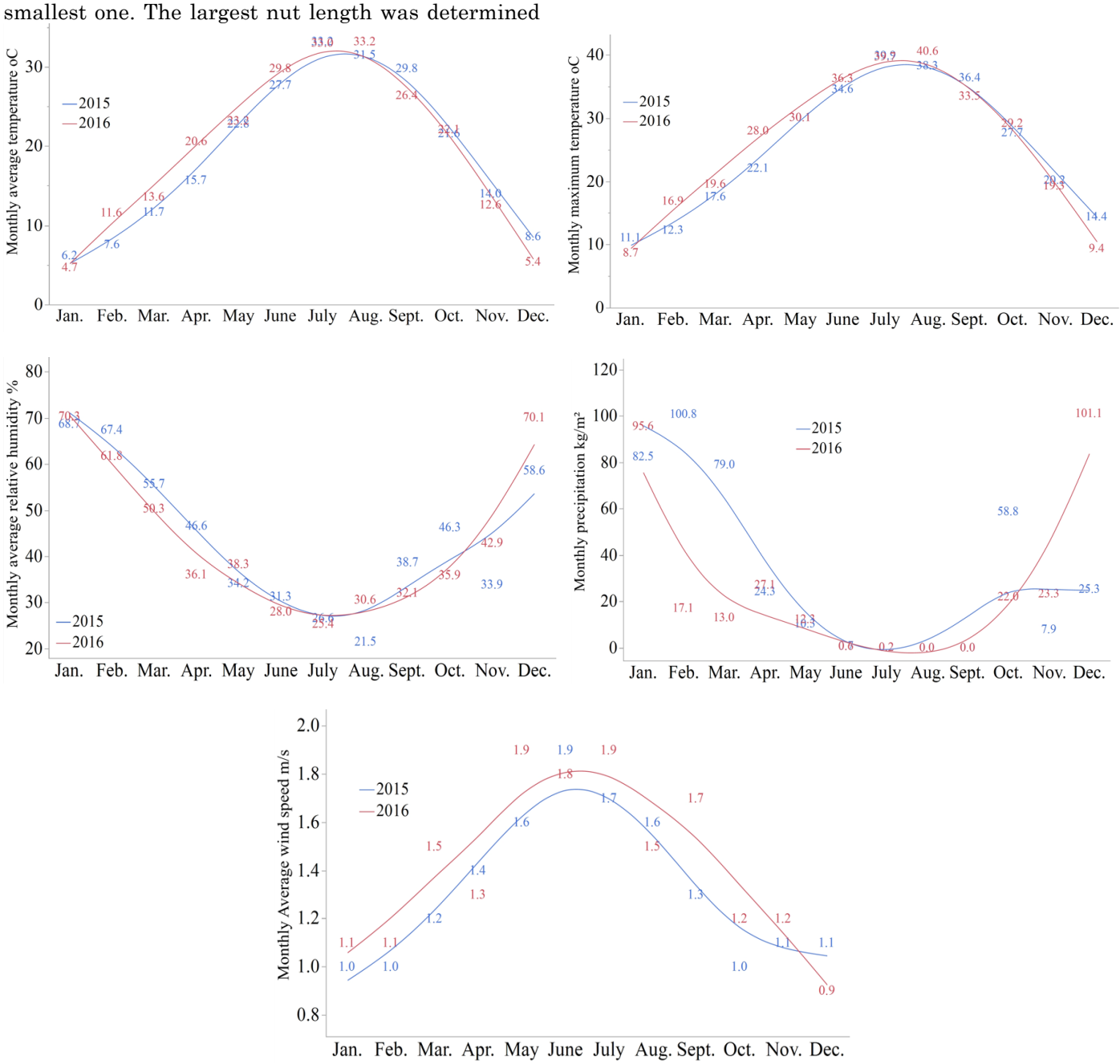

Figure 1. Meteorological data belonging to the research location
Resim 1. Araştırma alanına ait iklim verileri

The nut weight was between 3.66 and 10.35 g. The largest nut weight was measured in the 'Shoshoni' cultivar, while the 'Cherokee' cultivar was the smallest one. Seven cultivars were show good performance with 9 g and above kernel weight which was a preferable limit for markets. In addition, kernel weight was defined as 8 g and above in three cultivars, while ten cultivars were smaller than 8 g (Table 2). The nut weight and kernel ratio varied between 0.91 and 58.60 g, between 5.63 and 22.84% respectively, and for both the highest rate was calculated for 'Choctaw' while the lowest rate was for 'Cherokee'. Shell thickness varied from 0.61 mm 'Mahan' to 1.20 mm 'Shoshoni'. Kernel quality is relative to kernel ratio and desirable high kernel ratio of 50% and higher by customers. The 'Choctaw' and

'Western' cultivars yielded the highest kernel ratio, 58.60%, and 58.45%, respectively. However, the lowest kernel ratio (22.84%) was determined in 'Cherokee'. The high relationship between kernel weight and kernel ratio is a well-known phenomenon in nut crops (Khadivi et al., 2019). The regression plot illustrated in Figure 2 proves a significant positive association (r=0.80, P<0.0001) between kernel weight and kernel ratio was observed a significant positive association.

Table 1. İllustrative statistics for morphometric characters in the pecan cultivars.
Çizelge 1. Pikan çeşitlerinde morphometrik karekterlerin istatiksel tanımlanması.

Traits	Abbrev	Unit/code	Minimum	Maximum	Mean	SE	CV
Nut width	NW	mm	15.93	30.11	23.20	0.15	12.92
Nut length	NL	mm	25.51	54.42	40.77	0.21	10.68
Nut height	NH	mm	18.44	36.29	26.71	0.18	13.79
Nut weight	NWT	g	2.24	13.72	8.11	0.12	29.26
Kernel weight	KWT	g	0.35	8.51	4.17	0.08	40.48
Kernel color	KC	Code	1.00	3.00	2.10	0.02	20.35
Kernel ratio	KR	%	9.77	82.28	49.57	0.55	22.53
Shell color	SC	Code	1.00	4.00	1.95	0.04	43.27
Shell thickness	ST	mm	0.41	1.67	1.00	0.01	24.85
Harvest date	HD	Date	1.00	4.00	3.10	0.04	28.07
			Frequencies (%) *Frekanslar (%)*				
Kernel color	KC	Code	Light (4.76)	Medium (80.95)	Dark (14.29)		
Shell color	SC	Code	Extra light (28.57)	Light (57.14)	Medium (4.76)	Dark (9.52)	
Kernel ratio	KR	%	Small (47.61)	Medium (14.28)	Large (38.09)		
Shell thickness	ST	mm	Thin (19.04)	Medium (47.61)	Thick (33.33)		
Harvest date	HT	Date	Very early (9.52)	Early (4.76)	Medium (52.34)	Late (33.33)	

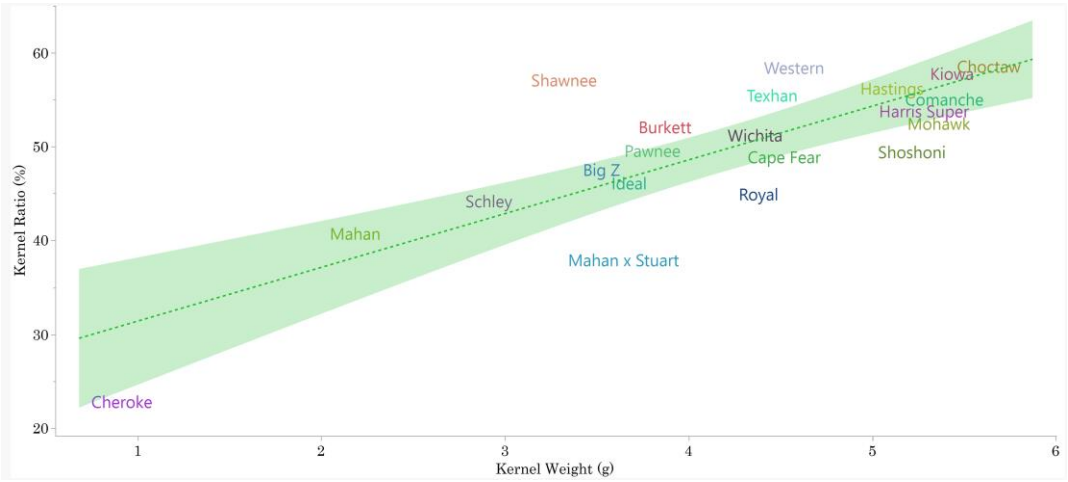

Figure 2. Regression plot for kernel ratio and kernel weight in pecan cultivars.
Resim 2. Pikan çeşitlerinde iç oranı ve iç ağırlığına ait regresyon grafiği.

Nut and kernel color are major parameters for pecan cultivars and desirable kernel color is 'light' for marketing and breeding value. In addition, previous works showed that the oxidation of polyphenols and fatty acids contents is relatively within the color sensory of walnut (Muradoglu and Balta, 2010; Fuentealba et al., 2017). A total of 6 cultivars (28.57%) had 'extra light', 12 cultivars had 'light', 1 cultivar 'medium', and only two cultivars had 'dark' skin color. The kernel color of 1 cultivar (4.76%) was 'light', 17 (80.95%) was 'medium', and 3 (14.29%) was 'dark'.

The cultivars were suitable for marketing in terms of the skin at a rate of 84.17% rate and kernel color at 38.09%. (Tables 1 and 2). The earliest harvested cultivars were 'Royal' and 'Wichita' followed by another early cultivar 'Shoshoni'. Seven cultivars were determined late in terms of harvest time. The average harvest date for very early in this region was 10 October and for the late cultivars was 15 November. Similar results were reported by Badyal and Upadhayay (2004) who reported nut length, width, weight, kernel weight, and kernel percentage varied from 31.6 to 54.0 mm, from 23.2 to 34.0 mm, from 3.59 to 5.71 g, from 2.10 to 3.90 g, from 8.30 to 55.2%, respectively. In another study conducted on 5

pecan cultivars, nut traits were investigated and reported as 5.42-10.84 g for nut weight, 34.51-51.83 mm for nut length, 21.99-26.18 mm for nut width, 23.20-25.40 mm for nut height, 2.42-6.10 g for kernel weight, and 0.58-1.10 mm for shell thickness. (Binici Akkuş et al., 2020). Similarly, nut weight and kernel ratio of pecan cultivars were reported as 3.98-8.40 g and 43.55-59.28% by Reid and Hunt (2000) and 0.8-6.9 g and 41.1-62.3% by Poletto et al., 2020.

Studied cultivars showed major diversity because of genetic factors. Also, morphometric traits of studied pecan cultivars were in close relationship with previous findings, but minor differences related to cultivars factors, edaphoclimatic conditions, different locations, maturity stages, and cultural practices have existed.

Table 2: Some nut traits of investigated Pecan cultivars.
Çizelge 2. İncelenen pikan çeşitlerinin bazı meyve özellikleri.

Cultivars	NW (mm)	NL (mm)	NH (mm)	NWT (g)	KWT (g)	ST (mm)	KR (%)	SC	KC	HD
Big Z	23.29±0.28^{fg}	40.93±0.41^{eh}	26.62±0.73^{be}	7.38±0.20^{fg}	3.52±0.12^{hj}	1.03±0.02^{be}	47.58±0.63^{eh}	L**	M	Late
Burkett	19.62±0.18^{j}	44.21±0.61^{c}	25.83±1.01^{be}	7.32±0.26^{fg}	3.87±0.25^{fi}	1.02±0.06^{bf}	52.12±1.94^{cf}	EL	M	Medium
Cape Fear	25.41±0.23^{bd}	35.86±0.73^{lm}	26.03±0.52^{be}	9.25±0.20^{ab}	4.52±0.12^{cg}	1.19±0.05^{a}	48.94±0.90^{eh}	L	M	Late
Cherokee	21.59±0.31^{hı}	37.16±0.47^{kl}	22.98±0.36^{f}	3.66±0.23^{j}	0.91±0.161^{l}	1.07±0.04^{ad}	22.84±2.48^{k}	EL	D	Late
Choctaw	26.23±0.21^{ab}	41.58±0.48^{df}	29.45±0.61^{a}	9.51±0.48^{ab}	5.63±0.35^{a}	1.02±0.03^{bf}	58.60±0.86^{a}	L	M	Medium
Comanche	26.56±0.40^{ab}	39.36±0.43^{hj}	29.43±0.55^{b}	9.76±0.39^{ab}	5.39±0.24^{ac}	1.15±0.05^{ac}	55.06±0.74^{ad}	M	M	Medium
Harris Super	21.21±0.68^{hı}	39.55±1.16^{fj}	23.31±0.40^{f}	9.71±0.34^{ab}	5.28±0.26^{ad}	0.88±0.02^{fg}	53.82±1.54^{ae}	D	M	Medium
Hastings	24.92±0.45^{ce}	39.72±0.41^{fj}	28.52±0.65^{ab}	8.95±0.34^{bd}	5.11±0.28^{ae}	0.90±0.03^{eg}	56.23±1.35^{ad}	L	M	Medium
Ideal	22.11±0.23^{gh}	40.78±0.60^{ei}	27.27±0.89^{ad}	7.98±0.29^{cf}	3.67±0.15^{fj}	1.16±0.03^{ab}	46.15±1.26^{eh}	L	M	Late
Kiowa	22.01±0.75^{h}	41.36±0.75^{dg}	27.71±1.05^{ac}	9.14±0.51^{ac}	5.43±0.42^{ab}	0.95±0.05^{dg}	57.81±1.84^{ab}	L	M	Medium
Mahan	22.48±0.27^{gh}	50.84±0.51^{a}	26.92±0.83^{be}	5.15±0.24^{i}	2.19±0.21^{k}	0.61±0.03^{h}	40.77±2.44^{ij}	L	M	Late
MahanxStuart	23.80±0.71^{ef}	43.25±0.76^{cd}	28.20±0.46^{ac}	8.64±0.78^{be}	3.65±0.50^{gj}	1.15±0.05^{ab}	37.94±2.92^{j}	L	D	Late
Mohawk	26.01±0.35^{bc}	37.94±0.36^{jk}	27.76±0.61^{ac}	10.18±0.30^{a}	5.36±0.19^{ac}	0.92±0.02^{eg}	52.49±0.62^{bf}	D	L	Late
Pawnee	25.31±0.30^{bd}	41.99±0.63^{de}	26.93±1.11^{be}	7.57±0.34^{eg}	3.80±0.28^{fi}	0.94±0.05^{dg}	49.59±2.62^{eh}	L	M	Medium
Royal	24.58±0.28^{de}	38.92±0.46^{ik}	27.89±0.88^{ac}	9.70±0.20^{ab}	4.38±0.16^{eh}	1.11±0.05^{ac}	44.95±1.17^{hı}	EL	M	Very early
Schley	24.09±0.24^{ef}	34.88±0.28^{m}	24.64±0.42^{ef}	6.44±0.32^{gh}	2.91±0.22^{jk}	0.99±0.06^{cf}	44.23±1.57^{hı}	L	M	Medium
Shawnee	19.44±0.25j	38.96±0.52^{ik}	24.98±0.63^{df}	5.73±0.25^{hı}	3.32±0.21^{ij}	0.82±0.03^{g}	57.11±1.70^{ac}	EL	M	Medium
Shoshoni	27.30±0.37^{a}	38.23±0.72^{jk}	27.92±0.36^{ac}	10.35±0.46^{a}	5.22±0.34^{ae}	1.20±0.04^{a}	49.46±1.52^{eh}	L	M	Early
Texhan	20.48±0.16^{ij}	46.23±0.37^{b}	26.88±1.13^{be}	7.94±0.28^{df}	4.45±0.25^{dg}	0.91±0.04^{eg}	55.49±1.84^{ad}	EL	M	Medium
Western	21.28±0.17^{hı}	42.35±0.47^{de}	25.15±0.92^{df}	7.81±0.22^{df}	4.57±0.16^{be}	0.89±0.05^{eg}	58.45±1.55^{a}	E	M	Medium
Wichita	19.43±0.71^{j}	41.90±0.68^{de}	26.35±0.58^{be}	8.07±0.61^{cf}	4.36±0.47^{eh}	1.00±0.06^{bf}	51.24±2.04^{dg}	L	D	Very early
Average	23.20±0.14	40.76±0.21	26.70±0.17	8.11±0.11	4.17±0.08	1.00±0.01	49.56±0.54	SC	KC	HD

*Different letters in lines indicate significantly different values at $p \leq 0.05$. NW: nut width, NL: nut length, NH: nut height, NWT: nut weight, KWT: kernel weight, ST: shell thickness, KR: kernel ratio, SC: shell color, KC: kernel color, HD: harvest date. **L: light, EL: extra light, M: medium, D: dark.

The correlation and distribution of the morphometric traits were exhibited with the scatterplot matrix (Figure 3). Morphometric traits showed a highly significant and highly positive correlation (P<0.0001) between NH and NW, NH, and NL (P<0.001). KWT had highly significant and positive correlations (P<0.0001) with NH, NWT, and KR. Nut weight exhibited a highly significant and positive correlation with Nut height, nut weight, as well as KR and NW (P<0.01) exhibited significant positive correlations. Furthermore, significant negative correlations were identified between ST and NL (P<0.001) and KR and NW (P<0.01). Previously, Poggetti et al. (2017) reported negative relationships between nut weight and kernel ratio and nut ratio and shell thickness, while there were positive correlations with kernel weight, shell thickness, and kernel skin color in walnut. Similar results were reported by Guler et al. (2020) in rosehip observing positive relationships between fruit width, fruit weight, and fruit shape index.

The principal component analysis (PCA) separated the pecan cultivars according to their morphometric traits. Nine principal components were significant (P<0.001), and four components explained 75.44% of the total variance (Figure 4). The first component (PC1) constituted 32.5 % of the total variance and was positively correlated with nut width and weight, kernel weight, and ratio while negatively correlated to kernel color. The second component (PC2) constituted 16.5 % of the total variance and was mostly defined by nut height, shell thickness, and negative kernel ratio. The remaining components also contained other variables and explained less variability (24.5 % of total variance). PCA is performed to identify the main factors and parameters that are discriminant among studied traits (Khadivi et al., 2015). PCA was successfully conducted to determine diversity among different fruit species or genotypes such as walnut (Özcan et al., 2020; Başak et al., 2022), hawthorn (Muradoglu et al., 2021), and rosehip (Güler et al., 2021).

The couples of the highest similarities among the cultivars were identified by the Hierarchical cluster analysis and presented in Figure 5. The highest similarity was between 'Big Z' and 'Ideal', while the farthest distance was between 'Big Z' and 'Burkett'.

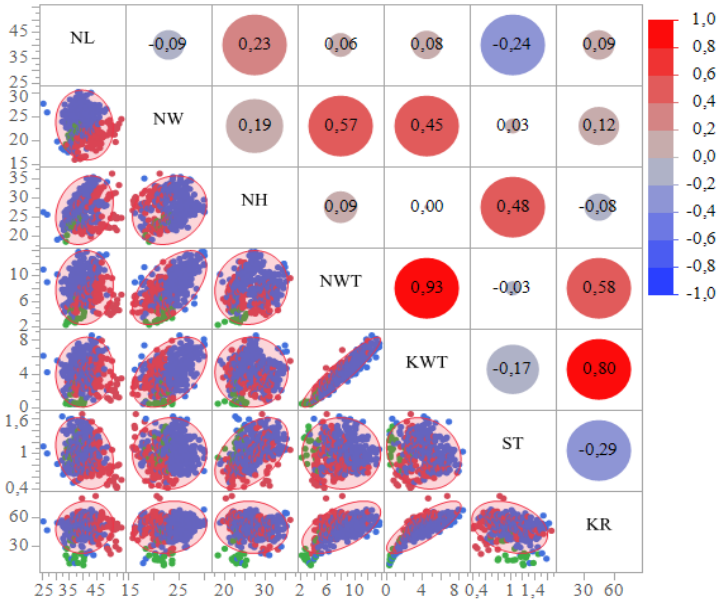

Figure 3. Scatterplot matrix demonstrating morphological traits pairwise. The color gradient (red to blue) and the size of the circles show the amount and significance of correlations.

Resim 3. Morfolojik özellikleri gösteren dağılım grafiği matrisi. Renk gradyanı (kırmızıdan maviye) ve dairelerin boyutu, korelasyonların miktarını ve önemini göstermektedir.

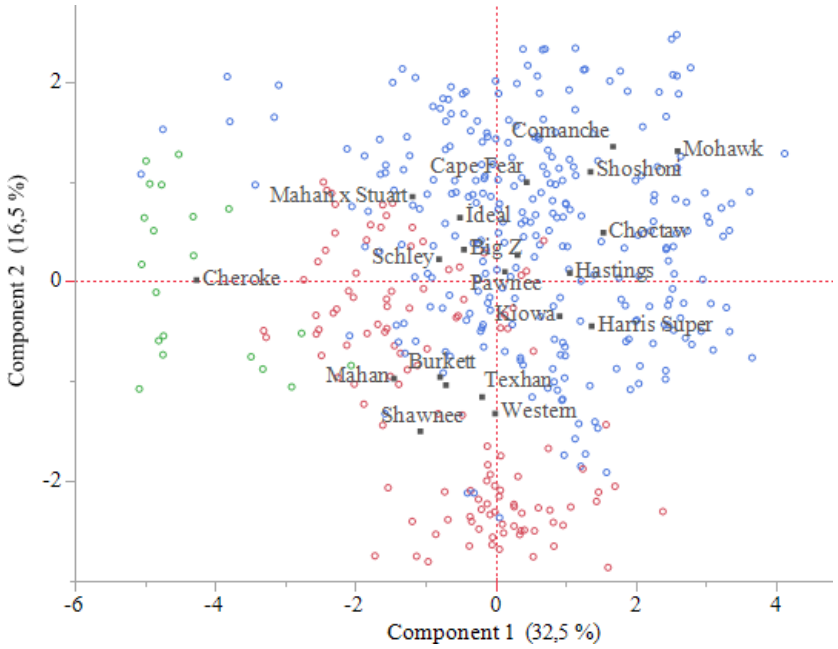

Figure 4. Distribution of pecan cultivars according to morphometric traits.

Resim 4. Pikan çeşitlerinin morfometrik özelliklerine göre dağılımı.

The cultivars were clustered into four main groups and the first group consisted of two subgroups with six cultivars. The first subgroup consisted of five cultivars 'Big Z', 'Ideal', 'Pawnee', 'Cape Fear', and 'Schley' while the second subgroup consisted of one cultivar, 'MahanxStuard'. The first cluster was characterized by low HD values. The second main group is comprised of eight cultivars in two subgroups. The first subgroup was containing 'Choctaw', 'Hasting', 'Kiowa', Comanche', Shoshoni', and 'Royal' cultivars. The second subgroup was formed by 'Harris Super' and 'Mohawk' cultivars. The second cluster was mostly characterized by, SC, NW, NH, KWT, NWT, and KR. Cluster three included 'Burket', Texhan', Western', 'Wichita', and 'Mahan' cultivars characterized by NL. The 'Cherokee' cultivar formed the fourth group by itself demonstrating low values of KC and HD traits. The HC and the PCA showed a similarity in terms of grouping cultivars according to morphometric traits. correlation

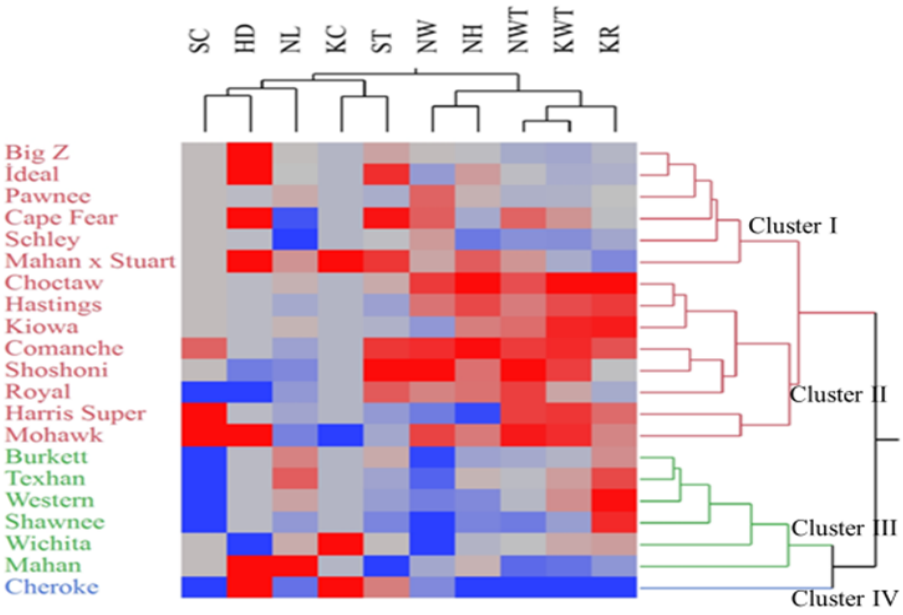

Figure 5. Hierarchical clustering and heatmap analysis based on the morphological characters studied for the twenty-one pecan cultivars. The highest and lowest morphological traits measurements belong to red and blue colors, respectively.

Şekil 5. Yirmi bir pikan çeşidi için incelenen morfolojik karakterlere dayalı hiyerarşik kümeleme ve ısı haritası analizi. En yüksek ve en düşük morfolojik özellik ölçümleri sırasıyla kırmızı ve mavi renkle gösterilmiştir.

CONCLUSION

This study, which was carried out on 21 pecan cultivars in Şanlıurfa province in southeastern Anatolia, revealed great variability in morphometric traits. In the study, only morphometric traits that could be reasonably observed were monitored. Traits that are valuable for marketing and future breeding programs such as nut appearance, skin color, nut weight, kernel color, kernel weight, kernel ratio, and harvest date were examined by Multivariate analysis. In this study, 'Choctaw', 'Hasting', 'Kiowa', 'Comanche', 'Harris Super', 'Mohawk', 'Shoshoni', 'Western', and 'Texhan' cultivars showed ideal values of nut size and kernel quality that are desirable in marketing. Considering these quality criteria, these cultivars are suggestable for cultivation in this region.

The results will contribute to the cultivation of pecan both in Şanlıurfa and other provinces of the Southeastern Anatolia Region. Moreover, the results will encourage growers to establish new orchards in the region. In addition, variations among cultivars and relationships of morphometric traits that were revealed by multivariate analysis can be used in future breeding programs.

Statement of Conflict of Interest

The authors declare that they have contributed equally to the article.

Author's Contributions

The authors of the article declare that there is no conflict of interest between them.

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Influence of Different Vermicompost Doses on Growth, Quality and Element Contents in Curly Lettuce (*Lactuca sativa* L. var. *crispa*)

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ABSTRACT
This study was conducted to determine the effects of different vermicompost doses on plant growth, quality properties and element contents in curly lettuce (*Lactuca sativa* L. var. *crispa*). The research was carried out in pots under plastic greenhouse conditions in Karabük province. In the study, 6 applications as 4 different doses of vermicompost (V), chemical fertilizer (CF) and control were examined. The applications were as follows: 1) Control (100% soil), 2) V1 (97.5% soil + 2.5% vermicompost, w/w), 3) V2 (95% soil + 5% vermicompost, w/w), 4) V3 (90% soil + 10% vermicompost, w/w), 5) V4 (80% soil + 20% vermicompost, w/w) and 6) CF (100% soil + chemical fertilizer). The experiment was established in completely randomized design with 3 replications. According to the research findings, vermicompost applications significantly increased plant height, plant fresh weight, plant dry weight, number of marketable leaves, chlorophyll, nitrogen, phosphorus, potassium, magnesium, calcium, sodium, iron, copper, and zinc contents of the plant compared with the control. When compared to the control, V1 application increased plant fresh weight by 13.25% and phosphorus content by 44.07%; V2 application increased potassium content by 24.29%; V3 application increased plant height by 13.77%, chlorophyll content by 22.30% and nitrogen content by 53.23%; V4 application increased magnesium content by 48.46%, calcium content by 14.36% and zinc content by 16.19%. It was detected that vermicompost had positive effects on plant growth, quality properties and element contents in curly lettuce. As a result, vermicompost can be used successfully as an alternative organic fertilizer for sustainable agriculture in curly lettuce cultivation.

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Quality properties
Elements

Farklı Vermikompost Dozlarının Kıvırcık Marulda (*Lactuca sativa* L. var. *crispa*) Büyüme, Kalite ve Element İçerikleri Üzerine Etkisi

ÖZET
Bu çalışma, farklı vermikompost dozlarının kıvırcık marulda (*Lactuca sativa* L. var. *crispa*) bitki gelişimi, kalite özellikleri ve element içerikleri üzerine etkilerini belirlemek amacıyla yapılmıştır. Araştırma, Karabük ilinde plastik sera koşullarında saksılarda yürütülmüştür. Çalışmada, vermikompostun (V) 4 farklı dozu, kimyasal gübre (CF) ve kontrol olmak üzere 6 uygulama incelenmiştir. Uygulamalar aşağıdaki gibidir: 1) Kontrol (%100 toprak), 2) V1 (%97.5 toprak + %2.5 vermikompost, w/w), 3) V2 (%95 toprak + %5 vermikompost, w/w), 4) V3 (%90 toprak + %10 vermikompost, w/w), 5) V4 (%80 toprak + %20 vermikompost, w/w) ve 6) CF (%100 toprak + kimyasal gübre). Çalışma tesadüf parselleri deneme desenine göre 3 tekerrürlü olarak kurulmuştur. Araştırma bulgularına göre, vermikompost uygulamaları kontrole göre bitki boyu, bitki yaş ağırlığı, bitki kuru ağırlığı, pazarlanabilir yaprak sayısı, klorofil, azot, fosfor, potasyum, magnezyum, kalsiyum, sodyum, demir, bakır ve çinko içeriğini önemli ölçüde artırmıştır.

Bahçe Bitkileri

Araştırma Makalesi

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Anahtar Kelimeler
Lactuca sativa
Organik gübre
Bitki gelişimi
Kalite özellikleri
Elementler

Kontrol ile karşılaştırıldığında, V1 uygulaması bitki yaş ağırlığını %13.25 ve fosfor içeriğini %44.07 oranında; V2 uygulaması potasyum içeriğini %24.29 oranında; V3 uygulaması bitki boyunu %13.77, klorofil içeriğini %22.30 ve azot içeriğini %53.23 oranında; V4 uygulaması magnezyum içeriğini %48.46, kalsiyum içeriğini %14.36 ve çinko içeriğini %16.19 oranında artırmıştır. Vermikompostun kıvırcık marulda bitki gelişimi, kalite özellikleri ve element içerikleri üzerine olumlu etkilerinin olduğu saptanmıştır. Sonuç olarak, vermikompost kıvırcık marul yetiştiriciliğinde sürdürülebilir tarım için alternatif bir organik gübre olarak başarılı bir şekilde kullanılabilir.

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INTRODUCTION

Fertilization is one of the most important factors determining yield and quality in agricultural production. Nowadays, it is known that human health and sustainability of soils are adversely affected because of excessive and unconscious chemical fertilization to obtain high yield from vegetables. On the other hand, it is reported that continuous use of chemical (inorganic) fertilizers causes pollution of soil and water, soil salinity, decline in organic matter content of soil, heavy metal accumulation, decrease in microbial activities of soil, nitrate accumulation, decrease in soil fertility, poor product quality and serious health problems (Savcı, 2012). For this reason, recently, organic fertilizers have gained great importance as an alternative to chemical fertilizers used in agricultural production all over the world with the new understanding focused on ensuring sustainability in agriculture due to the adverse effects of chemical fertilizers.

Vermicompost is the most popular among organic fertilizers in recent years due to its positive effects on plant growth, yield and soil properties. Additionally, vermicompost is intensely applied as a reliable, economical, and sustainable method in the processing and evaluation of solid organic wastes, which has become a major environmental problem (Shetinina et al., 2019). Vermicompost, which is obtained by composting organic wastes during digestion by earthworms, is an organic product with high economic value (Karmakar et al., 2012). It is also referred to as biohumus (Shetinina et al., 2019). The production and use of vermicompost have been increasing in recent years. Vermicompost is a type of fertilizer that contributes to the use of organic wastes and their recycling. Many organic wastes (plant wastes, animal manure, food wastes, urban solid waste, waste paper, sawdust, etc.) can be used in vermicompost production (Karmakar et al., 2012). Vermicompost is a well stabilized, finely divided peat-like material produced through a non-thermophilic process involving the biodegradation and stabilization of organic materials by interactions between earthworms and microorganisms. Vermicompost can be directly applied to the soil without any other treatment. Vermicompost has very high porosity, aeration, drainage, water holding capacity and microbial activity, and a low C:N ratio (Kumar and Topal, 2015). Vermicompost is very rich in macro and micronutrients, beneficial soil microorganisms, various enzymes, vitamins, humic acid, organic matter, and growth hormones (Özkan et al., 2016). Vermicompost is a highly nutritive organic fertilizer rich in nitrogen (N), phosphorus (P) and potassium (K). In addition, 97% of the plant nutrients (especially N, P and K) in the vermicompost are in forms that are readily taken up by the plants. Vermicompost has a vast surface area, providing strong absorbability and retention of nutrients. Vermicompost may influence plant growth directly via the supply of plant growth regulating substances (PGRs). Vermicompost has a wide range of uses as organic fertilizer and soil conditioner in both organic and conventional agriculture because of its advantageous properties like promoting plant growth, increasing the physical, chemical, and biological properties of soil, restoring and improving natural fertility of soil without polluting the environment (Shetinina et al., 2019). Vermicompost is a good and ideal organic fertilizer that reduces environmental pollution and restricts the use of chemical fertilizers for sustainable agriculture. Therefore, vermicompost is considered as a promising alternative to inorganic fertilizers used in agriculture and growing media used in the greenhouse (Mahmoud and Gad, 2020). Vermicompost plays a major role in improving growth and yield of different field crops, vegetables, fruits, and flowers. It was determined that growth, yield and quality of many vegetables increased with the application of vermicompost (Kashem et al., 2015; Degwale, 2016;

Durak et al., 2017; Adiloğlu et al., 2018; Kenea and Gedamu, 2018; Rekha et al., 2018). Vermicompost has a significant positive influence on seed germination, seedling vigor, plant growth, flowering, fruiting, root development, leaf length, number of leaves, colour, shelf-life and quality of vegetables (Peyvast et al., 2008). Vermicompost can also suppress plant diseases, pests, and plant parasitic nematodes. In addition, it was reported that vermicompost improved the physical, chemical, and biological properties of the soil and increased the nutrient content of the soil (Özkan et al., 2016).

As in the whole world, chemical fertilizers are mostly used in fertilization to increase the yield in vegetable growing in Turkey. To provide a sustainable agriculture system that protects human health, environment, and natural resources in the long term in vegetable growing where the use of chemical fertilizers is excessive, it is necessary to expand the use of organic fertilizers such as vermicompost instead of chemical fertilizers. The use of vermicompost fertilizer in vegetable growing is an agricultural activity that has become widespread in Turkey with the increase in environmental awareness and the adoption of high-quality product consumption in recent years, though it is widely used in many countries for many years. In addition, many agricultural wastes in Turkey are either incinerated or thrown away. The use of vermicompost is one of the best ways to evaluate agricultural wastes in the country. For this reason, it is of great importance to produce vermicompost, to promote the use, to conduct studies on its benefits and to share the obtained results with the producers in Turkey.

Lettuce (*Lactuca sativa* L.) belongs to the Compositae family and is an annual winter vegetable. Lettuce, which has been cultivated and consumed fondly for many years in the world, can be found in markets throughout the year. Lettuce, whose fresh leaves are used as vegetable, is among the species of high commercial importance. It is one of the most produced and consumed winter vegetables in Turkey and it has high economic value. It can be grown in the open field or greenhouse conditions using different cultivars all year round almost all over the country. The vegetation period of lettuce is short, and it can be grown in 2-3 months. In Turkey, lettuce was grown in an area of 21821 ha with a production of 520151 tons in 2020 (TÜİK, 2021). Lettuce develops rapidly in soils rich in organic matter and comes to harvest maturity in a short time. As with other vegetables, lettuce is usually grown using inorganic fertilizers. On the other hand, fertilization in lettuce must be performed carefully because it is a leafy vegetable and consumed uncooked. Lettuce is quite sensitive to N fertilization. However, excessive and unconsciously used chemical N fertilizers increase the nitrate accumulation in the plant, which is harmful to human health. Lettuce is one of the vegetables with the highest nitrate accumulation. Therefore, the use of organic fertilizers in lettuce cultivation should be expanded.

It is very important to examine the effects of vermicompost on soil and plant productivity to increase yield and quality, improve the physical and chemical structure of the soil and prevent environmental pollution in lettuce cultivation. Although there are many studies showing the effects of vermicompost on plant growth and yield, it has been found that these effects are not general or constant and show great variability. The variability in the effects of vermicompost may depend on the cultivation conditions, plant species and variety, physical, chemical, and biological properties of vermicompost, earthworm species used and age of vermicompost (Warman and AngLopez, 2010). Generally, it is known that vermicompost positively affects the soil properties, plant growth and health. However, there are not enough academic studies about how much the most suitable dose should be for which vegetable. Accordingly, knowledge and experience of producers on this issue is lacking. In this context, determining of optimum vermicompost doses for plant growth, quality and nutrient content of lettuce is necessary, and it will provide important contributions to further studies.

The objective of this study was to investigate the effects of different vermicompost doses on growth parameters, quality properties and element contents of curly lettuce. In addition, a comparison of vermicompost with chemical fertilizer was made.

MATERIAL and METHOD

The research was carried out in pots under plastic greenhouse conditions in Karabük province of Turkey (lat. 41°07' N, long. 32°41' E, alt. 311 m) during the autumn growing season of 2017. Curly lettuce (*Lactuca sativa* L. var. *crispa* cv. Maritima) was used as plant material in the study.

The soil used in the experiment was clay-loam, pH value of 7.4, organic matter content of 2.73%, lime ratio of 13.16% and EC value of 0.67 dS m^{-1}. The N, P and K contents of the soil were 0.16%, 7.70 mg kg^{-1} and 385.00 mg kg^{-1}, respectively. The vermicompost used in the experiment was pH value of 6.9, organic matter content of 20%, EC value of 3.7 dS $^{m-1}$, N content of 1.20%, P content of 1.09% and K content of 3.51%.

In the study, 6 applications as 4 different doses of vermicompost (V), chemical fertilizer (CF) and control were examined. The applications were as follows: 1) Control (100% soil), 2) V1 (97.5% soil + 2.5% vermicompost, w/w), 3) V2 (95% soil + 5%

vermicompost, w/w), 4) V3 (90% soil + 10% vermicompost, w/w) 5) V4 (80% soil + 20% vermicompost, w/w) and 6) CF (100% soil + chemical fertilizer). The experiment was performed in completely randomized design with 3 replications. There were 5 pots in each replication and 15 pots in each application. In total, 90 pots (6 x 3 x 5) were used, and 90 plants were grown in the greenhouse. In CF application, ammonium sulphate, triple superphosphate and potassium sulphate fertilizers as commercial fertilizer were applied at 15 kg N da^{-1}, 10 kg P_2O_5 da^{-1} and 15 kg K_2O da^{-1}. All of P and K fertilizers and half of the N fertilizer were given at the time of planting, while the other half of the N fertilizer was given two weeks after planting. No fertilizer was added to the control application.

The curly lettuce seedlings were grown in plastic greenhouse according to standard procedures. The curly lettuce seeds were sown in multi-pot plastic trays consist of 45 pots (4 × 4 × 4 cm), containing a mixture of peat and perlite (3:1, v/v) on 17 August, 2017. To grow plants, each plastic pot (26 x 24 x 17 cm) was filled with 5 kg of growing medium prepared with different doses of soil, vermicompost or chemical fertilizer. Twenty seven days after sowing, seedlings were transplanted into pots at the 4-5 true leaf stage on 13 September, 2017. Life water was given immediately after planting the seedling. All necessary cultural practices were carried out regularly until harvest. No plant protection product was used during the experiment. The plants were harvested 53 days after planting on 5 November, 2017. During the experiment, the temperature and relative humidity values in the greenhouse were recorded using a temperature and humidity recorder (Onset HOBO UX100-003 Data Logger, USA) (Figure 1). Necessary measurements and analyzes of the harvested plants were made in the laboratory of Bolu Abant Izzet Baysal University, Faculty of Agriculture, Department of Horticulture.

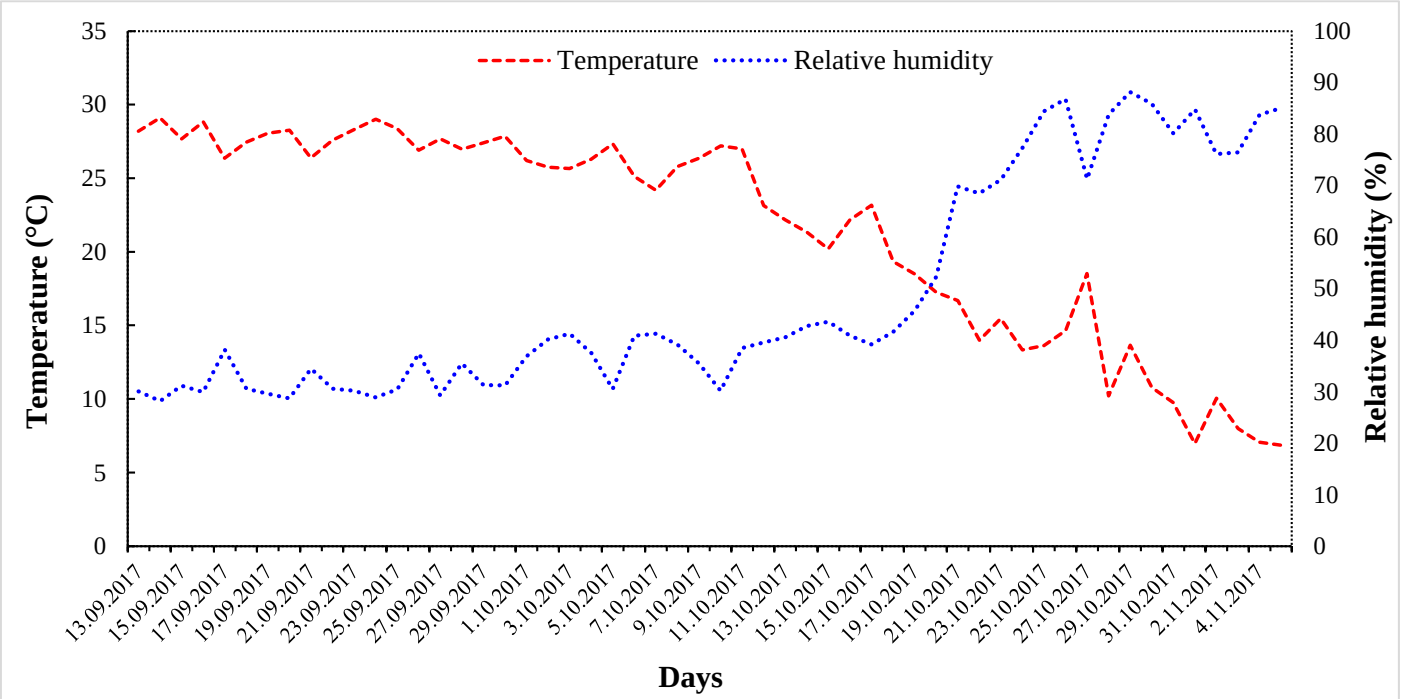

Figure 1. Temperature and relative humidity values measured in the greenhouse during the experiment
Şekil 1. Deneme süresince sera içerisinde ölçülen sıcaklık ve nispi nem değerleri

In this study, 23 properties related to plant growth and quality and 24 elements were determined. Plant height (cm), plant diameter (cm), root length (cm), leaf length (cm) and leaf width (cm) were determined by measuring with a ruler. Plant fresh weight (g), root fresh weight (g) and discard leaf weight (g) were detected by weighing with a precision balance. Plant dry weight (g) and root dry weight (g) were determined by weighing with a precision balance the samples after drying in an oven at 65 °C until they reach a constant weight. The number of marketable leaves (number $plant^{-1}$) was determined by counting the marketable leaves and the number of discard leaves was detected by counting the discard leaves. The root collar diameter (mm) was measured with a digital caliper. The dry matter content (%) was determined by using the procedures of AOAC (1990). The pH values of the samples were measured using a digital pH meter (Thermo Scientific, Orion Star A111). Total soluble solid content (%) was measured with a hand-held refractometer (ATC-1, Atago, Japan). The colour properties of the leaves (L*, a*, b*, C* and h°) were measured using a colorimeter (3NH NR60CP). The chlorophyll content of the leaves (spad)

was determined with a chlorophyll meter (Apogee Chlorophyll Concentration Meter, MC-100). The nitrate and P contents of the samples (mg kg^{-1}) were determined using UV-visible spectrophotometer (UV-1800, Shimadzu, Japan). The N and sulphur (S) contents of the samples (%) were detected using Elemental Analyzer CHNS-O (Thermo Scientific, Flash 2000). To determine element (aluminum-Al, arsenic-As, barium-Ba, boron-B, cadmium-Cd, calcium-Ca, chrome-Cr, cobalt-Co, copper-Cu, iron-Fe, lead-Pb, magnesium-Mg, manganese-Mn, mercury-Hg, nickel-Ni, potassium-K, selenium-Se, sodium-Na, thallium-Tl, tinnen-Sn and zinc-Zn) contents of the samples (mg kg^{-1}), dried samples were firstly ground by using a grinder (MC23200, Siemens, Germany) and then prepared for analysis according to the microwave digestion method. Element contents of the samples were detected using inductively coupled plasma-mass spectrometry ICP-MS X Series (Thermo Scientific, UK).

All chemical analyses were performed with three replications. Measurements for morphological properties carried out with ten replications. Mean values and standard errors were calculated, and the data were expressed as mean ± standard error. Data obtained in the study were subjected to analysis of variance (ANOVA) using SPSS statistical software (Version 23.0). Differences among means were evaluated by Duncan's multiple range test and the significance was accepted at $P < 0.05$ level.

RESULTS and DISCUSSION

Effects of the applications evaluated in the study on plant height, plant diameter, plant fresh weight and plant dry weight in curly lettuce are given in Table 1. The difference among the applications in terms of plant height, plant diameter and plant fresh weight was significant at the $P < 0.05$ level, while the difference among the applications in terms of plant dry weight was significant at the $P < 0.01$ level. Plant height, plant diameter, plant fresh weight and plant dry weight depending on the applications ranged from 19.02 to 21.64 cm, 27.14 to 30.64 cm, 187.46 to 235.11 g and 12.67 to 17.97 g, respectively. Among the applications, the highest plant height was determined in V3 application, and it was followed by CF, V4, V2 and V1 applications. The highest plant diameter was found in CF application closely followed by V4 application. Plant fresh weight is the most important yield parameter in curly lettuce. CF and V1 applications which there was no statistically significant difference between them possessed the highest plant fresh weight. In parallel with plant fresh weight, the highest plant dry weight was also observed in CF application followed by V3 and V1 applications. However, the lowest values in terms of plant properties mentioned above were observed in the control. In the present study, the higher values in terms of plant height, plant fresh weight and plant dry weight were obtained from all vermicompost applications (V1, V2, V3 and V4) in comparison with the control application. When compared to the control, V3 application increased plant height by 13.77% and plant dry weight by 32.68%. In addition, it was determined that V1 application increased plant fresh weight by 13.25% compared with the control (Table 1).

Table 1. Effect of different applications on plant height, plant diameter, plant fresh weight and plant dry weight in curly lettuce

Çizelge 1. Kıcırcık marulda farklı uygulamaların bitki boyu, bitki çapı, bitki yaş ağırlığı ve bitki kuru ağırlığı üzerine etkisi

Application	Plant height (cm)	Plant diameter(cm)	Plant fresh weight(g)	Plant dry weight(g)
Control	19.02±0.46b*	27.14±0.46b*	187.46±10.66b*	12.67±1.36d**
V1	19.41±0.59ab	27.44±0.90b	212.30±11.88ab	16.05±0.74abc
V2	19.47±1.54ab	26.73±0.68b	194.47±13.14b	14.64±0.81bcd
V3	21.64±0.96a	28.08±1.14b	199.84±9.60b	16.81±0.74ab
V4	20.05±0.69ab	28.64±0.93ab	194.46±7.54b	13.45±0.76cd
CF	21.46±0.35ab	30.64±0.44a	235.11±14.49a	17.97±0.89a

Means followed by different letters within the same columns are statistically different according to Duncan's multiple range test. *: significant at $P < 0.05$, **: Significant at $P < 0.01$.

Similar to the results in this study, Hernandez et al. (2010) investigated effect of vermicompost and compost on lettuce production and the highest plant fresh weight was determined in inorganic fertilizer. In another study, Durak et al. (2017) stated that yield and growth parameters in lettuce were improved by vermicompost application when compared to control and conventional fertilization. Likewise, Özkan and Müftüoğlu (2016) investigated the effects of different doses of vermicompost on lettuce and reported that vermicompost increased plant growth and yield. In the study conducted by Adiloğlu et al. (2018) on lettuce, significant increases in vermicompost applications were found in terms of plant height, plant diameter and plant fresh weight as compared with the control, which was agreed with the findings in this study.

According to Table 2, there were significant

differences ($P < 0.05$) in terms of root length and root collar diameter among the applications. On the other hand, no statistically significant difference was found among the applications in terms of root fresh weight and root dry weight. In the control application, the root length was found to be the highest with 12.41 cm, and it was followed by V4 and V1 applications which were not statistically different. However, the lowest values for root length was detected in CF, V3 and V2 applications. Root collar diameter varied from 12.74 (V4 application) to 16.79 mm (V3 application) depending on the applications (Table 2).

In a study conducted in lettuce, there was no significant difference in terms of root length among control and vermicompost applications (Adiloğlu et al., 2018). Getnet and Raja (2013) reported that vermicompost applications increased root length compared to control in cabbage, which was not compatible with the results of this study. In the study carried out by Özkan et al. (2016) in spinach, the root fresh weight in vermicompost applications was found to be significantly higher than the control, and the root fresh weight increased as the amount of vermicompost increased. Likewise, Kashem et al. (2015) reported that vermicompost applications significantly increased root dry weight as compared with the control in tomato. In another study, it was determined that vermicompost applications significantly increased root length, root fresh and dry weight compared to the control in eggplant (Kumari et al., 2017).

Table 2. Effect of different applications on root length, root collar diameter, root fresh weight and root dry weight in curly lettuce

Çizelge 2. Kıcırcık marulda farklı uygulamaların kök uzunluğu, kök boğazı çapı, kök yaş ağırlığı ve kök kuru ağırlığı üzerine etkisi

Application	Root length (cm)	Root collar diameter (mm)	Root fresh weight (g)	Root dry weight (g)
Control	12.41±0.52a*	15.31±1.21ab*	17.04±1.63^{ns}	4.65±0.79^{ns}
V1	11.70±0.30ab	16.12±1.53a	20.36±1.17	3.97±0.68
V2	11.29±0.43b	15.44±1.53ab	16.83±1.15	3.31±0.43
V3	11.24±0.32b	16.79±1.52a	16.28±0.99	3.57±0.14
V4	11.84±0.34ab	12.74±1.50b	17.44±0.89	3.10±0.28
CF	10.80±0.23b	14.30±1.38ab	17.50±2.07	4.25±0.71

Means followed by different letters within the same columns are statistically different according to Duncan's multiple range test. *: significant at $P < 0.05$, ns: non-significant.

The analysis of variance showed that there were significant differences ($P < 0.05$) among the applications in terms of all leaf properties examined except for leaf width and number of discard leaves. Among the applications, the highest leaf length was determined in CF application with 18.08 cm, and it was closely followed by V1, V3, control and V2 applications which were statistically in the same group with CF. However, the lowest leaf length was observed in V4 application with 16.62 cm. As in leaf length, the highest number of marketable leaves was found in CF application (25.58) followed by V1, V4, V3 and V2 applications, while the lowest number of marketable leaves was recorded in control application (21.67). Discard leaf weight ranged from 9.84 (V1 application) to 19.47 g (CF application). The lower values in terms of discard leaf weight were obtained from the vermicompost applications in comparison with control and CF. The lower number and weight of discard leaves is very important in terms of market value and quality in lettuce. With respect to number of marketable leaves, the higher values were obtained from vermicompost applications than the control application. It was determined that V1 application increased number of marketable leaves by 12.28% compared to the control (Table 3).

It was found that vermicompost applications increased leaf length, leaf width and number of leaves as compared with the control in lettuce and leaf width also increased as the dose of vermicompost increased (Özkan and Müftüoğlu, 2016). Similarly, it was reported that vermicompost applications significantly increased leaf length, leaf width and number of leaves compared to the control in cabbage (Getnet and Raja, 2013), spinach (Özkan et al., 2016) and lettuce (Adiloğlu et al., 2018). Kenea and Gedamu (2018) found that vermicompost applications increased leaf length in comparison with control in garlic. Rekha et al. (2018) reported that vermicompost application increased number of leaves compared to the control in pepper. In addition to, it was reported that the number of leaves increased as the dose of vermicompost increased in tomato (Kashem et al., 2015) and garlic (Degwale, 2016).

Significant differences ($P < 0.01$) were found among the applications with regards to chlorophyll content, dry matter content, total soluble solid content and nitrate content. However, no statistically significant difference was found among the applications in terms of pH value. The chlorophyll content in curly lettuce plants belonging to different applications varied from 19.69 to 24.08 spad. The highest values with regard to chlorophyll content were found in V3, V2, V4 and V1 applications, while the lowest value were observed in

the control application. The maximum and minimum dry matter contents were obtained from V3 (8.45%) and V1 (6.51%) applications, respectively. When the total soluble solid content is examined, the highest values were found in control, V3 and V2 applications (3.10, 3.08 and 2.98%, respectively). Whereas, the lowest total soluble solid content was detected in V1 application (2.39%). There was a wide range variation in nitrate content of the plants. The nitrate content varied between 516.78 and 2694.33 mg kg^{-1} depending on the applications. In the current study, V3, V2, V4 and CF applications had the highest nitrate contents, and they were statistically in the same group. Conversely, the lowest nitrate contents were observed in control and V1 applications. The nitrate content in the control were considerably lower than those of other applications. It was determined that vermicompost applications generally provide an increase in chlorophyll content when compared to control and CF. In the present study, V3 application increased chlorophyll content by 22.30% compared with the control (Table 4).

Table 3. Effect of different applications on leaf length, leaf width, number of marketable leaves, number of discard leaves and discard leaf weight in curly lettuce

Çizelge 3. Kıcırcık marulda farklı uygulamaların yaprak uzunluğu, yaprak genişliği, pazarlanabilir yaprak sayısı, ıskarta yaprak sayısı ve ıskarta yaprak ağırlığı üzerine etkisi

Application	Leaf length (cm)	Leaf width (cm)	Number of marketable leaves (number $plant^{-1}$)	Number of discard leaves (number $plant^{-1}$)	Discard leaf weight (g)
Control	17.04±0.38ab*	17.72±0.42^{ns}	21.67±0.53b*	2.83±0.51^{ns}	16.78±3.33ab*
V1	17.29±0.43ab	18.35±0.32	24.33±1.30ab	2.58±0.38	9.84±1.59c
V2	16.98±0.53ab	18.09±0.63	22.75±0.87ab	2.67±0.26	14.07±1.57abc
V3	17.16±0.41ab	17.98±0.40	23.58±0.82ab	3.42±0.43	16.16±1.71abc
V4	16.62±0.45b	17.81±0.34	24.17±1.30ab	2.42±0.38	10.70±2.10bc
CF	18.08±0.41a	18.41±0.46	25.58±1.03a	3.00±0.28	19.47±1.96a

Means followed by different letters within the same columns are statistically different according to Duncan's multiple range test. *: significant at $P < 0.05$, ns: non-significant.

Table 4. Effect of different applications on chlorophyll content, dry matter content, pH, total soluble solid content and nitrate content in curly lettuce

Çizelge 4. Kıcırcık marulda farklı uygulamaların klorofil içeriği, kuru madde içeriği, pH, suda çözünebilir kuru madde miktarı ve nitrat içeriği üzerine etkisi

Application	Chlorophyll content (spad)	Dry matter content (%)	pH	Total soluble solid content (%)	Nitrate content (mg kg^{-1})
Control	19.69±0.87c**	7.77±0.37ab**	5.99±0.02^{ns}	3.10±0.17a**	516.78±60.96b**
V1	21.68±0.61abc	6.51±0.30c	6.00±0.02	2.39±0.07c	1189.89±266.35b
V2	23.10±0.64ab	7.39±0.15b	6.01±0.02	2.98±0.04ab	2210.44±129.55a
V3	24.08±0.58a	8.45±0.48a	6.05±0.02	3.08±0.21a	2694.33±239.43a
V4	22.61±1.17ab	6.93±0.12bc	6.00±0.02	2.66±0.12bc	2130.89±178.86a
CF	20.67±0.85bc	7.37±0.27b	6.06±0.03	2.70±0.06bc	2054.56±437.44a

Means followed by different letters within the same columns are statistically different according to Duncan's multiple range test. **: Significant at $P < 0.01$, ns: non-significant.

In the study carried out by Baliah and Muthulakshmi (2017) in okra, the chlorophyll content in vermicompost applications was found to be higher than control, which was consistent with the findings in this study. Degwale (2016) determined that vermicompost increased dry matter content as compared with the control in garlic. Tavalı et al. (2014) reported that there was no significant difference in terms of pH value among control, chemical fertilization and vermicompost applications in cauliflower and cabbage, respectively, which was agreed with the findings in this study. Aminifard and Bayat (2016) observed that no significant difference was found for total soluble solids between control and vermicompost treatments in pepper. In the present study, the higher nitrate content detected in vermicompost applications compared to the control may be due to the high N content of vermicompost. In the study conducted by Vigardt (2012) on spinach, higher nitrate content in vermicompost application was found as compared with the control, and it was noted that the nitrate content also increased as the dose of vermicompost increased, which was consistent with the findings in this study. It was determined that the nitrate content determined in this study does not exceed the limit value reported for lettuce by the Türk Gıda Kodeksi (2008).

As seen in Table 5, effects of different applications on b* and C* (Chroma) colour values of curly lettuce were statistically significant ($P < 0.05$). On the other

hand, there was no statistically significant difference among the applications with respect to L*, a* and h° (Hue angle) colour values. The b* and C* colour values of the curly lettuce samples varied from 26.02 to 29.99 and 28.56 to 31.93, respectively. The higher values in terms of b* and C* colour properties were obtained from the control compared to other applications. On the contrary, the lowest b* and C* values were recorded in V1 application. An apparent effect of vermicompost applications on the colour properties of curly lettuce was not observed (Table 5).

Colour is one of the most important factors in terms of quality in lettuce. Similar to the results in this study, in the study carried out by Özen (2018) in lettuce, the effect of different organic materials (waste mushroom compost, leonardite and vermicompost) and their different doses on colour was found to be insignificant.

Table 5. Effect of different applications on colour properties of the leaves (L*, a*, b*, C* and h°) in curly lettuce
Çizelge 5. Kıcırcık marulda Marulda farklı uygulamaların yaprakların renk özellikleri (L, a*, b*, C* ve h°) üzerine etkisi*

Application	L*	a*	b*	C*	h°
Control	52.97±1.00^{ns}	-12.30±0.52^{ns}	29.99±0.91a*	31.93±0.94a*	112.29±0.42^{ns}
V1	53.36±1.27	-11.34±0.54	26.02±0.94b	28.56±1.02b	113.14±0.55
V2	52.70±1.53	-12.70±0.53	28.52±0.97ab	31.23±1.09ab	113.79±0.35
V3	51.64±1.24	-12.29±0.51	28.28±0.63ab	30.92±0.68ab	113.35±0.54
V4	53.03±1.70	-12.17±0.64	27.63±0.88ab	30.22±1.04ab	110.77±2.87
CF	53.83±1.23	-12.10±0.66	27.70±1.17ab	30.29±1.33ab	110.63±2.79

Means followed by different letters within the same columns are statistically different according to Duncan's multiple range test. *: significant at P < 0.05, ns: non-significant.

Effects of different applications on element contents of curly lettuce are shown in Table 6. The difference among the applications in terms of Al, Cd, Co, Cr, Cu, K, Mg, N, Na, Ni, P, S and Sn contents was significant at the P < 0.01 level, while the difference among the applications in terms of As, Ca, Fe, Mn, Pb and Zn contents was significant at the P < 0.05 level. On the other hand, no statistically significant difference was found among the applications in terms of B, Ba and Se contents. In addition to, it was determined that Hg and Tl contents in curly lettuce plants were not detected because of below the detection limits (Table 6).

In the present study, a considerable variation in terms of element contents was observed among the applications. K, Mg, Ca, P, Na, N and S contents depending on the applications varied from 18163.67 to 22574.78 mg kg^{-1}, 7846.83 to 11649.49 mg kg^{-1}, 4033.78 to 4880.33 mg kg^{-1}, 2253.33 to 3681.22 mg kg^{-1}, 1110.00 to 2910.00 mg kg^{-1}, 4.49 to 6.88% and 3.32 to 7.04%, respectively. Among the applications, the lowest values in terms of K, Mg, P, Na and N were observed in the control. The highest K content was obtained from all vermicompost applications (V1, V2, V3 and V4) and CF which were statistically in the same group. The plants in V4 application possessed the highest Mg content. Likewise, the highest Ca content was also found in V4 application, and it was closely followed by V3 application. The highest values regarding P and S contents were recorded in CF application. The highest N content was observed in V3 application followed by V1 application. Regarding Na content, the highest values were determined in V4, V3 and V2 applications which were not statistically different. The lowest values for Ca content were observed in V1, control, V2 and CF applications. V1 and V2 applications had the lowest S content. It was determined that vermicompost applications generally increased essential mineral contents such as K, Mg, Ca, P, Na and N in the plant compared with the control. When compared to the control, V4 application increased Mg content by 48.46%, Ca content by 14.36% and Na content by 162.16%. It was determined that V1 application increased P content by 44.07% compared to the control. V2 application increased K content by 24.29% as compared with the control. In addition to, it was found that V3 application increased N content by 53.23% compared to the control. On the contrary, the lower values in terms of S content were obtained from vermicompost applications in comparison with control and CF. In applications containing vermicompost (V1, V2, V3 and V4), Ca and Na contents increased as the dose of vermicompost increased (Table 6).

Fe, Zn, Mn, Cu and Se contents depending on the applications varied from 703.89 to 848.45 mg kg^{-1}, 49.11 to 79.79 mg kg^{-1}, 60.30 to 70.66 mg kg^{-1}, 14.53 to 21.88 mg kg^{-1} and 0.20 to 0.27 mg kg^{-1}, respectively. When the Fe content was examined, it was determined that there was no statistically significant difference among V4, CF, V3, V2 and control applications and they had the highest Fe content. However, the lowest Fe content was observed in V1 application. The highest Zn content was obtained in V4, V3, control, V1 and V2 applications which were not statistically different. Conversely, Zn content in CF application was found to be considerably lower than those of other applications examined in the study. Mn content was the highest in V3 application followed by CF, V4 and control applications, while it

was the lowest in V1 and V2 applications. The highest values in terms of Cu content were determined in vermicompost applications (V1, V2, V3 and V4) which were statistically in the same group, whereas the lowest Cu content was observed in the control. In applications containing vermicompost (V1, V2, V3 and V4), Fe content increased as the dose of vermicompost increased. Cu content in vermicompost applications was found to be higher than control and chemical fertilizer (CF). When compared to the control, V4 application increased Zn content by 16.19% and Fe content by 8.72%. V3 application increased Mn content by 8.77% as compared with the control. Additionally, V1 application increased Cu content by 50.58% compared to the control (Table 6).

Table 6. Effect of different applications on element contents in curly lettuce
Çizelge 6. Kıcırcık marulda farklı uygulamaların element içerikleri üzerine etkisi

Application	Al(mg kg^{-1})	As(mg kg^{-1})	B(mg kg^{-1})	Ba(mg kg^{-1})	Ca(mg kg^{-1})
Control	355.08±26.07bc**	0.41±0.02b*	59.35±2.32^{ns}	27.95±1.84^{ns}	4267.56±215.17b*
V1	311.89±15.74cd	0.39±0.03b	59.88±4.65	25.21±2.13	4033.78±89.90b
V2	365.69±49.54bc	0.40±0.03b	60.29±4.19	28.12±2.27	4303.89±286.71b
V3	268.14±45.36d	0.47±0.02ab	58.45±2.37	27.84±2.36	4514.78±151.20ab
V4	381.46±16.46b	0.49±0.02a	59.36±1.93	27.32±1.24	4880.33±125.59a
CF	454.22±19.51a	0.44±0.04ab	63.96±2.50	25.03±1.71	4322.33±127.79b
Application	Cd (mg kg^{-1})	Co (mg kg^{-1})	Cr (mg kg^{-1})	Cu (mg kg^{-1})	Fe (mg kg^{-1})
Control	0.63±0.02a**	0.34±0.01a**	1.90±0.16b**	14.53±0.32c**	780.41±51.50ab*
V1	0.47±0.03b	0.23±0.01d	1.87±0.04b	21.88±1.53a	703.89±11.92b
V2	0.51±0.05b	0.26±0.02cd	1.91±0.22b	19.01±0.99ab	788.06±61.35ab
V3	0.56±0.02ab	0.30±0.02abc	2.12±0.08b	19.05±0.84ab	798.69±46.87a
V4	0.55±0.03ab	0.29±0.01bc	2.25±0.12b	19.88±0.81ab	848.45±23.98a
CF	0.62±0.04a	0.31±0.01ab	3.02±0.48a	17.68±0.89b	844.06±10.89a
Application	Hg (mg kg^{-1})	K (mg kg^{-1})	Mg (mg kg^{-1})	Mn (mg kg^{-1})	N (%)
Control	nd	18163.67±503.14b**	7846.83±441.82d**	64.96±2.44ab*	4.49±0.04e**
V1	nd	21379.11±478.36a	9233.55±266.72bc	60.30±1.72b	6.86±0.03ab
V2	nd	22574.78±474.96a	8670.50±217.08cd	61.83±3.41b	6.77±0.05b
V3	nd	22023.56±902.81a	10116.68±234.49b	70.66±4.09a	6.88±0.03a
V4	nd	22073.67±556.00a	11649.49±655.51a	66.91±2.39ab	5.15±0.03d
CF	nd	21078.11±760.67a	8730.34±227.44cd	68.05±1.24ab	5.79±0.03c
Application	Na (mg kg^{-1})	Ni (mg kg^{-1})	P (mg kg^{-1})	Pb (mg kg^{-1})	S (%)
Control	1110.00±155.44d**	4.95±0.85a**	2253.33±9.37e**	2.39±0.09a*	5.39±0.12b**
V1	1839.56±140.20bc	2.94±0.51b	3246.33±9.90b	1.82±0.22b	3.33±0.03e
V2	2229.44±183.17ab	3.07±0.60b	2525.11±52.93c	2.19±0.23ab	3.32±0.02e
V3	2414.33±276.03ab	2.81±0.27b	2400.78±13.58d	2.43±0.10a	4.33±0.02c
V4	2910.00±388.83a	3.25±0.22b	2545.78±9.30c	2.09±0.05ab	3.53±0.04d
CF	1386.33±137.75cd	4.95±0.32a	3681.22±7.60a	2.09±0.14ab	7.04±0.06a
Application	Se (mg kg^{-1})	Sn (mg kg^{-1})	Tl (mg kg^{-1})	Zn (mg kg^{-1})	
Control	0.26±0.03^{ns}	0.94±0.14b**	nd	68.67±10.48ab*	
V1	0.22±0.01	1.27±0.27b	nd	68.46±5.26ab	
V2	0.20±0.01	0.65±0.02b	nd	59.67±5.44ab	
V3	0.21±0.02	0.86±0.10b	nd	76.04±12.78a	
V4	0.25±0.03	2.05±0.69b	nd	79.79±5.11a	
CF	0.27±0.02	5.57±1.86a	nd	49.11±2.60b	

Means followed by different letters within the same columns are statistically different according to Duncan's multiple range test. *: Significant at P < 0.05, **: Significant at P < 0.01, ns: non-significant, nd: not detected.

The Al contents of plants showed a wide range of variation from 268.14 to 454.22 mg kg-1. The minimum and maximum values for Al were determined in V3 and CF applications, respectively. The highest As content was found in V4, V3 and CF applications which were not statistically different, whereas the lowest As content was determined in V1, V2 and control applications. Cd content was found to between 0.47 (V1 application) and 0.63 mg kg^{-1} (control). Co content was in the range from 0.23 (V1 application) to 0.34 mg kg^{-1} (control). In the current study, Sn and Cr contents in the CF application (5.57 and 3.02 mg kg^{-1}, respectively) were considerably higher than those of other applications examined in the study. On the other hand, Sn and Cr contents were determined at the lowest level in control, V1, V2, V3 and V4 applications. The highest Ni content was found in control and chemical fertilizer (CF) applications with 4.95 mg kg^{-1}. On the contrary, Ni content was the lowest in vermicompost applications

(V1, V2, V3 and V4). The lowest value in terms of Pb content was obtained in V1 application, though the highest values were observed in V3, control, V2, V4 and CF applications which were not statistically different. In applications containing vermicompost (V1, V2, V3 and V4), As and Cr contents also increased as the dose of vermicompost increased. The Cd, Co, and Ni contents in vermicompost applications were found to be lower than control and CF. Additionally, Al, Cr and Sn contents in vermicompost applications were lower than that of chemical fertilizer (Table 6). This is very important considering that Cd, Co, Ni, Al, As, Pb, Cr and Sn are heavy metals.

It is thought that the K (3.51%), N (1.20%) and P (1.09%) contents of vermicompost used in the experiment is effective in the increase K, N and P contents of plant in vermicompost applications compared to the control in this study. Similar to the results in this study, Hernandez et al. (2010) reported that the highest Ca, Mg, Fe, Cu, and Mn contents in lettuce were determined in vermicompost among vermicompost, compost and inorganic fertilizer applications. In addition to, highest P content in the plant was found in inorganic fertilizer, which was also compatible with the results of this study. Durak et al. (2017) determined that Mg, P, Fe, Mn, Zn and Cu contents in vermicompost applications were higher than the control in lettuce. Researchers also reported that K, Ca, Fe and Mn contents in vermicompost applications were higher than the conventional fertilization. In another study carried out in lettuce, there was no significant difference in terms of K, Mg, P, Ca and N contents among control and vermicompost applications (Adiloğlu et al., 2018). In the study conducted by Özen (2018) on lettuce, it was determined that Na and Fe contents increased as the dose of vermicompost increased, which was compatible with the results of this study. In the previous studies, it was determined that vermicompost applications increased K, Ca, Mg, N, P, Fe, Mn, Zn, and Cu contents in the plant compared to the control in different vegetables (Peyvast et al., 2008; Tavalı et al., 2014). Tavalı et al. (2014) found that there was no significant difference in terms of Fe, Zn and Cu contents among control, chemical fertilizer and vermicompost applications in cabbage.

CONCLUSIONS

Nowadays, in parallel with the importance given to environment, the use of organic fertilizers such as vermicompost is becoming increasingly important. As in the whole world, the use of vermicompost in vegetable growing has been increasing in recent years in Turkey. According to findings obtained from the present study, the applications evaluated in the study significantly affected the plant growth, quality properties and element contents in curly lettuce. The results indicated that vermicompost applications generally increased plant height, plant fresh weight, plant dry weight, number of marketable leaves, chlorophyll, N, P, K, Mg, Ca, Na, Fe, Cu, and Zn contents of the plant compared to control application. In addition, as the dose of vermicompost increased in vermicompost applications, Ca, Na and Fe contents increased. In terms of heavy metals such as Cd, Co and Ni, vermicompost applications showed lower values than control and chemical fertilizer. Likewise, heavy metal contents such as Al, Cr and Sn in vermicompost applications were lower than that of chemical fertilizer. Consequently, all vermicompost applications (V1, V2, V3 and V4) in terms of plant height, number of marketable leaves, chlorophyll, K and Zn contents; V1 application in terms of plant fresh weight and P content; V1 and V3 applications in terms of plant dry weight and N content; V3 and V4 applications in terms of Ca and Mn contents; V4 application in terms of Mg content; V2, V3 and V4 applications in terms of Fe content were found to be the most successful vermicompost applications. It was concluded that vermicompost can be used successfully as an alternative organic fertilizer for curly lettuce cultivation. The results of this study will be beneficial in terms of raising the awareness of producers, reducing the use of chemical fertilizers in agricultural production, contributing to the widespread use of vermicompost, obtaining healthier and higher quality agricultural products in Turkey. Nevertheless, conducting of this study, which was carried out as a pot experiment in the greenhouse, under field conditions will reveal clearer results.

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Contribution Rate Declaration Summary of Researchers

The authors declare that they have contributed equally to the article.

Conflict of Interest Statement

The authors of the article declare that there is no conflict of interest between them.

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Şanlıurfa Koşullarında Yetişen Kültür ve Yabani Hünnap (*Ziziphus jujuba* Mill.) Genotiplerinin Meyve, Yaprak ve Stoma Özelliklerinin Belirlenmesi

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ÖZET
Bu araştırma, Güneydoğu Anadolu Bölgesi'nin kurak ve yarı-kurak ekolojik koşullarına sahip Şanlıurfa ilinde iki ayrı bahçede yetiştirilen hünnap (*Ziziphus jujuba* Mill.) genotiplerinin meyve, yaprak ve stoma özelliklerinin belirlenmesi amacıyla 2021 yılında yürütülmüştür. Araştırmada incelenen hünnap genotiplerinde meyve ağırlığı 2.78-20.28 g, meyve boyu 21.77-39.47 mm ve meyve eni 18.62-37.23 mm, yaprak alanı 3.11-7.01 cm², yaprak eni 1.31-2.65 cm ve yaprak boyu ise 3.29-5.09 cm arasında belirlenmiştir. Hünnap genotiplerinde stoma sayıları 323.30 adet mm^{-2} (kültür hünnap)-333.31 adet mm^{-2} (yabani hünnap), ortalama stoma boyu ve eni değerleri ise sırasıyla 27.22 (yabani hünnap)-28.00 µm (kültür hünnap) ve 18.21 (yabani hünnap)-20.05 µm (kültür hünnap) arasında tespit edilmiştir. Elde edilen bu sonuçlara göre daha küçük yaprak alanına sahip olan yabani hünnap genotipinin stoma sayısı, stoma eni ve boyu değerlerinin istatistiksel olarak kültür hünnap genotipinden farklılık göstermediği belirlenmiştir. Bu bakımdan, birim alandaki stoma yoğunluğunun diğer türlere göre daha fazla olması nedeniyle, hünnap bitkisinin kurak ve yarı-kurak ekolojilerde ağaçlandırma çalışmalarında başarılı olarak kullanılabileceği düşünülmektedir.

Bahçe Bitkileri

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Anahtar Kelimeler
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Ağaçlandırma
Yaprak alanı
Stoma sayısı
Stoma yoğunluğu

Determination of Fruit, Leaf and Stomata Characteristics of Culture and Wild Jujuba (*Ziziphus jujuba* Mill.) Genotypes Grown in Şanlıurfa Conditions

ABSTRACT
This research was carried out in 2021 to determine the fruit, leaf and stomata characteristics of jujube (*Ziziphus jujuba* Mill.) genotypes grown in two separate orchards in Şanlıurfa province, which has arid and semi-arid ecological conditions in the Southeastern Anatolia Region. In the jujube genotypes examined in the study; fruit weight was 2.78-20.28 g, fruit length was 21.77-39.47 mm, fruit width was 18.62-37.23 mm, leaf area was 3.11-7.01 cm², leaf width was 1.31-2.65 cm and leaf length was 3.29-5.09 cm. In the jujube genotypes, the number of stomata is 323.30 units mm^{-2} (culture jujube)-333.31 units mm^{-2} (wild jujube), mean stomatal length and width are 27.22 (wild jujube)-28.00 µm (culture jujube) and 18.21 (wild jujube)-20.05 µm (culture jujube). According to these results, it was determined that the number of stomata, stomatal width and length values of the wild jujube genotype, which has a smaller leaf area, did not differ statistically from the culture jujube genotype. In this respect, it is thought that the jujube plant can be used successfully in afforestation studies in arid and semi-arid ecologies, due to the fact that the stomatal density per unit area is higher than other species.

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GİRİŞ

Hünnap (*Ziziphus jujuba* Mill.); *Rhamnaceae* (Cehrigiller) familyasına ait 135'ten fazla türü bulunan sert çekirdekli meyvelere sahip bir bitkidir (Pandey ve ark., 2010). Hünnap, ilk olarak 7700 yıl önce Çin'de kültüre alınmış ve İpek Yolu aracılığıyla Hindistan, İran, Afganistan ve Orta Asya gibi dünyanın diğer bölgelerine yayılmıştır. Bazı hünnap türleri dünyada ılıman ile alt-tropikal iklim orijinli olup, doğal olarak kışları soğuk, yazları sıcak ve kuru iklimleri olan bölgelere uyum sağlamıştır (Tatari ve ark., 2016).

Z. jujuba; dünyanın çeşitli bölgelerinde Jujuba, Jujubier, Jujube, Juiba, Chinese date, Chinese jujube; Türkiyede ise Hünnap, Ünnap, Annep, Hinnâbi, İnnâbi, İğde, Çiğde ve Honaz iğdesi gibi yerel isimlerle adlandırılmaktadır. Yao (2012), Çin'de taze olarak tüketim, kurutma, şekerleme ve süs bitkisi dahil olmak üzere 700 ile 800 hünnap çeşidi bulunduğunu, kurutmaya uygun çeşitlerin, Çin'in hünnap üretiminin %90'ını oluşturduğunu belirtmiştir.

Hünnap bir ılıman iklim bitkisidir. Deniz seviyesinden 1700 m yükseklikte yetişmekte ve -20 ºC'ye kadar dayanmaktadır. Süzek ve verimli topraklarda aşırı yağışlara karşı dayanıklıdır (Ecevit ve ark., 2008; Kavas ve Dalkılıç, 2015). Rakımı 0-1500 m, yıllık ortalama sıcaklık kışın 7-13 °C ve yazın 37-48 °C, yıllık ortalama yağış miktarı 120-2200 mm olan bölgelerde, kumlu-tınlı, nötr veya hafif alkali topraklarda iyi yetişebilir (Anonim, 2017). Hünnap, su kıtlığı ve tuzluluk gibi bazı çevresel streslerin yanı sıra bazı zararlılara ve hastalıklara karşı oldukça toleranslıdır (Tatari ve ark., 2016).

Türkiyede Ege Bölgesi'nde Çanakkale ve Denizli; Akdeniz Bölgesi'nde Burdur, Isparta, Hatay ve Antalya; İç Anadolu Bölgesi'nde Kayseri; Marmara Bölgesi'nde ise Bursa illeri hünnap bitkisinin doğal yayılış alanlarını oluşturmaktadır (Karıncalı, 2003). Türkiyede, 2015 yılında 298 ton olan hünnap üretimi, 2020 yılında 1 229 tona çıkmıştır. Amasya (297 ton), Antalya (190 ton), Manisa (125 ton) ve Denizli (123 ton) illeri en çok hünnap üretimi yapılan illerdir (TÜİK, 2021).

Hünnap, morfolojik olarak dik ve tırmanıcı bir bitki olup, bitkiler ağaç ve çalı formunda, boyları 7-10 metreyi bulmaktadır. Bu türler içerisinde *Z. jujuba* ve *Z. mauritiana*'nın meyveleri tüketim için yaygın olarak yetiştirilmektedir. Üretilen meyveler çoğunlukla kurutulmuş ve taze olarak tüketilmektedir (Gao ve ark., 2003; Wang ve ark., 2016). Alternatif tıp üzerine eski bir Çin kitabı olan "Huangdi Neijing" adlı kitapta da belirtildiği gibi Çin'de şeftali, kayısı, erik ve armut gibi en değerli 5 meyveden biri de hünnap meyvesidir. Dünyadaki hünnap üretiminin yaklaşık %90'ını Çin karşılamaktadır (Li ve ark., 2005; Wang ve ark., 2016).

Hünnap meyvesinin Çin'de 4000 yılı aşkın süredir yetiştiği pek çok araştırıcı tarafından bildirilmiştir (Liu ve ark., 2003; Xue ve ark., 2009; Choi ve ark., 2011). Nitekim Liu ve ark. (2003), yapmış olduğu çalışmasında, Çin'in kuzey tarafında bulunan Situ bölgesinde hala 500-1300 yıllık hünnap ağaçlarının var olduğunu tespit etmiştir.

Hünnap bitkisi birçok avantaja sahiptir. Nitekim Liu ve Zhao (2009), hünnap bitkisinin dikim yılında ürün vermesi, özellikle C vitamini gibi zengin besin içeriği, birçok tüketim şekli, alternatif tıpta kullanımı, uzun çiçeklenme periyodu ve kuraklığa ve tuzluluğa yüksek toleransı gibi üstün avantajlara sahip olduğunu ifade etmişlerdir. Ayrıca; hünnap taze tüketiminin yanı sıra özellikle Uzak Doğu ülkelerinde daha çok kurutularak tüketilmektedir. Bunun yanında, yüksek besin içeriği ve biyo-fonksiyonel bileşimlerinden dolayı geleneksel tıpta ve gıda katkı maddesi olarak kullanılmaktadır (Pareek ve ark., 2002; Xue ve ark., 2009; Choi ve ark., 2011). Aynı zamanda hünnap meyveleri ekmek, kek, şeker, hoşaf ve reçel yapımında da kullanılmaktadır (Krška ve Mrshra, 2009). Bu özelliklerinden dolayı giderek popülerliği artmakta olan bir meyve türüdür (Liu ve Zhao, 2009). Bununla birlikte, hünnap meyveleri sağlığı geliştirici özelliklere sahiptir ve geleneksel Çin tıbbında detoksifikasyon, aneminin önlenmesi, analeptik, palyatif ve bağışıklık sistemini iyileştirmek için yaygın olarak kullanılmaktadır. Hünnap meyvesinin kabuğu ve meyve eti halk hekimliğinde öksürük ve soğuk algınlığının giderilmesi ve balgam söktürücü olarak kullanılmaktadır. Hünnap ağacının dal kabuğu şişkinlik önleyici, ishal önleyici ve kusma önleyici özellikler için kullanılırken, yaprakları çay olarak tüketilmekte ve antidiyabet de dahil olmak üzere soğuk algınlığı ve tıkanıklığı gidermek için kullanılmaktadır. Hünnap tohumlarının yatıştırıcı ve hipnotik etkileri bulunmaktadır (Siriamornpun ve ark., 2015).

Hünnap meyveleri, mineral maddeler bakımından oldukça zengindir. Yapılan bir çalışmada; hünnap meyvelerinin kalsiyum, potasyum, brom, rubidyum ve lantan elementleri bakımından zengin olduğu bulunmuştur (Zhumatov, 1996). Limondan yirmi kat daha fazla C vitaminine sahip olan hünnap meyvelerinin, vitamin B1 (tiamin) ve vitamin B2 (riboflavin) yönünden de oldukça zengin olduğu WHO tarafından onaylanmıştır (Kundi ve ark., 1989).

Dünyada tropik ve subtropik iklim koşulllarında yaygın olarak yayılış gösteren hünnap ağaçları, genel

olarak kurak koşullara uyum sağlamakta ve hatta şiddetli kuraklık koşullarında bile yeterli verim sağlamaktadır. Kuraklık stresine dayanıklı bir ağaç türü olması nedeniyle, dünyanın birçok kurak yöresinde yetişmektedir. Özellikle güçlü kök sistemleri vasıtasıyla toprak erozyonunu önleyebilme kabiliyetindedir. Diğer taraftan, sıcak ve kurak koşullar altında tuzlu topraklarda başarıyla yetişebilen, kurak bölgelerin dayanıklı bir ağacı olan hünnap, ışık isteği yüksek olan, daha çok kireççe zengin killi, kumlu, hafif alkali, asidik, drenajı iyi ve derin toprakları tercih eder (Meena ve ark., 2003; Anonim, 2017).

Türkiyede hünnap meyve türünün kültüre alınması veya yetiştirilmesi, hünnap çeşit ve genotipleri ile bu genotiplerinin meyve, yaprak ve diğer bitkisel özellikleri konusunda fazla sayıda bilimsel çalışma bulunmamaktadır. Bu çalışmada, Güneydoğu Anadolu Bölgesi'nin kurak ve yarı-kurak ekolojik koşullarında iki ayrı bahçede yetiştirilen hünnap genotiplerinin bazı meyve, yaprak ve stoma özellikleri belirlenerek, bunların karşılaştırılması amaçlanmıştır.

MATERYAL ve METOD

Materyal

Araştırma kapsamında materyal olarak, Şanlıurfa ilinde bulunan iki ayrı bahçede yetiştirilen 15-20 yaşlı hünnap (*Ziziphus jujuba* Mill.) ağaçlarından 2021 yılında alınan meyve ve yaprak örnekleri kullanılmıştır. Alınan meyve ve yaprak örneklerinin analiz ve incelemeleri Harran Üniversitesi, Ziraat Fakültesi, Bahçe Bitkileri Bölümü'ne ait laboratuvarlarda yapılmıştır.

Şanlıurfa Büyükşehir Belediyesi Park ve Bahçeler Müdürlüğü'ne ait meyve bahçesinde kapama bahçe olarak yetiştirilen hünnap ağaçlarının fidanları Ege Bölgesi'nden temin edilerek dikilmiştir. Yüksek boylu ağaçlara sahip, büyük yapraklı, daha az dikenli ve daha iri meyveli olan hünnap bitkileri, araştırmada "kültür hünnap" (Şekil 1.) olarak adlandırılmıştır. Öte yandan, Harran Üniversitesi Osmanbey Yerleşkesi Ar-Ge Meyve Bahçesi'nde, Denizli ilinden temin edilen küçük meyveli hünnap çekirdeklerinden yetiştirilen çöğürlerin dikilmesiyle oluşturulmuş bahçede yer alan hünnap ağaçları; daha kısa boylu, sıkı ve daha fazla dikenli dallara, küçük yaprak ve meyvelere sahip olup, "yabani hünnap" (Şekil 1) olarak adlandırılmıştır.

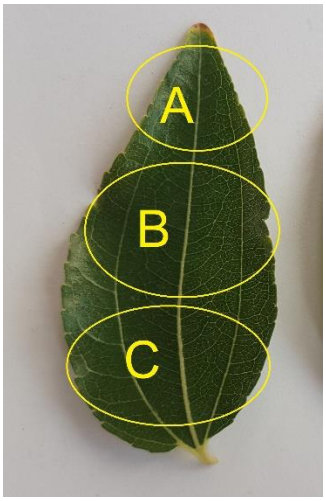
Yöntem

Çalışmada, 2021 yılı Temmuz ayı ortasında hünnap genotiplerine ait ağaçların 4 ayrı yönünden ve boy hizasındaki sürgünlerin orta kısımlarından olmak üzere, toplam 30 adet yaprak örneği alınmıştır. Alınan yaprakların arazide su kaybı nedeniyle kurumaması için şeffaf örnek torbalarına konulmuştur. Alınan örnekler, araç içi buzdolaplarına konularak, hızlıca analizlerin yapılacağı laboratuvara ulaştırılmıştır.

Şekil 1. Araştırmada incelenen hünnap genotiplerinin ağaç ve meyve görüntüleri
Figure 1. Tree and fruit images of jujube genotypes examined in the study

Alınan her yapraktan 3 bölge (üst, orta, alt dilim) tırnak cilasıyla cilalanıp, kurumaya bırakılmıştır (Şekil 2.). Tırnak cilaları kuruduktan sonra, şeffaf koli bandı yardımıyla kuruyan cila yapraktan alınarak, elde edilen kalıp lam üzerine aktarılmıştır (Bekişli, 2014; Durmaz, 2014; Bekişli ve Gürsöz, 2016). Hazırlanan preparatlar Leica 1000 marka mikroskopta incelenmiştir. Aynı marka mikroskoba takılı Leica EC3 marka dijital kamera ile fotoğrafları çekilmiş, çekilen fotoğrafların ölçümü Las v4.3 programında yapılmıştır.

Her bir hünnap genotipine ait ağaçlardan 20 Eylül 2021 tarihinde plastik poşetlere toplanan 50 adet meyve örneği, yine araç içi buzdolabı yardımıyla, ölçüm ve analizlerin yapılacağı laboratuvara ulaştırılmıştır.



Şekil 2. Hünnap yapraklarında stoma ölçüm ve sayımı yapılan alanlar
Figure 2. Stoma measurement and counting areas on jujube leaves

Meyvelerin bazı fiziksel özellikleri

Çalışmada, hünnap genotiplerinin meyve kalite özelliklerinden meyve ağırlığı (g), meyve eni (mm), meyve boyu (mm) ve meyve en/boy oranı belirlenmiştir. Her bir genotipe ait ağaçlardan toplanan meyve örneklerinde meyve kalite analizleri, 3 tekerrürlü ve her tekerrürde 10 meyve olacak şekilde, toplam 30 meyvede gerçekleştirilmiştir.

Yaprak veya stoma özellikleri

Ağaçların dört ayrı yönünden alınan ve laboratuvara getirilen yaprak örneklerinden rastgele seçilen 9 adet yaprağın cetvel yardımıyla ölçülmesiyle; yaprak boyu (cm), yaprak eni (mm) ve yaprak sapı uzunluğu (cm) belirlenmiştir. Yaprak yüzey alanı ise Image-J programı ile saptanmıştır.

Birim alandaki stoma sayısı (adet mm^{-2}): Her bir kalıpta çekilen fotoğrafta 10X büyütmede sayılan stomaların, 1 mm^2 alana göre hesaplanmasıyla, birim alandaki stoma sayısı belirlenmiştir (Bekişli, 2014). Gerçekleştirilen bu araştırmada, Şekil 2.'de görüldüğü gibi yapraklar üzerinde A, B ve C ile işaretlenmiş bölgelerde stoma ile ilgili ölçüm ve sayımlar yapılmıştır.

Stoma boyu ve eni (µm): Her bir stoma kalıbında, 3 farklı görüş alanının fotoğrafları çekilmiştir. Her bir bölgeden (A, B, C) çekilen kalıp fotoğraflarındaki 5'er adet stomanın boyu ve eni Las v4.3 paket programında ölçülerek belirlenmiştir.

Stoma por boyu ve por eni (µm): Her bir bölgeden (A, B, C) çekilen kalıp fotoğraflarındaki 3 farklı görüş alanında 5'er adet stomanın açıklıklarının boyu ve eni Las v4.3 paket programında ölçülerek belirlenmiştir.

İstatistik Analizler

Çalışmada, hünnap genotiplerinde incelenen meyve, yaprak ve stoma özelliklerine ait elde edilen veriler MINITAB 13.0 paket programı ile varyans analizine tabi tutulmuş ve farklılıkların $p<0.05$ önem seviyesinde tespit edildiği durumlarda, LSD testi ile farklılıklar ortaya konmuştur.

BULGULAR ve TARTIŞMA

Meyve kalite özellikleri

Çalışmada incelenen hünnap genotiplerinin ortalama meyve ağırlığı (g), meyve boyu (mm), meyve eni (mm) ve meyve en/boy değerleri Çizelge 1.'de verilmiştir. Meyve kalite özelliklerinden meyve ağırlığı, meyve boyu, meyve eni ve meyve en/boy oranı bakımından incelenen genotipler arasında istatistiki olarak farklılıklar olduğu belirlenmiştir. İncelenen hünnap genotiplerinde en yüksek ortalama meyve ağırlığı, meyve boyu, meyve eni ve meyve en/boy oranı değerleri sırasıyla; 20.28 g, 39.47 mm, 37.23 mm ve 0.94 ile kültür hünnap genotipinde belirlenmiştir (Çizelge 1.).

Türkiye'nin değişik yörelerinde hünnap çeşit ve genotiplerinde pomolojik özelliklerin belirlenmesi amacıyla yürütülen bazı araştırmalarda 2.73-28.85 g ortalama meyve ağırlığı, 16.86-39.58 mm ortalama meyve boyu ve 17.27-37.35 mm ortalama meyve eni değerleri tespit edilmiştir (Ecevit ve ark., 2008; Kavas ve Dalkılıç, 2015; Bektaş, 2019; Acarsoy Bilgin, 2020).

Romanya'da seleksiyonla elde edilmiş olan 'Jurilovca 1', 'Jurilovca 2'.ve 'Ostrov' genotiplerinde ortalama 1.30 (Jurilovca 1)-6.29 g (Ostrov) arasında meyve ağırlığı değerleri elde edilmiştir (Stănică, 2009). Grygorieva ve ark. (2014), Slovakya'da 28 hünnap (*Z. jujuba* Mill.) genotipi üzerinde yürütmüş oldukları çalışmada, ortalama meyve ağırlığını 2.90-28.99 g (ZJ-22), meyve boyunu 20.73 mm-44.84 mm, meyve enini 16.64-38.87 mm ve meyve en/boy oranını (şekil indeksi) 0.80-0.86 arasında saptamışlardır. Ghazaeian (2015), İran'ın Golestan eyaletinde bulunan 10 farklı

hünnap genotipinde 0.79-4.80 g arasında meyve ağırlığı, 15.3-21.6 mm arasında meyve boyu ve 14.6-21.3 mm arasında ise meyve eni değerleri tespit etmiştir. Tatari ve ark. (2016), İsfahan (İran) eyaletindeki hünnap (*Z. jujuba* Mill.) germplazmı içindeki çeşitliliği belirlemeyi amaçladıkları çalışmalarında, 18.4-42.0 g arasında meyve ağırlığı, 10-22 mm arasında meyve boyu ve 6-17 mm arasında ise meyve eni değerleri saptamışlardır. Ivanišová ve ark. (2017), Ukrayna'dan temin edilen 15 hünnap (*Z. jujuba* Mill.) genotipinde ortalama meyve ağırlığının 2.52-19.37 g, meyve boyunun 18.11-40.69 mm ve meyve eninin ise 16.66-35.60 mm arasında değiştiğini bildirmişlerdir. İran'da doğal olarak yetişen hünnap (*Z. jujuba* Mill.) genotiplerinde 0.36-3.83 g arasında meyve ağırlığı, 9.43-49.47 mm arasında meyve boyu ve 8.38-30.17 mm arasında meyve eni değerleri belirlenmiştir (Khadivi ve ark., 2021). Pakistan'ın Cholistan çölünde sulanan ve sulanmayan koşullarda yetiştirilen hünnap (*Z. jujuba* Mill.) genotipinde ortalama 39 mm meyve boyu ve 38 mm meyve eni değerleri elde edilmiştir (Riaz ve ark., 2021).

Çizelge 1. Şanlıurfa ilinde yetiştirilen hünnap genotiplerinin ortalama meyve ağırlığı, meyve boyu, meyve eni ve meyve en/boy oranı değerleri

Table 1. The mean fruit weight, fruit height, fruit width and fruit index values of jujube genotypes grown in Şanlıurfa province

Genotipler *Genotypes*	Meyve ağırlığı *Fruit weight* (g)	Meyve boyu *Fruit length* (mm)	Meyve eni *Fruit width* (mm)	Meyve en/boy oranı *Fruit index*
Kültür hünnap	20.28± 1.97 a	39.47±0.95 a	37.23±0.14 a	0.94±0 01 a
Yabani hünnap	2.78±0.29 b	21.77±0.54 b	18.62±0.16 b	0.86±0.02 b
LSD (%5)	0.80	3.51	3.04	0.06

Aynı sütunda farklı harflerle ifade edilen gruplar birbirlerinden istatistiki olarak farklılık göstermektedir.
Groups expressed with different letters in the same column differ statistically from each other.

Araştırmalardan elde edilen sonuçlar arasındaki farklılıklar, çevresel ve genetik faktörlere bağlı olarak değişkenlikler gösterebilmektedir (Riaz ve ark., 2021). Benzer coğrafi bölgelerden gelen meyvelerin ağırlıklarındaki farklılıklar, genotipik etkilerin bir sonucu olabilmektedir. Ancak, farklı agroekolojik bölgelerdeki *Z. jujuba* genotiplerinin meyve ağırlığındaki varyasyonların, yukarıda açıklandığı gibi çevresel ve genetiksel faktörlere bağlı olarak değişebileceği söylenebilir. Öte yandan, yüksek meyve ağırlığına sahip olan hünnap genotiplerinin, daha iri boyutlu tohumlardan elde edilebileceği belirtilmiştir (Markovski ve ark., 2015). Hünnap bitkisi, kuraklığa karşı yüksek toleransa sahiptir ve farklı iklimlerde yetişebilir. Doğal koşullarda yetiştiği alanlarda, doğal üreme sonucu meydana gelen hünnap bireyleri, o yöredeki insanlar tarafından seçilmiştir. Böylece, uzun evrim tarihi boyunca hünnap çeşitliliği artmış ve farklı genotipler ortaya çıkmıştır. *Ziziphu*s cinsinde ortak bir özellik olan tozlanmada kendi kendine ve karşılıklı uyuşmazlık durumu da bir popülasyonda artan genetik ve fenotipik varyasyona neden olabilmektedir (Khadivi ve ark., 2021).

Ecevit ve ark. (2008), daha büyük hacimli hünnap çeşitlerinin muhtemelen triploid olabileceğini belirtmişlerdir. Öte yandan, küçük meyveli hünnap çeşitlerinin, büyük meyveli çeşitlere göre daha yüksek askorbik asit, toplam şeker ve toplam suda çözünür madde miktarına sahip olduğu rapor edilmiştir (Gao ve ark., 2003; Ecevit ve ark., 2008). Gao ve ark. (2003), küçük meyveli çeşitlerin kurutmaya ve kuruyemiş olarak değerlendirmeye daha uygun olduğunu belirtmişlerdir.

Yaprak özellikleri

Araştırmada incelenen hünnap genotiplerinden alınan yaprak örneklerinde (Şekil 3.) belirlenen yaprak alanı, yaprak eni, yaprak boyu, pedisel boyu ve yaprak en/boy oranı değerleriyle ilgili bulgular Çizelge 2.'de verilmiştir. Hünnap genotipleri arasında yaprak alanı, yaprak eni, yaprak boyu ve yaprak en/boy oranı değerleri bakımından farklılıklar istatistiki olarak %5 düzeyinde önemli bulunmuştur.

Hünnap genotipleri arasında en büyük yaprak alanı kültür hünnap (7.01 cm^2) genotipinde belirlenirken, en küçük yaprak alanı ise 3.11 cm^2 ile yabani hünnap genotipinde tespit edilmiştir (Çizelge 2.). Şanlıurfa ilinde yetiştirilen hünnap genotiplerinde 1.31 (yabani hünnap)-2.65 mm (kültür hünnap) arasında ortalama yaprak eni, 3.29 (yabani hünnap)-5.09 mm (kültür hünnap) arasında ortalama yaprak boyu, 0.37 (yabani hünnap)-0.39 mm (kültür hünnap) arasında ortalama pedisel boyu ve 0.38 (yabani hünnap)-0.52 (kültür hünnap) arasında yaprak en/boy oranı değerleri elde edilmiştir (Çizelge 2.).

Aydın ilinde yetiştirilen üç farklı hünnap tipinde yaprak boyu 32.79-47.20 mm ve yaprak eni 17.46-27.05 mm arasında saptanmıştır (Kavas ve Dalkılıç, 2015). Romanya'da seleksiyonla elde edilmiş olan 'Jurilovca 1', 'Jurilovca 2'.ve 'Ostrov' hünnap (*Z. jujuba* Mill.) genotiplerinde ortalama 25-51 mm yaprak boyu, 14-22 mm yaprak eni ve 2.4-4.5 mm yaprak sapı uzunluğu belirlenmiştir (Stănică, 2009). İran'ın Golestan eyaletinde bulunan 10 farklı hünnap genotipinde ortalama yaprak boyu 25-56 mm, yaprak eni 13.6-24.3 mm ve yaprak sapı uzunluğu 13.6-24.6 mm arasında belirlenmiştir (Ghazaeian, 2015). İran'ın

Şekil 3. Hünnap genotiplerine ait yaprakların görünümü
Figure 3. The appearance of the leaves of the jujube genotypes

Çizelge 2. Şanlıurfa ilinde yetiştirilen hünnap genotiplerinde yaprak özellikleriyle ilgili elde edilen değerler
Table 2. The values obtained about the leaf characteristics of jujube genotypes grown in Şanlıurfa province

Genotipler *Genotypes*	Yaprak alanı *Leaf area* (cm^2)	Yaprak eni *Leaf width* (cm)	Yaprak boyu *Leaf length* (cm)	Pedisel boyu *Pedicel length* (cm)	Yaprak en/boy oranı *Leaf width/length ratio*
Kültür hünnap	7.01±1.09 a	2.65±0.19 a	5.09±0.43 a	0.39±0.08	0.52±0.04 a
Yabani hünnap	3.11±0.76 b	1.31±0.03 b	3.29±0.06 b	0.37±0.15	0.38±0.05 b
LSD (%5)	6.432	0.275	0.814	Ö.D.	0.065

Aynı sütunda farklı harflerle ifade edilen gruplar birbirlerinden istatistiki olarak farklılık göstermektedir.
Groups expressed with different letters in the same column differ statistically from each other.

İsfahan vilayetindeki hünnap genotipleri arasında 44-51 mm yaprak boyu ve 18-28 mm arasında ise yaprak eni tespit edilmiştir (Tatari ve ark., 2016). 'Maya' hünnap çeşidinden elde edilmiş olan diploid ve triploit bitkilerde yaprak alanının 3.84-6.73 cm^2, yaprak uzunluğunun 3.41-4.36 cm ve yaprak eninin 1.29-2.26 cm arasında değiştiği belirlenmiştir (Li ve ark., 2021). İran'da doğal olarak yetişen hünnap (*Z. jujuba* Mill.) genotiplerinin morfolojik özelliklerini belirlemek amacıyla yürütülen araştırmada, genotiplerin yaprak boyu 26.33 ile 84.05 mm, yaprak eni 11.02 ile 32.87 mm ve yaprak sapı uzunluğu 1.55 ile 10.60 mm arasında tespit edilmiştir (Khadivi ve ark., 2021). Pakistan'ın Cholistan çölünde sulanan ve sulanmayan koşullarda yetiştirilen hünnap (*Z. jujuba* Mill.) genotipinde ortalama 74 mm yaprak boyu, 42 mm yaprak eni ve 13 mm yaprak sapı uzunluğu değerleri elde edilmiştir (Riaz ve ark., 2021).

Hünnap genotipleri arasında yaprak özelliklerindeki varyasyonlar (yaprak boyutu ve yaprak şekli), ışık miktarı ve kalitesi yanında, yıllık ortalama yağış ile de güçlü bir şekilde ilişkilidir. Yüksek sıcaklık ve düşük yağış miktarı, *Z. jujuba* genotiplerinin morfolojik, fizyolojik ve biyokimyasal özelliklerini önemli ölçüde etkilemektedir (Riaz ve ark., 2021).

Stoma özellikleri

Araştırmada incelenen hünnap genotiplerinin yapraklarında belirlenen stoma özelikleri ile ilgili bulgular Çizelge 3.'te verilmiştir. Hünnap genotiplerinde belirlenen ortalama stoma boyu ve eni ile por boyu ve eni değerleri arasındaki farklılığın istatistiki olarak önemli olmadığı belirlenmiştir (Çizelge 3.).

Hünnap genotiplerinde ortalama stoma boyu 27.22 (yabani hünnap)-28.00 µm (kültür hünnap) arasında, ortalama stoma eni 8.21 (yabani hünnap)-20.05 µm (kültür hünnap) arasında, ortalama stoma por boyu 17.34 (yabani hünnap)-19.22 µm (kültür hünnap) arasında ve ortalama stoma por eni 8.10 (yabani hünnap)-8.90 µm (kültür hünnap) arasında olduğu tespit edilmiştir (Çizelge 3.).

Çizelge 3. Şanlıurfa ilinde yetiştirilen hünnap genotiplerinde saptanan ortalama stoma boyu ve eni ile por boyu ve eni değerleri
Table 3. The mean stomatal length and width and pore length and width values found in jujube genotypes grown in Şanlıurfa province

Genotipler *Genotypes*	Stoma boyu *Stoma length* (µm)	Stoma eni *Stoma width* (µm)	Por boyu *Pore length* (µm)	Por eni *Pore width* (µm)
Kültür hünnap	28.01±1.11	20.05±1.24	19.22±1.77	8.90±1.21
Yabani hünnap	27.22±1.24	18.21±1.54	17.34±1.74	8.10±1.04
LSD (%5)	Ö.D.	Ö.D.	Ö.D	Ö.D.

Aynı sütunda farklı harflerle ifade edilen gruplar birbirlerinden istatistiki olarak farklılık göstermektedir.
Groups expressed with different letters in the same column differ statistically from each other.

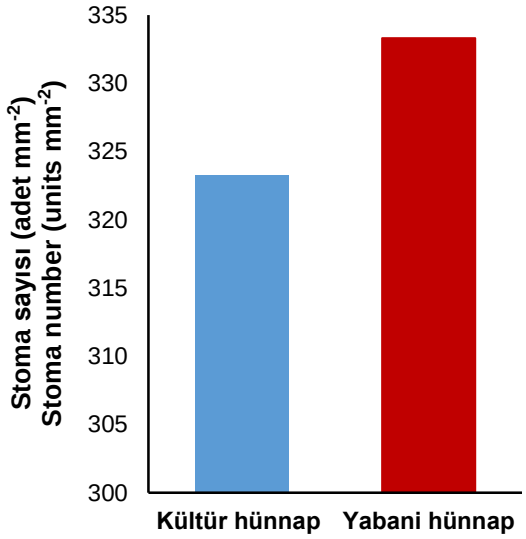
Çağlar ve ark. (2004), incelemiş oldukları Kahramanmaraş kökenli ceviz tiplerinin stoma boyunun 14-18 µm ve Hatay kökenli ceviz tiplerinde ise 21-28 µm arasında, incelenen ceviz tiplerinin stoma çaplarının ise 10-12 µm arasında değiştiğini tespit etmişlerdir. Ilgın ve Çağlar (2009), Kahramanmaraş ili koşullarında kayısı çeşitlerinde stoma uzunluğunun 64.1 µm ile 100.8 µm arasında değiştiğini, Orange Red kayısı çeşidinin en yüksek stoma yoğunluğuna (349 adet mm^{-2}) ve daha küçük stomalara (71.6 µm); Cnef çeşidinin ise en düşük stoma yoğunluğuna (182.2 adet mm^{-2}) ve daha büyük stomalara (100.8 µm) sahip olduğunu bildirmişleridir. Muradoğlu ve Gündoğdu (2011), 11 farklı kültür ceviz çeşidinde 10.65 -30.10 µm arasında stoma boyu belirlemişlerdir. 18 Türk fındığı çeşidinde 22.00-27.45 µm arasında stoma uzunluğu ve 17.00-22.61 µm arasında stoma eni saptanmıştır (Avcı ve Aygün, 2014). Kara ve ark. (2018), 41B asma anacında 26.33-29.47 µm arasında stoma uzunluğu ve 17.00-20.02 µm arasında ise stoma genişliği tespit etmişlerdir. Alpaslan (2019) ise Düzce yöresinden selekte edilen kestane genotiplerinin yapraklarındaki stoma boyunu 16.22-23.89 µm, stoma enini 13.99-19.82 µm, stoma por boyunu 7.00 -11.49

µm ve por enini ise 2.62-7.37 µm olarak saptamıştır. 'Maya' hünnap çeşidinden elde edilmiş olan diploid ve triploit bitkilerde stoma uzunluğunun 17.29-22.34 µm ve stoma eninin 15.83-17.61 µm arasında değiştiği belirlenmiştir (Li ve ark., 2021).

Yapılan araştırmalarda, yapraklardaki stoma yoğunluğu yüksek çeşitlerin, stoma genişliklerinin daha dar olduğu bildirilmiştir (Çağlar ve ark., 2004; Ilgın ve Çağlar, 2009; Mert ve ark., 2009; Avcı ve Aygün, 2014). Yürütülen bu araştırmada elde edilmiş olan sonuçlar, bu ifadeyi destekler niteliktedir.

Stoma yoğunluğu (adet mm^{-2})

Araştırmada kullanılan hünnap genotiplerine ait ağaçlardan alınan yaprak örneklerinde saptanan stoma yoğunluğu ile ilgili bulgular Şekil 4.'te verilmiştir. Yapraklardaki stoma yoğunluğu (Şekil 5.) bakımından çalışmada incelenen genotipler arasındaki farklılığın istatistiki olarak önemli olmadığı saptanmıştır. Stoma yoğunluğu en fazla olan yapraklar 323.297 adet mm^{-2} ile kültür hünnap'ında ve en düşük stoma yoğunluğuna sahip yapraklar ise 333.310 adet mm^{-2} ile yabani hünnap genotipinde belirlenmiştir (Şekil 5).



Şekil 4. Hünnap genotiplerinin ortalama stoma yoğunluğu

Figure 4. Average stomatal density of jujube genotypes

Li ve ark. (2021). 'Maya' hünnap çeşidinden elde edilmiş olan diploid ve triploit bitkilerde yaprak stoma yoğunluğunu 356.25-474.23 adet mm^{-2} arasında belirlemişlerdir. Türkiye'nin değişik yörelerinde farklı tür ve çeşitler üzerinde yürütülen bazı araştırmalarda; antepfıstığı yapraklarının alt yüzünde 171-221 adet mm^{-2} (Çağlar ve Tekin, 1999), bazı zeytin çeşitlerinde 388.242 (Gemlik)-464.023 adet mm^{-2} (Nizip Yağlık) (Demirkaya, 1999), Kahramanmaraş ve Hatay yörelerinden selekte edilen ceviz genotiplerinde 120-217 adet mm^{-2} (Çağlar ve ark., 2004), bazı kestane genotiplerinde 321.1-457.3 adet mm^{-2} (Kurt, 2008), Kahramanmaraş ili koşullarında yetiştirilen 20 kayısı çeşidinde 182.2-349 adet mm^{-2} (Ilgın ve Çağlar, 2009), 18 Türk fındığı çeşidinde 83.08 (Kalınkara)-117.73 adet mm^{-2} (Sivri) arasında (Avcı ve Aygün, 2014), 41 B asma anacında 410.57-550.45 (Kara ve ark., 2018), Düzce yöresinden selekte edilen ümitvar kestane genotiplerinde 243.81-729.61 adet mm^{-2} (Alpaslan, 2019), Şanlıurfa koşullarında SL 64 anacı üzerine aşılı Stella kiraz çeşidinde 235.91-251.03 adet mm^{-2} (Polat, 2019) arasında değişen stoma yoğunluğu saptanmıştır.

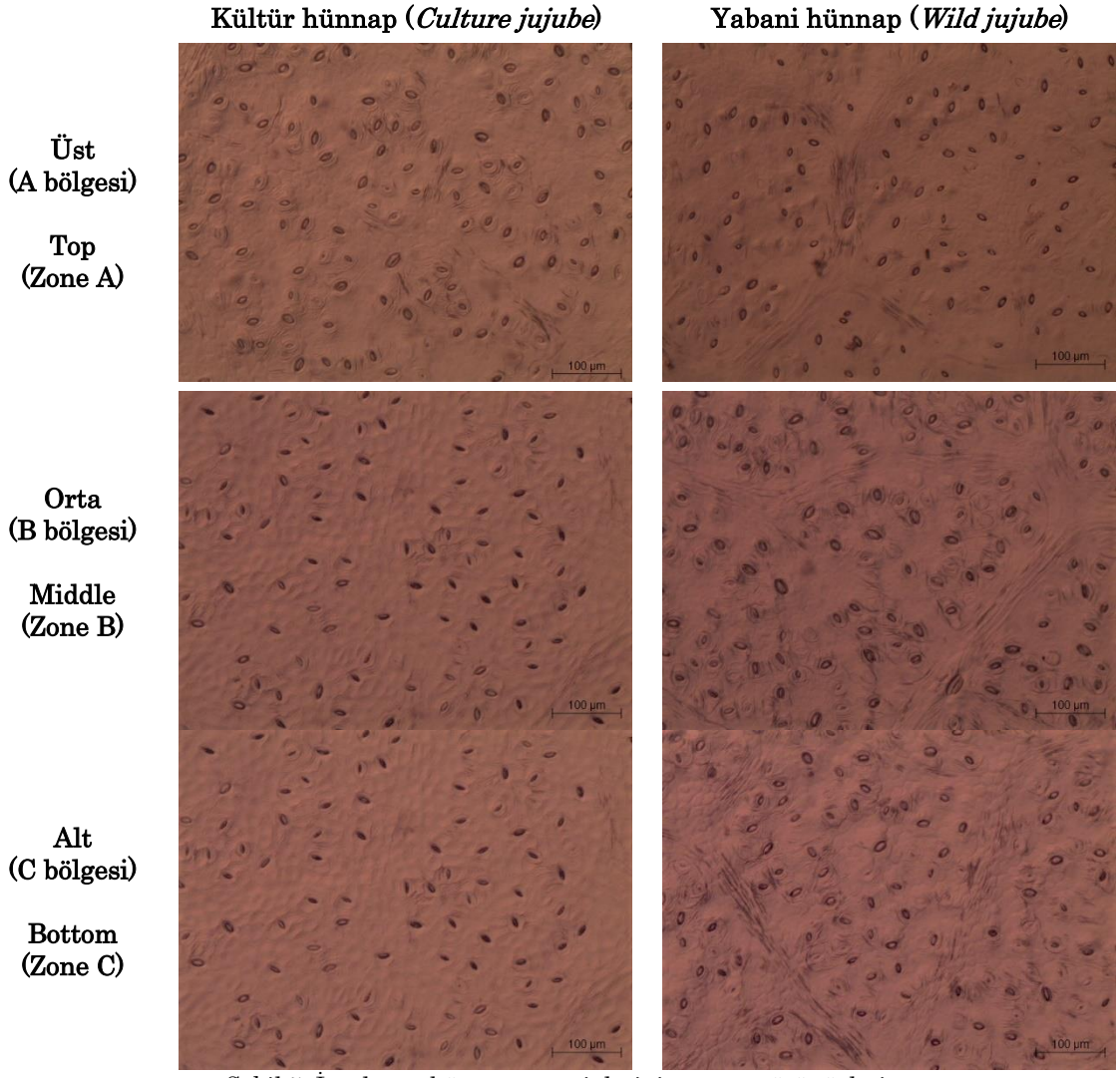
Bitki yapraklarındaki stomaların, değişen çevresel koşullara uyum sağlamada önemli bir rolü olduğu bilinmektedir. Stomaların yoğunluğunun bitki tür ve çeşitlerine, yaprakların genç veya yaşlı oluşlarına, ekolojiye, uygulanan bakım koşulları ile sürgün üzerindeki pozisyonlarına göre değişiklik gösterdiği belirlenmiştir (Düzenli ve Ağaoğlu, 1992). Stoma sıklığı türden türe, çeşitten çeşide ve yetiştirme koşullarına göre büyük değişkenlikler gösterir (Ryugo, 1988; Avcı ve Aygün, 2014). Farklı bölgelerde, yıllık yağış miktarına göre de stoma sıklığı değişebilmektedir (Mısırlı ve Aksoy, 1994). Çağlar ve ark. (2004), özellikle rakımın stoma sayısı üzerine çok önemli bir etkisinin bulunduğunu, deniz seviyesinden yukarılara çıkıldıkça, yani rakım arttıkça, stoma sayısının da arttığını bildirmişlerdir. Benzer şekilde, topraktaki su eksikliği ve ağır ışık koşulları da stoma sayısında artışa neden olabilmektedir (Avcı ve Aygün, 2014).

Yürütülmüş olan bu çalışmada elde edilen 323.30-333.31 adet mm^{-2} arasındaki stoma yoğunluğu değerlerinin (Şekil 4.), yurdumuzun değişik yörelerinde farklı tür ve çeşitler üzerinde gerçekleştirilen ve sonuçları yukarıda verilen araştırma bulgularıyla benzerlik gösterdiği belirlenmiştir.

SONUÇ ve ÖNERİLER

Araştırmada incelenen meyve ağırlığı, meyve boyu, meyve eni ve meyve en/boy oranı değerleri bakımından kültür hünnap genotipinin, yabani hünnap genotipinden daha yüksek değerlere sahip olduğu tespit edilmiştir. Kültür hünnap meyvelerinin, yabani hünnap meyvelerinden 7 kat daha ağır olduğu, aynı şekilde; kültür hünnap meyvelerinin meyve eninin, yabani hünnap meyvelerinin meyve eninin iki katı olduğu saptanmıştır. Kültür hünnap'ın yaprak alanının, yabani hünnap'ın yaprak alanının iki katından daha büyük olduğu, incelenen bazı yaprak özellikleriyle ilgili saptanan değerler bakımından da (yaprak eni, yaprak boyu, yaprak en/boy oranı) kültür hünnap yapraklarının, yabani hünnap yapraklarının iki katı daha yüksek değerlere sahip olduğu saptanmıştır. Stoma boyu, stoma eni, por boyu ve por

eni değerleri bakımından hünnap genotipleri arasındaki farklılığın istatistiksel olarak önemli olmadığı saptanmıştır. İncelenen stoma ve por en-boy değerlerinin her iki hünnap genotipinde de birbirine oldukça benzer olduğu tespit edilmiştir. Birim alandaki stoma yoğunluğu bakımından incelenen her iki hünnap genotipi arasında önemli bir fark belirlenememiştir. Genotiplerinin birbirine yakın sayıda stoma yoğunluğuna sahip olduğu tespit edilmiştir.



Şekil 5. İncelenen hünnap genotiplerinin stoma görünüşleri
Figure 5. Stomata views of the examined jujube genotypes

Sıcak iklimlerde ve kurak koşullarda yetişebilen, tuzluluk stresine yüksek toleransı olan, öte yandan bazı zararlılara ve hastalıklara karşı da oldukça toleranslı bir bitki olan hünnabın (Meena ve ark., 2003; Liu ve Zhao, 2009; Tatari ve ark., 2016) Güneydoğu Anadolu Bölgesi'ndeki bazı illerde ağaçlandırma çalışmalarında ve erozyonla mücadele alanlarında başarıyla kullanılabileceği düşünülmektedir. Ayrıca, Harran Ovası'nın güneyinde yer alan tuzlanma nedeniyle çoraklaşmış toprakların değerlendirilmesinde de hünnap çeşit ve genotiplerinin gelecekte denenmesi yararlı olacaktır.

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Yazarlar; makaleye eşit oranda katkı sağlamış olduklarını beyan eder.

Çıkar Çatışması Beyanı

Makale yazarları aralarında herhangi bir çıkar çatışması olmadığını beyan ederler.

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Seed and Fruit Fatty Acid Compositions of *Crambe orientalis* and *Crambe tataria* Oils Collected from Three Different Provinces in Turkey

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ABSTRACT
Crambe is an annual or perennial plant belonging to the Brassicaceae family. The interest in Crambe is mainly due to its unique fatty acid profile, low input management, and broad adaptability to the environment. Thus, Crambe seed and fruit fatty acids were investigated by GC / MS in order to examine the chemical variability which correlates with the environmental variability. In the study, *Crambe orientalis* var. *orientalis* and *Crambe tataria* var. *tataria* were used and they were collected from the different areas of three provinces of Türkiye. Fifteen compounds were found, representing 84.6-100% of the total seed and fruit oils. The major components in both species were erucic acid (24.7-44.7%), gondoic acid (19.0-28.3%), oleic acid (14.9-28.5%), and linoleic acid (8.1-17.6%). As a consequence, it is needed to increase the production of erucic acid (C22:1) and gondoic acid (C20:1) in Crambe by using wild populations via plant breeding. These two promising fatty acids may be an alternative to meet industrial fatty acid market demand in a more environmentally friendly way compared to using fatty acids from fossil oil.

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Türkiye'de Üç Farklı İlden Toplanan *Crambe orientalis* ve *Crambe tataria* Tohum ve Meyvelerinin Yağ Asidi Kompozisyonu

ÖZET
Crambe, Brassicaceae familyasına ait tek yıllık veya çok yıllık bir bitkidir. Crambe'ye olan ilgi, temel olarak benzersiz yağ asidi profili, düşük girdi yönetimi ve çevreye geniş ölçüde uyum sağlama yeteneğinden kaynaklanmaktadır. Bu nedenle, çevresel değişkenlik ile ilişkili olan kimyasal değişkenliği incelemek için Crambe tohumlarının ve meyvelerinin yağ asidi bileşenleri GC/MS ile araştırılmıştır. Çalışmada *Crambe orientalis* var. *orientalis* ve *Crambe tataria* var. *tataria* kullanılmış ve Türkiye'nin üç ilinin farklı bölgelerinden toplanmıştır. Toplam tohum ve meyve yağlarının %84.6-100'ünü temsil eden on beş bileşen saptanmıştır. Her iki türdeki lokasyona göre ana bileşenler erusik asit (%24.7-44.7), gondoik asit (%19.0-28.3), oleik asit (%14.9-28.5) ve linoleik asit (%8.1-17.6) olarak belirlenmiştir. Sonuç olarak Crambe'de yabani populasyonlar kullanılarak erusik asit (C22:1) ve gondoik asit (C20:1) üretiminin bitki ıslahı yoluyla artırılmasına ihtiyaç duyulmaktadır ve bu iki ümitvar yağ asidi, fosil yağlardan elde edilen yağ asitlerinin kullanılmasına kıyasla daha çevre dostu bir şekilde piyasanın talebi olan endüstriyel yağları karşılamak için bir alternatif olabilir.

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INTRODUCTION

Crambe is an oilseed plant from the Brassicaceae family and it is native to the Mediterranean region (Comlekcioglu et al., 2008). Although widespread in the flora of Türkiye, it is a little-known species as it has not yet been grown commercially and has no traditional use among the people. (Tansı et al., 2003). Davis (1965) reported 4 taxa, 2 species (*C. orientalis* and *C. tataria*) registered in the flora of Türkiye, and 2 varieties belonging to the species. However, with subsequent flora studies, 4 more species were added to the flora of Türkiye (*C. maritima* L., *C. hispanica* L., *C. grandiflora* DC., and *C. alutacea* Hand.-Mazz.) and the number of taxa was determined as 10 with a total of 6 species and subspecies (Arslan et al., 2015). It was initially grown as a crop in 1933 at the Boronez Botanical Station in the Soviet Union, and it was included in a Swedish breeding program in 1949 (Oplinger et al., 1991). *Crambe* came to the forefront with its high erucic acid content among 8000 plant species with the examination of new potential species as a result of 16 years of systematic studies of USDA in 1957 (Glaser, 1996).

Cultivation experiments of Crambe in Türkiye were started for the first time in Çukurova, and seed yields and oil rates were determined (Tansı et al., 2003; Köybaşı and Tansı, 2008). It is still in its early stages of development as an agricultural crop and is not commonly cultivated in Türkiye, but due to potential uses of the seed oil for the production of many valuable products, many developed countries have shown a scientific interest in it. Presently, *C. abyssinica* Hochst. is the only species of the Crambe genus utilized in the industry.

Crambe is a cool-climate plant and can withstand temperatures as low as 5 °C. (Oplinger et al., 1991; Adamsen et al., 2005). It is cultivated as both a spring and a winter crop. It can be cultivated in regions with annual precipitation ranging from 350 to 1200 mm, an annual average temperature ranging from 5.7 °C to 16.2 °C, and soils with a pH ranging from 5.0 to 7.8 (Falasca et al., 2010). Its oil-bearing seeds, high yield capacity, and adaptability to regional conditions make it an appealing alternative oil crop for Eurasian regions (Rudloff and Wang, 2011). It requires modest agricultural inputs compared to many other oilseed crops that may be grown all over the world and provides the opportunity to minimize the consumption of inputs such as fertilizers and water (Rogério et al., 2013). Therefore, *C. abyssinica* cultivation has less environmental impacts than other crops such as canola or maize (Costa et al., 2019).

Erucic acid and gondoic acid have been in fighting with petroleum alternatives for many years. Priorly, the cost has been a major problem for the development of new plant-derived oils. But now people are concerned about finite supply. Because of these reasons, there is a need to develop renewable products from plant oils. Erucic acid (C22:1) and gondoic acid (C20:1) is monounsaturated, non-edible, long-chain fatty acid and it is found only in seed oil from plants belonging to Brassicaceae and Tropaeolaceae families (Qi et al., 2018). In higher plants, gondoic acid is a precursor of erucic acid (Kikukawa et al., 2015). Gondoic acid has several advantages, for example, it improves the skin permeation of indomethacin which is an anti-inflammatory drug, and is used as raw material for medical material and moisturizing ingredient in cosmetic creams (Morimoto et al., 1996; Kikukawa et al., 2015).

The ability to withstand high temperatures and remain liquid at low temperatures makes Crambe oil a good lubricant in plastics manufacturing (Nelson et al., 1993). Moreover, it is a chemical compound that is used in the manufacturing of inks, pharmaceuticals, and other products. Also, Crambe fruit oil has the potential to be used as an acaricide against the Cattle Tick (*Rhipicephalus microplus*) in the pesticide industry (Mattos et al., 2021). Additionally, the oxidative stability of Crambe oil-derived biodiesel is higher than soybean oil-derived biodiesel, which opens another potential usage for Crambe (Wazilewski et al., 2013). Crambe oil is not used as cooking oil due to the presence of erucic acid, which has been found to cause health problems in humans. For instance, food contaminated with erucic acid can increase cardiovascular disease in humans (Hebard, 2016). Alternative erucic acid sources, such as Crambe, can help to solve the current risks of growing high erucic acid rapeseed. To avoid the entry of erucic acid into the food chain, the USA, Canada, and EU cultivate as an identity-preserved crop under contract. These strict limitations can result in time-consuming and expensive cultivation, transportation, processing and storage, and traceability. Hence, there is a need to find alternative and possibly cheaper sources of erucic acid.

Germplasm collection and ex situ conservation in gene banks are two of the most important tools for conservation biological diversity. As wild relatives of crops, several Crambe species are of additional interest with a view to using them to improve crops by plant breeding. All across the world, 1.117 Crambe accessions are present and *C. hispanica* shares 44% and *C. abyssinica* 22% of these accessions, respectively (Genesys, 2021). North Central Regional Plant Introduction Station (NCRPIS), USDA-ARS, United States, conserves the world's largest and most diverse collection of Crambe. NCRPIS contains 524 Crambe accessions and it shares 47 % of the conserved Crambe germplasm. When checked on the basis of countries, the USA (605), Spain (100), Germany (79), Australia

(72), and the UK (42) are the top five countries that conserve Crambe accessions.

Given the increasing pollution and global warming from the petroleum industry, it is important today to bring environmentally friendly plants as raw materials to the industry where environmental awareness is increasing. Biodegradability and naturality of the oil have increased the number of studies on Crambe in recent years. Because of the climatic unpredictability, the plant resources have to be diversified to a greater variety of crops to achieve a higher resistance. However, there is not enough information about the high erucic and gondoic acid Crambe species demanded by the industry, which originates from wild populations. Therefore, the study aimed to select the accessions with high oil quality potentials from Crambe species, which are naturally distributed in the Central Anatolian and Mediterranean regions.

MATERIAL and METHOD

The areas where some Crambe species naturally spread were determined in the flora of Niğde, Karaman, and Kahramanmaraş provinces (Table 1, Figure 1). Crambe plants were identified according to the identification keys of Davis (1965) by the taxonomist Prof. Dr. Ahmet İlçim. *C. orientalis* L. var. *orientalis* and *C. tataria* Sebeök var. *tataria* were collected in June-July 2020. *C. tataria* Sebeök var. *tataria* is distributed in Central Anatolia, stony slopes, and fallow areas. Its distribution altitude is 900-1400 m (Davis, 1965). *C. orientalis* L. var. *orientalis* is an Iran Turan element and is distributed in arid, hilly areas, cultivated field edges, and fallow areas in Central Anatolia, Eastern, and Southeastern Anatolia. The distribution altitude for *C. orientalis* is 500-2800 m according to Davis (1965).

Figure 1. Collection centers of the investigated Crambe species (1: Niğde 2: Karaman 3: Kahramanmaraş) (Google Earth, 2021)

Şekil 1. İncelenen Crambe türlerinin toplama merkezleri (1: Niğde 2: Karaman 3: Kahramanmaraş) (Google Earth, 2021)

Sample Preparation

For the determination of the fatty acid composition of the oils, fatty acid methyl esters were prepared from Crambe oil, using a methylation method (Stefanoudaki et al., 1999). The fatty acids were converted to fatty acid methyl esters before analysis by shaking a solution of 0.5 ml oil and 5 mL of hexane for 5 mins and added 0.5 mL of 2 N methanolic potassium hydroxide and shaken for 5 mins more and centrifuged 5 mins. 1 µl of the samples given to the GC-MS device.

Gas Chromatography±Mass Spectrometry (GC/MS) Analysis

GC-MS analyses were conducted in the Plant Physiology Laboratory in Biology Dept. of Kahramanmaras Sutcu Imam University, Kahramanmaras, Türkiye. Qualification of the oil was analyzed on an Agilent 5975C Mass Spectrometer coupled with an Agilent GC-6890II series. The GC was equipped with an HP-88 capillary column (100 m x 250 µm m x 0.20 µm film thickness) and He was used as

carrier gas with a flow rate of 0.8 mL/min. The GC oven temperature was programmed as follows: 170 °C (1 min), 230 °C at 15 °C/min and then kept at 230 °C at 20 min. The injector temperature was 250 °C. The mass spectrometer was operating in EI mode at 70 eV. The split ratio was 20:1. Mass range 35-400m/z; scan speed (amu/s): 1000. Wiley7n.1, Famdbwax.L, Famedb23.L libraries were used for identifying the compounds. The chemical analyses were performed in triplicate.

Statistical analysis

Principal components analysis on correlations was performed using statistical software JMP® (version 14.0, SAS Institute Inc., Cary, NC, 1989-2019). The heat map was constructed using Flourish studio.

Table 1. List of investigated *Crambe* germplasm and localities
Çizelge 1. İncelenen Crambe gen kaynakları ve lokasyon listesi

Sample No (*Örnek No*)	Name of Accessions (*Aksesyon*)	Taxon (*Takson*)	Organ (*Organ*)	Collection Province (*Toplanan İl*)	District (*İlçe*)	Village (*Köy*)	Altitude (m) (*Rakım (m)*)	Coordinates (*Koordinantlar*)
S1	K-1	*C. tataria*	fruit	Karaman	Ayrancı	Hüyükburun	1124	37°21'17.0"N 33°40'06.2"E
S2	K-2	*C. orientalis*	fruit	Karaman	Merkez	Bölükyazı	1022	37°11'59.4"N 33°07'00.9"E
S3	K-3	*C. orientalis*	fruit	Karaman	Merkez	Ağılönü	1030	37°14'29.5"N 33°21'38.2"E
S4	K-4	*C. tataria*	fruit	Karaman	Kazımkarabekir	Yollarbaşı	1033	37°12'40.4"N 33°01'27.5"E
S5	K-5	*C. tataria*	fruit	Karaman	Ayrancı	Hüyükburun	1125	37°21'15.7"N 33°40'02.3"E
S6	K-6	*C. orientalis*	fruit	Karaman	Merkez	Çakırbağ	1023	37°11'46.2"N 33°08'03.3"E
S7	K-7	*C. tataria*	fruit	Karaman	Kazımkarabekir	Pazar	1077	37°12'55.4"N 32°59'04.5"E
S8	K-8	*C. tataria*	fruit	Karaman	Merkez	Merkez	1036	37°10'51.3"N 33°14'56.4"E
S9	K-9	*C. orientalis*	fruit	Karaman	Merkez	Merkez	1040	37°10'49.1"N 33°15'35.1"E
S10	K-10	*C. tataria*	fruit	Karaman	Merkez	Ağılönü	1029	37°15'01.7"N 33°23'04.8"E
S11	N-1	*C. orientalis*	fruit	Niğde	Merkez	Ovacık	1340	38°04'29.5"N 34°47'45.8"E
S12	N-1	*C. orientalis*	seed	Niğde	Merkez	Ovacık	1340	38°04'29.5"N 34°47'45.8"E
S13	N-2	*C. orientalis*	fruit	Niğde	Merkez	Ovacık	1346	38°04'40.6"N 34°48'04.9"E
S14	N-2	*C. orientalis*	seed	Niğde	Merkez	Ovacık	1346	38°04'40.6"N 34°48'04.9"E
S15	N-3	*C. orientalis*	fruit	Niğde	Merkez	Çarıklı	1340	38°05'02.8"N 34°48'51.8"E
S16	N-3	*C. orientalis*	seed	Niğde	Merkez	Çarıklı	1340	38°05'02.8"N 34°48'51.8"E
S17	N-4	*C. orientalis*	fruit	Niğde	Merkez	Çarıklı	1368	38°10'52.5"N 34°57'29.5"E
S18	N-4	*C. orientalis*	seed	Niğde	Merkez	Çarıklı	1368	38°10'52.5"N 34°57'29.5"E
S19	N-5	*C. tataria*	fruit	Niğde	Merkez	Karaatlı	1368	38°10'44.3"N 34°57'10.5"E
S20	N-6	*C. orientalis*	fruit	Niğde	Merkez	Karaatlı	1362	38°09'11.9"N 34°54'38.3"E
S21	N-7	*C. orientalis*	fruit	Niğde	Merkez	Yeşilgölcük	1340	38°10'24.3"N 34°47'26.5"E
S22	N-7	*C. orientalis*	seed	Niğde	Merkez	Yeşilgölcük	1340	38°10'24.3"N 34°47'26.5"E
S23	N-8	*C. orientalis*	fruit	Niğde	Merkez	Yaylayolu	1360	38°03'56.4"N 34°43'17.6"E
S24	N-8	*C. orientalis*	seed	Niğde	Merkez	Yaylayolu	1360	38°03'56.4"N 34°43'17.6"E
S25	N-9	*C. orientalis*	fruit	Niğde	Merkez	Aktaş	1312	38°02'39.8"N 34°44'07.1"E
S26	N-9	*C. orientalis*	seed	Niğde	Merkez	Aktaş	1312	38°02'39.8"N 34°44'07.1"E
S27	N-10	*C. orientalis*	seed	Niğde	Merkez	Edikli	1356	38°13'22.9"N 34°57'51.2"E
S28	N-10	*C. orientalis*	fruit	Niğde	Merkez	Edikli	1356	38°13'22.9"N 34°57'51.2"E
S29	M-1	*C. orientalis*	fruit	Kahramanmaraş	Dulkadiroğlu	Ulutaş	1588	37°37'25.2"N 37°00'54.0"E
S30	M-1	*C. orientalis*	seed	Kahramanmaraş	Dulkadiroğlu	Ulutaş	1588	37°37'25.2"N 37°00'54.0"E

RESULTS and DISCUSSION

The chemical composition of fatty acids was analyzed by GC/MS, and obtained results were summarized (Table 2). The chemical composition of Crambe oil varied according to location and species. Fifteen compounds were found, representing 84.6-100% of the total seed oils. The major components based on the location and the species were erucic acid (24.7-44.7%), gondoic acid (19.0-28.3%), oleic acid (14.9-28.5%), and linoleic acid (8.1-17.6%). In the study, seed and fruit of Crambe accessions collected from three provinces were examined separately and no statistical difference was found in terms of fatty acid compounds. Similarly, Mridula et al. (2015) revealed that a minor change in whole and dehulled flaxseed oil samples were observed, although it was not statistically significant. This result will provide convenience by reducing the need for additional labor during oil extraction.

The erucic acid content of *C. tataria* ranged from 25.6 to 29.7% based on the location and the species with a mean of 27.5%. When we compared the erucic acid content of *C. tataria* with the earlier studies, diverse results were found; 27.0% (Miller et al., 1965), 29.9% (Comlekcioglu et al., 2008), 20.7% (Dolya et al., 1977), 30.8% (Subasi, 2020). The erucic acid content of *C. orientalis* ranged from 30.9 to 44.7% with a mean of 35.1%. Diverse results based on the erucic acid content of *C. orientalis* were also reported by the researchers; 36.0% (Miller et al., 1965), 39.4% (Comlekcioglu et al., 2008), 34.7% (Dolya et al., 1977), 37.8% (Subasi, 2020).

These diverse results are thought to be due to differences between plant material and climatic conditions. The erucic acid content was reported for *C. abyysinica* seeds; 50-58.6% (Warwick et al., 2003), 50.0-60.0% (Yaniv et al., 1998), 57.2% (Oliveira et al., 2013), and for *C. hispanica* seeds; 48.5-57.9% (Warwick et al., 2003) and 45.05-56.25% (Arslan et al., 2015). Although the differences in the findings vary according to the plant species, it has been stated that it may vary according to the plant organs, development periods, time of collection, ecological factors, and genetic structure (Yaniv et al., 1991; Mastebroek et al., 1994). However, some researchers have pointed out that the erucic acid content is quite stable and is not affected by the environment (Bondioli et al., 1998; Fontana et al., 1998). Compared to other oil plants of the Brassicaceae family, which are rich in erucic acid, it was reported in *Brassica oleraceae*, 45.7%; in *Brassica napus*, 46.4%; in *Sinapis alba*, 37.6% and in hybrids of both species with 44.5% (Ayaz et al., 2006). In the findings, it can be observed that the erucic acid content of *C. orientalis* and *C. tataria* does not reach the erucic acid content of *C. hispanica* and *C. abyysinica*. In addition, as in annual species, the main component was determined as erucic acid in this study which was perennial species.

The gondoic acid content of *C. tataria* ranged from 20.4 to 28.0% with a mean of 24.6%. Differences in the gondoic acid content were also reported by the researchers; 16.5% (Dolya et al., 1977), 7.7% (Comlekcioglu et al., 2008), 21.0% (Miller et al., 1965), 19.4% (Subasi, 2020). Gondoic acid content of *C. orientalis* ranged from 19.0 to 28.3% with a mean of 25.3%. When we compared the gondoic acid content of *C. orientalis* with the earlier studies, diverse results were found; 19.4% (Dolya et al., 1977), 11.3% (Comlekcioglu et al., 2008), 20.0% (Miller et al., 1965), 16.0% (Subasi, 2020). The gondoic acid content was reported for *C. abyysinica* seeds; 2.8% (Dolya et al., 1977), 3.7-5.6% (Warwick et al., 2003), and for *C. hispanica* seeds; 2.6-6.6% (Warwick et al., 2003). The gondoic acid findings are higher when compared to the annual Crambe species. Compared to other oil plants of the Brassicaceae family, which are rich in erucic acid, it was reported in *Brassica oleraceae*, 45.7%; in *Brassica napus*, 46.4%; in *Sinapis alba*, 37.6% and in hybrids of both species with 44.5% (Ayaz et al., 2006).

The oleic acid content of *C. tataria* ranged from 15.3 to 28.5% with a mean of 19.4%. Previous studies have reported that the oleic acid content of *C. tataria* varied; 28.7% (Dolya et al., 1977), 21.0% (Miller et al., 1965), 1.4% (Comlekcioglu et al., 2008), 34.7% (Dolya et al., 1977), 23.8% (Subasi, 2020). The oleic acid content of *C. orientalis* ranged from 14.9 to 24.9% with a mean of 20.4%. Variations in the oleic acid content have also been reported by other researchers; 1.6% (Comlekcioglu et al., 2008) 18.0% (Miller et al., 1965) 18.1% (Dolya et al., 1977) 21.7% (Subasi 2020). The difference in the oleic acid rate observed across these studies may simply reflect the environmental influences. The oleic acid content was reported for *C. abyysinica* seeds; 16.0-22.8% (Warwick et al., 2003), 15% (Yaniv et al., 1998), 17.2% (Oliveira et al., 2013), and for *C. hispanica* seeds; 17.0-20.6% (Warwick et al., 2003). The findings are higher when compared to the annual Crambe species.

The linoleic acid content of *C. tataria* ranged from 12.5 to 17.6% with a mean of 13.6%. The linoleic acid content results are close or higher than the other results; 22.1% (Dolya et al., 1977), 9.0% (Comlekcioglu et al., 2008), 15.0% (Miller et al., 1965), 12.4% (Subasi, 2020). The linoleic acid content of *C. orientalis* ranged from 8.1 to 13.6% with a mean of 10.5%. Similar results based on the linoleic acid content of *C. orientalis* were also reported by the researchers; 13.0% (Dolya et al., 1977), 12.4% (Comlekcioglu et al., 2008), 11.0% (Miller et al., 1965), 9.38% (Subasi, 2020). The linoleic acid content was reported for *C. abyysinica* seeds; 6.9-8.5% (Warwick et al., 2003), 9% (Yaniv et al., 1998), 8.2% (Oliveira et al., 2013), and for *C. hispanica* seeds; 5.4-8.4% (Warwick et al., 2003). The available studies show that the fatty acid composition of *Crambe* varies greatly depending on the origin, genotype, and climatic conditions of the growing areas. Moreover, the oil composition of *C. tataria* and *C. orientalis* is found to be less than 3% palmitic acid, palmitoleic acid, heptadecanoic acid, cis-10- heptadecenoic acid, stearic acid, arachidic acid, 8,11-eicosadienoic acid, behenic acid, 13z 16z-docosadienoic acid, lignoceric acid and nervonic acid.

Principal Component Analysis Biplot of the Samples According to Fatty Acid Compounds

The biplot (PCAbiplot) format is used to represent a data source with a few components that best reflect the data's variance. It allows to zoom out on the data and see the relationships among the variables that make up the data. There is a positive correlation among the lines that are in the same direction and close to each other, while there is a negative correlation among the opposite and distant lines. PCAbiplot on correlations was performed to visualize the effect of locations and the species (Fig. 2). The experimental groups were separately discriminated with principal component analysis on correlations. In terms of the seed oil components, clear discrimination was revealed on the plotted scores, where component 1 and component 2 accounted for 52.1% of the total variance. The first axis and second axis explained 37.5% and 14.6% of the total variance, respectively. As seen in the figure, PCAbiplot indicates that erucic acid was negatively correlated with gondoic acid and oleic acid.

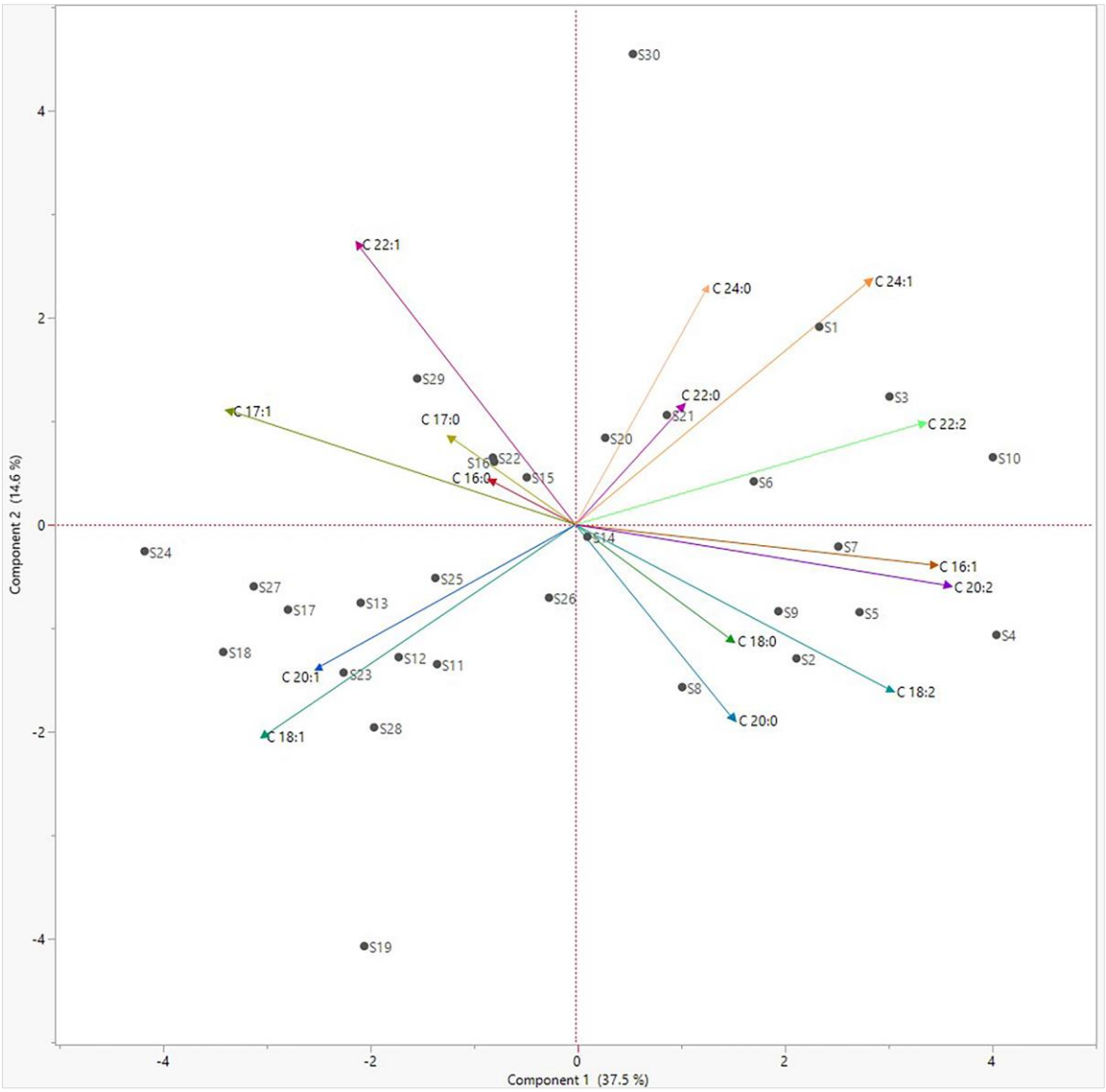

Figure 2. Principal component analysis on correlations of the samples according to fatty acid compounds (C 16:0 (Palmitic acid), C 16:1 (Palmitoleic acid), C 17:0 (Heptadecanoic acid), C 17:1 (cis-10-Heptadecenoic acid), C 18:0 (Stearic acid), C 18:1 (Oleic acid), C 18:2 (Linoleic acid), C 20:0 (Arachidic acid), C 20:1 (Gondoic acid), C 20:2 (8,11-Eicosadienoic), C 22:0 (Behenic acid), C 22:1 (Erucic acid), C 22:2 (13z 16z-docosadienoic acid), C 24:0 (Lignoceric acid), C 24:1 (Nervonic acid)).

Şekil 2. Aksesyonlardaki yağ asitlerinin korelasyona bağlı temel bileşen analizi, (C 16:0 (Palmitik asit), C 16:1 (Palmitoleik asit), C 17:0 (Heptadecanoic asit), C 17:1 (cis-10-Heptadecenoic asit), *C 18:0 (Stearik asit), C 18:1 (Oleik asit) C 18:2 (Linoleik asit), C 20:0 (Araşidik asit), C 20:1 (Gondoik asit), C 20:2 (8,11-Eikosadienoik), C 22:0 (Behenik asit), C 22 :1 (Erusik asit), C 22:2 (13z 16z-dokosadienoik asit), C 24:0 (Lignoserik asit), C 24:1 (Nervonik asit)).*

Heat Map for the Samples According to Main Fatty Acid Compounds

The changes in fatty acid components were structured using a heat map. According to the heat map (Fig. 3), the fatty acids were visualized under the samples. The differences among treatments can be observed easily with the help of the heat map. For instance; the highest erucic acid content was noted on S30, the highest gondoic acid content was noted on S-17 and the highest oleic acid content was noted on S-19. The differences can be observed clearly among the samples.

CONCLUSIONS

Crambe is an alternative plant for the production of biofuels, especially biodiesel, among the renewable energy sources instead of fossil fuels in our world threatened by global warming and climate change. Increasing genetic variability and discovering genotypes that are better suited to different environmental conditions can be beneficial, as future agriculture will have to adapt to climate change.

Table 2. Fatty acid compounds of Crambe accessions collected from different collection sites
Çizelge 2. Farklı bölgelerden toplanan Crambe aksesyonlarının yağ asidi bileşenleri

Fatty Acid Compounds (Yağ Asidi Bileşenleri)				C 16:0	C 16:1	C 17:0	C 17:1	C 18:0	C 18:1	C 18:2	C 20:0	C 20:1	C 20:2	C 22:0	C 22:1	C 22:2	C 24:0	C 24:1
Retention Time (min) *(Çıkış Zamanı (dakika))*				13.12	13.76	14.55	14.73	15.07	15.86	16.96	17.43	18.58	19.52	20.92	21.92	23.45	24.16	25.59
Sample No *(Örnek No)*	Accessions *(Aksesyonlar)*	Taxon *(Takson)*	Organ *(Organ)*	Area % *(Alan %)*														
S1	K-1	*C. tataria*	fruit	2.22	0.37	0.03	0.07	0.52	17.16	12.53	0.03	24.82	1.12	5.88	29.06	0.75	0.17	1.14
S2	K-2	*C. orientalis*	fruit	2.32	0.37	0.02	0.03	0.81	17.77	12.75	1.15	22.47	0.95	0.46	31.34	0.66	0.02	1.05
S3	K-3	*C. orientalis*	fruit	2.43	0.41	0.03	0.07	0.55	14.92	12.25	1.22	21.89	0.94	0.45	32.97	0.80	0.27	1.25
S4	K-4	*C. tataria*	fruit	2.31	0.44	0.05	0.08	1.47	16.49	12.89	1.13	23.85	1.25	0.42	27.51	0.67	0.20	1.40
S5	K-5	*C. tataria*	fruit	2.60	0.43	0.05	0.08	0.78	19.91	13.39	1.07	25.69	1.13	0.40	27.12	0.72	0.22	1.30
S6	K-6	*C. orientalis*	fruit	2.32	0.36	0.04	0.06	0.53	17.57	12.30	0.95	27.66	1.07	0.41	31.22	0.54	0.28	1.37
S7	K-7	*C. tataria*	fruit	2.12	0.40	0.02	0.08	0.50	15.25	17.56	-	20.35	0.98	0.29	25.60	0.39	0.13	0.88
S8	K-8	*C. tataria*	fruit	2.33	0.37	0.03	0.07	0.62	20.59	13.20	0.86	27.02	1.10	0.31	29.16	0.39	0.11	1.06
S9	K-9	*C. orientalis*	fruit	2.65	0.43	0.02	0.02	0.63	19.05	13.63	0.99	22.88	0.97	0.35	30.87	0.43	0.11	1.18
S10	K-10	*C. tataria*	fruit	2.45	0.41	0.03	0.05	0.62	18.16	12.86	1.17	22.62	1.29	0.43	29.72	1.03	0.29	1.43
S11	N-1	*C. orientalis*	fruit	2.43	0.30	0.05	-	0.60	22.68	10.55	0.88	25.62	0.64	0.31	34.55	0.36	0.11	0.19
S12	N-1	*C. orientalis*	seed	2.58	0.27	0.04	-	0.67	24.90	9.54	0.87	26.30	0.56	0.32	32.80	0.28	0.10	0.74
S13	N-2	*C. orientalis*	fruit	2.42	0.31	-	-	0.55	20.15	10.61	0.92	25.60	-	0.37	37.47	0.29	-	0.69
S14	N-2	*C. orientalis*	seed	2.37	0.31	0.04	-	0.57	19.44	10.57	1.14	25.13	0.81	0.45	37.20	0.45	0.14	0.89
S15	N-3	*C. orientalis*	fruit	2.65	0.36	0.03	-	0.48	20.71	9.78	0.88	25.57	0.63	0.32	36.82	0.43	0.16	1.02
S16	N-3	*C. orientalis*	seed	2.64	0.37	0.04	-	0.49	19.90	9.31	0.78	25.97	0.61	0.31	38.07	0.38	0.13	1.00
S17	N-4	*C. orientalis*	fruit	2.06	0.17	0.03	-	0.43	21.60	8.32	0.66	28.33	0.62	0.21	36.51	0.23	-	0.70
S18	N-4	*C. orientalis*	seed	1.90	0.09	-	-	0.86	21.76	8.13	0.52	27.77	0.39	0.22	36.42	0.16	-	0.44
S19	N-5	*C. tataria*	fruit	3.04	0.28	-	-	0.59	28.46	12.88	0.84	28.02	0.59	0.08	24.67	0.17	-	0.38
S20	N-6	*C. orientalis*	seed	2.06	0.20	-	-	0.54	20.38	9.67	0.82	24.75	0.86	-	34.67	0.56	0.17	1.59
S21	N-7	*C. orientalis*	fruit	2.77	0.37	0.04	-	0.46	17.30	11.17	0.78	25.39	0.69	0.53	35.39	0.75	0.14	1.25
S22	N-7	*C. orientalis*	seed	2.94	0.37	0.45	-	0.46	18.04	11.39	0.76	26.87	0.74	0.21	36.31	0.44	0.09	0.90
S23	N-8	*C. orientalis*	fruit	2.76	0.28	-	-	0.46	22.66	10.21	0.63	27.32	0.57	0.23	34.10	0.24	-	0.52
S24	N-8	*C. orientalis*	seed	2.66	0.16	-	-	0.41	22.82	9.92	-	26.16	-	0.20	34.49	0.15	-	0.47
S25	N-9	*C. orientalis*	fruit	2.48	0.31	-	-	-	22.70	10.96	0.93	25.02	0.61	0.37	34.87	0.34	0.10	0.62
S26	N-9	*C. orientalis*	seed	2.53	0.32	0.04	-	0.68	22.58	11.15	1.04	24.02	0.66	0.46	34.87	0.40	0.15	0.73
S27	N-10	*C. orientalis*	fruit	2.59	0.23	-	0.60	0.52	21.95	9.71	0.79	26.86	0.70	0.23	34.63	0.29	-	0.70
S28	N-10	*C. orientalis*	seed	2.42	0.30	-	-	0.46	24.37	10.35	0.71	26.89	0.59	0.20	32.67	0.28	-	0.50
S29	M-1	*C. orientalis*	fruit	2.48				0.67	19.48	9.48	0.48	23.83	0.63	0.43	33.40	0.26	0.24	1.17
S30	M-1	*C. orientalis*	seed	2.87	0.29			0.53	15.85	9.89	0.45	18.97	0.51	0.42	44.68	0.41	0.23	2.11

C 16:0 (Palmitic acid), C 16:1 (Palmitoleic acid), C 17:0 (Heptadecanoic acid), C 17:1 (cis-10-Heptadecenoic acid), C 18:0 (Stearic acid), C 18:1 (Oleic acid), C 18:2 (Linoleic acid), C 20:0 (Arachidic acid), C 20:1 (Gondoic acid), C 20:2 (8,11-Eicosadienoic), C 22:0 (Behenic acid), C 22:1 (Erucic acid), C 22:2 (13z 16z-docosadienoic acid), C 24:0 (Lignoceric acid), C 24:1 (Nervonic acid)

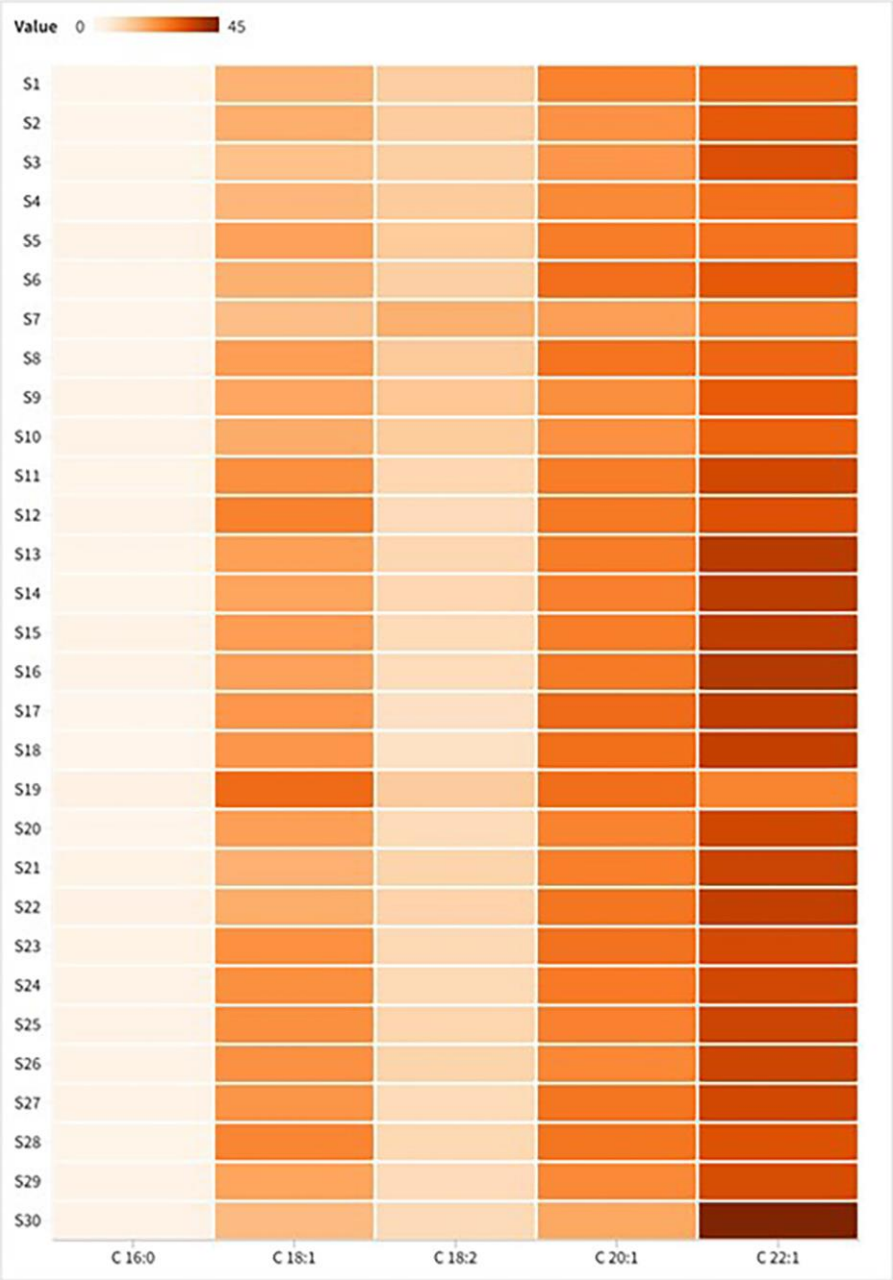

Figure 3. Heatmap for the fatty acids of Crambe samples (Value=%)
Şekil 3. Crambe örneklerinin yağ asitleri bakımından ısı haritası (Değer=%)

Crambe is one of the important genera for industrial uses of its oil. It is a cost-effective oil plant compared to many others. In the study, different Crambe plants were collected at various locations in the Central Anatolian and Mediterranean regions, the species were identified and the seed and fruit oil composition were evaluated. As a result of the identification, two different species, *C. orientalis* and *C. tataria*, were determined. However, the amount of erucic acid of both species was lower than the annual *C. abyssinica*, which is cultivated in different parts of the world. This is due to the varieties improved for the higher amount of erucic acid demanded by the industry, in addition to the genetic species characteristic of *C. abyssinica*. *Crambe*, which has a high production potential due to its natural presence in Türkiye, is not well-known commercially. In recent years, increasing awareness around the world about agriculture and the environment has led to an intensification of studies on this plant. Since it is a crop that can be grown taking advantage of winter and spring rainfall, it has good potential as an alternative oil crop for industrial purposes in those regions that oppose the increase in drought. Different species of Crambe, stand out with their biodegradable feature, and the determination of morphological and quality characteristics of these

species have gained importance in this respect. Further studies are recommended to focus on studies aimed at achieving the higher levels of erucic and gondoic acid required by the industry and examining the yield and quality of Crambe species in different regions.

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Author's Contributions

The contribution of the authors is equal.

Statement of Conflict of Interest

The authors have declared no conflict of interest.

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Evaluation of Oat (*Avena sativa* L.) Genotypes for Green Forage, Hay Yield and Some Quality Parameters in Trakya-Marmara Region

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ABSTRACT
This study was carried out during the 2016-17 and 2017-18 growing seasons in Edirne. This study was conducted in a randomized complete block design with four replications. The five varieties (Kirklar, Kahraman, Kucukyayla, Yeniceri and Sebat) and 10 lines were used as material in the study. It was aimed to determine the genotypes suitable for animal nutrition. The traits such as green forage (GFY) and hay yield (HY), plant height (PH), acid detergent fiber (ADF), neutral detergent fiber (NDF) and crude protein content (CP) as well as dry matter digestibility (DMD), dry matter consumption (DM) and relative feed value (RFV) quality parameter performances of the genotypes were investigated. There were statistically significant differences among genotypes for green forage, hay yield and plant height. The effects of genotype x year interaction on green forage, hay yield and plant height were found statistically significant. According to the results of two years of research, correlations between hay yield with green forage (0.8865**) and plant height (0.6141**) were determined as significant and positive. In terms of two years average, GFY, HY, PH, ADF, NDF, CP and RFV of oat lines ranged between 39.90-56.69 (50.84 t ha^{-1}), 10.52-15.09 (12.93 t ha^{-1}), 84.4-105.4 (95.8 cm), 36.0-44.0 (39.7%), 50.6-59.0 (55.1%), 8.9-17.2 (12.6%) and 86.1-108.3 (98.2%). The oat G6 had the highest hay yield with 15.18 t ha^{-1} and G8 had highest RFV with 107.8%. G2 (Kahraman), the oat G9 and G8 were suitable for hay yield and RFV in Trakya-Marmara region.

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Trakya-Marmara Bölgesinde Yulaf (*Avene sativa* L.) Genotiplerinin Yeşil Ot, Kuru Ot ve Bazı Kalite Özellikleri Yönünden Değerlendirilmesi

ÖZET
Bu çalışma, 2016-17 ve 2017-18 üretim sezonlarında Tesadüf Blokları Deneme Deseninde dört tekerrürlü olarak Edirne'de yürütülmüştür. 15 yulaf genotipin kullanıldığı denemede, 5 standart çeşit (Kırklar, Kahraman, Küçükyayla, Yeniçeri ve Sebat) yer almıştır. Araştırmada, yulaf genotiplerin yeşil ot ve kuru ot verimi ile bazı kalite özellikleri incelenerek hayvan beslemesi için bölgeye uygun genotiplerin belirlenmesi amaçlanmıştır. Bu kapsamda genotiplerin yeşil ve kuru ot verimi, bitki boyu ile kalite özelliklerinden ADF(Asit Deterjan Lif), NDF (Nötral Deterjan Lif), HP (Ham Protein), KMS (Kuru Madde Sindirebilirliği), KMT (Kuru Madde Tüketimi) ve NYD (Nispi Yem Değeri) incelenmiştir. Yapılan araştırma sonucunda iki yılda da yeşil ot verimi, kuru ot verimi ve bitki boyları arasında genotipler arasındaki fark istatistiki olarak önemli bulunmuştur. Ayrıca ADF, NDF, HP, KMS, KMT ve NYD yönünden genotipler arasında farklılıklar belirlenmiştir. Genotip x yıl interaksiyonunun genotiplerin yeşil ot, kuru ot ve bitki boyu üzerine etkilerinin istatistiki olarak önemli bulunmuştur. İki yıllık çalışma sonucuna göre genotiplerin kuru ot verimi ile yeşil ot verimi (r=0.8865**) ve bitki boyu (r=0.6141**) arasında pozitif ve önemli bir

Tarla Bitkileri

Araştırma Makalesi

Makale Tarihçesi
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Anahtar Kelimeler
Ham Protein
Yem Kalitesi
Kuru ot verimi
Nisbi Yem değeri
Çeşit

ilişki belirlenmiştir. İki yıl ortalamasına göre genotiplerin yeşil ot verimi; 39.90-56.69 (50.84) t ha^{-1}, kuru ot verimi; 10.52-15.09 (12.93) t ha^{-1}, bitki boyu; 84.4-105.4 (95.8) cm, ADF; %36.0-44.0 (39.7), NDF; %50.6-59.0 (55.1), HP; %8.9-17.2 (12.6), KMS; %2.0-2.4 (2.2), KMT; %54.6-60.8 (58.0) ve NYD; %86.1-108.3 (98.2) arasında değişim göstermiştir. İki yıllık çalışma sonucunda 15. 18 t ha^{-1} kuru ot verimi ile 6 nolu genotip, %107.8 NYD ile 8 nolu genotip en kaliteli olarak öne çıkmıştır. Kuru ot verimi ve nispi yem değeri yönünden Kahraman, 9 ve 8 nolu genotipler bölge için en uygun olarak öne çıkmıştır.

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INTRODUCTION

Oat (*Avena sativa* L.) is an important cereal for human nutrition and animal feed across the world (Buerstmayr, 2007). Oat stems are softer and leaves are more abundant, so they are rich in organic and mineral substances from wheat and barley straw. Besides, oat is used to support the mixture of legumes such as grain, green forage, silage, straw and vetch-feed peas. Among the cereals, oat has the highest protein content and quality in the feeding of domestic animals. The oat also shows the highest oil content. Oat green were grown on an area of 82.551 ha in 2012 and 214.257 ha in 2018 in Turkey. At the same time, green forage production was recorded 934.157 t in 2012 and as 2.843.686 t in 2018. The yield per area should be increased to fill the gap of higher quality forage in Turkey (Avcıoğlu et al., 2000). 17 oat varieties (Faikbey, Seydisehir, Sebat, Yeniceri, Sari, Fetih, Kirklar, Kahraman, Haskara, Albatros, Bc Marta, Dirilis, Arslanbey, Kucukyayla, Kehlibar, Kayi and Kupa) have been registered in Turkey (Anonim, 2019). All of these varieties are cultivated for grain. Oat is important in the feeding of farm animals due to the high protein content (Wood, 2001). Cheap and easily available feed sources are required in order to increase animal production, and oat is an important alternative plant. Oat is a priority product in the world as animal food, and it is inevitable to increase its production if the importance of oats in animal nutrition is taken into account in our country (Serin and Tan, 2009). Koçer and Albayrak (2012), investigated feed peas mixtures with oat and barley. They reported hay yield as 13.52 t ha^{-1}, ADF value as 34.6%, NDF value as 59.1% and CP as 10.87%. and RFV as 97.45% for monoculture oat and RFV as 167.27% for monoculture feed peas. As the feed pea ratio increased, the relative feed value of the feed increased. Avci (2017), used 13 oat genotypes and reported green forage yield 55.65 t ha^{-1} oat sowing during winter and 37.39 t ha^{-1} in summer. While they obtained hay yield as 12.64 t ha^{-1} in winter, they obtained hay yield as 6.88 t ha^{-1} in the summer. Mut et al. (2015), tested 100 oat genotypes and reported CP ranged from 5.88-13.64%, ADF values ranged from 33.32-42.48% and NDF values ranged from 52.25-65.24%. Çeri and Acar (2019), used 12 oat genotypes and they obtained green forage yield between 23.42-31.09 t ha^{-1}, hay yield between 6.14-9.94 t ha^{-1}, ADF value varied between 37.82-41.75%, NDF between 52.79-57.80% and CP between 9.64-11.53%. This study tested the 15 oat genotypes (10 oat lines and 5 varieties) developed by Trakya Agricultural Research Institute for green forage and hay yield, as well as some quality characteristics to determine the accessions suitable for the Trakya-Marmara region.

MATERIALS and METHODS

This research was conducted in Edirne Trakya Agricultural Research Institute during the 2016-17 and 2017-18 growing seasons. Five standard varieties (Kirklar, Kahraman, Kucukyayla, Yeniceri and Sebat) and ten oat lines were used. According to the results of some physical and chemical analysis of the soil in which the research was conducted, the texture class was silty-clay loam, organic matter content 1.07%, lime content 0.00%, salt 0.05%, pH 6.20, available phosphorus amount 279.2 kg ha^{-1}, potassium content was 968.0 kg ha^{-1}. The climatic values of the research site for trial years were given in Table 1. While the total rainfall was 417.2 mm in 2016-2017, 833.8 mm in 2017-2018. The mean temperature of the trial was 12.0 ^{0}C in 2016-17, 10.2 ^{0}C in 2017-18. Due to the high temperature in the first year, the flowering date of plants were 10-15 days earlier than in the second year. However, in the second year of the trial, especially April rainfall was insufficient (3 mm). The lack of rainfall in this period negatively affected the plant height, green forage and hay yield. Although the flowering date was delayed in the first year of the experiment, as a result of sufficient rainfall in April (65.6 mm) and May (85 mm), the plant height, green

forage and hay yields were the same as in the second year. The experiment was carried out with four replications according to the Randomized Complete Block Design. Each plot consisted of six rows of 7 m length and 1 m width and line spacing 17 cm. Seeding rate and field management were determined according to the results of regional research, with about 600 seeds per m^2.

Table 1. Rainfall (mm), mean temperature (^{0}C) and relative humidity (%) of the research site*
Çizelge 1. Araştırma yerinin yağış miktarı (mm), ortalama sıcaklık (8) ve nispi nem (%) değerleri

	Total Rainfall (mm)		Mean Temperature (^{0}C)		Relative Humidity (%)	
	2016-17	2017-18	2016-17	2017-18	2016-17	2017-18
September	9.2	34.2	20.8	21.3	57.5	57.8
October	44.4	135.2	14.3	13.6	69.5	77.1
November	3.2	71.6	0.7	9.5	72.9	75.7
December	3.2	119.6	0.7	7.4	72.9	85.1
January	67.8	55.6	-1.9	4.3	83.7	88.1
February	43.4	101.8	5.3	5.7	80.0	89.5
March	51.0	145.6	10.2	8.9	73.0	88.8
April	65.6	3.0	12.5	16.6	63.1	61.3
May	85	18.8	17.9	20.3	65.4	76.3
June	44.4	148.4	21.2	22.6	74.4	66.4
Total	417.2	833.8				
Mean			10.2	12.0	71.2	76.6

* Values were taken from Edirne Meteorology Directorate
** Veriler Edirne Metereoloji Müdürlüğünden alınmıştır*

The experiment was carried out with four replications according to the Randomized Complete Block Design. Each plot consisted of six rows of 7 m length and 1 m width and line spacing 17 cm. Seeding rate and field management were determined according to the results of regional research, with about 600 seeds per m^{-2}. The trials were planted on 19 October 2016 in the first year and on 18 October 2017 in the second year. Sowing was done with a specific seeder for plots. Before planting, 20-20-0 composite fertilizer (about 40 kg ha^{-1} of P2O5, 4 kg ha^{-1} of N) was broad-casted and incorporated. An additional of N was top-dressed 70 kg ha^{-1} at tillering stage and 40 kg ha^{-1} stem elongation stage. Weeds were controlled by Glean herbicide (about 10 cc ha^{-1}) before germination and Lancelot super herbicide (about 30 cc ha^{-1}) at the end of tillering stage. The plants were cut with a special rice machine at 50% flowering in each plot (6 m^{-2} area). The trials were cut between 1 May and17 May 2017 in the first year and between 22 April and 4 May 2018 in the second year. In the harvest, plots were evaluated as 6mx1m = 6 m^{-2} area.

Green forage yield (t ha^{-1}): The plants are cut and weighed when there is 50% flowering in each plot.

Hay yield (t ha^{-1}): After weighing the green forage harvested from each plot, samples of 0.5-1 kg green forage were dried in a drying cabinet at 70 ^{0}C for 48 h (Ünal, 2011), dried plants are kept at room temperature for 24 hours, and then weighted with precision balance (0.05 g).

Crude Protein Content (%): Crude protein content was determined by AOAC method (nitrogen multiplied by 6.25 was determined by device LECO FP 528) (Anonymous, 2009).

Insoluble Fiber in Neutral Detergent Solution (NDF) (%): It forms the insoluble part of neutral detergent in oat forage samples. It contains hemicellulose, cellulose, lignin and silica. Oat samples were determined by Spectrastar 2400D, Unity Scientific, USA NIR brand method (Van Soest et al., 1991).

Insoluble Fiber in Acid Detergent Solution (ADF) (%): It is composed of insoluble parts of oat grass samples under acid detergent conditions. It contains cellulose, lignin and silica. Oat samples were determined using the Spectrastar 2400D, Unity Scientific, USA NIR device according to the method (Van Soest et al., 1991).

Relative Feed Value (RFV) (%): It was calculated by using the formula (120)/NDF) x ((88.9- (0.779 x ADF)) x (0.775)).

Total Digestible Food (TDN) (%): It was calculated by using the ((-1.291 x ADF) +101.35) formula. DMD and DM of feeds were determined using below the equations (Van Dyke and Anderson, 2000). DMD value were determined using ADF values (Kaya, 2008).

DMD = 88.9 - (0.779 x ADF), DM = 120 / NDF, NYD = DMD x DM x 0.775

The data were analyzed with JMP (5.0) statistical software. According to the variance analysis results, statistically significant factor averages were compared using the Least Significant Difference (LSD) (Kalaycı, 2005).

RESULTS and DISCUSSION

Green forage yields of genotypes were 37.88-63.50 t

ha^{-1} in the first year and 41.92-55.54 t ha^{-1} in the second year, while the average green forage yield was 51.28 t ha^{-1} in the first year and 50.40 t ha^{-1} in the second year (Table 2-3). Based on the two-year averages of results, green forage yields of genotypes ranged from 39.90- 56.69 t ha^{-1}. While the highest green forage yield were obtained from G4 (Sebat) (56.69 t ha^{-1}) and G6 (56.60 t ha^{-1}) the lowest green forage yield were obtained from G5 (Yeniceri) (39.90 t ha^{-1}) and G7 (41.83 t ha^{-1}). Acar (1995), obtained oat yield as 11.49 t ha^{-1}, Gül et al. (1999) reported oat forage yield 16.82-28.48 t ha^{-1}. Uzun and Aşık (2012), reported the highest of green forage yield 47.34 t ha^{-1}.

Table 2. Mean square (MS) from the coşıkined analysis of variance for green forage yield, hay yield and plant height of oat genotypes

Çizelge 2. Yulaf genotiplerinin yeşil ot verimi, kuru ot verimi ve bitki boylarının birleştirilmiş varyans analizlerinin ortalama kareleri

Source of Variation	DF	GFY	HY	PH
Years (Y)	1	8.39523	0.13213	385.208
Replication [Yrs]	6	114.214	7.7099	441.608
Genotypes (G)	14	79.7125**	5.01841**	336.619**
Y x G	14	34.9702**	6.06818**	97.119**
Error	84	7.8713	0.52366	15.388
Total	119	24.87754	2.06379	87.39321

Significant at *p<0.05. and **p<0.01 levels. Respectively, **GFY**: Green forage yield, **HY**: Hay yield, **PH:** Plant height
*Önemlilik *p<0.05 ve **p<0.01 seviyeleri. Yeşil ot verimi, Kuru ot verimi, Bitki boyu*

Table 3. Yields of green forage and hay of the 15 oat genotypes during the 2016-17 and 2017-18 growing seasons

Çizelge 3. Yulaf genotiplerinin 2016-17 ve 2017-18 yetiştirme sezonlarındaki yeşil ot ve kuru ot verimi ortalama değerleri ve gruplar

	Genotypes	Green forage yield (t ha^{-1})			Hay yield (t ha^{-1})		
G. No	Genotype or Pedigree	2016-2017	2017-2018	2016-2018	2016-2017	2017-2018	2016-2018
G6	IL 3555-0BD-0T-5T-0T	61.92±3.70 a	51.29±3.50 a	56.60±6.58 a	17.28±1.03 a	13.08±0.89 b-e	15.18±2.41 a
G4	Sebat (st)	57.83±5.36 a-c	55.54±7.79 a	56.69±6.31 a	14.92±1.38 b	14.33±2.01 a-c	14.63±1.63 ab
G13	MN06130-0BD-0T-1T-0T	63.50±3.48 a	49.00±9.30a-c	56.25±10.1 a	16.89±0.93 a	11.42±2.17 ef	14.15±3.31 a-c
G2	Kahraman (st)	44.13±3.55 gh	52.00±11.3 a	48.06±8.80 de	12.93±1.04 cd	14.72±3.19 ab	13.82±2.39 b-d
G10	FL0507-0BD-0T-0T-9T-0T	53.0.0±5.54 c-e	53.71±8.47 a	53.35±6.64 a-c	13.94±1.46 bc	13.64±2.15 a-d	13.79±1.71 b-d
G9	FL0507-0BD-0T-0T-1T-0T	49.96±4.03 d-f	54.67±3.56 a	52.31±4.33 a-d	11.49±0.93 e-g	15.09±0.98 a	13.29±2.12 c-e
G12	MN06203-0BD-0T-2T-0T	57.92±0.88 a-c	55.13±6.95 a	56.52±4.82 a	12.86±0.19 c-e	13.34±1.68 a-e	13.10±1.14 c-f
G8	FL0522-0BD0T-0T-10T-0T	58.92±3.49 ab	48.67±11.9 a-c	53.79±9.82 ab	13.49±0.80 b-d	12.70±3.12 c-e	13.10±2.15 c-f
G1	Kirklar (st)	48.85±3.47 e-g	48.67±8.10 a-c	48.76±5.77 c-e	12.95±0.92 cd	13.09±2.18 b-e	13.02±1.55 c-f
G11	MN05131-0BD-0T-5T-0T	55.67±3.14 b-d	49.54±6.01 ab	52.60±5.52 a-d	14.14±0.80 bc	11.40±1.38 ef	12.77±1.80 d-f
G3	Kucukyayla (st)	44.58±6.05 f-h	50.54±8.44 ab	47.56±7.51 e	10.39±1.41 gh	13.85±2.31 a-d	12.12±2.56 e-g
G15	FL0534-0BD-0T-0T-1T-0T	43.04±3.00 hı	51.08±7.15 a	47.06±6.65 e	9.94±0.69 h	13.95±1.95 a-d	11.94±2.53 fg
G14	FL06020-0BD-0T-0T-3T-0T	51.88±9.00 de	50.83±5.68 ab	51.35±6.99 b-e	12.29±2.13 d-f	10.52±1.18 f	11.41±1.86 gh
G7	FL04167-0BD-0T-0T-9T-0T	40.21±4.77 hı	43.46±8.28 bc	41.83±6.49 f	11.14±1.32 f-h	11.39±2.17 ef	11.26±1.67 gh
G5	Yeniceri (st)	37.88±2.92 ı	41.92±7.30 c	39.90±5.58 f	8.48±0.65 ı	12.28±2.14 d-f	10.38±2.50 h
Average		51.28±8.77	50.40±7.88	50.84±8.31	12.88±2.56	12.99±2.24	12.93±2.39
CV (%)		7.86	10.4	9.20	7.79	10.6	9.33
LSD (% 5)		5.75	7.48	4.65	1.43	1.96	1.20
		Year: 7.96 not significant Yıl : Önemsiz			Year: 2.07 not significant, Yıl: Önemsiz		
		Genotype x Year: Significant, Genotip x Yıl: Önemli			Genotype x Year: Significant, Genotip x Yıl: Önemli		

*Means marked with the same letter are no different from each other.
**Aynı harfli olanlar birbirinden farklı değildir*

Avcı (2017), used 13 oat genotypes and reported as 55.65 t ha^{-1} oat sowing during winter and 37.39 t ha^{-1} in summer. On the other hand, Çeri and Acar (2019), used 12 oat genotypes and they obtained green forage yield as 23.42-31.09 t ha^{-1}. As it is seen, summer yields have declined of Çeri and Acar (2019) because oat plant likes cool and rainy weathers.Our results are similar to results of Avcı (2017), Uzun and Aşık (2012). However, our results were not similar to the other studies of Acar (1995), Gül et al. (1999), because the material used was different and the studies were conducted in different locations.

Hay yields of genotypes varied between 8.48-17.28 t ha^{-1} in the first year and 10.52-15.09 t ha^{-1} in the second year. In the first year, genotype G6 reached the highest hay yield (17.28 t ha^{-1}), followed by G4 and number G13 as 16.89 t ha^{-1} and 14.92 t ha^{-1}, respectively. According to the average of two years, the highest hay yield was reached in G6 with 15.18 t ha^{-1}. It is followed by G4 variety with 14.63 t ha^{-1} and G13 with 14.15 t ha^{1}.

There was statistically significant difference in green forage and hay yields of genotype and genotype x year interaction, while there was no statistically significant difference in green forage and hay yields of genotypes between years. According to the results of two years of research, correlations between hay yield with green forage (0.8865**) were determined as significant and positive. Gül et al. (1999), stated hay yield as 7.05-8.27 t ha^{-1}, Koçer and Albayrak (2012) stated as 13.52 t ha^{-1} in their study. Avcı (2017) used 13 oat genotypes for winter sowing. While they obtained hay yield as 12.64 t ha^{-1} in winter, they obtained hay yield as 6.88 t ha^{-1} in the summer. Çeri and Acar (2019) used 12 oat genotypes and they ranged from hay yield between 6.14-9.94 t ha^{-1} in their study. Our results were similar to those of Avcı (2017), Koçer and Albayrak (2012), but were different from those of Gül et al. (1999) and Çeri and Acar (2019). The reason why the results were not similar was that the materials and experiments used were conducted in different regions. The data on plant height and flowering dates of genotypes are given (Table 4).

Table 4. Values of plant height and flowering date of the 15 oat genotypes during the 2016-17 and 2017-18 growing seasons.
Çizelge 4. Yulaf genotiplerinin 2016-17 ve 2017-18 yetiştirme sezonlarındaki bitki boyu ortalama değerleri, grupları ve başaklanma tarihleri

G. No	Genotype or Pedigree	Plant Height (cm) 2016-2017	2017-2018	2016-2018	Flowering Date (day/month) 2016-2017	2017-2018
G1	Kirklar (st)	91.8±4.92 d	101.3±10.30 b-d	96.5±9.04 bc	3/5	24/4
G2	Kahraman (st)	87.8±2.22 e	96.5±7.94 c-f	92.1±7.14 d	4/5	25/4
G3	Kucukyayla (st)	80.8±2.98 f	88.0±8.52 g	84.4±7.07 e	1/5	24/4
G4	Sebat (st)	88.8±2.22 de	87.3±5.31 g	88.0±3.85 e	15/5	04/5
G5	Yeniceri (st)	83.3±2.75 f	89.8±8.77 fg	86.5±6.94 e	6/5	30/4
G6	IL 3555-0BD-0T-5T-0T	105.0±1.41 b	101.5±4.43 b-d	103.3±3.57 a	9/5	27/4
G7	FL04167-0BD-0T-0T-9T-0T	89.5±2.64 de	96.3±7.41 d-f	92.9±6.29 cd	4/5	27/4
G8	FL0522-0BD0T-0T-10T-0T	97.8±3.30 c	92.0±1.87 e-g	94.9±9.37b-d	17/5	03/5
G9	FL0507-0BD-0T-0T-1T-0T	102.5±5.74 b	107.8±3.30 ab	105.1±5.16 a	5/5	26/4
G10	FL0507-0BD-0T-0T-9T-0T	96.3±0.95 c	109.3±10.04 a	102.8±9.58 a	3/5	22/4
G11	MN05131-0BD-0T-5T-0T	97.3±0.48 c	93.3±6.55 e-g	95.3±4.94b-d	14/5	03/5
G12	MN06203-0BD-0T-2T-0T	97.3±1.89 c	98.8±16.58 c-e	98.0±10.95 b	15/5	02/5
G13	MN06130-0BD-0T-1T-0T	109.0±2.58 a	101.8±8.18 b-d	105.4±6.82 a	12/5	28/4
G14	FL06020-0BD-0T-0T-3T-0T	95.5±1.29 c	97.0±8.04 c-e	96.3±5.39 bc	4/5	24/4
G15	FL0534-0BD-0T-0T-1T-0T	87.8±0.25 e	103.5±6.24 a-c	95.6±9.34b-d	5/5	28/4
Average (Year)		94.0±8.12	97.6±10.2	95.8±9.34		
CV (%)		2.66	5.08	4.10		
LSD (% 5)		3.56	7.07	3.90		

Year: No significant, Genotype x Year: Significant
Yıl: Önemsiz, Genotipx Yıl: Önemli

There was statistically significant difference on plant height of genotype and genotype x year interaction, while there was no statistically significant difference on plant height of genotypes between years. According to the results of two years of research, correlations between hay yield with plant height (0.6141**) were determined as significant and positive. Taller oat plants are preferred for green forage production. The taller the plant height, the higher the yield of green forage and hay yield. Like plant height, stem thickness and amount of leaves are very important in green forage and hay yield. However, too tall plants can lodge and this leads to loss of yield. Moderate tall plants should be preferred for resisting to lodging. In addition, yield losses occur due to lodging during seed production of tall varieties.

Plant height of the genotypes varied from 83.3-109.0 cm in the first year and 87.3-109.3 cm in the second year. According to the year averages plant height of genotypes varied from 84.4-105.4 cm. The tallest plant length were recorded G13 (105.4 cm), G9 (105.1 cm) and G6 (103.3 cm) while the shortest plant height were recorded G3 (Kucukyayla) (84.4 cm) and G5 (86.5cm) according to the average of two years. The genotypes flowered between May 1 and May 17 in the first year and between April 22 and May 3 in the second year. G3, G10, G1 (Kirklar) and G2 (Kahraman) varieties were determined as the earliest flowering while G8, G11, G12 and G4 were determined as the latest flowering. Early flowering is very important in oat green forage and hay yield especially for second crop farming. The ADF, NDF and CP values of the genotypes are shown in Table 5. ADF refers to the amount of cellulose, lignin and insoluble protein in the plant cell wall structure. It provides the digestibility of the feed and the energy intake of the animal.

Table 5. Values of ADF, NDF and CP of the 15 oat genotypes during the 2016-17 and 2017-18 growing seasons
Çizelge 5. Yulaf genotiplerinin 2016-17 ve 2017-18 yetiştirme sezonlarındaki ADF (Asit Deterjan Lif) , NDF (Nötral Deterjan Lif) ve HP (Ham Protein) değerleri

		ADF (%)		NDF (%)		CP (%)	
G. No	Genotype or Pedigree	2016-17	2017-18	2016-17	2017-18	2016-17	2017-18
G1	Kirklar (st)	41.4	40.8	57.4	57.3	14.6	13.4
G2	Kahraman (st)	39.2	39.2	53.9	55.8	17.2	10.9
G3	Kucukyayla (st)	39.6	37.3	53.9	54.4	17.2	8.9
G4	Sebat (st)	40.4	41.7	58.0	56.5	11.8	10.0
G5	Yeniceri (st)	36.0	38.1	52.6	53.3	14.2	10.1
G6	IL 3555-0BD-0T-5T-0T	44.0	39.4	59.0	54.5	12.2	10.3
G7	FL04167-0BD-0T-0T-9T-0T	40.5	39.2	55.3	55.8	16.3	13.1
G8	FL0522-0BD0T-0T-10T-0T	38.5	37.3	50.6	51.8	14.4	13.0
G9	FL0507-0BD-0T-0T-1T-0T	37.5	40.2	54.5	57.2	15.2	11.1
G10	FL0507-0BD-0T-0T-9T-0T	41.3	43.3	57.9	57.5	11.8	12.7
G11	MN05131-0BD-0T-5T-0T	38.0	41.0	52.1	53.0	11.9	10.3
G12	MN06203-0BD-0T-2T-0T	40.6	40.5	55.4	52.7	12.6	11.5
G13	MN06130-0BD-0T-1T-0T	39.9	39.9	57.3	53.5	11.9	11.9
G14	FL06020-0BD-0T-0T-3T-0T	40.6	39.3	54.6	55.0	12.7	12.4
G15	FL0534-0BD-0T-0T-1T-0T	37.9	38.2	56.5	54.0	11.2	13.2
Minimum		36.0	37.3	50.6	51.8	11.2	8.9
Maximum		44.0	43.3	59.0	57.5	17.2	13.4
Average		39.7	39.7	55.3	54.8	13.7	11.5

High content feeds have low digestibility and energy (Kaya, 2008). ADF values of genotypes ranged from 36.0-44.0% and 37.3-43.3% in the first and the second year, respectively. G5 showed the lowest ADF value

(36.0%), while G6 showed the highest ADF value (44.0%) in the first year. G3 had the lowest (37.3%) and G10 had the highest (43.3%) ADF value in the second year. Feed quality of forage is better if ADF is low. Therefore, G8, G15 and G3 are considered good in terms of forage quality. Koçer and Albayrak (2012), stated that ADF value is 34.6%, Mut et al. (2015), used 100 oat genotypes in their study and they founded that ADF values ranged from 33.32-42.48%. Çeri and Acar (2019), used 12 oat genotypes and they determined that ADF value varied between 37.82-41.75%. Our results were similar to Çeri and Acar (2019), Koçer and Albayrak (2012), Mut et al. (2015). NDF value is considerably important because the amount of metabolizing energy in cereal depends on its concentration. The soluble substances in NDF consist mostly of starch, sugar, crude protein and fat. These substances are 98% digestible. However, as the amount of NDF increases, soluble substances contained in NDF decrease. G8 had the lowest NDF (50.6%) and G6 had the highest NDF (59.0%) in the first year. G8 had the lowest NDF (51.8%) and G10 had the highest (57.5%) in the second year. Similar to ADF value, if NDF value is low, forage quality is better. Thus, G8, G11, G5 and G3 varieties were considered to be good. Koçer and Albayrak (2012) reported 59.1% NDF. Mut et al. (2015) found 52.25-65.24% NDF value. Çeri and Acar (2019) investigated 12 oat genotypes and reported NDF as 52.79-57.80%. Our results were similar to Ceri and Acar (2019), Koçer and Albayrak (2012), while Mut et al. (2015) results were slightly different. These differences could be due to the genetic structure of the genotypes, growing conditions and nitrogen fertilizer applications. Oats used as human nutrition and animal feed should have a high protein content.

Table 6. Values of DMD, DM and RFV the 15 genotypes during the 2016-17 and 2017-18 growing seasons.
Çizelge 6. Yulaf genotiplerinin 2016-17 ve 2017-18 yetiştirme sezonlarındaki KMS (Kuru Madde Sindirebilirliği), KMT (Kuru Madde Tüketimi) ve NYD (Nispi Yem Değeri) değerleri

		DMD (%)		DM (%)		RFV (%)	
G. No	**Genotype or Pedigree**	**2016-17**	**2017-18**	**2016-17**	**2017-18**	**2016-17**	**2017-18**
G1	Kirklar (st)	2.1	2.1	56.7	57.1	91.9	92.7
G2	Kahraman (st)	2.2	2.1	58.4	58.3	100.9	97.2
G3	Kuçukyayla (st)	2.2	2.2	58.1	59.9	100.2	102.4
G4	Sebat (st)	2.1	2.1	57.5	56.4	92.2	93.0
G5	Yeniceri (st)	2.3	2.2	60.8	59.2	107.7	103.2
G6	IL 3555-0BD-0T-5T-0T	2.0	2.2	54.6	58.2	86.1	99.4
G7	FL04167-0BD-0T-0T-9T-0T	2.2	2.2	57.4	58.3	96.5	97.3
G8	FL0522-0BD0T-0T-10T-0T	2.4	2.3	58.9	59.8	108.3	107.4
G9	FL0507-0BD-0T-0T-1T-0T	2.2	2.1	59.7	57.6	102.0	93.7
G10	FL0507-0BD-0T-0T-9T-0T	2.1	2.1	56.7	55.1	91.2	89.2
G11	MN05131-0BD-0T-5T-0T	2.3	2.3	59.3	57.0	105.9	99.9
G12	MN06203-0BD-0T-2T-0T	2.2	2.3	57.3	57.3	96.2	101.3
G13	MN06130-0BD-0T-1T-0T	2.1	2.2	57.8	57.8	93.9	100.6
G14	FL06020-0BD-0T-0T-3T-0T	2.2	2.2	57.3	58.3	97.5	98.7
G15	FL0534-0BD-0T-0T-1T-0T	2.1	2.2	59.4	59.2	97.8	101.8
Minimum		2.0	2.1	54.6	55.1	86.1	89.2
Maximum		2.4	2.3	60.8	59.9	108.3	107.4
Average		2.2	2.2	58.0	58.0	97.9	98.5

While the crude protein values of genotypes ranged between 11.2-17.2% in the first year and 8.9-13.4% in the second year, the mean crude protein content was 13.7% in the first year and 11.5 5 in the second year. In the first year, G2 and G3 varieties reached the highest value with 17.2% protein content, while G15 reached the lowest value with 11.2% protein. In the second year, G2 was the highest with 13.4% crude

protein content. There have been high differences in the amount of crude protein in the genotypes. Koçer and Albayrak (2012), stated 10.87% crude protein content. Mut et al. (2015), tested 100 oat genotypes and reported 5.88-13.64% crude protein content. Çeri and Acar (2019), used 12 oats genotypes and defined crude protein content between 9.64-11.53%. Our results were similar to Çeri and Acar (2019), Koçer and Albayrak (2012) and Mut et al. (2015) studies.

Data related to DMD, DM and RFV values of genotypes are given (Table 6). While the DMD values of genotypes were 2.0-2.4% in the first year, and 2.1-2.3% in the second year, there was no difference between the genotypes considering the years. DM values of genotypes varied between 54.6-60.8% in the first year and 55.1-59.9% in the second year. While there were differences between genotypes in terms of DM, there was no difference between years. Koçer and Albayrak (2012) reported similar results in their study.

The relative feed value (RFV), which is included in different quality indices for the determination of forage quality, is based on the ADF and NDF contents. RFV in feed is of great importance in determining the quality and marketing of feeds. High RFV value indicates that the quality of forage is good. RFV values of genotypes ranged from 86.1-108.3% in the first year and 89.2-107.4% in the second year. G8 showed the highest quality in the first year because had 108.3% RFV followed by G5 with 107.7%, G11 with 105.9%, G9 with 102.0% and G2 variety with 100.9% RFV. In the second year, G8 with 107.4% RFV reached the highest quality feed value, followed by G5 with 103.2 %, G3 with 102.4% and G15 with 101.8% RFV. According to the two-year RFV average value G8, G5, G11 and G3 had the highest forage quality, while G10, G1, G4 and G6 genotypes had the lowest.

Koçer and Albayrak (2012), investigated feed peas mixtures with oat and barley, and reported 97.45% RFV for monoculture oat and 167.27% RFV for monoculture feed peas. As the feed pea ratio increased, the relative feed value of the feed increased. Koçer and Albayrak's (2012) results were similar to our results. Conslusion to the two-year results of our study, G6 (15.18 t ha^{-1}), G4 (14.63 t ha^{-1}), G13 (14.15 t ha^{-1}) and G2 (13.82 t ha^{-1}) genotypes had the highest hay yield. The G8 (107.8%), G5 (105.5%), G11 (102.9%) and G3 (101.3%) showed highest RFV and forage quality. G2, G9 and G8 were suitable in terms of hay yield and RFV for Trakya-Marmara region. Besides, G2 and G9 with high forage quality and hay yield and early flowering characteristics are recommended for the second crop planting places.

Author's Contributions

The contribution of the authors is equal

Statement of Conflict of Interest

Authors have declared no conflict of interest

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The Investigation of the Effect of the Salicylic Acid on the Antioxidant Potential, Vitamin C Content and DNA Protection Activity of Wheatgrass (*Triticum aestivum* L.)

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ABSTRACT
Wheatgrass is considered as a superfood because of its high antioxidant potential and beneficial ingredients. Especially in recent years, wheatgrass juice and its powder have been tested *in vivo* animal models and clinical studies against some diseases. Salicylic acid (SA) one of the important phytohormones controlling plant growth is used as an exogenous elicitor to increase plant bioactive compounds. The aim of this study was to investigate antioxidant potential, vitamin C content and DNA protection of wheatgrass grown from SA pre-treated seeds. For this purpose, total antioxidant statue, total oxidant statue, oxidative stress index and vitamin C level were determined. Additionally, pUC19 plasmid was incubated with Fenton's solution to determine DNA protection activity of lyophilized wheatgrass extract. Bread wheat caryopses were imbibed in different concentrations of SA for 2 hours. Wheatgrass grown from seeds pre-treated with 10^{-8} M SA had significantly higher total antioxidant statue, vitamin C and soluble protein content than control. It was observed that wheatgrass extracts had a DNA protective role against hydroxyl radicals. It was concluded that SA pretreatment of seeds could be a good approach to increase their antioxidant potential, soluble protein content and vitamin C level of plants used as antioxidant sources by people.

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Salisilik Asitin Buğday Çiminin (*Triticum aestivum* L.) Antioksidan Potansiyeline, C Vitamini İçeriğine ve DNA Koruyucu Aktivitesine Etkisinin Araştırılması

ÖZET
Buğday çimi yüksek antioksidan potansiyeli ve yararlı içeriğinden dolayı bir süper besin olarak görülmektedir. Özellikle son yıllarda, buğday çim suyu ve tozu bazı hastalıklara karşı *in vivo* hayvan modellerinde ve klinik çalışmalarda test edilmiştir. Bitki büyümesini kontrol eden önemli bitki hormonlarından biri olan salisilik asit (SA) bitki biyoaktif maddelerini artırmak için dış kaynaklı bir elisitör olarak kullanılır. Bu çalışmanın amacı, SA ile ön uygulama yapılmış tohumlardan büyüyen buğday çiminin antioksidan potansiyelini, C vitamini içeriğini ve DNA koruyucu aktivitesini araştırmaktır. Bu amaç için, toplam antioksidan seviye, toplam oksidan seviye, oksidatif stres indeksi ve C vitamini seviyesi belirlendi. Bunlara ek olarak, liyofilize buğday çimi ekstrelerinin DNA koruyucu aktivitesini belirlemek için pUC19 plazmiti Fenton solüsyonu ile muamele edildi. Ekmeklik buğday karyopsis meyveleri farklı konsantrasyonlarda ki SA içinde 2 saat şişirildiler. 10^{-8} M SA ile şişirilen tohumlardan büyüyen buğday çimi kontrole göre anlamlı olarak daha yüksek toplam antioksidan seviyeye, C vitamini ve çözünen protein içeriğine sahiptir. Buğday çimi ekstresinin hidroksil radikallerine karşı DNA koruyucu rolüne sahip olduğu gözlendi. İnsanlar tarafından antioksidan kaynağı olarak tüketilen bitkilerin antioksidan potansiyelini, çözünen protein içeriğini ve C vitamini

Bitki Fizyolojisi

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Anahtar Kelimeler
Buğday çimi
Salisilik asit
C vitamini
Elisitör
DNA koruma

seviyesini artırmak için tohumlarına SA ön uygulamasının yapılmasının iyi bir yaklaşım olabileceği sonucuna varıldı.

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INTRODUCTION

Wheat is one of the most important crop plants consumed by human. Bread wheat (*Triticum aestivum* L.) and durum wheat (*Triticum turgidum durum* L.) are mostly cultivated species for food source in the world. In recent years, wheatgrass juice and powder have been popular as a functional food which is used for human health (Bar-Sela et al., 2015). Wheatgrass is harvested 10-15 days after germination. It has been used for some disease treatments for several years because of its rich source of chlorophyll, vitamins, amino acids and mineral content and antioxidant potential (Rana et al., 2011; Thakur et al., 2019). Wheatgrass juice has 70 % of chlorophyll also called as green blood because of its similar chemical structure to hemoglobin, only central magnesium is present at porphyrin ring of chlorophyll instead of iron in hemoglobin (Padalia et al., 2010). Similarly, the treatment with wheatgrass is called as green blood therapy. Wheatgrass has chlorophyll, tannins, flavonoids, phytic acid, saponins, protein, crude fat, ascorbic acid, beta carotene, magnesium, calcium, iron, magnesium, selenium, zinc, copper, chromium and cobalt (Singh et al., 2012; Devi et al., 2019; Thakur et al., 2019). Harvesting of wheatgrass between seven and thirteen days after planting was suggested for high free radical scavenging activity (Devi et al., 2020).

There are several studies reporting wheatgrass use in some diseases such as cancer, oxidative stress, immunologic disorders, bone diseases, metabolic and cardiovascular diseases (Rana et al., 2011; Bar-Sela et al., 2015). Nephroprotective effect of wheatgrass juice was reported ethanol-induced oxidative damage in rats (Hebbani et al., 2020). It was reported that cereal grass juice had the wound healing potential (Karbarz et al., 2019). Oh et al., (2019) investigated that ethanolic bread wheat extract contributed to protection of liver damage in mice fed a choline deficient or high fat diet. Two novel compounds isolated from wheatgrass had the cytotoxic activities against some cancer cell lines (pancreatic, breast, colon, prostate and lung) (Save et al., 2019). It was suggested that wheatgrass might have had an important role in kids with thalassemia receiving chronic blood transfusion (Mutha et al., 2018). The healing effects of wheatgrass juice were presented in a study conducted with type 2 diabetic patients (Shakib et al., 2017). The protective role of wheatgrass on liver damage was investigated alcohol administered rats and heated polyunsaturated fatty acids (Durairaj et al., 2014).

Plant bioactive compounds also known as secondary metabolites are specific products of primary biological pathways and intermediates (Bourgaud et al., 2001). Plants and mushrooms have some bioactive compounds playing important role against biotic or abiotic environmental stresses (Dogan et al., 2018). Plants or plant cells adapt to physiological and morphological changes by producing bioactive compounds against biotic or abiotic stress factors (Isah, 2019). Additionally, plant bioactive substances can be used in pharmaceutical fields, food additives and other industries (Balandrin et al., 1985). Elicitors are used to manipulate to production of bioactive compounds presence in plants (Guerriero et al., 2018). Some chemicals such as calcium, silver nitrate, iron, magnesium, or some macromolecules such as proteins, carbohydrates and fatty acids or allelopathic relationships may have an elicitor effect (Bhatia and Bera, 2015). Chemical elicitors can be applied *in vitro* by creating callus in plant tissue culture or applied directly to plants (Dias et al., 2016). Salicylic acid, methyl salicylate, benzoic acid and chitosan are widely studied chemicals their effects on the production of phenolic acids and activity of defence enzymes (Patel and Krishnamurthy, 2013). Salicylic acid is a colourless and crystalline organic acid. Foliar spraying of common purslane (*Portulaca oleracea* L.) with different concentration with SA improved photosynthetic pigments, respiration and its bioactive content (Saheri et al., 2020). Exogenous SA treatment of wheat seeds or seedlings had protective role against different stresses (Fardus et al., 2018; Azeem et al., 2019; Loutfy et al., 2020).

In recent years, the popularity of wheatgrass is increasing as a functional food for human health. Studies about enrichment of food ingredients are also being hot topic in plant research. The aim of this study was to explore how pretreatment of wheat caryopses with different concentrations of SA affected germination rate, its antioxidant capacity (TAS and (TOS) and its vitamin C content. Additionally, another aim was to observe *in vitro* DNA protection role of lyophilized wheatgrass extracts against hydroxyl radicals generated by Fenton reaction.

Furthermore, we wondered how different concentrations of the salicylic acid imbibition of bread wheat caryopses affected to physiological parameters (length and fresh weight of shoot and root), biochemical parameters (proline, malondialdehyde, hydrogen peroxide, chlorophyll and soluble protein content) and antioxidant enzyme activities (catalase, ascorbate peroxidase and superoxide dismutase) of wheatgrass.

MATERIALS and METHOD

Plant Material

Bread wheat caryopses (*Triticum aestivum* L. 'Bezostaja-1') were used as a plant material. It was kindly provided from Transitional Zone Agricultural Research Institute, Eskişehir, Turkey.

Sterilization

Seeds were surface sterilized by 3 % of sodium hypochlorite for 5-10 minutes shaking at 200 rpm. After washing with sterile distilled water for several times, seeds were treated with 70% of ethanol for 1 minute. Washing with sterile distilled water was repeated.

Preparation of Salicylic Acid Concentrations

Different concentrations of salicylic acid (SA) were tested in this study. 10^{-2} M (SA-2), 10^{-4} M (SA-4), 10^{-6} M (SA-6), 10^{-8} M (SA-8), 10^{-10} M (SA-10) and 10^{-12} M (SA-12) salicylic acid were prepared from 1 M stock solution.

Seed Pretreatment with Salicylic Acid and Growth of Seedlings

Surface sterilized seeds were imbibed with different concentrations of SA for 2 hours. Control seeds were imbibed with sterile distilled water for 2 hours. Imbibed bread wheat caryopses were cultivated on hydroponic culture under controlled growth room (25±1 °C and 16/8 light and dark photoperiod). Hoagland's medium was used to grow wheat seedlings (Hoagland and Arnon, 1950). This study was conducted under semi-sterile conditions. Hoagland's medium was prepared from stock solutions and pH was adjusted between 5.7-5.8. After autoclaving, Fe-EDTA solution was added. Ten days old seedlings were harvested and stored ultra-freezer until analysis.

Wheatgrass Homogenization

Ten days old wheatgrass samples were homogenized with liquid nitrogen using mortar and pestle. Samples were stored at ultra-freezer. Homogenized wheatgrasses were used to determine malondialdehyde (MDA) content, proline content, hydrogen peroxide (H_2O_2) content, total antioxidant statue (TAS), total oxidant statue (TOS) oxidative stress index (OSI), vitamin C content and some antioxidant enzyme activities including ascorbate peroxidase (APX), catalase (CAT) and superoxide dismutase (SOD).

Germination Rate

Seeds were sown in petri dishes with wet two-layer filter paper for 3 days. Germination rate was calculated for SA treated and control wheat seeds according to formula given below.

Germination Rate (%) = (Germinated seeds / Cultivated Seeds) X 100

Determination of Antioxidant Capacity of Wheatgrass

The effects of salicylic acid pre-treatment of wheat seeds were evaluated for antioxidant potential of wheatgrass. Total antioxidant statue (TAS) and total oxidative statue (TOS) were measured and oxidative stress index (OSI) were calculated for this aim. Commercially available TAS and TOS kits (Rel Assay Diagnostics, Gaziantep, Turkey) were used to determine TAS and TOS levels according to Erel (2004; 2005). TAS kit is based on reduction of dark-blue color of ABTS radical (2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) to colorless form of ABTS. Extracted wheatgrass was homogenized with 140 mM potassium chloride buffer (1:10, w/v) to determine total antioxidant statue (TAS) and total oxidative statue (TOS). After vortex, samples were filtrated and centrifuged to collect supernatants. 18 µL of sample or standard (1 mmol/L Trolox) or water put into spectrophotometer cuvette. After that, 300 µL of reagent 1 (buffer solution, acetate buffer 0.4 mol/L, pH:5.8) was added into cuvette and mixed well. Absorbance 1 (Abs1) was spectrophotometrically measured at 660 nm. 45 µL of reagent 2 (pro chromogenic solution, ABTS 30 mmol/L) was put into cuvette and mixed well. Cuvette was incubated at room temperature for 10 minutes and absorbance 2 (Abs2) at 600 nm was measured. Results were presented as µmol Trolox equivalent g^{-1} FW. TAS level was calculated according to commercial kit guideline.

TOS test is based on oxidizing the ferrous ion chelator complex to ferric ion by oxidants in the sample. 45 µL of sample or standard (10 µL/L hydrogen peroxide) and 300 µL of reagent 1 (buffer solution, sulphuric acid 25mM pH:1.25) were mixed in a cuvette and measured at 530 nm for absorbance 1 (Abs1). 15 µL of reagent 2 (substrate solution, sulphuric acid 25 mM pH:1.75, ferrous ion 5mM, o-dianisidine 10nM) was added into cuvette and mixed well before incubation at room temperature for 10 minutes. After incubation, intensity of color change was spectrophotometrically measured as absorbance 2 (Abs 2) at 530 nm to determine total oxidant level in the sample or

standard. Results were presented as µmol hydrogen peroxide g^{-1} FW. TOS level and oxidative stress index (OSI) were calculated according to commercial kit guideline.

Determination of Vitamin C Content of Wheatgrass

Vitamin C content of shoot tissue was determined with colorimetric method using commercial kit protocol (E-BC-K034, Elabscience Biotechnology, USA). Samples were read at 536 nm and vitamin C content was calculated as µg g^{-1} FW.

DNA Protection Activity

Lyophilized extract preparation

3 g of shoot tissue ground by liquid nitrogen was extracted with 80 % of ethanol at a ratio 1:20. Samples were incubated in a shaker at 200 rpm for 2 hours. After that, samples were centrifuged at 3500 rpm for 10 minutes. Supernatant was collected and ethanol was evaporated. Water was evaporated by a freeze dryer. Extract yield was about 3.8 %. Lyophilized extract was used as 10 mg/mL final concentration after filter sterilization.

Bacterial growth

Escherichia coli having pUC19 plasmid was inoculated in Luria Broth (LB) medium. After overnight incubation at 37 °C, 100 µL of cultivation was spread onto nutrient agar medium. Ampicillin was added to bacterial growth media to select colonies with pUC19 plasmid having an ampicillin resistance gene. Plasmid isolation was performed using commercial plasmid isolation kit (K0502, Thermo Fisher Scientific). Plasmid was run in an agarose gel (1 %) to observe plasmid. Plasmid was digested with restriction enzymes for correction. Additionally, plasmid concentration and purity were determined by spectrophotometrically.

In vitro DNA damage protection assay

This assay is based on the principle that plasmid DNA is damaged by hydroxyl radicals formed by incubation of Fenton's solution at 37 °C and this damage is visualized on agarose gel (Lee et al., 2002; Locatelli et al., 2018). 10 mg/mL of lyophilized wheatgrass extracts obtained from bread wheat leaves grown from seeds imbibed with different concentrations of SA were tested for DNA damage protection. Fenton's solution (30 mM hydrogen peroxide, 50 mM ascorbic acid, 80 mM iron chloride) was used for *in vitro* hydroxyl radical source (Locatelli et al, 2018). Quercetin known as an antioxidant was used as a positive control as 50 µg/mL (Lee et al., 2002). 8 µL of pUC19 plasmid, 3 µL of lyophilized wheatgrass extract or quercetin and 9 µL of Fenton's solution were added into an eppendorf tube in order. 8 µL of pUC19 plasmid and 12 µL of nuclease free water were used as control. 8 µL of pUC19 plasmid, 9 µL of Fenton's solution and 3 µL of nuclease free water were used as negative control. Samples were run agarose gel (1 %) after incubation at 37 °C for 30 minutes and visualized with a transilluminator.

Physiological Parameters

The length of shoots and roots were measured with ruler. Also, fresh weight, dry weight and turgor weight were noted for shoot and root tissues. While shoots directly were weighted with analytical balance for fresh weight (FW), roots were rinsed with running water and were dried with tissue paper.

Biochemical Parameters

Malondialdehyde (MDA) content of shoots were determined. Shoot tissue was weighted and recorded. Tissue was extracted with 1 mL of 5 % trichloroacetic acid (TCA) in eppendorf tubes. Supernatants were collected after 12000 rpm centrifugation for 15 min. at room temperature. Equal amounts of supernatant and 0.5 % thiobarbituric acid (TBA) in 20 % TCA were added in a new tube. Tubes were incubated at 96°C for 25 min. in a hot block after brief vortex. After that, tubes were cooled into ice until they reached to room temperature. At the last step, tubes were centrifuged at 10000 rpm for 5 min. The absorbance of supernatant was read at 532 nm and 600 nm. 0.5 % TBA in 20 % TCA was used as blank (Ohkawa et al., 1979). The MDA content was calculated as nmol MDA g^{-1} FW.

The effect of SA imbibition of seed was evaluated for proline content. Shoot tissue was extracted wit 1 mL of 3 % sulphosalicylic acid. Extracts were transferred into eppendorf tubes and centrifuged at 14000 rpm for 5 min at 4°C. Acid ninhydrin (0.2 mL), acetic acid (0.2 mL), 0.1 mL sulphosalicylic acid and 0.1 mL of supernatant were added into a new tube in order. After briefly vortex, tubes were incubated at 96°C for 1 hour in a hot block. Toluene (1 mL) was added tubes to stop reaction at the end of the incubation. Vortexed tubes were centrifuged at 14000 rpm for 5 min at 4°C. Upper red phase was read at 520 nm absorbance against toluene (Bates et al., 1977). The proline content was calculated as µmol proline g^{-1} FW.

Shoot tissue (0.5 g) was homogenized with 100 mM K-PO_4 buffer (pH:6.8). After filtration, samples were centrifuged 18000 g at 4°C for 20 minutes to determine H_2O_2 content. Supernatant (0.25 mL) was put into a new tube and peroxidase solution (1.25 mL) including o-dianisidine (0.005 % (w/v)) and peroxidase (40 µg/mL) was added into tube. Tubes were incubated in a water bath at 30°C for 10 min. Lastly, 1N perchloric acid (0.25 mL) was added into to tubes to stop reaction. After centrifugation at 5000 g for 5

min., samples were read at 436 nm against blank which was peroxidase solution. The H_2O_2 content was calculated as nmol H_2O_2 g^{-1} FW (Bernt and Bergmeyer, 1974).

Total protein extraction was performed from ground shoot tissue. Shoot samples were extracted in 1 mL of extraction buffer (50 mM K-PO_4 including 1 mM EDTA and 2 % of PVP). Supernatants were collected after centrifugation at 13000 g for 20 min. at 4°C. Bradford method was used to determine protein concentration (Bradford 1976). Protein content of samples was calculated as mg protein g^{-1} FW. Total chlorophyll (Chl) content of wheatgrass was measured according to Lichtenthaler (1987).

Determination of Antioxidant Enzymes

Catalase activity was measured with spectrophotometer at 240 nm according to Aebi (1974). 900µL of assay solution (50 mM KH_2PO_4, pH 7.00), 100µg of crude protein and 100µL of 100 mM H_2O_2 were added into a quartz cuvette in order. Cuvette was read at 240 nm for 120 second with 10 second intervals. CAT activity of samples was calculated as U mg^{-1} protein.

Ascorbate peroxidase activity was measured according to Nakano and Asada method (1981). Quartz cuvette including (800µL of 50 mM K-PO4, pH 6.60, 100µL of 2.5 mM ascorbate, 100µg of crude protein and 100µL of 10 mM H_2O_2) was read at 290 nm for 120 second with 10 second intervals. APX activity of samples was calculated as U mg^{-1} protein.

Superoxide dismutase activity was measured with nitro blue tetrazolium (NBT) method (Govinda et al., 2017). Reaction mix was prepared with 2.9 mL of 50 mM K-PO_4 (pH: 7.80) buffer having 10 mM methionine, 168 µM NBT, 0.025 % Triton X-100 (w/v) and 1.17 µM riboflavin) and 0.1 mL of crude protein in tubes. Tubes were incubated with 15 watts fluorescent light for 15 minutes. Samples were read at 560 nm with a spectrophotometer. SOD activity of samples was calculated as U g^{-1} FW.

Statistical Analysis

Each experiment was performed at least three times. Results were presented as mean $\pm$ SEM and analyzed by one-way analysis of variance (ANOVA) using MINITAB package program. Statistically significance difference was accepted as $p < 0.05$.

RESULTS and DISCUSSION

Wheatgrass is one of the important superfoods which have protective role in human health because of its high antioxidant potential, chlorophyll content, amino acid content, vitamin and mineral content. Young wheat seedlings are used to produce wheatgrass juice or powder (Singh et al., 2012). The protective role of wheatgrass against some diseases such as cancer, diabetes, oxidative stress and thalassemia was investigated *in vitro* and *in vivo* studies (Bar-Sela et al., 2015). There are a lot of reports that SA causing increase of the secondary metabolites and antioxidant enzymes of wheatgrass had protective role in wheat plants under environmental stresses (Fardus et al, 2018; Azeem et al., 2019; Loutfy et al., 2020).

Germination Rate

While imbibition of bread seeds with SA-2 application prevented germination, germination rates of other SA concentrations were more than control (Fig. 1). However, only SA-12 application caused significantly increase (8.3 %) in germination rate compared to the control. The increase in germination rate was between 2.8 – 5.6 % for other concentrations (Fig. 1). Germination is the most important process for plants. SA priming of wheat seeds with different concentrations of SA for 12h promoted seed germination under salt stress (Azeem et al., 2019). Similarly, the positive effects of SA to germination of wheat seeds were reported under salinity stress (Fardus et al., 2018). Yanik et al., (2018) found that high concentrations of SA prevented seed germination of rye plant, while the lowest SA application promoting seed germination. We also found similar results to Yanik et al., (2018), the highest concentration of SA (SA-2) inhibited seed germination. On the other hand, the lowest SA concentration (SA-12) promoted seed germination (Fig. 1).

Antioxidant Potential

SA-8 application caused significantly increase (30.2 %) in TAS compared to the control (Fig. 2a). Additionally, we found that SA-8 application caused significantly increase in TAS compared to SA-4, SA-6 and SA-10 treatments. SA application did not cause increase in TOS (Fig. 2b). According to OSI results, SA-8 shoot tissue had the lowest OSI value and significantly lower (31.1 %) than control (Fig. 2c). Additionally, OSI of SA-8 treated wheatgrass was the importantly lower than SA-4 and SA-10 applications. Wheatgrass has high antioxidant potential because of its high content of antioxidants, vitamins (Vitamin E, C and A) and minerals (iron, magnesium and calcium) (Aydos et al., 2011). Kamat et al., (2000) demonstrated that chlorophyllin a derivative of chlorophyll had a protective effect on mitochondria against oxidative damage. Antioxidant activity of wheatgrass was not affected from different drying methods (Devi et al., 2019). Indoor growth of wheat young leaves for seven or ten days resulted with higher free radical scavenging activity than outdoor growth ones (Devi et al.,2020). Virdi et al., (2021) demonstrated that antioxidant potential of

wheatgrass was affected from different photoperiod regime and incubation media. Additionally, using spring water in cultivation of wheat seedlings resulted with increase in radical scavenging activity and phenolic content (Fortună et al., 2018). We found that SA-8 application caused significantly increase in TAS compared to the control, SA-4, SA-6 and SA-10 treatments. Additionally, SA did not cause increase in TOS. Moreover, OSI of SA-8 treated wheatgrass was the importantly lower than control, SA-4 and SA-10 (Figure 2c).

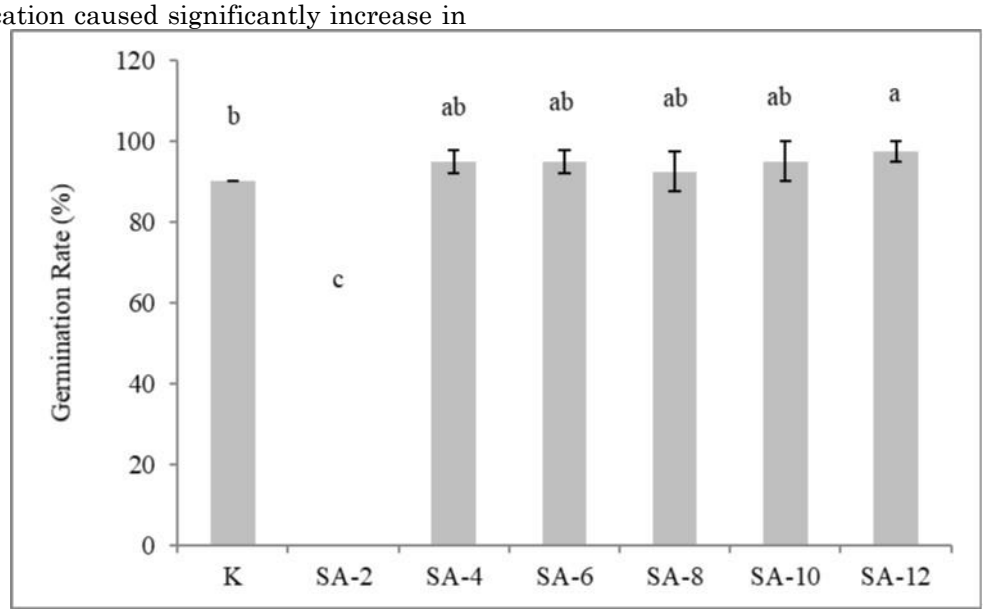

Figure 1. The effect of salicylic acid pre-treatment on germination rate. Different letters in the each column indicate significant difference (P<0.05).

Şekil 1. Salisilik asit uygulamasının çimlenme oranına etkisi. Her kolondaki farklı harfler anlamlı farkı temsil eder (P<0.05).

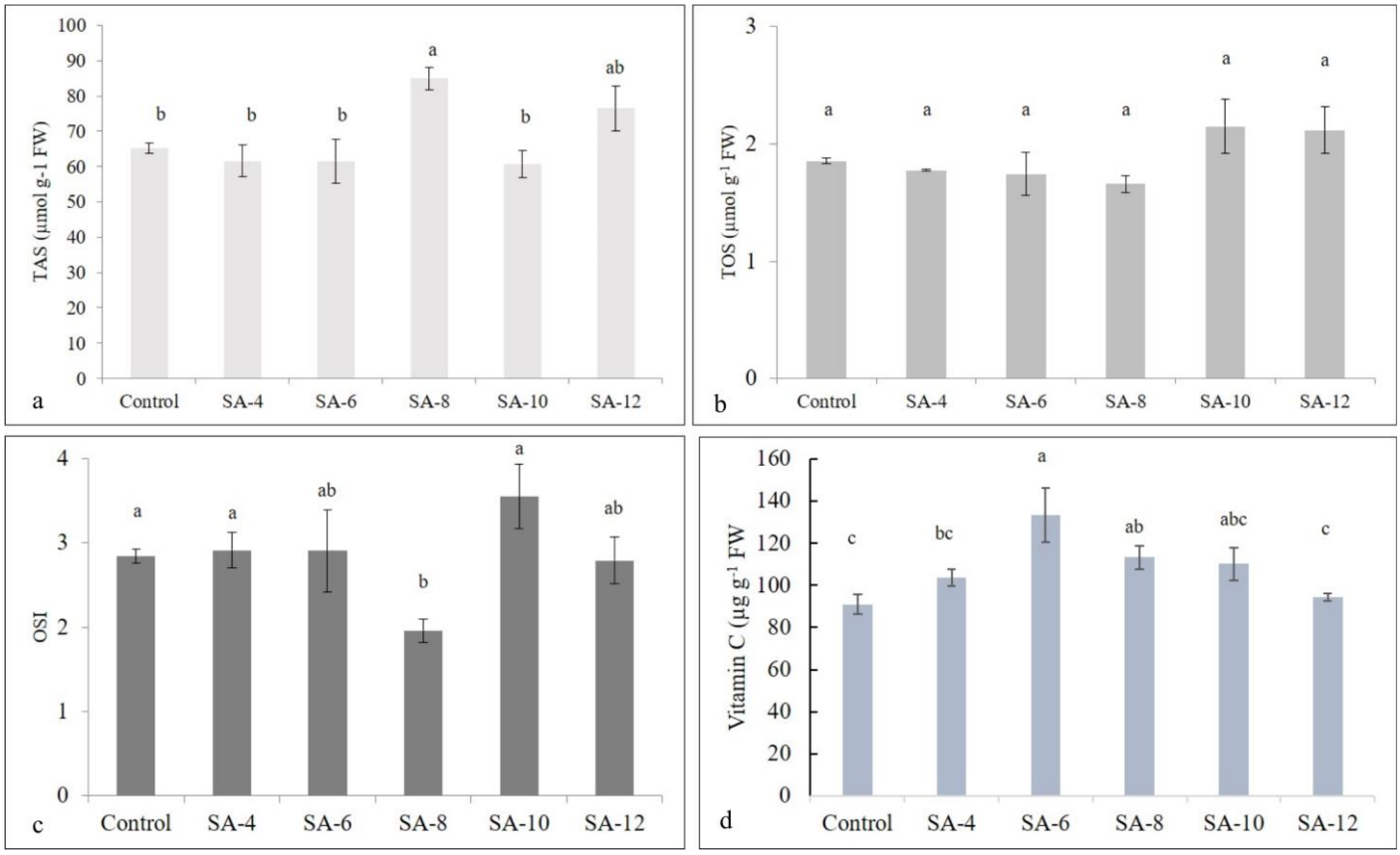

Figure 2. The effect of SA on TAS, TOS, OSI and vitamin C content. Different letters in the each column indicate significant difference (P<0.05).

Şekil 2. SAnın TAS, TOS, OSI ve C vitamini içeriğine etkisi. Her kolondaki farklı harfler anlamlı farkı temsil eder (P<0.05).

The Effect of SA on Wheatgrass Vitamin C Content

We evaluated vitamin C content of shoot tissue of SA treated wheat seeds. According to results, the highest vitamin C content was measured at SA-6 treatment. SA-6 treated wheat shoot had significantly more (46.7 %) vitamin C content than control (Fig. 2d). Additionally, SA-8 treatment caused significantly increase (24.7 %) in vitamin C content compared to the control (Fig. 2d). While both of SA-6 and SA-8 applications had significantly more vitamin C content than SA-12, the vitamin C content of SA-6 treatment was higher than SA-4. Vitamin C (ascorbic acid) is one of the vitamins present in wheatgrass juice powder (Thakur et al., 2019). Drying methods affected ascorbic acid content of wheatgrass, especially oven drying method (Devi et al., 2019). Harvesting of wheatgrass in different days after sowing indoor and outdoor affected vitamin C content in wheatgrass, especially thirteenth day harvesting of indoor planted wheat seedlings was significantly higher than outdoor ones (Devi et al., 2020). Thakur et al., (2019) found high amount of vitamin C (9.3 mg/100 g) in wheatgrass juice powder. Spraying of SA caused increase in ascorbic acid content of wheat plants and protected plants from negative effects of fenoxaprop-p-ethyl herbicide (Yaman and Nalbantoğlu 2020). We found that SA caused increase in vitamin C content of the wheatgrass. Additionally, vitamin C content of SA-6 and SA-8 pretreated plants was significantly higher than control (Fig. 2d). The high level of vitamin C in SA-8 treated plants could be a reason for the highest TAS level in this application.

DNA Protective Role of Lyophilized Wheatgrass Extracts

Lyophilized ethanol extracts were prepared from the homogenized wheatgrass powder. The prepared extracts were dissolved at 10 mg/mL for DNA protection. The *pUC19* plasmid was used to investigate the DNA protective effect of lyophilized wheatgrass extract. After growing the bacteria with the plasmid overnight, plasmid isolation was made and its purity and concentration were determined (Fig. 3a-b). The purity of the isolated plasmid was measured as OD260/280 ratio between 1.8-2.1. The plasmid concentration was diluted to 62.5 ng/µL.

Plasmids are mostly in supercoil form and to a lesser extent in linear form. However, when exposed to stress conditions for a long time, the phosphodiester bonds in both chains are broken and converted to the nicked form. It was observed that DNA was in two different forms, namely supercoiled and linear form, in the well loaded only with plasmid. In order to determine the DNA protective effect, three different plasmid forms were observed in the wells containing plasmid DNA in which oxidative damage was induced in vitro by generating hydroxyl radical by Fenton reaction. In addition to the natural forms of the plasmid, it was observed that nicked plasmid forms were formed by the effect of hydroxyl radicals.

When the intensity of the bands was observed, it was observed that while the brightness of the linear form decreased in the well containing only the plasmid, especially in the wells containing only Fenton reaction (Well 2) and quercetin for control (Well 3), the nicked form was formed. In addition, although the nicked plasmid DNA form was formed in the wheatgrass extracts, it was observed that the nicked form band density was less than the well 2 and well 3 samples (Fig. 3c). However, no difference was observed in the band density of the wheatgrass extracts. Based on these observations, it appears that wheatgrass extracts have a DNA protective effect by reducing the nicked form formation of plasmid pUC19.

DNA protective effects of methanolic extracts of ten medicinal plants belonging to six families were investigated and it was reported that they had protective activity related with their phenolic and flavonoids content (Kumar et al., 2010). In a study investigating the antioxidant properties ethanolic extract of the stem of the *Opuntiaficus indica* var. Saboten, it was reported that the extract protected plasmid DNA from the harmful effects of hydroxyl radicals (Lee et al., 2002). It was investigated that the *Polyalthia longifolia* leaf extract strongly inhibited hydrogen peroxide induced DNA damage (Jothy et al., 2013). It was found that water-methanol extract of shade dried tuber of *Eulophia nuda* Lindl. had the highest protective role against DNA damage caused by free radicals (Kumar et al., 2013). It was found that lyophilized ethanol extracts (10 mg/mL) of the wheatgrass prevented damage on the pUC19 plasmid from the hydroxyl radicals formed by Fenton reaction because of its high content of antioxidants and vitamins.

Physiological Parameters

The effects of SA imbibition of bread seeds on shoot and root length and FW were evaluated. The highest shoot length was measured at SA-6 application (23.040 cm), however not significant than control (22.413 cm) (Table 1). SA-6 application caused significant increase in shoot length compared to SA-12 application (21.100 cm). SA application affected significantly root length of bread wheat. SA-10 application had significantly longer root length according to other applications. The lowest root length was measured at control. SA-4, SA-6 and SA-8 applications caused significantly longer roots than control (Table 1). There was no significant increase in

shoot FW. The highest root FW was weighted at SA-8 application. Additionally, SA-8 application caused significantly weightier at root tissue compared to control, SA-4, SA-6 and SA-10 applications (Table 1). Moreover, SA-10 and SA-12 root tissues had importantly root weight than control, SA-4 and SA-6 roots. Saheri et al., (2020) found that foliar spraying of SA of common purslane plants under drought stress resulted with increase in length, fresh weight and dry weight. Foliar spraying of SA caused increase in shoot and root length and shoot fresh and dry weight of wheat seedlings in greenhouse (Behnam et al., 2018). It was reported that shoot and root fresh mass of 15 days old wheat plants growth from seeds soaked with SA was weightier than control (Loutfy et al., 2020). According to our results, while SA application caused significant increase in root length compared to control, it did not affect significantly in shoot length according to control plants. SA-8, SA-10 and SA-12 treatments caused importantly increase in root fresh weight according to control (Table1).

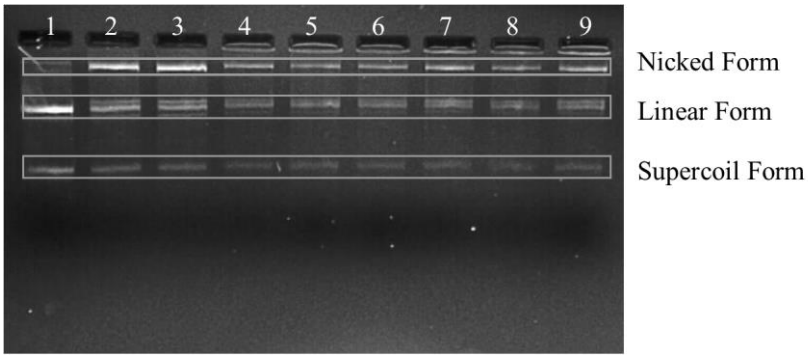

Figure 3. DNA protective activity of wheatgrass extracts. Well-1: Control (Plasmid), Well-2: Fenton solution (FS)+Plasmid, Well-3: FS+Plasmid+Quercetin (50 µg/mL), Well-4: FS+Plasmid+Control wheat grass extract (WGE), Well-5: FS+Plasmid+SA-4 WGE, Well-6: FS+Plasmid+SA-6 WGE, Well-7: FS+Plasmid+SA-8 WGE, Well-8: FS+Plasmid+SA-10 WGE, Well-9: FS+Plasmid+SA-12 WGE.

Şekil 3. Buğday çimi ekstrelerinin DNA koruyucu etkisi. Kuyucuk-1: Kontrol (Plazmit), Kuyucuk-2: Fenton solüsyonu (FS)+Plazmit, Kuyucuk-3: FS+Plazmit+Kersetin (50 µg/mL), Kuyucuk-4: FS+Plazmit+Kontrol buğday çimi ekstresi (BÇE), Kuyucuk-5: FS+Plazmit+SA-4 BÇE, Kuyucuk-6: FS+Plazmit+SA-6 BÇE, Kuyucuk-7: FS+Plazmit+SA-8 BÇE, Kuyucuk-8: FS+Plazmit+SA-10 BÇE, Kuyucuk-9: FS+Plazmit+SA-12 BÇE.

Table 1. The effects of SA pretreatment of bread wheat caryopses on some physiological parameters.
Tablo 1. Ekmeklik buğday karyopsis meyvelerinin SA ön uygulamasının bazı fizyolojik parametrelere etkileri.

	Shoot		Root	
Treatment	Length (cm)	FW (g)	Length (cm)	FW (g)
Control	22.413±0.336^{ab}	0.268±0.008^{a}	15.707±0.194^{d}	0.162±0.006^{c}
SA-4	21.827±0.531^{ab}	0.285±0.009^{a}	16.840±0.178^{b}	0.164±0.007^{c}
SA-6	23.040±0.546^{a}	0.280±0.006^{a}	16.387±0.165^{bc}	0.169±0.006^{c}
SA-8	22.040±0.545^{ab}	0.284±0.006^{a}	16.633±0.153^{b}	0.223±0.009^{a}
SA-10	21.767±0.836$_{ab}$	0.262±0.011^{a}	17.607±0.210^{a}	0.192±0.006^{b}
SA-12	21.100±0.750^{b}	0.264±0.011^{a}	15.993±0.225^{cd}	0.223±0.011^{ab}

Different letters in the each column indicate significant difference (P<0.05).
Her kolondaki farklı harfler anlamlı farkı temsil eder (P<0.05).

Biochemical Parameters

The highest MDA content was determined at control. While we calculated decrease in MDA level of SA applications compared to control, there was no significant decrease. The highest proline content was at SA-8 treatment, however not significant (Table 2). The highest proline content of wheat shoot tissue was at SA-8 treatment however not significantly different from other treatments and control (Table 2). The highest hydrogen peroxide contents were at SA-12 and SA-8 applications and significantly higher than SA-4, SA-6 and SA-10. However, they were not significantly different compared to control and SA-12 (Table 2). According to results of Saheri et al., (2020), foliar spraying of SA caused increase in proline content and decrease in MDA level and hydrogen peroxide content of common purslane under drought stress. While pretreatment of *Artemisia aucheri* BOISS with SA did not affect proline content of plants under normal conditions, proline content of plants under osmotic stress was higher than control (Abbaspour and Ehsanpour, 2020). Loutfy et al., (2020) demonstrated that SA pretreatment of wheat seeds caused decrease in proline content of

wheatgrass. It was reported that different concentration of SA caused decrease in MDA level and fluctuations of hydrogen peroxide content in foliar sprayed wheat seedlings (Alsahli et al., 2019). In the same study, proline content was not affected from spraying of SA. In our study, SA pretreatment did not significantly affect the MDA, proline and H_2O_2 content compared to control. However, pretreatment of SA-8 and SA-12 caused increase in H_2O_2 content compared to other SA treatments (Table2). Results found in this study for MDA, proline and H_2O_2 content are similar to previous studies.

SA applications did not cause significantly change in total Chl content. Plants are affected environmental changes and media composition. It was reported that different photoperiod and media caused change in wheatgrass height (Virdi et al., 2021). In the same study, it was reported that indoor and outdoor growth of wheatgrass caused significantly change in chlorophyll content and outdoor growth wheat seedlings had significantly more chlorophyll than indoor ones. Additionally, different cultivation conditions affected Chl content of wheatgrass (Fortună et al., 2018).

Table 2. Some biochemical parameters and antioxidant enzyme activities of wheatgrass growth from bread wheat caryopses pretreated with different concentrations of SA.

Tablo 2. Farklı SA konsantrasyonları ile ön uygulama yapılmış ekmeklik buğday karyopsis meyvelerinden büyümüş buğday çiminin bazı biyokimyasal parametreleri ve antioksidan enzim aktiviteleri.

	Control	SA-4	SA-6	SA-8	SA-10	SA-12
Biochemical Parameters						
MDA	25.76±4.36^{a}	19.40±1.41^{a}	19.81±1.37^{a}	17.13±2.86^{a}	22.56±1.96^{a}	17.69±2.41^{a}
Pro	6.69±0.40^{a}	7.04±0.20^{a}	7.40±0.40^{a}	7.28±0.10^{a}	6.93±0.24^{a}	6.86±0.38^{a}
H_2O_2	157.20±5.89^{ab}	149.74±2.36^{b}	149.13±2.51^{b}	167.86±5.13^{a}	149.67±1.58^{b}	169.39±1.06^{a}
SPC	9.15±0.34^{c}	14.33±1.06^{ab}	13.03±0.47^{b}	13.88±0.57^{ab}	13.86±0.33^{b}	15.16±0.18^{a}
Chl	358.68±19.62^{a}	363.55±29.24^{a}	353.44±37.00	364.88±41.54^{a}	346.35±30.00^{a}	356.16±27.66^{a}
Antioxidant Enzyme Activities						
CAT	226.90±10.90^{b}	252.79±13.19^{ab}	271.07±11.74^{a}	263.20±28.77^{ab}	254.32±12.97^{a}	257.61±15.01^{ab}
APX	552.50±45.53^{ab}	642.86±37.64^{ab}	583.21±36.82^{ab}	638.21±11.02^{ab}	648.57±8.98^{a}	573.21±27.04^{b}
SOD	100.01±9.53^{a}	96.15±4.89^{a}	99.94±8.16^{a}	95.56±7.21^{a}	90.17±8.65^{a}	90.24±5.91^{a}

Different letters in the each row indicate significant difference (P<0.05).
Her kolondaki farklı harfler anlamlı farkı temsil eder (P<0.05).

Seed imbibition with SA caused significantly increase in soluble protein content (SPC) of bread wheat shoot tissue. The highest soluble protein content was at SA-12 treatment (Table 2). Wheatgrass has high amount of some amino acids such as histidine, glutamic acid, threonine, arginine and leucine (Ghumman et al., 2017). Different growth conditions affected protein content of wheatgrass (Devi et al., 2020). Additionally, photoperiod, media composition and genotype affected protein content in wheatgrass shoot powder, especially photoperiod (Virdi et al., 2021).

We investigated that the effect of SA on some antioxidant enzymes (CAT, APX and SOD). While SA application did not affect SOD enzyme activity, SA caused significantly changes on APX and CAT enzyme activities (Table 2). According to Table 2, there was increase in CAT activities of SA treatments compared to control. However, SA-6 and SA-10 treatments had significantly more CAT activity than control. The highest APX activity was at SA-10 treatment however only importantly higher than SA-12 treatment. Additionally, APX activities of other SA treatments were higher than control but not significant (Table 2). Plant antioxidant defense enzymes CAT, APX and SOD are important enzymes to adapt environmental changes. In this study, we investigated that how SA pretreatment affected CAT, APX and SOD enzyme activities. SOD enzyme presence in wheatgrass reduces negative effects of radiations and toxins (Cao et al., 1996; Bar-Sela et al., 2007). According to Alsahli et al., (2019), foliar spraying of wheat seedling with different concentration of SA resulted with increase in CAT and APX enzyme activities while it did not affect SOD activity. We found similar results in our study. Seed imbibition with SA resulted with fluctuations in APX and CAT enzyme activities (Table2). However, we did not find increase in SOD activity.

CONCLUSION

We concluded that the highest concentration of SA prevented bread wheat germination. Imbibition of wheat seeds with SA resulted with increase in TAS level and vitamin C content of wheatgrass. Additionally, lyophilized wheatgrass extract had a protective role against DNA damage induced by hydroxyl radicals. Additionally, SA caused increase in root length and FW compared to control. Moreover, we determined increase in APX and CAT antioxidant enzyme activities according to control. SA pretreatment seeds before planting could be used to enrich antioxidant potential and vitamin C content of plants.

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Researchers Contribution Rate Declaration Summary

AB contributed to the study conception and design. Material preparation, data collection and analysis were performed by SD and AB. The first draft of the manuscript was written by SD and AB commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Conflict of Interest

Authors declare that there is no conflict of interest.

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A Survey on Egg Parasitism and Overwintered Adult Population of Sunn Pest [(*Eurygaster* spp.) (Hemiptera: Scutelleridae)] in Usak Province, Türkiye

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ABSTRACT
Sunn Pest, *Eurygaster* spp. (Hemiptera: Scutelleridae), is the most important pest being able to cause significant losses on wheat yield and quality in Türkiye. To monitor the overwintered adult population, this study was carried out in 41 sites in 19 locations during two consecutive years. The samplings were performed in April and May in both years. During the study, the economic threshold, which is 0.8 overwintered adults per square meter, was obtained only in Yesildere location, and the number of adults in other locations remained quite low. Moreover, it was determined that the parasitization rates varied between 48.2% and 70.1% in the locations where egg masses were collected. As a result of this study, it was found that the number of adults was higher in locations with high altitudes and close to overwintering areas. Therefore, more attention should be paid to survey studies in similar locations.

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Uşak İlinde Kışlamış Süne [(*Eurygaster* spp.) (Hemiptera: Scutelleridae)] Ergin Populasyonu ve Yumurta Parazitlenmesi Üzerine Sürvey

ÖZET
Türkiye'de buğdayın verim ve kalitesi üzerine önemli kayıplara neden olan en önemli zararlı Süne, *Eurygaster* spp. (Hemiptera: Scutelleridae) dir. Bu çalışma kışlamış ergin popülasyonunun takibi amacıyla, iki yıl süresince 19 lokasyona ait 41 ünitede gerçekleştirilmiştir. Örneklemeler her iki yılda da nisan ve mayıs aylarında yapılmıştır. Çalışma boyunca ekonomik zarar eşiği olan 0.8 kışlamış ergin/m² sadece Yeşildere bölgesinde tespit edilmiş diğer alanlardaki ergin sayıları oldukça düşük olmuştur. Parazitlenme oranının tespiti için yumurta paketi toplanan lokasyonlardaki parazitlenme oranları %48.2 - %70.1 arasında değiştiği tespit edilmiştir. Ayıca bu çalışma sonucunda ergin sayılarının rakımı yüksek ve kışlaklara yakın olan alanlarda daha yüksek olduğu belirlenmiştir. Dolayısıyla bu ve buna benzer bölgelerdeki sürvey çalışmalarına özen gösterilmelidir.

Bitki Koruma

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Tahıl
Yükseklik
Ekonomik eşik
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Trissolcus

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INTRODUCTION
Cereals, which include products such as barley, wheat, corn and rice, are a rich source of carbohydrates and play an important role in dietary habits all over the world. Grain is produced in 50.6% (724.2 million hectares) of the world's agricultural land (1.43 billion hectares). This rate was 59.1% (10.7 million ha) in Türkiye, which has 18.1 million hectares of agricultural land (Faostat, 2021). Among the cereals, the most grown crop has been wheat due to its high adaptability both in the world and in Türkiye. Besides utilizing in human nutritional flour, bulgur (cracking and precooking of wheat grains), pasta and starch obtained from wheat, its stem is used in the paper-cardboard industry and animal nutrition (Kan, 2000; Flowers, 2012).

Sunn pest (*Eurygaster* spp.) (Hemiptera:Scutelleridae) is one of the most

important pests affecting the yield and quality of wheat, which is an indispensable resource in our daily diet and also has a strategic position economically. While there are 15 species of *Eurygaster* genus in the world, only seven species of them have been identified in Türkiye. Among them, *E. integriceps* (Put.), *E. maura* (L.) and *E. austriaca* (Schr.) have been economically damaging and are the most common species (Simsek et al., 1996). It feeds on wheat, barley, rye and oats belonging to the Poaceae family and their wild forms. Commercially, the most important damage is found on the wheat plant, which it prefers more than other grain types. Although Sunn pest is univoltine and active for only 3 months a year, it is a pest that can cause up to 100% product loss if control measures are not taken (Davari and Parker, 2018).

For the management of this pest, the use of synthetic insecticides is very common owing to their short-term effect and easy application. In Türkiye, the pest management method, which started with physical control in 1928, has turned into chemical control as of 1955. The chemical application, which was made with aircraft and ground tools until 2005, was carried out only with ground tools in the following years (Kocak, 2007). The negative effects of these chemicals on the environment, natural enemies and human health have been increasing as a result of unconscious and unnecessary use. Hence determining the population density of the pest plays a key role in deciding on the use of chemicals against pests.

Egg parasitoids are one of the most important actors suppressing population growth of Sunn Pest (Kivan, 1998). As such, *Trissolcus* spp. belonging to Scelionidae (Hymenoptera) family have come into prominence. In some locations of Türkiye, due to the effectiveness of Sunn Pest egg parasitoids, either no insecticide is applied or partially done (Cetin et al., 2012).

In this study, which was conducted in 2020 and 2021, the overwintered adult population and egg parasitism of Sunn pests was observed and their density were compared to the studied locations.

MATERIAL and METHODS

Survey area and Sampling method

The studies were carried out in 19 locations of Usak province, where barley and wheat are cultivated intensively, with altitudes varying between 851-1043 m. Each location was divided into sites ranging in size from 100 to 500 ha, and overwintered adults (OA) were sampled in 41 sites (7000 ha) in total. Controls were made in the wheat and barley fields from the beginning of April, when the daily temperatures reached 15°C in both years, and the counting of the overwintered adults was started 10-15 days after the first adults were detected. The samplings were carried out between 10-26 April in 2020, while in 2021 it was launched on 20 April due to rainy weather and were completed on 7 May (Table 1). OA were counted with the help of 0.25 square meter frames.

Table 1. The areas and dates on which the overwintered adult were counted in 2020-2021
Çizelge 1. 2020-2021 yıllarında kışlamış ergin sayımı yapılan bölge ve tarihler

Location	Sampling date		Altitude (m)	No.of thrown frame
	2020	2021		
Bozkus	10-13 Apr	21 Apr	881	240
Bolme	24 Apr	27 Apr	884	160
Carıkkoy	13 Apr	03 May	953	160
Gucer	10 Apr	20 Apr	911	160
Demiroren	22 Apr	26 Apr	951	160
Elmacik	22 Apr	28 Apr	878	160
Hocalar	17 Apr	29 Apr	863	160
Ikisaray	14 Apr	21 Apr	869	160
Kalfa	15 Apr	22 Apr	868	160
Karahasan	22 Apr	28 Apr	853	160
Karlik	14 Apr	04 May	1043	240
Kayagil	24 Apr	27 Apr	914	80
Muharremsah	15 Apr	22 Apr	879	240
Ovademirler	20 Apr	29 Apr	884	160
Ortakoy	13-14 Apr	07 May	991	240
Selikler	20 Apr	30 Apr	861	160
Susuzoren	17 Apr	06 May	913	240
Yavi	26 Apr	05 May	851	160
Yesildere	25 Apr	26 Apr	968	80

Forty frames were randomly tossed to each site and the number of adults found within the frame was recorded. Also, parasitization rates in egg masses of the pest were determined in the locations where the mean number of OA per square meter was above economic threshold (0.8 OA/m²), and in four locations close to overwintering areas (Carikkoy, Gucer, Karlik and Kayagil) (Adiguzel, 1981). For this purpose, at least 20 egg masses were collected from each location, when 20-30% of the eggs were in the anchor-sign stage. The collected egg masses were placed in glass tubes and kept at room temperature for 5 days. The eggs that turned black in color were considered as parasitized and the parasitization rates of the locations were determined (Simsek et al., 1985).

Data analaysis

The number of OA collected from the sites belonging to the locations was divided by the number of frame thrown, and the mean number of OA of that location was calculated. As the values of each location based on the years were not normally distributed, Mann–Whitney test was performed to evaluate whether the difference between the mean number of OA obtained from each location in the two survey years was significant. In addition, one-way analysis of variance was applied to the mean numbers of OA of the studied locations, which providing homogeneity of variance and normality, and the different groups were detected by the Tukey multiple comparison test. All statistical analyzes were performed in SPSS 24.0 software package.

The number of adult Sunn pest collected from the locations based on their altitude were mapped using the Surfer 22.2 software program. On the wireframe map, the z value represented the altitude of the locations, while the z value on the Contour map expressed the number of OA. Also the inverse distance method was used for interpolation in contour map.

RESULTS and DISCUSSION

Throughout the study, OA were counted in a total of 3280 frames and 223 adult insects were obtained. Except for Yesildere, no significant difference was found between the number of OA of the locations in 2020 and 2021 (Fig.1).

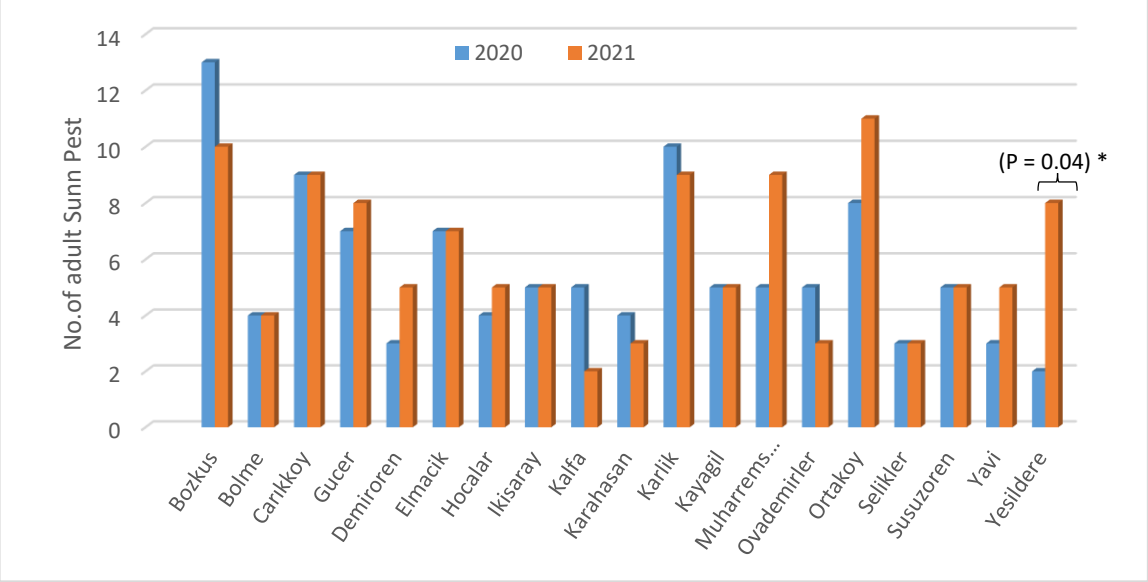

Figure 1. Number of adult Sunn Pest collected from studied locations in 2020 and 2021. The bars labeled with an asterisk (*) means that the data between the two survey years differ at the signifcance level of 0.05.

Şekil 1. 2020 ve 2021 yıllarında toplanan ergin süne sayıları. Yıldız ile işaretlenmiş çubuklar iki sürvey yılı arasında 0.05 önem seviyesinde farklılık olduğunu göstermektedir.

In 2020, mean number of adult sunn pests per frame were the lowest in Demiroren, Muharremsah, Selikler, Susuzoren and Yavi (0.04±0.02), while it was the highest in Kayagil with a mean of 0.13±0.05 ($F_{18, 1621} = 75.85$; $P < 0.001$). As for 2021, the locations with the lowest and highest means were Kalfa (0.03±0.02) and Yesildere (0.2±0.07), respectively ($F_{18, 1621} = 210.35$; $P < 0.001$) (Fig. 2). Additionally, it was determined that the difference between the mean number of OA in 2020 (0.065±0.01) and 2021 (0.07±0.01) was not statistically significant ($P = 0.57$). In 2020, the mean number of OA per square meter (four frames) obtained from the locations did not exceed 0.8. In 2021, it was determined a mean of 0.8 (0.2 per frame) in only Yesildere, while the means in other locations were low.

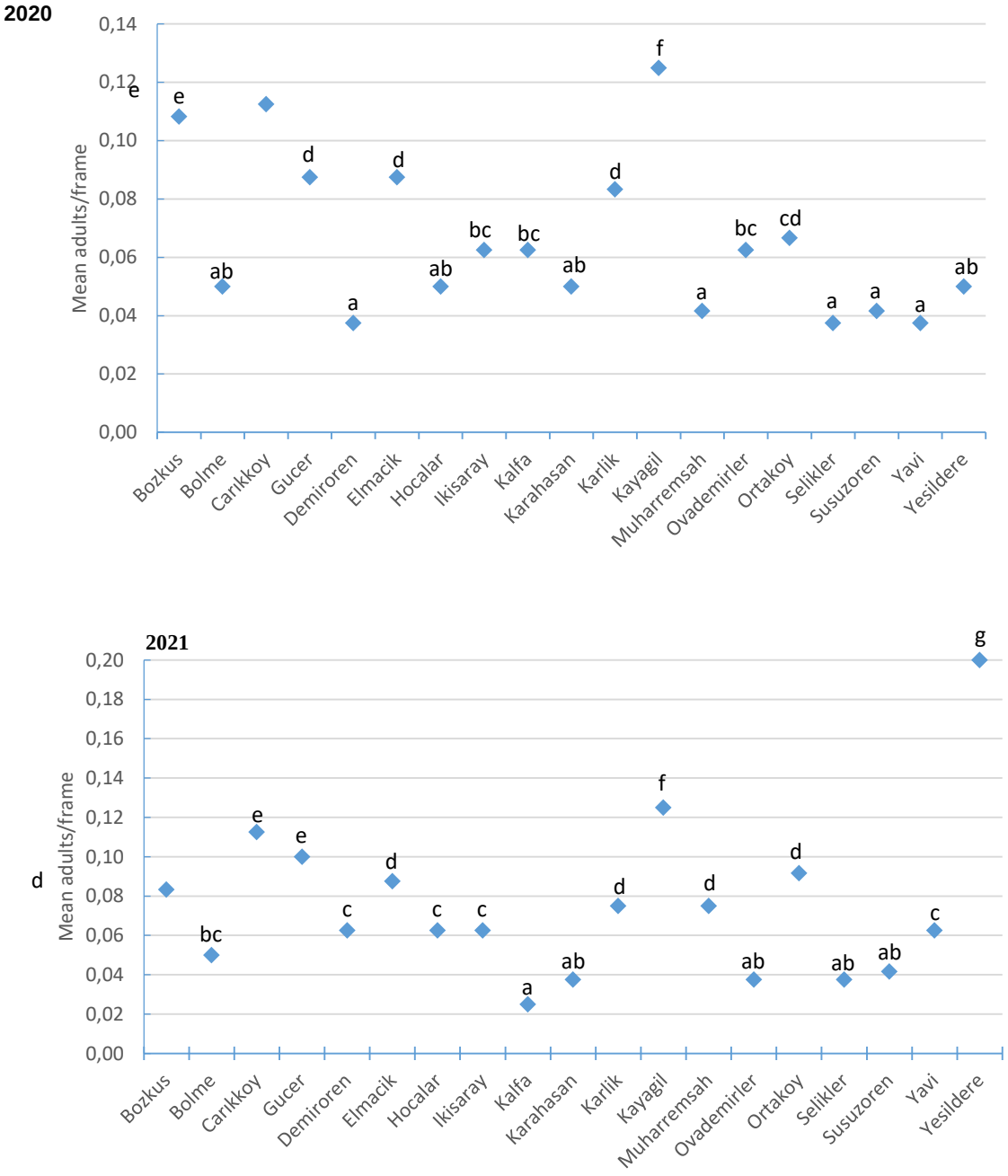

Figure 2. Mean numbers of adult sunn pest per frame during the survey. The difference between the averages with the same latter is not statistically significant (Tukey $p \geq 0.05$)

Şekil 2. Sürvey boyunca her çerçevedeki ergin süne ortalaması. Aynı harf ile gösterilen ortalamalar arasındaki fark istatiksel olarak önemli değildir (Tukey $p \geq 0.05$)

In May, 33 egg masses containing a total of 462 eggs were collected from the location in question. While 224 of these eggs were found to be parasitized, no parasitization was observed in 238 of them. The parasitization rate of the location was calculated as 48.5%, and since this rate was over 40%, spraying was not recommended (Kutuk et al., 2009). Furthermore, parasitization rates in Carikkoy (21 egg masses), Gucer (29 egg masses), Karlik (21 egg masses) and Kayagil (27 egg masses), where egg masses were collected, were 55%, 56.1%, 70.1% and 48.2% respectively. As the number of overwintered adults per square meter in the locations, where the egg masses were collected, increased, the parasitization rate decreased. However this rate was not below 48%. Therefore, while the efficiency of egg parasitoids is sufficient in this study, the parasitization rates should be meticulously controlled

in cases where the number of overwintered adults per square meter is one or more. The number of OA per square meter determined in studies conducted in different locations of Türkiye and in other countries was higher than that of the present study (Simsek et al., 1985; Popov et al., 2003; Canhilal et al., 2005; Kutuk et al., 2009; Salis et al., 2013; Kapustkinaa and Khilevskiy, 2019). Despite the presence of suitable overwintering areas for Sunn pests and not applying insecticide in the studied areas, the low number of overwintered adults found shows the effectiveness of natural enemies. Especially the egg parasitoid *Trissolcus* spp. (Hymenoptera: Scelionidae) is one of the most important species among these natural enemies. So far, 17 *Trissolcus* species have been identified as egg parasitoid of Sunn pest in Türkiye (Kocak, 2007) whereas 3 of them were reported in the studied locations dominanted by *T.semistriatus* Nees (Kocak and Kilincer, 2001). *E. integriceps*, which is one of the two common species in Türkiye, was the dominant species in southeastern Anatolia and Thrace, while *E. maura* was the main species in Central and Western Anatolia (Kocak and Babaroglu, 2005; Koçak et al., 2014). Therefore, *T. semistriatus*, which mostly preferred eggs of *E. maura*, was more efficient in the study area (Brown, 1962a). It was reported that this parasitoid species spends two to three generations on the eggs of the Sunn pest during their oviposition period, while it prefers the eggs of other scutellerid and pentatomid species for the remaining six generations (Kivan, 1998; Kocak, 2007; Kodan and Gurkan, 2016). In the survey areas, uncultivated areas in the field boundaries, alfalfa and vetch fields as well as orchards have created natural habitats and shelters for alternative species that host *Trissolcus* species. As such, Zatyamina et al. (1976) and Zomorrodi (1979) found that *Trissolcus* species moved on to sunflower, corn and paddy fields following grain harvest. Moreover, Oak, juniper, almond and pear trees, which are abundant in the location, provide a suitable environment for overwintering of these parasitoids thanks to their thick bark, as well as being an important food source with the nectar they produce. Similarly, Gozuacik (2011) pointed out that alternative host eggs for *Trissolcus* spp. appeared for 6 months from April to September in areas with natural woodland and annual plants. In addition, Simsek and Ozer (2001) ascertained that parasitization rates were higher in fields near such areas.

It was reported that *E. integriceps* was dominant species in the Southeastern part of Türkiye and some areas of Thrace location, where the Sunn pest population density was above the economic threshold with lower parasitization (Koçak et al., 2014). Therefore, population density of *T.vassilievi* Mayr, which mostly prefers *E.inetgriceps* eggs in such locations, should be increased (Kocak and Kilincer, 2001; Iranipour et al., 2010). In addition, monoculture and scarce woodland in these locations are issues that need to be corrected in terms of the continuity of the parasitoid population.

The number of OA was lower Bolme, Demiroren, Hocalar, Karahasan, Seliks, Susuzoren and Yavi, where are farther from the overwintering areas when compared to Karlik, Carikkoy, Kayagil and Ortakoy locations, where are closer to the overwintering areas and with higher altitudes. In this context, Karimzadeh et al. (2011) emphasized that the migration of Sunn pest from overwintering areas to fields was not randomly occurred while wind direction and speed had a significant effect on migration behavior, thus the edge effect was an important parameter in the distribution of the pest.

It was found that there was a moderate, positive, linear relationship between the number of OA obtained from the locations and the location altitudes ($R = 0.52$; $P < 0.05$). The number of OA was higher in the locations with high altitudes (Fig.3). It was reported that in locations where do not have high summer temperatures like survey locations, some groups of Sunn pest do not migrate to higher altitude from overwintering areas and spend their aestivation and overwintering periods under suitable vegetation at the edge of the harvested fields (Brown, 1962b; Parker, 2011). It has been considered that this situation is one of the reasons for the correlation between altitude and the number of OA.

CONCLUSION

Sunn pest populations remained below the economic threshold in the studied years in Usak province of Türkiye. It has been concluded that if woodland and green belts present in the survey areas and providing shelter for parasitoids are preserved, there will be no need for chemical control against Sunn pest in the following years. Moreover, in terms of follow up of the sunn pest population, surveys should be continued primarily starting from locations with high OA populations in this study. The observations should be made for the E. integriceps species, which is not found in the study area and is a problem for other locations, and if detected, its density and parasitization rates should be examined. The essential points such as host selection of parasitoids and host susceptibility should be well evaluated, and wrong practices should not be made in the study area where the natural enemy-host balance has been established.

Author's Contributions

The contribution of the authors is equal.

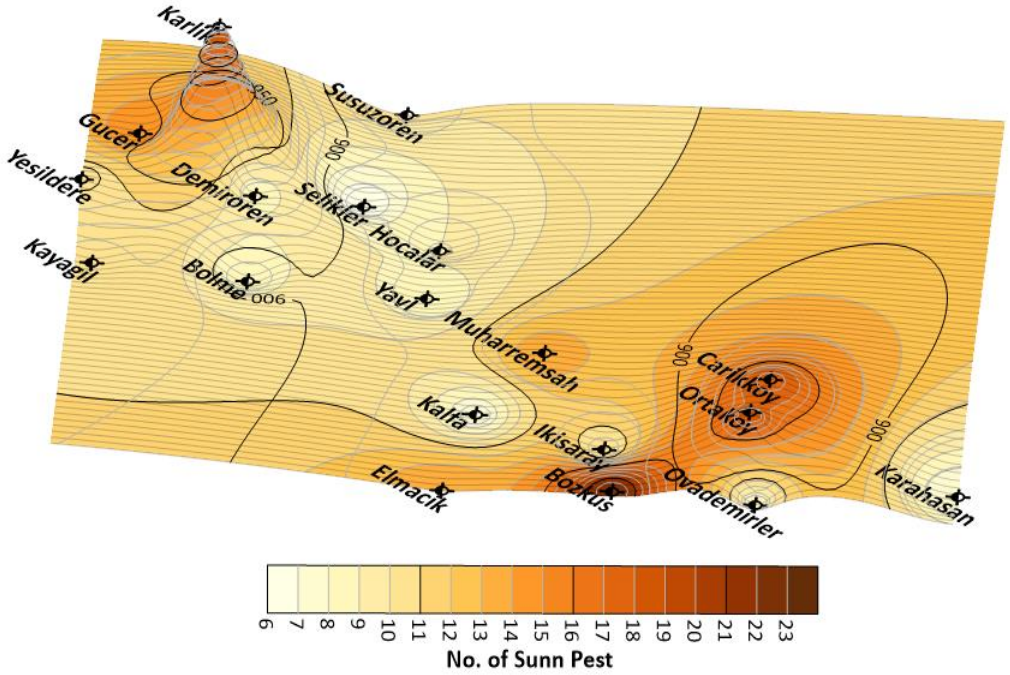

Figure 3. 3D-wireframe plots of the location elevation and Contour map of number of adult Sunn Pest obtained during the study.

Şekil 3. Çalışma süresince elde edilen ergin süne sayılarına ait kontur harita ve bölge yükseltilerinin 3D tasarımı

Statement of Conflict of Interest

Authors have declared no conflict of interest.

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Detection and Molecular Characterization of *Tobacco mild green mosaic virus* Isolates Infecting Peppers in Turkey

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ABSTRACT

In this study, *Tobacco mild green mosaic virus* (TMGMV) infection was investigated in pepper crops from two regions having high economical importance in Turkey. A total of 397 leaf and fruit samples showing mosaic, yellowing, mottling, and pitting symptoms were collected to test by double antibody sandwich ELISA (DAS-ELISA) using polyclonal antiserum. DAS-ELISA results indicated that 97 out of all tested samples (24.4%) were found to be infected with TMGMV. Samples resulting positive for TMGMV infection were used to amplify the virus coat protein (CP) gene sequences with specific primers by RT-PCR for further molecular characterization of the virus isolates. A comparison of the CP sequences of the virus isolates revealed that the identity of nucleotides ranged between 97.2 and 100%, and the amino acid similarity ranged between 96.8 and 100% among themselves. To the best of our knowledge, this is the first report of TMGMV infection in *Capsicum annuum* in Turkey.

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Türkiye'de Biberde Enfeksiyon Oluşturan *Tobacco mild green mosaic virus* İzolatlarının Belirlenmesi ve Moleküler Karakterizasyonu

ÖZET

Bu çalışmada, Türkiye'de biber yetiştiriciliğinin ekonomik olarak önemli olduğu iki bölgede *Tobacco mild green mosaic virus* (TMGMV) enfeksiyonu incelenmiştir. Mozaik, sararma, beneklenme ve çukurlaşma simptomu gösteren toplam 397 yaprak ve meyve örneği toplanmış ve double antibody sandwich ELISA (DAS-ELISA) ile poliklonal antiserum kullanılarak testlenmiştir. DAS-ELISA testinde testlenen örnekler içerisinde 97 örneğin (%24.4) TMGMV enfeksiyonuna sahip olduğu belirlenmiştir. TMGMV ile enfekteli pozitif örnekler, gen spesifik primerler kullanılarak RT-PCR yöntemiyle virüs kılıf protein (KP) geninin çoğaltılması ve izolatların moleküler karakterizasyonu için kullanılmıştır. KP nükleotid dizilerinin karşılaştırması sonucunda, izolatların kendi aralarında nükleotid benzerliğinin %97.2-100, amino asit benzerliğinin ise %96.8-100 arasında değiştiği belirlenmiştir. Bildiğimiz kadarıyla, bu Türkiye'de TMGMV'nin enfeksiyonunun *Capsicum annuum*'daki ilk raporudur.

Fitopatoloji

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 27.12.2021
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Anahtar Kelimeler
Tobamovirus
TMGMV
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DAS-ELISA
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INTRODUCTION

Pepper (*Capsicum annuum* L.) is the second most cultivated vegetable crop (2.636.905 tonnes) in Turkey (Faostat, 2020) where pepper is cultivated both in open field and greenhouse. Turkey is the world's third-largest producer of green pepper, so pepper plays important role in global trade. Viral diseases are significant constraint in pepper cultivation affecting the yield and quality of pepper fruits (Green and Kim, 1994). In the last 30 years, the

number and prevalence of infecting virus species in pepper growing areas in tropical and subtropical regions have increased remarkably. To date, more than 70 virus species have been reported infecting pepper throughout the world (Pernezny et al., 2003; Kenyon et al., 2014). Among these, about 20 virus species belonging to 15 different taxonomic groups cause economic damage to pepper cultivation in the Mediterranean basin (Moury and Verdin, 2012). The most important of these viruses are seed-borne tobamoviruses, insect-transmitted potyviruses, cucumoviruses, and tospoviruses (Moury and Verdin, 2012). Tobamoviruses are highly contagious and stable group of viruses causing significant yield losses in commercial pepper crops (Alonso et al., 1989; Smith and Dombrovsky, 2020). They are found all over the world and can be spread through infected seeds, infected soil, and mechanical contact between plants, but not transmitted by vectors (Broadbent, 1965; Lanter et al., 1982; Pares et al., 1996). There are 37 members within the *Tobamovirus* genus, and out of 8 are known to infect peppers (Kenyon et al., 2014; Fidan et al., 2021). Among them, *Tobacco mosaic virus* (TMV), *Tomato mosaic virus* (ToMV), and *Pepper mild mottle virus* (PMMoV) are the most prevalent viruses in pepper production in Turkey (Ozaslan et al., 2006; Şevik, 2011; Şimsek et al., 2015).

Tobacco mild green mosaic virus (TMGMV) belongs to the genus *Tobamovirus,* and its genome consists of a positive-sense single-stranded RNA. Its genomic organization is similar to that of TMV and ToMV. The genomic RNA of TMGMV is 6355 nt long that encodes four proteins, namely; the 126K and 183K replicases, 28.5K protein homologous to TMV and ToMV the movement proteins (MP), and 17.5K coat protein (CP) (Solis and Garcia-Arenal, 1990). TMGMV has been recently isolated from tobacco plants in Turkey (Karanfil et al., 2020). In the current study, the occurrence of TMGMV was investigated in pepper plants in Turkey, and molecular experiments were carried out to have a preliminary result for the characterization of its isolates based on the coat protein gene. To our knowledge, this is the first report of natural infection by TMGMV in pepper crops.

MATERIALS and METHOD

Survey and sample collection

Surveys were conducted in commercial greenhouses and open fields in the Eastern Mediterranean (Mersin, Adana, Kahramanmaraş, Hatay), and Southeastern (Gaziantep, Kilis) regions of Turkey, where pepper production is economically important, during 2017-2020 growing seasons. A total of 397 leaf and fruit samples showing vein clearing, mosaic and discoloration of young leaves, and fruit distortion symptoms (Figure 1) were collected during surveys.

DAS-ELISA

All samples were ground in four volumes of 0.03 M phosphate buffer (pH 7.0) (wt/vol) supplemented with 0.2% diethyldithiocarbamate in extraction bags. The extracts were tested in DAS-ELISA (Clark and Adams, 1977) for the presence TMV, ToMV, *Paprika mild mottle virus* (PaMMV), PMMoV, and TMGMV using polyclonal antiserum (Loewe Biochemica GmbH, Germany). Absorbance at 405 nm was determined using a plate reader (Seac Sirio S). The samples were considered as positive if their average absorbance value were equal to or higher than twice that of the negative control.

Mechanical transmission of TMGMV

The sap from pepper source carrying TMGMV infection only was mechanically inoculated onto *Nicotiana tabacum* cv. Samsun plants at 2-4 leaf stage by rubbing infected leaf extract prepared in four volumes of 0.03 M phosphate buffer (pH 7.0) containing 0.2% (wt/vol) diethyldithiocarbamate, active charcoal at 20 mg/ml, and carborundum at 20 mg/ml (Moury et al., 2004). Negative control plants were prepared with mock inoculation and carborundum. Each plant species had three replicates. Inoculated plants were kept in a growth chamber with 16 h photoperiod and constant temperature of 25°C, and the symptom expression was monitored for 3 week-post inoculation.

Total RNA extraction and reverse transcription-polymerase reaction (RT-PCR)

Total RNA was isolated from TMGMV-infected *Nicotiana tabacum* cv. Samsun plants according to Chomczynski and Sacchi (1987). RNAs were reverse-transcribed with the specific reverse primer using M-MLV reverse transcriptase (Promega) for the synthesis of the first-strand cDNA. PCR amplification was carried out with the primer combination (TMGMVspec/Tob-Uni1) (Letschert et al., 2002) specific to the complete coat protein (CP) region. PCR amplicons were directly sequenced by the Sanger method with both primers in two directions (Medsantek, Turkey).

Nucleotide sequences and phylogenetic analysis

Among the TMGMV isolates, 19 were sequenced, and have been deposited in the GenBank database with the accession numbers OK182752- OK182770 (Table 1). Multiple nucleotide alignments were conducted using CLUSTALX 1.8 (Thompson et al., 1994). Phylogenetic tree was constructed with Maximum likelihood method using MEGA 7 software (Kumar et al., 2016) with 1000 bootstrap replicates to assess the robustness of the nodes.

Table 1. The accesion numbers of *Tobacco mild green mosaic virus* (TMGMV) isolates obtained in this study
Çizelge 1. Çalışmada elde edilen Tobacco mild green mosaic virus (TMGMV) izolatlarının erişim numaraları

Accession no	Isolate	Location	Year
OK182752	TMGMV-Adn26	Adana	2017
OK182753	TMGMV-Adn32	Adana	2017
OK182754	TMGMV-Adn16	Adana	2017
OK182755	TMGMV-Adn40	Adana	2018
OK182756	TMGMV-Adn45	Adana	2018
OK182757	TMGMV-Adn21	Adana	2017
OK182758	TMGMV-Adn53	Adana	2018
OK182759	TMGMV-Mrs61	Mersin	2018
OK182760	TMGMV-Mrs80	Mersin	2018
OK182761	TMGMV-Mrs84	Mersin	2018
OK182762	TMGMV-Mrs86	Mersin	2018
OK182763	TMGMV-Mrs116	Mersin	2018
OK182764	TMGMV-Mrs49	Mersin	2018
OK182765	TMGMV-Mrs62	Mersin	2018
OK182766	TMGMV-Gza125	Gaziantep	2020
OK182767	TMGMV-Kls280	Kilis	2020
OK182768	TMGMV-Khr180	Kahramanmaraş	2018
OK182769	TMGMV-Khr3	Kahramanmaraş	2020
OK182770	TMGMV-Khr261	Kahramanmaraş	2020

RESULTS and DISCUSSION

Symptom observation and TMGMV incidence

Infected plants showing severe symptoms consisting of chlorosis, curling, necrotic lesions, and mottling were occasionally found in the field (Figure 1). The plants were small and stunted, compared with healthy plants. Under open-field conditions, symptoms were recognized at an early stage of plant development; however, under greenhouse conditions, symptoms were observed at the advanced growth stage. Tobacco plants mechanically inoculated with each isolate showed mild green and chlorotic mosaic symptoms by 3–5 days post-inoculation (Figure 2).

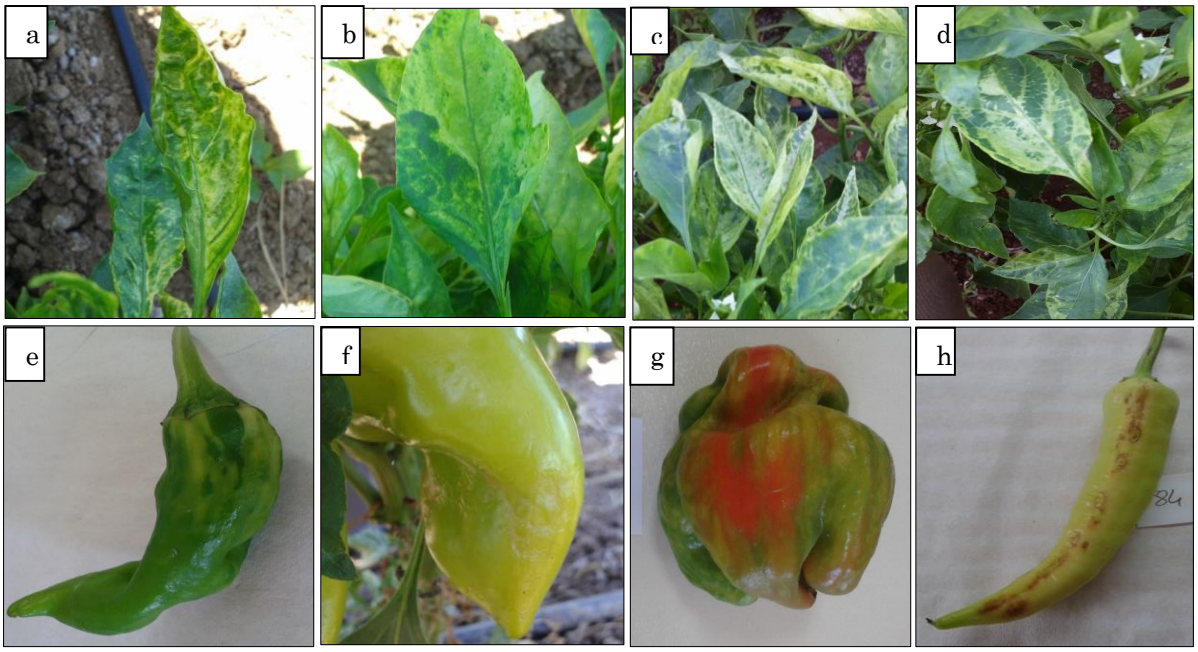

Figure 1. Field symptoms of TMGMV-infected pepper leaves and fruits. (a-d: mosaic and yellowing on pepper leaves, e: mosaic, f: pitting, g: mottle, and h: necrosis on pepper fruits)
Şekil 1. TMGMV ile enfekteli biber yapraklarının ve meyvelerinin simptomları. (a-d: yapraklarda mozaik ve sararma, meyvelerde e: mozaik, f: çukurlaşma, g: beneklenme, h: nekroz

Figure 2. Symptoms consisted of mild green and mosaic on *N. tabacum* cv. Samsun leaves
Şekil 2. N. tabacum cv. Samsun yapraklarında oluşan açık yeşil ve mozaik simptomları

DAS-ELISA results revealed that 97 samples out of 397 tested (24.4%) were infected with TMGMV. TMGMV was found in 8 samples from 49 collected in Adana, 14 samples from 81 collected in Gaziantep, 24 samples from 62 collected in Hatay, 15 samples from 112 collected in Kahramanmaraş, 26 samples from 77 collected in Kilis, and 10 samples from 16 collected in Mersin (Table 2).

Table 2. The presence of viruses in pepper samples collected during surveys according to DAS-ELISA results
Çizelge 2. DAS-ELISA sonucuna göre surveylerde toplanan biber örneklerinde virüslerin bulunma durumu

Location	Number of tested plants	Number of TMGMV infected plants (infection rate %)	Number of mixed infected plants			
			TMGMV+ TMV	TMGMV+ ToMV	TMGMV+P aMMV	TMGMV+P MMoV
Adana	49	8 (16.3%)	5	1	-	6
Mersin	16	10 (62.5 %)	4	3	-	8
Hatay	62	24 (38.7 %)	5	7	-	10
Kahramanmaraş	112	15 (13.4 %)	3	2	2	7
Gaziantep	81	14 (17.3 %)	9	4	-	11
Kilis	77	26 (33.8 %)	5	8	5	16
Total	**397**	**97 (24.4 %)**				

Multiple sequence comparisons and phylogenetic relationships

The nucleotide sequences of the CP gene derived from the 19 TMGMV isolates were subjected to sequence alignment and phylogenetic analysis. The DNA fragment (480 bp) containing the full length of CP was used to determine sequence identity at both the nucleotide and amino acid levels. The Turkish TMGMV isolates shared nucleotide identity of 97.2 to 100% with each other, and 96.6 to 100% with the sequences registered in GenBank (Figure 3a) while the amino acid similarities were between 96.8-100% and 95.5-100% (Figure 3b), respectively. The phylogenetic analysis showed that TMGMV-Adn26, TMGMV-Khr3, TMGMV-Adn16, TMGMV-Mrs49, and TMGMV-Adn45 isolates were closely related to the pepper isolates from Spain (FN594860.1). The isolates (TMGMV-Mrs80, TMGMV-Mrs84, TMGMV-Gza125) had the highest nucleotide identity (100%) with the pepper isolates from China (MF139550.1, JX534224.2) and Vietnam (MW012408.1). It should be noted that TMGMV-Adn40 isolate clustered with Turkish tobacco isolate (MK944273.1) was distantly related to the other pepper isolates (Figure 3c).

In recent years, increasing epidemics of tobamoviruses have started to be reported in commercial pepper fields in Turkey (Çağlar et al., 2013; Buzkan and Arpacı, 2017). The mixed infections of TMGMV with TMV, ToMV, (PaMMV), and PMMoV were detected during this survey (Table 2). Due to their high stability, tobamoviruses remain infectious in contaminated plant debris, compost, soil, and irrigation water. As mentioned by Salamon and Kaszta (2000), pollen grains also play an imported role in the distribution of tobamoviruses throughout the world. In terms of the importance of pepper growing in Turkey, there is a high risk of devastating consequences both in greenhouses and in open fields. However, the infected seeds and plantlets could provide an inoculum source for the initiation of disease epidemics in the crops. Especially, the use of population seeds (taking seeds from the fruits of the previous year) in open pepper cultivation in Turkey plays a very effective role in the spread of these seed-borne viruses. To prevent introductions of tobamoviruses, seeds can be disinfected by using 2% NaOH (Salamon and Kaszta, 2000) or with 10% trisodium phosphate (Na_3PO_4) for 2.5 hours at room temperature (Rast and Stijger, 1987; Jarret et al., 2008). Virus-contaminated disposable materials should be destroyed by burning out or deep burial. Because of the potential for this virus to cause significant losses in other susceptible plants, the hosts and distribution of the agent should be carefully monitored. Host resistance, phytosanitary and cultural control measures should be combined for the management of virus diseases.

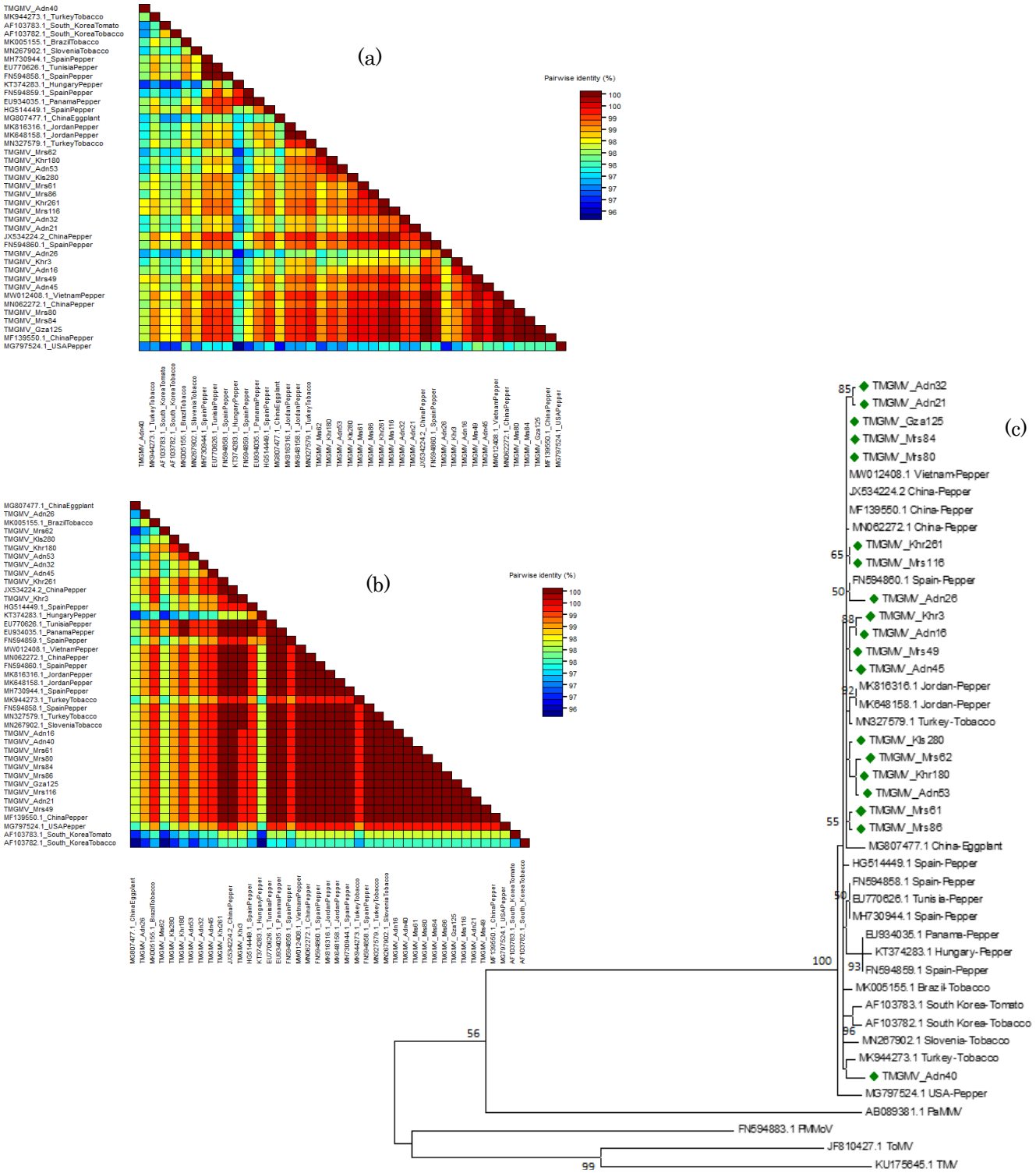

Figure 3. Nucleotide (a) and amino acid (b) sequence identity matrix generated using Sequence Demarcation Tool (SDT). Phylogenetic analysis of TMGMV isolates (c). The KU175645.1 (TMV), AB089381.1 (PaMMV), FN594883.1 (PMMoV), JF810427.1 (ToMV) pepper isolates were used as an outgroup. TMGMV isolates obtained in this study are labeled with symbol (◆).

Şekil 3. Sequence Demarcation Tool (SDT) kullanılarak oluşturulan nükleotid (a) ve amino asit (b) dizin benzerliği matriksi. TMGMV izolatlarının filogenetik analizi (c). KU175645.1 (TMV), AB089381.1 (PaMMV), FN594883.1 (PMMoV), JF810427.1 (ToMV) biber izolatları dış grup olarak kullanılmıştır. Bu çalışmada elde edilen TMGMV izolatları (◆) sembolü ile işaretlenmiştir.

CONCLUSION

In this study, the DAS ELISA test results showed that the TMGMV had a remarkable prevalence in open-field and greenhouse pepper-growing areas in the Eastern Mediterranean region (Mersin, Adana, Kahramanmaraş, Hatay) and the Southeastern region of Turkey (Gaziantep, Kilis). It has been determined that nucleotide sequences of TMGMV isolates showed high sequence identity with each other and pepper isolates from different countries, except for TMGMV-Adn40 isolate.

Statement of Conflict of Interest

Authors have declared no conflict of interest.

Author's Contributions

The contribution of the authors is equal.

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Investigation of Aflatoxin M1 Residue in Raw Cow Milk Samples in Burdur

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ABSTRACT
This study was conducted to research the aflatoxin M_1 (AFM_1) level in food due to the economic losses and public health concerns resulting from its presence. A total of 82 raw cow milk samples were randomly obtained from dairy farms in Burdur and they were examined in terms of AFM_1 using Enzyme-Linked Immunosorbent Assay (ELISA) method. It was found that the AFM_1 level was between 5.06 and 50.63 ng kg^{-1} in 48 (58.5%) of 82 raw cow milk samples analyzed and the average contamination rate was 15.53 ±1.49 ng kg^{-1}. In 1 (1.2%) of the milk samples, AFM_1 level was found to be over the legal limits specified by Turkish Food Codex and European Union's Regulation. As a result, the AFM_1 level determined in the raw milk samples was below the maximum residue limits and was suitable for human consumption. In addition, the estimated daily intake (EDI) of AFM_1 was determined for the adult consumer in Türkiye. The average EDI (0.19 ng kg^{-1} body weight day^{-1}) of the adult consumer was found to be close to the proposed value of tolerable daily intake (0.2 ng kg^{-1} body weight day^{-1}) for AFM_1. However, it is recommended to repeat the studies on this subject within a regular program and inform both the producers and the consumers about the issue.

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Burdur İli Çiğ İnek Sütü Örneklerinde Aflatoksin M1 Kalıntısının İncelenmesi

ÖZET
Bu çalışma, gıdalarda aflatoksin bulunmasından kaynaklanan ekonomik kayıplar ve halk sağlığı endişeleri nedeniyle aflatoksin M_1 (AFM_1) düzeyini belirlemek amacıyla yapılmıştır. Burdur ilindeki çiftliklerden toplam 82 çiğ inek sütü örneği rastgele alındı ve Enzyme-Linked Immunosorbent Assay (ELISA) yöntemi kullanılarak AFM_1 açısından analiz edildi. İncelenen 82 çiğ inek sütü örneğinin 48'inde (%58,5) AFM_1 seviyesinin 5,06 ila 50,63 ng kg^{-1} arasında ve ortalama kontaminasyon oranının 15,53 ±1,49 ng kg^{-1} olduğu tespit edildi. Süt örneklerinin 1'inde (%1,2) AFM_1 düzeyi Türk Gıda Kodeksi ve AB Yönetmeliği'nde belirtilen yasal sınırların üzerinde olduğu belirlendi. Sonuç olarak çiğ süt örneklerinde belirlenen AFM_1 seviyesi maksimum kalıntı limitlerinin altında ve insan tüketimine uygun bulunmuştur. Ayrıca Türkiye'deki yetişkin tüketici için AFM_1'in tahmini günlük alım miktarı (EDI) belirlenmiştir. Yetişkin tüketicinin ortalama EDI'si (0,19 ng kg^{-1} vücut ağırlığı $gün^{-1}$), önerilen günlük 0,2 ng kg^{-1} vücut ağırlığı $gün^{-1}$ AFM_1 alımına yakın bulunmuştur. Ancak bu konudaki çalışmaların düzenli bir program dahilinde tekrarlanması ve hem üreticilerin hem de tüketicilerin konu hakkında bilgilendirilmesi önerilmektedir.

Gıda Bilimi

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INTRODUCTION

Aflatoxins (AF) are a group of mycotoxins produced by the fungi species of *Aspergillus* genus, especially *A. flavus, A. parasiticus* and *A. nominus*, as secondary metabolites. The optimum growth temperature of *Aspergillus* spp. is 25ºC and the minimum water activity is 0.75. The fungi start to produce secondary metabolites at 10-12ºC. However, the most toxic ones are produced at 25°C with water activity of 0.95 (Lizárraga-Paulín et al., 2011; Kagera et al., 2018). The well-known aflatoxin species are aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1) and aflatoxin G_2 (AFG_2) (Kagera et al., 2018). Aflatoxin M_1 (AFM_1) and aflatoxin M_2 (AFM_2) are the hydroxylated metabolites of aflatoxin B_1 and B_2 and they are found in milk of the animals fed with mouldy forage (Creppy, 2002; Yitbarek and Tamir, 2013). AFM_1 amount in milk depends on the AFB_1 concentration in the contaminated forage. Animals fed with feed containing AFB_1, these toxins are metabolized in the liver and secreted to milk as AFM_1. This is the only way to convert AFB_1 into AFM_1 (Creppy, 2002; Hassan and Kassaify, 2014). Roughly 0.3-6.2% of AFB_1 in animal feed is converted to AFM_1 in milk (Creppy, 2002; Karakaya and Atasever, 2010).

Milk and its products are a food with high nutritional value for all people, especially babies and children (Tekinsen and Ucar, 2008; Shundo et al., 2009; Yitbarek and Tamir, 2013; Kamkar et al., 2014). In addition to being consumed in liquid form, milk is used in the production of baby foods, dairy products and milk desserts and is widely consumed by people of all age groups (Tekinsen and Ucar, 2008; Kamkar et al., 2014).

Aflatoxins pass through the human placenta and they cause growth disorders in the young children exposed to aflatoxin (IARC, 2002). Especially infants are at a higher risk compared to adults due to their low body weight, high metabolic rates, insufficient detoxification, and underdevelopment of their organs and tissues (Kamkar et al., 2014). It has been reported that aflatoxins cause renal damage, cirrhosis, hepatitis, hepatocellular carcinoma, chronic gastritis, and Reye's syndrome (Henry et al., 2001; Lizárraga-Paulín et al., 2011; Li et al., 2018). AFB_1 and AFM_1 have genotoxic activity due to its potential accumulation and as it is linked to DNA (Shibahara et al., 1995). Aflatoxins also cause a decrease in milk production. The fact that AFM_1 exists in milk and dairy products is a serious food hygiene problem (Yitbarek and Tamir, 2013). AFB_1 and AFM_1 are described as carcinogenic to humans (Group 1) (IARC, 2002). Due to high toxicity and health concerns, many countries have determined maximum residue limits for AFM_1 to prevent or reduce aflatoxin risks. According to Turkish Food Codex (TFC, 2011) and European Union's Regulation (EC, 2010), the maximum AFM_1 level in milk to be used in the production of raw milk, heat-treated milk and milk-based products must be 50 ng kg^{-1} or less. According to FDA standards, the AFM_1 level in milk in the United States must be 500 ng kg^{-1} or below (FDA, 2005). For this reason, it is necessary to define the AFM_1 levels in milk and its products for the purpose of protection the health of consumers in various age groups from the possible risks (Tekinsen and Ucar, 2008; Yitbarek and Tamir, 2013; Kamkar et al., 2014).

It has been statemented that AFM_1 in milk and its products has resistance against the pasteurization, boiling, sterilization, processing at ultra-high temperature, cooling, and freezing conditions (Galvano et al., 1996; Henry et al., 2001; Awasthi et al., 2012; Iha et al., 2013; Hassan and Kassaify, 2014). A study on AFM_1 concentration changes in white cheese, it was determined that there was no important alteration in AFM_1 concentration even after a 3-month storage (Deveci, 2016). It has been reported that the AFM_1 content of milk and dairy products is not affected during cool and frozen storage. If aflatoxin-contaminated milk is utilized in the making of dairy products, the chances of AFM_1 reaching consumers significantly increase (Wiseman and Marth, 1983; Iqbal et al., 2015).

ELISA is a laboratory screening method that is preferred in food analysis as it has advanced qualifications such as high sensitivity, ease of use, rapid, low cost, and on-site monitoring (Var and Kabak, 2008; Hassan and Kassaify, 2014; Matabaro et al., 2017). Food mycotoxin detoxification processes are still ineffective in terms of food safety and cost. Monitoring programs are by far the most important strategy for reducing the risk of aflatoxins exposure in both animals and humans (Hassan and Kassaify, 2014).

The annual cow milk production throughout Burdur city is 378935 tone (TSI, 2018). It is an important centre for the country in terms of its raw milk production and most of milk is transferred to industry and processed and offered for consumption. Because of the public health concerns and the economic losses caused by the presence of aflatoxin in food, this study was conducted to detect the AFM_1 level in the milk samples gathered from dairy farms in Burdur province, assess the exposure of consumers, and estimate the risk.

MATERIAL and METHOD

Sample collection

In this study, 82 raw cow milk samples were

randomly collected from farms in Burdur province of Türkiye, between September and December 2019. The samples were taken to the laboratory for analysis in covered sterile containers, under aseptic conditions and cold chain and kept at -18ºC further analysis.

Sample preparation

The presence of AFM_1 in the milk samples was detected by ELISA method, using Bio-Shield M_1 ES B2048/B2096 ELISA test kit according to the manufactures' instructions (Prognosis Biotech S.A., Larissa, Greece, 2019). The samples were centrifuged at 10°C, 3000xg for 10 minutes. After centrifuging, the upper cream layer was separated by a Pasteur pipet. The fat-free supernatant was used in the analysis. The dilution factor was calculated to be 1 for the milk samples prepared in this way.

Analysis procedure

The AFM_1 standards ranged in concentration from 0 to 250 ng kg^{-1}, and the milk samples were added at a rate of 100 µL per well in the microplate using an automated pipet. Then, the wells were covered with a transparent film and shaken for 30 seconds and were kept at room temperature for 45 minutes. Later, the liquid in the wells was discharged and the wells were irrigated 4 times with an irrigation buffer solution (Wash Buffer 1X). After each irrigation, the micro-plates were turned upside down, an absorbing paper was tapped on it in order to remove the liquid completely from the wells. After these steps, 100 µL AFM_1 Detection solution (AFM_1-HRP) was added to all of the wells. Then, the wells were covered with a transparent film and shaken for 30 seconds and were kept for 15 minutes at room temperature. The wells were emptied again and they were irrigated 4 times with irrigation solution (Wash Buffer 1X) and after each irrigation, the microplates were turned upside down and tapped. Subsequently, 100 µL of TMB Substrate was added to all of the wells. The wells were covered with transparent film and they were manually shaken for a few seconds incubated in the the dark at room temperature for 15 minutes. At the end of this process, 100 µL stop solution (15% H_3PO_4) was added to all of the wells. The plate was shaken by hand again and mixed slightly. The blue colour became yellow by adding a stopping solution to the wells. Then, it was measured in absorbance ELISA reader at 450 nm and in 60 minutes.

Assessment

The results obtained were assessed according to the computer program (Prognosis-Data-Reader) designed by Bio-Shield. The levels of the aflatoxin standards ranged from 0 to 250 ng kg^{-1}. According to the analysis preparation document, LOD value was 2 ng kg^{-1} and LOQ value was 5 ng kg^{-1} for milk. For milk, the recovery rate was 99.4% and the satisfactory range was 79-119% (Prognosis Biotech S.A., Larissa, Greece, 2019). The statistical analyses were conducted using Minitab for Windows Version Release 16.1. (Minitab Inc., 2011). Occurrence the AFM_1 in raw cow milk is reported as the mean±standard error, range (minimum–maximum), frequency distribution of samples and percentage of samples exceeding maximum limits of Regulations.

The assessment of the estimated exposure levels of consumers to AFM1

While assessing the exposure levels of population to AFM_1 in Türkiye, the calculations were made by considering the report published by World Health Organization (WHO, 2005). Estimated of daily intake (EDI) of population by milk consumption was calculated using the formula: EDI (ng kg^{-1} b.w. day^{-1}) = toxin (ng kg^{-1}) x milk consumption (kg $person^{-1}$ day^{-1})/body weight (kg).

The milk consumption quantity used to calculate the EDI was obtained according to the National Milk Council (NMC, 2018). Despite a lack of statistics on milk consumption by age group in Türkiye, a per capita milk consumption of roughly 270 kg $person^{-1}$ $year^{-1}$ is estimated (NMC, 2018). The daily consumption amount was estimated by dividing the total annual amount by 365. In this study, the mean body weight (b.w.) of 60 kg for adults population in Türkiye was used for calculating the EDI.

RESULTS and DISCUSSION

In this study, a total of 82 milk samples were analysed to determine AFM_1 concentration and the results obtained are shown in Table 1. It was determined that the AFM_1 level was over 5 ng kg^{-1} in 48 (58.5%) and the AFM_1 level was below 5 ng kg^{-1} in 34 (41.5%) among 82 milk samples gathered from several dairy farms in Burdur. The AFM_1 level was between 5.06 and 50.63 ng kg^{-1} in 48 (58.5%) of 82 raw cow milk samples analysed and the average contamination rate was 15.53±1.49 ng kg^{-1}. The AFM_1 contamination was 50.63 ng kg^{-1} in 1 (1.2%) of the milk samples.

Various studies have been conducted in different countries using different techniques in order to determined the presence and level of AFM_1 in milk. Different AFM_1 levels in raw milk samples found in the previous studies conducted in Türkiye and different countries were summarized in Table 2 and Table 3.

In this study, AFM_1 was detected in 58.5% of the raw milk samples. When compared to the previous studies conducted in Türkiye, this result was determined to be higher than the values determined by Keskin et al. (2009), and Aksoy and Sezer (2019), and lower than

the values determined by Oruc et al., (2011), Ertas et al., (2011), Buldu et al., (2011), Sahindokuyucu Kocasari et al., (2012), Bakirdere et al., (2014), Temamogullari and Kanici (2014), Isleyici et al., (2015), Yildirim et al., (2018), Eker et al., (2019), Turkoglu and Keyvan (2019), and Guven et al., (2020).

Table 1. Occurrence and distribution of AFM_1 in raw cow milk samples collected from Burdur.
Çizelge 1. Burdur'dan toplanan çiğ inek sütü örneklerinde AFM_1'in varlığı ve dağılımı.

	Tested *Test edilen* n	Positive *Pozitif* n (%)	Distribution of samples *Örneklerin dağılımı* ng kg^{-1} (%)				Exceed legal limit** *Yasal sınırı aşan*** n (%)	AFM_1 concentration *AFM_1 konsantrasyonu* (ng kg^{-1})		
			<5*	5-25	26-50	>50		Mean *Ortalama* ± SE	Min.	Max.
cow milk *inek sütü*	82	48 (58.5)	34 (41.5)	41 (50)	6 (7.3)	1 (1.2)	1 (1.2)	15.53 ± 1.49	5.06	50.63

n: number of samples, *Distribution of negative samples, **TFC and EU Regulation legal limits (50 ng kg^{-1}) for AFM_1 in milk, SE: Standard Error

Table 2. AFM_1 levels in raw cow milk analyzed in Türkiye.
Çizelge 2. Türkiye'de analiz edilen çiğ inek sütlerindeki AFM_1 düzeyleri

Location *Yer*	n	Positive *Pozitif* n (%)	Range *Aralık* (Mean) (*Ortalama*)	Exceed legal limit *Yasal sınırı aşan* n (%)*	Reference *Kaynak*
İstanbul	60	20 (33.3)	5.40-300.20 (166.80) ng L^{-1}	5 (8.3)	Keskin et al. (2009)
Bursa	30	30 (100)	2.48-18.93 (7.23) ng kg^{-1}	0 (0.00)	Oruc et al. (2011)
Kayseri	50	43 (86)	1-30.0 (8.73) ng kg^{-1}	0 (0.00)	Ertas et al. (2011)
Kayseri	90	90 (100)	54.4-65.5 (59.93) ng L^{-1}	63 (70)	Buldu et al. (2011)
Burdur	45	41 (91.1)	15.3-80 (45.3) ng L^{-1}	16 (35.5)	Sahindokuyucu Kocasari et al. (2012)
Kocaeli, Sakarya, Düzce	77	61 (79.22)	0.005-0.410 (0.031) µg L^{-1}	4 (n.r.)	Bakirdere et al. (2014)
Şanlıurfa	38	n.r. (94.7)	0.82-125.70 (56.74) ng kg^{-1}	21 (55.3)	Temamogullari and Kanici (2014)
Van	100	85 (85)	<5->80 (n.r.) ng L^{-1}	12 (12)	Isleyici et al. (2015)
Kırıkkale	154	154 (100)	0.08-10.11 (1.73) ng L^{-1}	0 (0.00)	Yildirim et al. (2018)
Kars	50	28 (56)	0-21.57 (10.02) ng L^{-1}	0 (0.00)	Aksoy and Sezer, (2019)
Çanakkale	120	107 (89.2)	5.14-78.69 (16.70) ng kg^{-1}	4 (3.3)	Eker et al. (2019)
Burdur	35	35(100)	n.r. (25.45) ng L^{-1}	5 (14.28)	Turkoglu and Keyvan (2019)
Kars	80	80 (100)	0.00-17.86 (9.28) ng kg^{-1}	0 (0.00)	Guven et al. (2020)

n: No. of raw cow milk samples, *TFC and EU Regulation legal limits (50 ng kg^{-1}) for AFM_1 in milk, n.r.: results not reported by author.

Table 3. AFM_1 contamination in raw cow milk analysed by ELISA in different countries.
Çizelge 3. Farklı ülkelerde ELISA ile analiz edilen çiğ inek sütündeki AFM_1 kontaminasyonu.

Country *Ülke*	n	Positive *Pozitif* n (%)	Range (*Aralık)* (mean) (*Ortalama*)	Exceed legal limit *Yasal sınırı aşan* n (%)*	Reference *Kaynak*
Serbian	40	38 (95)	0.005-0.90 (0.19) µg kg^{-1}	5 (12.5)	Kos et al. (2014)
Iranian	45	22 (48.88)	6.3-23.3 (11.61) ng L^{-1}	0 (0.00)	Zanjani et al. (2015)
Iranian	288	163 (56.59)	0.01-0.25 (0.95) ppb	113 (69.32)	Mahmoudia and Norian (2015)
Macedonia	3635	1538 (42.4)	<6.6-408 (14.3) ng kg^{-1}	105 (2.9)	Dimitrieska-Stojkovi et al. (2016)
Egypt	15	5 (33.3)	6.40-70 (35.68) ng L^{-1}	2 (13.3)	Tahoun et al. (2017)
Italy	416	51 (12.3)	n.r. (0.037 µg kg^{-1})	1 (n.r.)	De Roma et al (2017)
Pakistan	156	143 (91.7)	20-3090 (317.4) ng L^{-1}	125 (80.1)	Asghar et al. (2018)
China	133	100 (75.2)	5.3 -36.2 (15.9) ng L^{-1}	0 (0)	Xiong et al. (2020)
Lebanon	701	412 (58.8)	0.011-0.440 (0.035) µg L^{-1}	196 (28)	Daou et al. (2020)

n: No. of raw cow milk samples,*EU Regulation legal limits (50 ng kg^{-1}) for AFM_1 in milk, n.r.: results not reported by author.

When comparing the AFM_1 level determined in the current study with the studies conducted in the other countries, the AFM_1 level determined in the current study was found to be lower than the values determined by Kos et al., (2014), Asghar et al., (2018), Xiong et al., (2020), and Daou et al. (2020) and higher than the values determined by Zanjani et al., (2015), Mahmoudia and Norian (2015), Dimitrieska-Stojkovi et al., (2016), Tahoun et al., (2017), and De Roma et al., (2017).

In this study, the average contamination level of AFM_1 in the milk samples was determined to be 15.53±1.49 ng kg^{-1}. The result obtained in this study was similar to the average contamination levels determined by Xiong et al., (2020). It was determined to be contaminated with high level of AFM_1 in comparison with the results obtained by Oruc et al., (2011), Ertas et al., (2011), Zanjani et al., (2015), Dimitrieska-Stojkovi et al., (2016), Yildirim et al., (2018), Aksoy and Sezer (2019), and Guven et al., (2020) and with low level of AFM_1 in comparison with the results obtained by Keskin et al., (2009), Buldu et al., (2011), Sahindokuyucu Kocasari et al., (2012), Bakirdere et al., (2014), Kos et al., (2014), Temamogullari and Kanici (2014), Mahmoudia and Norian (2015), Tahoun et al., (2017), De Roma et al., (2017), Asghar et al., (2018), Eker et al., (2019), Turkoglu and Keyvan (2019), and Daou et al., (2020). In comparison with the previous studies, it was determined that the AFM_1 levels in raw milk were variable. The AFM_1 residue levels in milk vary significantly based on the species of animal from which milk is obtained, milking type and time, lactation period, type, growing and keeping method of forage, geographical conditions (local weather, humidity and temperature), seasonal changes and the development levels of the countries (Galvano et al., 1996; Tajkarimi et al., 2008; Kamkar et al., 2014; Milićević et al., 2017; Akbar et al., 2019).

The presence of AFM_1 in milk and its products consumed in developing countries is a serious problem (Prandini et al., 2009). Due to high toxicity and health concerns, many countries have determined maximum residue limits for AFM_1 to prevent or reduce aflatoxin risks. According to Turkish Food Codex (TFC, 2011) and European Union's Regulation (EC, 2010), the maximum AFM_1 level in milk to be used in the production of raw milk, heat-treated milk and milk-based products must be 50 ng kg^{-1} or less. In the USA, according to FDA regulations, the AFM_1 level in milk must be 500 ng kg^{-1} or lower (FDA, 2005). In this study, it was determined that the AFM_1 amount of 1 (1.2%) of the milk samples exceeded the level of 50 ng kg^{-1} specified by Turkish Food Codex and European Union's Regulation, although 58.53% of the milk samples gathered from dairy farms were contaminated with AFM_1. In contradistinction to this study, it has been reported that the AFM_1 levels in the milk samples tested by Oruc et al., (2011), Ertas et al., (2011), Zanjani et al., (2015), Yildirim et al.,

(2018), Aksoy and Sezer (2019), Guven et al., (2020), and Xiong et al., (2020) have not exceeded the acceptable limits determined by European Union's Regulation. Some other researchers have declared that the AFM_1 levels (2.9-80.1%) determined in milk samples are higher than the level specified by the regulation (Keskin et al., 2009; Buldu et al., 2011; Sahindokuyucu Kocasari et al., 2012; Temamogullari and Kanici 2014; Bakirdere et al., 2014; Kos et al., 2014; Mahmoudia and Norian 2015; Isleyici et al., 2015; Dimitrieska-Stojkovi et al., 2016; Tahoun et al., 2017; Asghar et al., 2018; Eker et al., 2019; Turkoglu and Keyvan 2019; Daou et al., 2020). The fact that 48 milk samples (58.5%) determined to be contaminated with AFM_1 in this study were lower than the maximum residue limits did not mean that they were safe.

According to the current survey, the average level of AFM_1 is 15.53 ng kg^{-1}. Although the lack of data regarding the consumption of milk according to different age groups in Türkiye, drinking milk consumption is estimated to be approximately 270 kg $person^{-1}$ $year^{-1}$, which equates to 0.74 kg of milk per day (NMC, 2018). In the light of these, based on the results obtained in the present study, the EDI of AFM_1 for Turkish adults was calculated 0.19 ng kg^{-1} b.w. day^{-1}, assuming an adult body weight of 60 kg.

There are no sufficient data available about the EDI values and exposure risks to AFM_1 by adult consumers in Türkiye. In the this study, the EDI of AFM_1 for adults was calculated 0.19 ng kg^{-1} b.w. day^{-1}. JECFA (2001) stated that the mean EDI of AFM_1 in milk was 0.11 ng kg^{-1} b.w. day^{-1} in European, 0.058 ng kg^{-1} b.w. day^{-1} in Latin America, 0.20 ng kg^{-1} b.w. day^{-1} in Far East, 0.10 ng kg^{-1} b.w. day^{-1} in Middle East, and 0.0020 ng kg^{-1} b.w. day^{-1} in Africa. This value in present study is 1.73, 3.28, 0.95, 1.90, and 9.5 times higher than the EDI determined for European, Latin America, Far East, Middle East, and Africa, respectively. The most of the previously EDI of AFM_1 through milk consumption in some countries were summarized in Table 4.

Tablo 4. Consumption of milk in different countries, average contamination, and exposure level to AFM_1.
Çizelge 4. Farklı ülkelerde süt tüketimi, ortalama kontaminasyon ve AFM_1'e maruz kalma düzeyi.

Country *Ülke*	Consumption of milk *Süt tüketimi*	Body weight *Vücut ağırlığı* (kg)	Mean AFM_1 concentration *Ortalama AFM_1 konsantrasyonu* (ng kg^{-1})	Mean EDI of AFM_1 *AFM1'in Ortalama EDI'si* (ng kg^{-1} b.w. day^{-1})	Reference *Kaynak*
Brazil	350 mL day^{-1}	60	31	0.188	Shundo et al. (2009)
Spain	adult male: 0.305 kgday^{-1} adult female: 0.305 kg day^{-1}	adult male: 80.83 adult female: 66.42	9.69	adult male: 0.036 adult female: 0.043	Cano-Sancho et al. (2010)
Portuguese	87 kg $year^{-1}$	69	23.4	0.08	Duarte et al. (2013)
Türkiye	71 g day^{-1}	60	46	0.054	Golge (2014)
Serbia	adult male: 0.21 L day^{-1} adult female: 0.18 L day^{-1}	adult male: 90 adult female: 69	210	adult male: 0.49 adult female: 0.56	Kos et al. (2014)
Brazil	350 mL day^{-1}	60	21	0.120	Santili et al. (2015)
Pakistan	adult male: 0.39 L day^{-1} adult female: 0.40 L day^{-1}	adult male: 79.3 adult female: 52.6	summer:94.9 winter: 129.6	adult male: 0.63 adult female: 1	Iqbal et al. (2017)

In comparison with previous studies, this result is higher than those detected by Shundo et al. (2009), Cano-Sancho et al., (2010), Duarte et al. (2013), Golge (2014), and Santili et al. (2015). However, this result is lower than reported by Kos et al. (2014), Skrbic et al. (2014), and Iqbal et al. (2017). Skrbic et al. (2014) reported that the mean AFM_1 exposure level for Serbian population through milk consumption, estimated at 1.420, 0.769 and 0.503 ng kg^{-1} b.w. day^{-1} in February, April and May, respectively. Kuiper-Goodman (1990) stated that the tolerable daily intake of AFM_1 was 0.2 ng kg^{-1} b.w. day^{-1}. However, the mean EDI of AFM_1 by adult consumer (0.19 ng kg^{-1} b.w. day^{-1}) was close to the calculated tolerable daily intake of 0.2 ng kg^{-1} b.w. day^{-1} of AFM_1. The international expert committees (JECFA, 2001) have

concluded that the daily exposure causes the risk of liver cancer even in the concentration lower than 1 ng kg^{-1} although they have not determined a tolerable daily intake for aflatoxins. Considering this information, it is seen that the AFM_1 taken through milk causes a high risk in all age groups. Therefore, it is recommended to take the AFM_1 at the lowest levels "*As Low As Reasonably Achievable*" principle by experts on the subject (EFSA, 2004). Milk is not only source of AFM_1. Additionally, it can be found in commonly consumed dairy products, such as yogurt, cheese, and milk-based desserts. Therefore, additional studies are required about commonly consumed dairy products to accurately predict consumers' exposure to AFM_1. New strategies are needed to reduce exposure to aflatoxins, especially AFM_1.

CONCLUSION

Consequently, it was satisfactory that the AFM_1 level determined in the milk samples analyzed in this study was lower than the maximum tolerance level determined in TFC and EU Regulation. However, the presence of AFM_1 in 58.5% of the milk samples poses a risk to public health. For this reason, continuous monitoring of AFM_1 at every stage should be performed for animal health, public health and the economy of the country. In order to prevent the formation of aflatoxin, the forage and foodstuff must be produced and kept under appropriate conditions. Also, it is essential to train the producers and consumers about the dangers of aflatoxins and the measures to be taken to minimize the contamination.

Author's Contributions

The contribution of the authors is equal.

Statement of Conflict of Interest

No potential conflict of interest was reported by the authors.

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Küresel İklim Değişikliğinin Ardahan İlinde Tahıl ve Yem Bitkileri Verimliliği Üzerine Etkilerinin ARDL Modeli ile Analizi

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ÖZET

Atmosferde sera gazı yoğunluğunun artmasıyla birlikte küresel ısınma ve buna bağlı olarak iklimde değişiklikler gözlenmeye başlamıştır. Küresel ısınma, insan faaliyetleri ile birlikte sera etkisi yapan gazların atmosferdeki oranlarının artmasından kaynaklanmaktadır. Artan dünya nüfusu, sanayinin gelişmesi, sıcak hava dalgası, aşırı hava olayları ve bunlara bağlı olarak gerçekleşen sel ve kuraklık gibi doğal afetlerdeki artışlar tarımsal üretim arzında istikrarsızlığa neden olmaktadır. Tarımsal teknolojilerin gelişmesine rağmen tarım sektörünün en önemli girdi bileşenlerinin doğal koşullara bağlı olması, sektörün istikrarını olumsuz etkilemektedir. Bu çalışmada, 1990-2020 zaman periyodunda, Ardahan ilinde küresel iklim değişiminin tarım sektörü üzerine etkilerinin ortaya konulması amaçlanmıştır. Bu amaçla Ardahan ilinde hayvancılık sektörü için üretilen tahıl ve yem bitkilerinin verimlilikleri, ortalama sıcaklık ve toplam yağış miktarı arasındaki uzun ve kısa dönemli ilişki Sınır testi ve Toda ve Yamamoto nedensellik testi ile araştırılmıştır. Elde edilen bulgular verimlilik ile iklim verilerinin uzun dönemde birlikte hareket etmediğini göstermiştir. Kısa dönemde ise ot ve yem bitkileri üzerinde yağışların etkili olduğu, sıcaklığın ise etkili olmadığı gözlenirken, tahıl verimliliği üzerinde söz konusu iklimsel değişimlerin etkisinin olmadığı gözlenmiştir.

Tarım Ekonomisi

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Anahtar Kelimeler
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Tahıl
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Sınır testi
Nedensellik

Analyzing the Effects of Global Climate Change on Grain and Forage Crops Productivity in Ardahan Province with ARDL Model

ABSTRACT

Global warming and changes in the climate have been observed as a result of increased greenhouse gas density in the atmosphere. Global warming is due to the increased greenhouse gas proportion in the atmosphere along with human activities. Increased world population, industrial development, heat waves, extreme weather events and increased natural disasters such as floods and droughts resulting from these factors cause instability in agricultural production supply. Despite the development in the agricultural technologies, the most important input components of the agricultural sector are dependent on natural conditions and affects the stability of the agricultural sector negatively. In this study, it was aimed to reveal the effects of global climate change on the agricultural sector in the province of Ardahan in the 1990-2020 time period. Boundary test and the Toda and Yamamoto causality test were used to investigate the long and short term relationships between the grain yield and forage crops, produced in Ardahan province for the livestock sector and productivity, average temperature and total precipitation. Results showed that productivity and climate data did not move together in the long time period. In the short term, it was observed that precipitation was effective on grass and forage crops, while temperature was not effective. On the other hand, the climatic changes had no effect on grain productivity.

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GİRİŞ

İklim değişikliği, günümüzde önemli çevresel felaketlerden biri olarak kabul edilmekte ve içinde bulunduğumuz süreçte etkileri giderek daha belirgin hale gelmektedir. Özellikle, insan faaliyetlerine bağlı olarak sera gazı emisyonlarının yoğunlaşarak artması küresel ısınmayı hızlandırmaktadır. Isınmayla birlikte yağış rejimindeki değişiklikler, nemlilik oranlarının değişimi, ani hava olayları, kuraklık vb. gibi yaşanan iklimsel hareketler ile küresel iklim değişimi meydana gelmektedir (Doğan, 2005). Sera gazı miktarının atmosferde artması sonucu meydana gelen ısınma, canlı yaşamını tehdit ederken yol açtığı ekonomik, sosyal ve çevresel problemler küresel ölçekte piyasa başarısızlığı yaşanmasına neden olmaktadır.

Yapılan çalışmalar gösteriyor ki sıcaklık artışının 2°C'nin üzerine çıkması birçok alanda olumsuzlukları artıracaktır. Bu tehditlerin yaşanmasının muhtemel olduğu sektörlerin başında tarım sektörü gelmektedir. Mevcut sıcaklık artışları, ani hava olaylarına bağlı kuraklık, sel veya yetersiz yağışlar tarımsal üretimi olumsuz etkilemektedir. Apata (2011) çalışmasında Nijerya'da tahıl üretiminin, olumsuz iklim koşulları altında nüfus artışına uyum sağlayamadığı takdirde gıda yetersizliği ve açlığa bağlı ölümlerin artabileceğini belirtmiştir. Dasgupta (2013) tarafından yapılan çalışmada, 1971-2002 yılları aralığında 66 ülkede iklim değişikliğinin mısır ve pirinç üretimini olumsuz etkilediği vurgulanmıştır.

İklim değişimindeki olaylar, büyük ölçekte doğal koşullara bağlı olan tarım sektörünü ve özellikle ekonomisi tarım sektörüne dayalı ülkeleri çok daha kırılgan hale getirmektedir. İklim olayları tarımsal üretim üzerinde verimlilik kayıplarına yol açarak ekonomiler üzerinde bir tehdit olarak görülmektedir. Ancak ülkeler, iklim değişiminden farklı düzeylerde etkilenmektedirler. Sıcaklık artışı tarımsal üretimi olumsuz etkilerken yağışlardaki değişimler, bazı bölgelerde tarımsal üretimi olumlu etkilemiştir. Nepal'de iklimde meydana gelen değişikliğin tarımsal katma değer üzerindeki etkisi 1975-2010 yılları baz alınarak değerlendirilmiş ve çalışmada, yağışların tarımsal GSYH üzerinde olumlu etkisinin olduğu görülmüştür (Acharya & Bhatta, 2013). Sekiz Asya ülkesinde yapılan bir başka çalışmada, yağıştaki değişimlerin, tarım sektörünün GSYİH içindeki payı artırdığı, buna karşılık sıcaklık değişimlerinin negatif olarak etkilediği sonucuna ulaşılmıştır (Akram, 2013). Güney Afrika'da 1961-2011 yılları arasında sıcaklıktaki genel artışın tarımsal üretimi olumsuz etkilediği, yağışların ise olumlu etkilediği gözlenmiştir (Belloumi, 2014).

Sıcaklık ve yağışta meydana gelen mevsimsel farklılıklar da tarımsal üretimi farklı boyutlarda etkilemektedir. Kış sıcaklıklarındaki artış tarımsal üretimi olumlu etkilerken yaz sıcaklık artışı olumsuz etkilemektedir. Deressa ve ark. (2005) tarafından Güney Afrika'nın 11 bölgesinde 1977-1998 yılları aralığında yapılan çalışmada, sıcaklıkların şeker kamışı üretimi üzerindeki etkisi incelenmiş ve sıcaklık değişimlerinin kış aylarında şeker kamışı üretimini negatif, yaz aylarında ise pozitif etkilediği sonucuna ulaşılmıştır. Van Passel ve ark. (2017), 15 Avrupa Birliği ülkesinde yaptıkları çalışmada 2007 yılı için sıcaklık değişimlerinin tarımsal gelir düzeyini yaz ve kış mevsimlerinde negatif, ilkbahar ve sonbahar mevsimlerinde ise pozitif etkilediği görülmüştür. Yağıştaki değişikliklerin yaz ve kış mevsiminde tarımsal geliri pozitif, ilkbahar ve sonbahar aylarında ise negatif yönde etkilediği gözlenmiştir. 1981-2012 yıllarında Pakistan'ın Pencap şehrinde iklim değişikliğinin buğday, pamuk ve pirinç üzerindeki etkisinin araştırıldığı çalışmada ise sıcaklık değişimlerinin ekim ve hasat döneminde buğday verimini olumlu düzeyde, çiçeklenme döneminde ise olumsuz düzeyde etkilediği ortaya konulmuştur. Bunun yanında yağış, minimum sıcaklık düzeyi ve nem miktarının ekim döneminde pirinç üretimini olumlu, hasat döneminde ise olumsuz etkilediği görülmüştür (Afzal ve ark., 2016).

İklim değişikliğine ilişkin yapılan tahmin çalışmaları gösteriyor ki bugüne kıyasla gelecekte toprak, su, doğal kaynaklar ve tarım sektörü olumsuz etkilenebilir. İklim değişikliğine bağlı olarak kuraklık yaşanan bölgelerin daha kuraklaşacağı, yağışlı bölgelerde ise ani yağış miktarının artacağı, tüm bölgelerde yağış rejimlerinin değişeceği ve mevsimsel temelli ani hava olaylarının miktarının ve şiddetinin artacağı yönünde çeşitli tahminler yapılmaktadır. Son 10 yılda iklim değişikliği küresel ölçekte tarımsal üretimi %1-5 aralığında azaltmıştır (Thornton ve ark., 2015). Bu azalışın devam edeceği yönünde çeşitli varsayımlar yapılmaktadır. Cline (2007)'in yaptığı çalışmada, bu verim azalışının devam edeceği ve dünyada 2080 yılında tarımsal üretimin, karbon gübreleme yapılırsa %3.2 oranında, karbon gübresi olmaksızın ise %15.9 oranında azalacağı beklenmektedir.

Türkiye ise iklim değişikliğinin oluşturabileceği

olumsuzluklardan en fazla etkilenecek ülkeler arasında yer almaktadır. Türkiye'de yağışların azalacağı, sıcaklık düzeylerinin artacağı bunlara bağlı olarak gerçekleşecek doğal afetlerin (sel, kuraklık gibi) sıklığının ve şiddetinin artacağı tahmin edilmektedir (Türkeş, 2012). Kuraklık artışına bağlı olarak verimlilik kayıplarının yaşanacağı, su problemi olmayan bölgelerde sıcaklığa bağlı bitkilerin strese gireceği, yeterli sulama olsa da verim kaybının yaşanabileceği öngörülmektedir. Bu bağlamda, Türkiye'de iklim değişikliğinin tarım sektörü üzerindeki etkisini görmek için 1972-2011 yılları arasında yapılan çalışmada, yağış rejiminde meydana gelen değişikliklerin tarımın GSYİH içindeki payını pozitif olarak etkilerken, sıcaklıkta meydana gelen değişimlerin tarımın GSYİH içindeki payını negatif olarak etkilediği görülmüştür (Başoğlu & Telatar, 2013). Yapılan bir başka çalışmada ise 1980-2013 yılları aralığında kısa dönem için yağış ve tarımsal verimlilik artışının tarımın GSYİH içindeki payını pozitif etkilerken, CO_2 ve sıcaklıktaki artışların tarımın GSYİH içindeki payını negatif olarak etkilediği gözlenmiştir. Uzun döneme ilişkin bulgularda ise CO_2 seviyesinin tarımsal verimlilik düzeyinden daha yüksek oranda arttığı ve tarımsal verimliliği olumsuz etkilediği sonucuna ulaşılmıştır (Bayraç & Doğan, 2016).

İklim göstergelerine ilişkin tahminlere göre, gelecek yüzyıl için yıllık ortalama sıcaklıkların 1.5 ℃ artması beklenirken yağışların ise 1.5 mm gün^{-1} azalması öngörülmektedir (Pachauri ve ark., 2014). Öngörülen ısınma oranları 20.yüzyılın ısınma oranlarından daha fazla olacağıdır. Kuzey Amerika'nın kuzey bölgelerinde ve Orta Asya'nın kuzeyinde küresel ortalamadan %40 daha fazla ısınma beklenmektedir. Yaz aylarında Güney ve Güneydoğu Asya, kış aylarında ise Güney Amerika için küresel ortalama sıcaklık artışlarından daha düşük olacağı öngörülmektedir (Türkeş, 2008). Dünyanın farklı bölgelerinin küresel ısınmadan farklı düzeylerde etkileneceği tahmin edilmektedir.

Diğer taraftan kurak ve yarı kurak alanlarda daha fazla kuraklaşma beklenirken, yağışlarda mevsimlik ve enlemsel kaymaların olabileceği diğer taraftan yüksek enlemlerde sürekli, orta enlemlerde ise kış aylarında artışların olacağı beklenmektedir. Avustralya, Orta Amerika ve Güney Afrika bölgelerinde kış yağışlarında bir azalma olacağı, özellikle Doğu Akdeniz havzası ve Orta Doğu için yağışlarda ve su kaynaklarında önemli düzeyde azalmaların olacağı tahmin edilmektedir (Türkeş, 2008). Türkiye'nin, Doğu Akdeniz havzası içerisinde yer alması iklim değişimine ve kuraklığa karşı hassas bir bölge olduğunu göstermektedir.

Yapılan bir başka çalışmada, Türkiye'nin 2030 yılında kuru ve sıcak iklimin etkisine gireceği tahmin edilmektedir (Özçatalbaş (2014). Bu değişimlerin, kuraklığı, sel ve heyelan riskini artıracağı özellikle su stresi oluşturabileceği öngörülmektedir (Şen, 2013). Gelecekte Türkiye'nin tarımsal üretimde %15 ile %25 arasında verimlilik kaybı yaşayacağı beklenmektedir (Cline, 2007). 2050 yılına kadar ise buğday, arpa, mısır, ayçiçeği ve pamuk gibi tarımsal ürünlerin üretiminde %3.8 ile %10.1 arasında düşüş yaşanacağı ve her yıl yaklaşık olarak 0.1 milyon dolar düzeyinde refah kaybının gerçekleşebileceği tahmin edilmektedir (Dellal ve ark., 2011). Diğer taraftan su kaynaklarının azalması ve doğal kaynakların dengesinin bozulacağı yönünde çeşitli çalışmalar yapılmaktadır. Kuraklığa uyum sağlayamayan birçok bitki türü ve canlının yok olması da söz konusu olabilecektir.

İklimde meydana gelecek dalgalanmalar sonucu, tarım sektöründe verim ve kalite kayıpları yaşanırken, insan yaşamı da sosyal ve ekonomik yönden etkilenmektedir. İklime bağlı değişimler tarım ekonomisini doğrudan etkilerken diğer yandan gelir kaybı, gıda enflasyonu, tarım sanayine ilişkin kayıplar, ekonomik kalkınmanın duraklaması, arazi fiyat artışları, işsizlik, çiftçilerin sektörden kopması, tarıma dayalı ihracatın azalması gibi olumsuzluklara yol açabilmektedir (Koç ve ark., 2016; Özçatalbaş, 2014; TAGEM, 2001). Tüm bu nedenler, iklim değişikliğinin tarımsal üretim üzerindeki etkisinin incelenmesi gerekliliğini ortaya koymaktadır.

Bitkisel üretimlerin genel olarak, dünyada ve Türkiye'de açık tarım alanlarında yapılması nedeniyle bitkisel üretimde temel değişken iklim olmaktadır. Diğer taraftan bitkisel üretimde verimlilik düzeyi, ekim alanı, sulama potansiyeli gibi faktörlerde doğrudan iklim değişikliğinden etkilenen alanlardır (Soylu & Sade, 2012). Dolayısıyla iklim değişikliği tarım sektörünü çok boyutlu etkilemektedir.

Bitkisel üretim içinde önemli bir alan olarak yer alan yem bitkileri, hayvancılık sektörünün en önemli girdisidir. Hayvancılık sektörü açısından stratejik düzeyde ürün olan yem bitkileri, düşük maliyetli girdi olması nedeniyle talebi yüksektir. Hayvan beslemesi için birçok alternatif varken (endüstri atıkları, küspe, posa ve bazı tahıl taneleri gibi) genel olarak besleme yöntemlerinde bunların miktarı, yem bitkilerine göre daha düşük düzeyde kalmaktadır. Yem bitkileri üretimi, yüksek verimlilik ve düşük maliyet avantajı sağladığından hayvancılığın geliştiği ülkelerde, yem bitkileri, çayır ve mera tarımı önemsenmektedir (Açıkgöz ve ark., 2005).

Kuzeydoğu Anadolu Bölgesi'nde özellikle Ardahan ilinde hayvansal üretim için ihtiyaç duyulan yem üç kaynaktan elde edilmektedir. Bunlar doğal çayır ve meralar, yem bitkileri ve tahıllardır. Ancak yemin elde edildiği kaynaklara bakıldığında, ağırlığın meralar ve maliyeti yüksek kesif yemlerden oluştuğu anlaşılmaktadır (Sayar ve ark., 2010). Kaba yemler ise hayvansal çayır mera alanları ve yem bitkilerinden karşılanmaktadır. Bitkisel üretim içerisinde önemli

bir paya sahip olan yem bitkileri bitkisel ve hayvansal üretimin sigortası konumundadır ve sürdürülebilir güvenli yem üretiminin önemli bir kaynağıdır (Açıkgöz, 2001).

Ardahan ilinde halkın önemli bir kısmının, geçimini hayvancılıktan sağlaması nedeniyle büyükbaş hayvan beslenmesi hayvancılığın gelişmesi açısından önem taşımaktadır. Ardahan 4 milyon 934 bin dekar arazi varlığına sahip ve bu alanın 2 milyon 458 bin 230 dekarı mera alanıdır. Çayır ve mera alanları yaklaşık %59 ile Türkiye ortalamasının (%18) üç katı kadardır. Bu durum mera hayvancılığının yapılmasını olanaklı kılmaktadır. Ancak son yıllarda sıcaklık artışları ve bölgede yağışların azalması kaynaklı mevcut meralarda otlatma için elverişli dönemin çok kısa olması, beslenme kaynağı ağırlıklı olarak kaba yem ihtiyacını doğurmaktadır. Kaba yemin temininde, yem bitkileri ve tahıl önemli yer tutmaktadır. Hayvancılığın geleceği açısından mevcut meralardan en yüksek faydayı sağlayacak şekilde kullanmak ve yem bitkilerinin verimliliğini sürdürülebilir kılmak büyükbaş hayvancılık açısından önem taşımaktadır.

Bu kapsamda çalışmada, bölgede büyükbaş hayvan yetiştiriciliğinin sürdürülebilirliğinin sağlanmasında önemli yer tutan yem bitkileri üretimi ve tahıl üretiminin iklim değişikliğinden etkilenip etkilenmediğinin araştırılması amaçlanmıştır.

Çalışmanın ilerleyen kısmı, araştırmaya konu serilerin ve yöntemin tanıtıldığı materyal ve metod, analiz sonuçlarının yorumlandığı bulgular ve tartışma, son olarak ve sonuç ve önerilerden oluşmaktadır.

MATERYAL ve METOD

Çalışmada Ardahan ili genelinde iklim değişikliğinin tahıl ürünleri ile ot ve yem verimlilikleri üzerindeki etkisi 1995-2020 dönemi itibariyle araştırılmıştır. Çalışmanın konusuna ilişkin yapılacak ekonometrik analizlerde kullanılan veri setine ait bilgiler Çizelge1'de verilmiştir.

Çizelge 1. Değişkenlerin tanımı
Table 1. Definition of variables

Değişken *Variable*	Tanım *Definition*	Kaynak *Source*
LTV	Tahıl Verimliliği	TUİK
LOYV	Ot ve Yem Verimliliği	TUİK
LOS	Ortalama Sıcaklık	MGM
LTY	Toplam Yağış	MGM

L, serilere logaritmik dönüşüm uygulandığını göstermektedir.

Bağımlı değişken olarak tahıl (LTV), ot ve yem verimliliği (LOYV) verilerinden, iklim değişikliğine yönelik açıklayıcı değişken olarak ise ortalama sıcaklık (LOS) ve toplam yağış (LTY) verilerinden yararlanılmıştır.

Çalışmada yer alan serilerin durağan olup olmadıkları geleneksel birim kök testlerinden Genişletilmiş Dickey-Fuller (ADF) ve Phillips-Perron (PP) testlerinin yanında yapısal kırılmayı dikkate alan Zivot ve Andrews (ZA) birim kök testi ile araştırılmıştır. Dickey & Fuller (1979), hata terimlerinin istatistiksel olarak bağımsız ve homojen olduklarını; Phillips-Perron (1988) ise hata terimlerinin zayıf bağımlı ve heterojen olduklarını varsaymaktadır. ADF modellerinde seride yüksek korelasyonun önüne geçmek amacıyla AR(p) sürecinden yararlanmakta ve modele p gecikmeli fark terimlerini ilave etmektedir. PP testinde test istatistiği modifiye edilerek asimptotik dağılımının serisel korelasyondan etkilenmemesi sağlanmaktadır.

Çalışmada geleneksel birim kök testlerine ek olarak yapısal kırılmaları dikkate alan Zivot ve Andrews'in (ZA) tek kırılmalı birim kök testinden yararlanılmıştır. ADF tipi test stratejisine dayanan ZA testinde kırılma zamanı içsel olarak kabul edilmekte ve önceden bilinmemekle birlikte, en düşük t-istatistiğine denk gelen gözlem kırılma dönemi olarak alınmaktadır.

Değişkenler arasında uzun dönemli olarak gerçekleşecek ilişkiyi test etmek amacıyla Pesaran ve ark. (2001)'in sınır testi yaklaşımı kullanılmıştır. Diğer eşbütünleşme testlerine kıyasla bazı üstünlükleri olan sınır testi, serilerin I (1) veya I (0) olup olmadıklarını yani bütünleşme derecelerine bakılmadan seriler arasında uzun dönemli ilişkilerin test edilmesi amacıyla kullanılan oldukça esnek bir yöntemdir.

İki aşamadan oluşan sınır testinin ilk aşamasında değişkenler arasında uzun dönem ilişkinin olup olmadığı sınanmakta; değişkenlerin eşbütünleşik olmaları durumunda ikinci aşamada ise kısa dönem ve uzun dönem katsayıları elde edilmektedir. Seriler arasında uzun dönemli ilişkiyi tespit etmek amacıyla (1) numaralı denklemler tahmin edilmiştir.

(1) numaralı modellerde β, δ, λ ve γ regresyon katsayılarını göstermektedir. Modellerin optimal gecikme uzunlukları belirlenerek tahmin edilen ve sonra değişkenler arasındaki uzun dönemli ilişki asimptotik dağılımı standart t ve F dağılımlarına uyan test istatistikleri aracılığıyla araştırılır. F_{III} istatistiği sabitli model için düzey değişkenlerin gecikmeli değerlerinin bir bütün olarak sıfıra eşit olup olmadığı ($H_0: \beta_1 = \beta_2 = \beta_3 = 0$) test edilirken, t_{III} istatistiği ise (1) numaralı modellerdeki bağımlı değişken gecikme katsayısının ($LOYV_{t-1}$ ve LTV_{t-1}) sıfıra eşit olup olmadığı ($H_0: \beta_1 = 0$) test edilmektedir.

$$\text{Model1: } \Delta LOYV_t = \beta_0 + \beta_1 LOYV_{t-1} + \beta_2 LTY_{t-1} + \beta_3 LOS_{t-1} + \sum_{i=1}^{p} \delta_i \Delta LOYV_{t-i} + \sum_{i=0}^{q_1} \lambda_i \Delta LTY_{t-i} + \sum_{i=0}^{q_2} \gamma_i \Delta LOS_{t-i} + \varepsilon_t$$

$$\text{Model2: } \Delta LTV_t = \beta_0 + \beta_1 LTV_{t-1} + \beta_2 LTY_{t-1} + \beta_3 LOS_{t-1} + \sum_{i=1}^{p} \delta_i \Delta LTV_{t-i} + \sum_{i=0}^{q_1} \lambda_i \Delta LTY_{t-i} + \sum_{i=0}^{q_2} \gamma_i \Delta LOS_{t-i} + \varepsilon_t \quad (1)$$

Karar aşamasında hesaplanan test istatistikleri Pesaran ve ark. (2001) tarafından belirlenmiş alt ve üst sınır değerleri karşılaştırılır. Ancak hesaplanan F_{III} ve t_{III} istatistikleri tablo alt sınır değeri altında kaldığında seriler arasında eş bütünleşme ilişkisinin olmadığını ileri süren sıfır hipotezi reddedilememektedir. Eğer hesaplanan test istatistikleri üst sınır değerlerini aşıyorsa seriler arasında uzun dönemli ilişkinin olduğu sonucuna varılmaktadır. Test istatistiklerinin alt ve üst kritik sınırlar arasında olması durumunda ise uzun dönem ilişkinin varlığı hakkında bir karar verilememektedir.

Bağımlı ve bağımsız değişkenlerin gecikme uzunluklarının bilgi kriterleri aracılığı ile tespit edilmesinin ardında en uygun ARDL modeli belirlenir ve daha sonra tahmin sonucunda uzun dönem katsayıları elde edilir. ARDL(p,q,r) modeli (2) numaralı modellerde gösterilmiştir.

$$LOYV_t = \beta_0 + \sum_{i=1}^{p} \delta_i LOYV_{t-i} + \sum_{i=0}^{q} \lambda_i LTY_{t-i} + \sum_{i=0}^{r} \gamma_i LOS_{t-i} + u_t$$

$$LTV_t = \beta_0 + \sum_{i=1}^{p} \delta_i LTV_{t-i} + \sum_{i=0}^{q} \lambda_i LTY_{t-i} + \sum_{i=0}^{r} \gamma_i LOS_{t-i} + u_t \quad (2)$$

(2) numaralı denklemlerde β, δ, γ ve λ regresyon katsayılarını; p, q ve r bağımlı değişken ve açıklayıcı değişkenlere ait en uygun gecikme uzunluklarını ifade etmektedir. Sınır testi sonuçlarına göre seriler arasında bir uzun dönem ilişkinin tespit edilmesi durumunda ARDL(p,q,r) modelinden yararlanılarak uzun ve kısa dönem katsayıları tahmin edilir.

Çalışmada değişkenler arasındaki nedensellik ilişkisinin tespitinde Toda-Yamamoto nedensellik analizinden yararlanılmıştır. Toda & Yamamoto (1995), bir ekonometrik model kurulurken ilgili değişkenlerin birim kök içermeleri durumunda da bu değişkenlerin seviye değerleri kullanılarak VAR analizine tabi tutulabileceğini ifade etmişlerdir. VAR sisteminde çalışmaya konu değişkenlere ilişkin aşağıdaki denklemler kurularak Toda-Yamamoto testi uygulanmıştır.

$$LOYV_t = \alpha + \sum_{i=1}^{k+d_{max}} \theta_i LOYV_{t-i} + \sum_{i=0}^{k+d_{max}} \omega_i LTY_{t-i} + e_{1t} \quad (4)$$

$$LOYV_t = \alpha + \sum_{i=1}^{k+d_{max}} \varphi_i LOYV_{t-i} + \sum_{i=0}^{k+d_{max}} \eta_i LOS_{t-i} + e_{2t} \quad (5)$$

$$LTV_t = \alpha + \sum_{i=1}^{k+d_{max}} \nu_i LTV_{t-i} + \sum_{i=0}^{k+d_{max}} \pi_i LTY_{t-i} + e_{3t} \quad (6)$$

$$LTV_t = \alpha + \sum_{i=1}^{k+d_{max}} \rho_i LTV_{t-i} + \sum_{i=0}^{k+d_{max}} \xi_i LOS_{t-i} + e_{4t} \quad (7)$$

(4)-(7) numaralı denklemlerde e_{it} hata terimlerinin beyaz gürültü süreci sergilediği ve otokorelasyonun olmadığı varsayılmaktadır. Toda-Yamamoto testinde ilk aşama olarak serilerin maksimum bütünleşme dereceleri birim kök testleri ile tespit edilerek dmax düzeyleri belirlenir. Daha sonra VAR sisteminde tüm istikrar koşullarının sağlandığı uygun gecikme uzunluğu k belirlenir. (k+dmax) gecikme dışsal değişken gecikmesi olarak modele eklenerek VAR modeli elde edilir. Son aşamada ise elde edilen VAR modelinden Granger nedensellik sonuçları yorumlanır.

BULGULAR ve TARTIŞMA

İklim değişikliği ile tahıl ürünleri ve ot ve yem verimlilikleri arasında uzun dönemli ilişkinin var olup olmadığına bakılmadan önce çalışmaya konu serilerin birim kök özellikleri ADF, PP ve tek kırılmalı ZA birim kök testleri aracılığıyla incelenmiştir. Çizelge 2'de sunulan ADF ve PP birim kök testi sonuçlarına göre tahıl verimliliği ile ot ve yem verimliliği değişkenleri birinci farkında (I(1)), yıllık ortalama sıcaklık ve yıllık yağış değişkenleri ise seviyede (I(0)) durağan olarak tespit edilmiştir.

Çizelge 2. Birim kök analizi
Table 2 Unit root analysis

	ADF		PP	
Değişkenler *Variables*	Sabitli *Constant*	Sabitli ve Trendli *Constant and Trend*	Sabitli *Constant*	Sabitli ve Trendli *Constant and Trend*
LTV	-2.3490	-2.6903	-2.1907	-2.6615
DLTV	-5.0221***	-4.9408***	-6.0684***	-6.6434***
LOYV	-1.3974	-1.7019	-1.6681	-1.5382
DLOYV	-4.5143***	-3.6796***	-4.4796***	-5.7897***
LOS	-1.6560	-4.1778**	-3.2538**	-4.1575**
DLOS	-4.7727***	-5.4024***	-15.4835***	20.1577***
LTY	-4.0899***	-3.9738**	-4.0745***	-3.9512**
DLTY	-8.7894***	-4.8617***	-11.6849***	-27.3968***

Yıldızlı değerler Akaike Bilgi Kriteri'ne göre belirlenmiş olup optimal gecikme uzunluklarıdır. *** ve ** sırasıyla serinin %1 ve %5 anlamlılık seviyesinde durağan olduğunu göstermektedir. D ise serilerin birinci farklarının alındığını ifade etmektedir.

Çizelge 3'te ZA birim kök testinin sabitli ve sabitli-trendli modellerine ilişkin bulgular raporlanmıştır. Yapısal kırılmalı birim kök testi sonuçlarına göre de tahıl verimliliği ile ot ve yem verimliliği değişkenleri birinci farkında (I(1)), yıllık ortalama sıcaklık ve yıllık yağış değişkenleri ise seviyede (I(0)) durağan olarak tespit edilmiştir.

Kullanılan değişkenlerin durağanlık düzeylerinin

farklı olması (I(0 ve I(1)) çalışmada uzun dönemli ilişki tespitinde Pesaran ve ark. (2001)'in sınır testi yaklaşımının kullanılmasını gerekli kılmıştır.

Çizelge 3. ZA birim kök analizi
Table 3. ZA unit root analysis

	Model A		Model C	
	t	TB	t	TB
LTV	-3.9683	2010	-3.6120	2010
DLTV	-6.2478***	2009	-6.4702***	2010
LOYV	-3.1066	2003	-3.9609	2012
DLOYV	-4.9667**	2003	-4.9809*	2003
LOS	-5.8614***	2010	-6.8255***	2010
DLOS	-7.1094***	2013	-5.3789**	2012
LTY	-6.3248***	2002	-6.2885***	2002
DLTY	-5.0849**	2006	-5.2226**	2009

Yıldızlı değerler optimal gecikme uzunluklarıdır. ***, ** ve * sırasıyla serinin %1, %5 ve %10 anlamlılık seviyesinde durağan olduğunu göstermektedir. D serilerin birinci farklarının alındığını ifade etmektedir.

Çizelge 4. Sınır testi F ve t istatistiği
Table 4. Bounds test F and t statistic

		İstatistik *Statistic*	Kritik Değer (0.05) *Critical Value*		Kritik Değer (0.01) *Critical Value*	
			I (0)		I (1)	I (1)
Model1	F-İstatistiği	3.801	5.55	6.747	7.977	9.413
	t-istatistiği	-2.6936	-3.41	-3.95	-3.96	-4.53
Model2	F-İstatistiği	4.2083	5.55	6.747	7.977	9.413
	t-istatistiği	-3.0617	-3.41	-3.95	-3.96	-4.53

Gecikme uzunluklarının tespitinde AIC bilgi kriterinden yararlanılmıştır.

Çizelge 4'de Model 1 ve Model 2'ye ait sınır testi sonuçlarına ilişkin F ve t istatistik tahminleri raporlanmıştır. Buna göre her iki modele ait F ve t istatistikleri tablo kritik alt sınır değerinin altında yer alması dolayısı ile serilerin eş bütünleşik olmadığı tespit edilmiştir. Yani verimlilik ve iklim serileri uzun dönemde birlikte hareket etmemektedir.

Çizelge 5'te Toda-Yamamoto Nedensellik analizi sonuçları raporlanmıştır. Toda-Yamamoto nedensellik testinin ilk aşaması olarak serilerin düzey değerleri standart VAR modeli sistemine

ve Hannan-Quinn (HQ) bilgi kriterlerinden faydalanılarak optimum gecikme uzunluğu belirlenmeye çalışılmıştır. Tüm istikrar koşullarını sağlayan gecikme uzunluklarının belirlenmesinin ardından tüm modeller için dışsal değişken gecikmeleri k+dmax=2 olarak belirlenip VAR(1) modelleri tahmin edilmiştir.

Çizelge 5. Toda-Yamamoto nedensellik testi
Table 5. Toda-Yamamoto causality test

Nedensellik *Casuality*	k	dmax	LM(1)	White	Jarque-Bera	χ ^2	Karar *Decision*
LTY→LOYV	1	1	1.2794	66.9235	1.2883	7.1963***	Nedensellik var.
			(0.8649)	(0.3440)	(0.8634)	(0.0073)	
LOS→LOYV	1	1	0.6176	71.4922	4.8985	0.3916	Nedensellik yok.
			(0.9611)	(0.2166)	(0.2979)	(0.5315)	
LTY→LTV	1	1	2.5459	70.1046	1.2518	0.5169	Nedensellik yok.
			(0.6364)	(0.1748)	(0.8695)	(0.4722)	
LOS→LTV	1	1	4.4427	58.7499	5.3965	0.4725	Nedensellik yok
			(0.3494)	(0.5215)	(0.2490)	(0.4918)	

Parantez içindeki değerler olasılık (p-value) değerlerini göstermektedir. ***, %1 hata payı ile anlamlılığı ifade etmektedir.

Çizelge 5'teki Toda-Yamamoto nedensellik test sonuçlarına göre, yıllık toplam yağışlardan ot ve yem verimliliğine doğru bir nedensellik ilişkisi olduğu görülmektedir. Buna göre Ardahan ili genelinde toplam yağışlar kısa dönemde ot ve yem verimliliğini etkilemektedir. Ancak yıllık ortalama sıcaklıklardan ot ve yem verimliliğine doğru kısa dönemde bir nedensellik ilişkisinin olmadığı tespit edilmiştir. Diğer yandan yıllık toplam yağış miktarı ve yıllık ortalama sıcaklık değişkenlerinden tahıl verimliliğine doğru bir nedensellik ilişkisinin olmadığı da görülmektedir.

Yani, kısa dönemde çalışmaya konu iklimsel değişkenlerin tahıl verimliliği üzerinde etkisi olmadığı söylenebilir.

SONUÇ ve ÖNERİLER

İklim değişikliği küresel bir sorundur. Bu nedenle iklim değişikliğine bağlı yaşanacak tüm olaylar tüm dünyayı etkilemektedir. Literatürde yapılan çalışmalar incelendiğinde, iklim değişikliği nedeniyle meydana gelen iklimsel olaylar tarım sektörünü olumsuz etkilemekte ve ekonomik kayıplara yol açmaktadır.

Türkiye, içinde bulunduğu coğrafyada iklim değişikliğinden en fazla etkilenecek ülkeler arasında yer almaktadır. Çalışmaya konu Ardahan ilinde küresel ısınmaya bağlı olarak sıcaklık artışları ve ani hava olayları gözlenmeye başlanmıştır. İlin ekonomisi içinde önemli yer tutan hayvancılığın gelişmesinde, yem bitkileri ve tahıl üretimine ilişkin mevcut durum ve geleceğe ilişkin tahminler önem taşımaktadır. İlde, son yıllarda yaz yağışlarının azalması ve sıcaklık artışları ile kuraklık gözlenmekte ve meralarda otlatma dönemi kısa sürmektedir. Bu durum kaba yem ihtiyacını doğurmakla birlikte, yem bitkileri ve tahıl üretimine olan talebi artırmaktadır.

Çalışmada, iklim değişiminde meydana gelen sıcaklık artışları ve yağış değişimlerinin, yem bitkileri ve tahıl verimliliği üzerindeki etkileri incelenmiştir. Elde edilen bulgulara göre, Ardahan ilinde toplam yıllık yağışlar, kısa dönem için ot ve yem verimliliği üzerinde etkili iken, ortalama sıcaklık değişimlerinin bir etkisinin olmadığı gözlenmiştir. Diğer taraftan tahıl verimliliği üzerinde kısa dönemde yıllık toplam yağışların ve sıcaklık değişimlerinin bir etkisinin olmadığı sonucuna ulaşılmıştır. İklim değişiminin olumsuz etkileri kısa dönemde yem verimliliğini etkilerken tahıl üretiminde henüz hissedilmemiştir.

Literatürde, sıcaklık ve yağış değişimlerinden her ülkenin veya ülkelerin farklı bölgelerinin tarımsal üretiminin farklı düzeylerde etkilendiği görülmüştür. Hatta sıcaklık ve yağış değişimleri mevsimler arasında tarımsal üretimi farklı düzeylerde etkilemektedir. Yağışlardaki değişimler tarımsal üretimi genel olarak olumlu yönde etkilerken sıcaklık değişimleri kimi ülkelerde tarımsal üretimi olumsuz etkilemiş ancak bu durum mevsimlere göre farklılık oluşturmuştur. Kış sıcaklıklarının artması, bazı ülkelerde bitkisel üretime olumlu yansımıştır.

Ardahan, yıllık ortalama sıcaklıklar bakımından Türkiye'nin en soğuk ili olması ve kış aylarının uzun geçmesinden dolayı bitkisel üretim çeşitliliği düşük bir ildir. Hava sıcaklıklarındaki artışların devam etmesi ile birlikte (yapılan tahmin çalışmaları artışların devam edeceğini göstermektedir) Ardahan ilinde bu durum tarım sektöründe yeni fırsatlar oluşturabilir. Sıcaklık artışları ile bitkilerin büyüme döneminin uzaması, ürün çeşitliliğini artırabilir. Bu kapsamda iklim değişikliğinin oluşturacağı olumsuz etkileri minimum düzeye indirecek ya da olumluya dönüştürecek, iklim değişikliğine adaptasyonu sağlayacak çalışmalara ihtiyaç duyulmaktadır. Dolayısıyla, iklim değişikliğinin oluşturacağı olumsuzlukların minimum düzeye indirilmesi için yerelde ve ulusal düzeyde alınabilecek önlemler ve uygulanabilecek politikalarla ilgili bazı öneriler geliştirilmiştir.

- İklim değişikliğinin kısa ve uzun dönemde olumsuz etkileri kaçınılmaz dolayısıyla, iklim değişikliğine uyum konusunda ülke genelinde seferberliğe gidilmeli,
- İklim değişikliğine adaptasyonu yüksek tür ve ırklar yetiştirilmeli,
- Üniversiteler bünyesinde iklim değişikliği araştırma merkezleri kurulmalı,
- Çiftçilere iklim değişikliğine uyum ve farkındalık konusunda destekler sağlanmalı,
- Kuraklığa dayanıklı ıslah çalışmaları yapılmalı,
- Sıcaklık artışı veya yağışa dayanıklı, iklim değişikliğine duyarlı tohum, gübre veya üretim yöntemleri geliştirilmeli,
- Tarım sigortaları daha yaygın olarak kullanılmalı ve kuraklığa, doğal afetlere, hastalıklara karşı devlet güvencesi sağlanmalı,
- Kuraklığa karşı etkin su yönetim sistemleri kurulmalı,
- Kuraklığa, ani iklim olaylarına ve doğal afetlere karşı eylem planları hazırlanmalı,
- Üreticilerin örgütsel ve kurumsal kapasiteleri artırılmalı,
- İklim değişikliğinin olası etkilerine yönelik çiftçiler ve aileleri eğitim programları ile desteklenmeli.

Sonuç olarak; Ardahan ilinin ekonomisi ve sürdürülebilir gıda arzı için iklim değişimine uyum çabaları artırılmalı ve bu konuda kamu, özel sektör ve üniversite işbirliği içinde iklim değişimiyle mücadele yaklaşımı kapsamlı olarak uygulanmalıdır. İklim değişikliğine uyum çabaları, ülke geleceği ve sürdürülebilir gıda arzı açısından stratejik düzeyde önemlidir.

Araştırmacıların Katkı Oranı Beyan Özeti

Yazarlar makaleye eşit oranda katkı sağlamış olduklarını beyan eder.

Çıkar Çatışması Beyanı

Makale yazarları aralarında herhangi bir çıkar çatışması olmadığını beyan ederler.

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Determination of Fish Consumption Habits of Students in Maritime High Schools: The Case of Turkey

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ABSTRACT
In this study, it has been aimed to determine the fish consumption habits of the students studying at high schools in the field of Maritime. This research is cross-sectional in nature. It has been found that the participants give importance to fish consumption, they prefer to eat fish because it is healthy, and it is the recommendation of family and friends that make them willing to consume a new fish species. In the consumption of fish, they prefer to consume fresh, fried fish, which are hunted and purchased from the fishermen's stall. It is thought that the necessity of health education for the development of fish consumption habits and increasing the fish consumption rate and the carrying out of these training with family and peer support can increase fish consumption.

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Denizcilik Alanındaki Liselerde Öğrenim Gören Öğrencilerin Balık Tüketim Alışkanlıklarının Belirlenmesi: Türkiye Örneği

ÖZET
Bu çalışmada, Denizcilik alanındaki liseler bünyesinde öğrenim görmekte olan öğrencilerin balık tüketim alışkanlıklarının belirlenmesi amaçlanmıştır. Bu araştırma kesitsel niteliktedir. Katılımcıların balık tüketimine önem verdikleri, balık yemeyi sağlıklı olduğu için tercih ettikleri, yeni bir balık türünü tüketmeye istekli hale getiren durumun aile ve arkadaş tavsiyesinin olduğu bulunmuştur. Balık tüketiminde avlanmış, balıkçı tezgahından satın alınan, taze, kızartılmış balık tüketmeyi tercih etmektedirler. Balık tüketim alışkanlıklarının geliştirilmesi ve balık tüketim oranının artırılmasına yönelik sağlık eğitiminin gerekliliğini ve bu eğitimlerin aile ve akran desteği ile yapılmasının balık tüketimini arttırabileceği düşünülmektedir.

Su Ürünleri

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INTRODUCTION
In many countries of the world, the leading cause of death in humans as a result of disease are, cardiovascular diseases, high blood pressure, sugar, and cholesterol. In addition to hereditary factors, nutrition regimen also has a very important place in the basis of these diseases. The therapeutic role of fish meat in these diseases has been studied for a long time and positive results have been obtained in this regard. In addition, seafood is the only source of polyunsaturated long-chain fatty acids in the n-3 series, which has proven beneficial to health (Erci, 2020).

The fact that some members of the profession require continuity of health status in order to be able to work in the profession is also important for shipmen (captain, officers, crew members, and other people working on the ship) to work at sea. Candidates for shipmen and shipmen who are currently working can obtain work permits by evaluating their health status, subject to the Shipmen Health Directive, which covers the requirements for health status and health

examinations. According to this directive, shipmen are not allowed to work at sea in case of diseases such as endocrine and metabolic diseases, cardiovascular system diseases, which are closely related to nutritional status (Anonymous, 2021). In order to prevent these diseases, changes in lifestyle and eating habits are needed. Considering that healthy lifestyle behaviors that are effective in improving health are acquired during the adolescence period, which is accepted as the 10-19 age group according to the World Health Organization (Erci, 2020) and considering that this period determines what kind of adult the individual will be in the future, it is also important to acquire these behaviors in secondary and high school ages (Erci, 2020). Therefore, determining the habit of consumption of fish will be a guide for the studies to be carried out.

When the studies conducted around the world on fish consumption are examined, various studies have been found examining; the effects of demographic and socioeconomic characteristics of consumers on seafood consumption at home (Bashimov, 2017; Sen and Sahin, 2017; Islam et al., 2018; Terin, 2019; Cantillo et al., 2021; Dasgupta et al., 2021), the most preferred cooking methods (Gundogdu and Buyruk, 2021), awareness of quality and safety of fish products, purchasing behaviors and willingness to pay (Terin et al., 2016; Bao et al., 2018; Terin, 2019), fresh and frozen (Yigit et al., 2020; Ferfolja et al., 2022; Selvi et al., 2022) convenience and weight control (Thong and Solgaard, 2017), being delicious and having high nutritional value (Kirici et al., 2018; Sari et al., 2019). It is seen that studies conducted both in the world and in Türkiye are generally conducted on adults. It is a study in which fish consumption habits and preferences of high school students (Yigit et al., 2020). However, we could not find any study that questioned the fish consumption habits of shipmen and shipmen candidates.

Evaluating the nutritional habits of our young people, who are the guarantee of our future, is an important indicator in terms of determining health risks. The importance of fish consumption for chronic diseases that are considered to be related to nutrition is emphasized and the consumption among young people should be determined. According to TURKSTAT 2020 data, annual fish consumption per capita in our country is 6.7 kg was lower than the global consumption value of 20.5 kg (Anonymous, 2020).

It is necessary to increase the effectiveness of the studies to be done and the programs to be developed in secondary education institutions, considering that this value can be raised higher and while doing this, health behaviors that can be sustained in adulthood can be gained in adolescence. In this study, it was aimed to determine the fish consumption habits of the students studying at high schools in the field of Maritime throughout Türkiye.

MATERIAL AND METHOD

Study Design

This cross-sectional study was carried out in secondary education institutions providing education in the field of Maritime under the Ministry of National Education between 15 march-15 June 2018 in Türkiye. It is divided into seven regions that show similar characteristics in terms of climate and cultural characteristics in Türkiye. Only five of these regions have high schools providing maritime education. The schools to be included in the study were determined by lottery method to take one school from each region using cluster sampling method from schools in five regions throughout Türkiye (Group 1: Black Sea, Group 2: Aegean, Group 3: Marmara, Group 4: Mediterranean and Group 5: Eastern Anatolia regions). 1050 students in the maritime field in five regions formed the study universe of the research. Sample size; was determined as at least 823 with a misconception level of 0.01, a 95% confidence interval, and an 80% ability to represent the universe by using OpenEpi, version 3, publicly available statistical software (http://www.openepi.com). The study was completed with 832 participants (Group 1 n:148; Group 2 n:202; Group 3 n:143; Group 4 n:270; Group 5 n:69) who agreed to participate in the study selected by the nonprobability random sampling method. Reporting of the study was structured according to the STROBE checklist.

Data Collection and Tools

The data of the study were collected by the researchers through a questionnaire form in 15-20 minutes by making school visits to the students and using face-to-face interview method. In the research, a questionnaire consisting of 21 questions prepared in the light of the relevant literature was used as a data collection tool. The questionnaire consists of a descriptive features form and a form that questions fish consumption habits.

Descriptive features form: Form; consists of questions that question the age, gender, class, monthly income, height, and weight of the students.

Fish consumption habit form: Consists of questions like; what kind of meat groups and how often the students consume, the importance of fish consumption in the family, preferred meat group, why fish consumption is preferred, fish type, things to consider when buying fish, fish preparation methods, consumption frequency, consumption time, situations that make new fish consumption desirable, any training status about the fish on health and where it is taken from, to think that it is important for health, the duration of taking/using fish oil support and by whom

it is recommended to use it for what purpose (Bashimov, 2017; Sen and Sahin, 2017; Anonymous 2019; Sari et al., 2019; Terin et al., 2019; Yigit et al., 2020; Cantillo et al., 2021).

Analysis of Data

The data of the study were evaluated with SPSS 23.0 Windows software (SPSS Inc., Chicago, IL, USA). In the study, descriptive statistics (frequency, percentage, mean and median) and chi-square analysis were used to determine the descriptive characteristics of the participants and their relationship with their fish consumption habits. Statistically, $p<0.05$ was accepted as the significance level.

Ethical Principles of Research

Before starting the research, written permission and ethics committee approval were obtained from the institutions where the research was conducted (dated 16.06.2017, numbered 40465587-98). In addition, written and verbal consent was obtained from the students and their parents.

Limitations of the Research

The study is limited with students actively studying in high school of maritime in five regions included in the study. As the study is cross-sectional and depends on self-reported assessment, under-reporting is more likely to occur. This cross-sectional study cannot infer about causality.

RESULTS

It was determined that the mean age of participants is 16.88 ±0.88, they are 85.7% male, 40.0% of them are in the 11th grade in maritime field, 29.1% of them had a monthly income status of 324-405 €, 24.6% were trained in the importance of fish in terms of health and received this training by 58.0% through audio and visual media, and the participants' BMI average was 21.60 ±2.99 (Table 1).

Table 1 Distribution of participants by groups according to their introductory characteristi
Çizelge 1. Katılımcıların gruplara göre tanıtıcı özelliklerine göre dağılımı

	Total	Group 1	Group 2	Group 3	Group 4	Group 5
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Gender						
Female	119(14.3)	9(6.1)	20(9.9)	17(11.9)	58(21.5)	15(21.7)
Male	713(85.7)	139(93.9)	182(90.1)	126(88.1)	212(78.5)	54(78.3)
Maritime field						
10th Grade	282(33.9)	48(32.4)	69(34.5)	65(45.5)	78(28.9)	22(31.9)
11th Grade	333(40.0)	46(31.1)	79(39.1)	61(42.7)	122(45.2)	25(36.2)
12th Grade	217(26.1)	54(36.5)	54(26.7)	17(11.9)	70(25.9)	22(31.9)
Monthly ıncome status						
324€ and less	144(17.3)	32()21.6)	43(21.3)	7(4.9)	36(13.3)	26(37.7)
325-405€	242(29.1)	49(33.1)	61(30.2)	38(26.6)	72(26.7)	22(31.9)
406-608€	211(25.4)	29(19.6)	46(22.8)	44(30.8)	82(30.4)	10(14.5)
≥609 €	235(28.2)	38(25.7)	52(25.7)	54(37.8)	80(29.6)	11(15.9)
Status of receiving education on the importance of fish in terms of health						
Educated	205(24.6)	48(32.4)	44(21.8)	23(16.1)	68(25.2)	22(31.9)
Uneducated	627(75.4)	100(67.6)	158(78.2)	120(83.9)	202(74.8)	47(68.1)
Education institution or persons**						
Health workers	86(42.0)	25 (52.1)	16(36.4)	11 (47.8)	24 (35.3)	10 (45.5)
Visual-audio media***	119(58.0)	23 (47.9)	28(63.6)	12(52.2)	44(64.7)	12(54.5)
		M±SD	M±SD	M±SD	M±SD	M±SD
Age	16.88±0.88	17.01±0.89	17.01±0.89	16.51±0.84	16.87±0.85	17.03±0.86
BMI	21.60±2.99	23.07±3.50	22.10±2.80	21.80±2.72	21.86±2.82	20.64±2.86

Group1: Blacksea Region, Group 2: Aegean Region, Group 3: Marmara Region, Group 4: Mediterranean Region, Group 5: East Anatolia Region
* Column percentage is taken
** From health workers/teachers within the scope of the lesson
*** Visual-audio media: television-radio, computer-internet, school's fishing club

When looking at the comparison of the consumption habits of the participants' meat group foods by groups, it was found; participants preferred the mostly red meat varieties (49.3%, Group 4) and preferred fish meat the least (5.6% Group 5) (Table 2).

When the distribution of the participants' opinions about fish consumption by group is examined; it was determined that fish consumption was emphasized in the family (71.6% Group 1), that they preferred to eat fish because it was healthy (71.4% Group 5), that they

became willing to consume a new type of fish with the advice of family and friends (60.9% Group 2), and that opinions about fish consumption differed statistically by group ($p<0.05$) (Table 3).

Table 2 Comparison of the meat group food consumption habits of the participants according to the groups
Çizelge 2. Katılımcıların et grubu besinleri tüketim alışkanlıklarının gruplara göre karşılaştırılması

	Total	Group 1	Group 2	Group 3	Group 4	Group 5	Test and p value
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	
Preferred meat type*							
Red meat	414(46.5)	82(48.8)	108(48.2)	58(39.5)	138(49.3)	28(38.9)	X^2=27.524
White meat	365(41.0)	66(39.3)	80(35.7)	69(46.9)	110(39.3)	40(55.6)	p=0.006
Fish	11(12.6)	20(11.9)	36(16.1)	20(13.6)	32(11.4)	4(5.6)	
Frequency of red meat consumption							
Once a week	347(41.7)	42(28.4)	91(45.0)	58(40.6)	126(46.7)	30(43.5)	X^2=29.958
2-3 times a week	194(23.3)	40(27.0)	36(17.8)	41(28.7)	60(22.2)	17(24.6)	p=0.003
More than 3 times a week	48(5.8)	11(7.4)	8(4.0)	3(2.1)	22(8.1)	4(5.8)	
Non-opinionated	243(29.2)	55(37.2)	67(33.2)	41(28.7)	62(23.0)	18(26.1)	
Frequency of white meat consumption							
Once a week	295(35.5)	36(24.3)	78(38.6)	57(39.9)	106(39.3)	18(26.1)	X^2=41.335
2-3 times a week	268(32.2)	44(29.7)	63(31.2)	48(33.6)	84(31.1)	29(42.0)	p=0.000
More than 3 times a week	90(10.8)	24(16.2)	8(4.0)	13(9.1)	32(11.99	13(18.8)	
Non-opinionated	179(21.5)	44(29.7)	53(26.2)	25(17.5)	48(17.8)	9(13.0)	
Frequency of fish consumption							
Once a week	426(51.2)	54(36.5)	109(54.0)	83(58.0)	146(54.1)	34(49.3)	X^2=34.720
2-3 times a week	109(13.1)	33(22.3)	29(14.4)	12(8.4)	26(9.6)	9(13.0)	p=0.001
More than 3 times a week	28(3.4)	11(7.4)	3(1.5)	3(2.1)	10(3.79	1(1.4)	
Non-opinionated	269(32.3)	50(33.8)	61(30.2)	45(31.5)	88(32.6)	25(36.2)	

Group1: Black sea Region, Group 2: Aegean Region, Group 3: Marmara Region, Group 4: Mediterranean Region, Group 5: East Anatolia Region * Calculated over multiple responses

Table 3 Distribution of participants' views on fish consumption by groups
Çizelge 3. Katılımcıların balık tüketimi ile ilgili görüşlerinin gruplara göre dağılımı

	Total	Group 1	Group 2	Group 3	Group 4	Group 5	Test and p value
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	
Thinking that fish consumption is important in the family							
Yes	510(61.3)	106(71.6)	127(62.9)	66(46.2)	166(61.5)	45(65.2)	X^2=21.135
No	322(38.7)	42(28.4)	75(37.1)	77(53.8)	104(38.5)	24(34.8)	p=0.000
Reasons for preferring to eat fish**							
Economic	37(4.3)	2(1.4)	12(6.4)	7(4.9)	10(3.7)	6(8.6)	X^2=24.138
Healthy	430(52.6)	75(50.7)	94(50.0)	67(46.9)	144(53.7)	50(71.4)	p=0.002
Tasty	352(43.1)	71(48.0)	82(43.6)	69(48.3)	114(42.5)	14(20.0)	
The condition that makes it desirable to consume a new fish type**							
Television-radio	168(22.3)	43(32.3)[2,4]	24(13.0)	34(26.2)[2]	42(17.2)	25(40.3)[2,4]	X^2=37.875
Computer-internet, newspaper-magazine	204(27.1)	40(30.1)	48(26.1)	33(25.4)	66(27.0)	17(27.4)	p=0.000
Recommendation of family and friends	381(50.6)	50(37.6)	112(60.9)[1,5]	63(48.5)	136(55.7)[5]	20(32.3)	

Group1: Blacksea Region, Group 2: Aegean Region, Group 3: Marmara Region, Group 4: Mediterranean Region, Group 5: East Anatolia Region
[1-5] It shows which group in the column related to fish consumption habits originates from the difference
* Column percentage is taken ** Calculated over multiple responses

When looking at the comparison of fish consumption habits of the participants; it was determined that they prefer the type of fish caught at the weight (52.9% Group 3), that the fishing stall is preferred when purchasing fish (85.5% Group 1), that the freshness aspect is taken into consideration when buying fish (52.9% Group 2), that frying method was preferred for fish preparation (38.8% Group 1), that the frequency of fish consumption is once a week (51.5% Group 2), that the winter season is preferred for fish consumption (55.8% Group 3) and that the use of fish oil support is at a low (29.7% Group 1), that this period is 2 months and below (67.6% Group 4) and that the recommendation to use fish oil was received from a health professional (79.5% Group 1) and that there was a statistical difference between the groups ($p<0.05$) (Table 4).

Table 4 Comparison of fish consumption habits of the participants according to the groups
Çizelge 4. Katılımcıların gruplara göre balık tüketim alışkanlıklarının karşılaştırılması

	Total	Group 1	Group 2	Group 3	Group 4	Group 5	Test and p
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	value
Preferred fish kind*							
Culture (Farm breeding)	371(25.9)	66(26.1)	87(25.7)	62(24.3)	130(27.0)	26(24.8	X^2=1.486
Caught	728(50.8)	125(49.4)	174(51.5)	135(52.9)[5]	242(50.2)	52(49.5	p=0.993
Frozen	334(23.3)	62(24.5)	77(22.8)	58(22.7)	110(22.8)	27(25.7)	
Preferred places to buy fish*							
Fishermen's stalls	561(70.9)	118(85.5)[2,4]	116(63.7)	113(85.6)[2,4]	168(65.6)	46(73.0)	X^2=66.925
Market	48(6.1)	3(2.2)	8(4.4)	3(2.3)	28(10.9)[1,3]	6(9.5)	p=0.000
Fish market	112(14.2)	4(2.9)	47(25.8)[1,3]	8(6.1)	46(18.0)[1,3]	7(11.1)	
Other/fish farm , fishing	70(8.8)	13(9.4)	19(10.4)	13(9.8)	20(7.8)	5(7.9)	
Considerations when buying fish*							
Freshness	616(48.2)	109(38.8)	137(52.9)	102(51.3)	214(49.5)	54(50.9)	X^2=12.585
Cheapness	158(12.4)	47(16.7)[2,3]	26(10.0)	18(9.0)	52(12.0)	15(14.2)	p=0.127
Season	442(34.69	79(28.1)	90(34.7)	75(37.7)	162(37.5)	36(34.0)	
Preferred methods of fish preparation							
Grilled	448(32.0)	88(37.9)	105(33.7)	69(27.3)	146(30.2)	40(34.2)	X^2=22.036
Oven baked	304(21.7)	36(15.5)	66(21.2)	50(19.8)	120(24.8)[1]	32(27.4)[1]	p=037
Fried	503(36.0)	90(38.8)	111(35.6)	98(38.7)	168(34.7)	36(30.8)	
Steamed	143(10.2)	18(7.8)	30(9.6)	36(14.2)[1]	50(10.3)	9(7.7)	
Fish consumption frequency							
Never	80(9.6)	8(5.5)	29(14.4)	9(6.3)	26(9.6)	8(11.6)	X^2=40.570
Once a week	376(45.2)	53(35.8)	104(51.5)	71(49.7)	120(44.4)	28(40.6)	p=0.000
More than once a week	113(13.6)	36(24.3)	26(12.9)	15(10.5)	30(11.1)	6(8.7)	
Once a month	263(31.6)	51(34.5)	43(21.3)	48(33.6)	94(34.8)	27(39.1)	
Preferred season for fish consumption*							
Winter	606(40.8)	115(41.4)[5]	141(35.6)	121(55.8)[4,5]	190(38.8)	39(36.8)	X^2=41.740
Spring	265(17.8)	48(17.3)[3]	83(21.0)[3]	20(9.2)	88(18.0)[3]	26(24.5)[3]	p=0.000
Summer	317(21.3)	55(19.8)[3]	93(23.5)[3]	29(13.4)	114(23.3)[3]	26(24.5)[3]	
Fall	299(20.1)	60(21.6)	79(19.9)	47(21.7)	98(20.0)	15(14.2)	
The status of using fish oil supplements							
User	206(24.8)	44(29.7)	45(22.3)	38(26.6)	74(27.4)	5(7.2)	X^2=15.260
Non-user	626(75.8)	104(70.3)	157(77.7)	105(73.4)	196(72.6)	64(92.8)	p=0.004
Fish oil use time (n=206)							
2 months and less	110(53.4)	15(34.1)	20(44.4)	20(52.6)	50(67.6)	5(100.0)	X^2=18.384
More than 2 months	96(46.6)	29(65.9)	25(55.6)	18(47.4)	24(32.4)	-	p=0.001
Person(s) from whom the recommendation to use fish oil was taken (n=206)**							
Health professional	115(55.8)	35(79.5)	24(53.3)	24(63.2)	30(40.5)	2(40.0)	X^2=18.499
Non-health-professional sources	91(44.2)	9(20.5)	21(46.7)	14(36.8)	44(59.5)	3(60.0)	p=0.001

Group1:Blacksea Region, Group 2:Aegean Region, Group 3: Marmara Region, Group 4: Mediterranean Region, Group 5: East Anatolia Region

[1-5] It shows which group in the column related to fish consumption habits originates from the difference.

[*] Health professionals: Doctor, dietitian, nurse-midwife, pharmacist
Non-health-professional sources: Friends, relatives, media

DISCUSSION

In this section, the findings of the research conducted to determine the fish consumption habits of future sailors (shipmen), the training status of the participants on the importance of fish in terms of health, their meat consumption habits, their opinions on fish consumption, and their fish consumption habits were discussed.

When the education status of the participants on the importance of fish in terms of health was examined, it was determined that one out of four people received education and more than half of them received this education through visual and audio media. According to the Turkish Nutrition and Health Survey, individuals between the ages of 15-18 are affected by food and beverage advertisements at a rate of 37.9%, with the group most affected by advertisements compared to other age groups (Anonymous, 2014). This suggests that advertisements can be an effective way to raise awareness about the importance of fish for health.

In the study, when the meat group food consumption habits were examined; it was found that the participants preferred red meat in the first place and fish meat in the third place, that red meat was consumed mostly in the Black Sea Region and fish

meat was consumed in the Aegean Region. When the studies are examined, it is stated that fish consumption preference comes after chicken and red meat and fish meat is preferred in the third place (Kirici et al., 2018; Sarı et al., 2019; Yigit et al., 2020; Selvi et al., 2022). Local studies show that fish meat is preferred the least. It is stated that the reasons for the less consumption of fish meat may be due to the "difficult to eat fish and cleaning, difficulty of accessing to fresh fish, price, taste and smell of fish meat, not availability of wanted fish type" (Kirici etal., 2019; Yigit et al., 2020; Selvi et al., 2022). These situations can also be explained by the low rate of fish consumption in this study.

In the study, when the consumption habits of meat and meat products were examined, it was found that the rate of consumption of fish meat two or three times a week was 13.1%. It is stated that the frequency of fish consumption 1-2 times a week in individuals over the age of 20 in Türkiye is 17.2% (Anonymous, 2014). In local studies, it is also seen that it is consumed every fifteen days and one times a month (Terin et al., 2016; Selvi et al., 2022). According to the nutrition guide specific to Türkiye, it is stated that it is very important for children between the ages of 15-18 to consume 2 servings of fish per week in terms of growth and development (Anonymous, 2015). In addition, according to TURKSTAT 2020 data, it is stated that the average fish consumption per capita is 6.7 kg, and this rate is quite low in this group. It is accepted that the energy content of fish, especially rich in protein, is lower than red and white meat, and that "omega-3 (n-3), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)" contents are high (Anonymous, 2015). Due to the beneficial effects of omega-3 fatty acids in the prevention and treatment of diseases such as heart disease, cancer, diabetes, and high blood pressure that affect human health, the low consumption rate requires more emphasis on awareness studies in this regard.

When the opinions of the participants about fish consumption were examined, it was seen that the Black Sea Region gave the highest importance to fish consumption in the family, and the Marmara Region thought that fish was important for health. It is seen that the Black Sea Region takes the first place in the production made with seafood fishing in Türkiye, followed by the Aegean and Marmara regions (Anonymous, 2020). The fact that individuals are available regionally suggests that it increases the level of awareness about fish consumption.

Looking at the participants in general, it was seen that one out of every two participants preferred to eat fish because it is healthy and they became willing to consume a new type of fish with the advice of family and friends. Studies have found that fish meat is considered important because it is healthy, because of nutritional value and taste (Bashimov, 2017; Sen and Sahin, 2017; Kirici et al., 2018; Sari et al., 2019) and one out of four people think that advertisements affect fish consumption (Bashimov, 2017). This brings to mind that commercials can be an effective way for studies of awareness about the significance of fish in terms of health. Also it is stated that study carried out in order to reveal the habits and preferences of seafood consumption of high school students, reported that they consume fish meat for healthy and balanced diet (Yigit et al., 2020). It is an indication that families, which have positive or negative effects on the shaping of children's behaviors, have important effects on the acquisition of nutritional behaviors and peer interaction also has an undeniable effect.

When the fish consumption habits of the participants were examined, it was found that they preferred to buy the fish caught, fresh, and from the fishermen's stalls.

According to the literature, it is stated that consumers prefer caughted and freshness or frozen, price, tasty, local fish market, fishbone(Terin et al., 2016; Bashimov, 2017; Sen and Sahin, 2017; Kirici et al., 2018; Sari et al., 2019; Rahman and Islam, 2020; Yigit et al., 2020). It is seen that while buying fish abroad, consumers were willing to pay more for domestic to imported production and fresh to frozen fish (Alam and Alfnes, 2020).

It was found that almost four out of ten people preferred the frying method in fish preparation in all groups, the most preferred method of grilling and frying was in the Black Sea region, the method of cooking in the oven was preferred in Eastern Anatolia, and the method of steaming was preferred in the Marmara region. In a study conducted with high school students, similar results were obtained with this study. As a method of cooking fish, it has been determined; that the first choice was fried (86.41%) and the second choice was grill (31.84%) (Yigit et al., 2020). In the literature, it is recommended to use fat-free and low-fat cooking methods such as "boiling, steaming, oven and grilling" as cooking techniques in order to be able to eat healthily and prevent health problems that may arise due to eating habits.

Regional studies also show that frying is the most prefered cooking method (Bashimov, 2017; Gundogdu and Buyruk, 2021; Selvi et al., 2022) It is stated that throughout Türkiye, 51.8% of the fish is consumed by frying and 38% by baking, grilling, cooking in a teflon pan (Anonymous, 2014; Anonymous, 2019). For this reason, it is clear that awareness studies should be focused on fish cooking methods and their effects on health.

When the preferred season for fish consumption was examined, it was found that the winter season (40.8%) was preferred the most. In the local studies, it is known that the rate of fish consumption in the winter season

varies between 51.3% and 84.1% (Terin et al., 2016; Bashimov, 2017; Kirici et al., 2018; Dasgupta et al., 2021; Selvi et al., 2022). According to the results of the local study on fish consumption habits, the high rate of consumption in the winter season is similar throughout Türkiye. The fact that processed products are not very common in Türkiye leads to consumption in the winter season, when hunting is the highest, which offers the opportunity to consume fresh.

It was found that the use of fish oil supplementation of the participants was 24.8% in all groups, and the recommendation to use fish oil supplement was 55.8% recommended by health professionals. In addition, in the study, it was determined that the rate of use in the Mediterranean region was high. In the study conducted to determine the nutritional support usage status of individuals aged 12 and over throughout Türkiye, it is stated that omega-3 fatty acids are used at the rate of 0.3% in the last 7 days, 0.4% in urban areas, and 0.1% in rural areas, and it is mostly in the Mediterranean region (1.0%) (Anonymous, 2014). The fact that the production made by seafood fishing in Türkiye is lower in the Mediterranean region suggests that fish oil supplement usage rate increases due to the lack of access to fresh fish.

CONCLUSION

It was determined that future sailors (shipmen) are aware of the importance of fish in their fish consumption habits in terms of health, they care about the advice of family and friends in the consumption of a new fish species, and the number of fish consumed is not in the amount, shape and frequency recommended for adequate and balanced nutrition. This study presents beginning, generalizable data about maritime students' knowledge about fish consumption in Türkiye. The results of the study show that maritime students in Türkiye are not adequately educated with appropriate knowledge for fish consumption. School health nursing, which is one of the fields of Public Health Nursing, reveals the necessity of health education for the importance of fish in terms of health. With the support of family and peers in these training, fish consumption can be increased among students in secondary education institutions. Particularly in primary and secondary schools, it is recommended to conduct lectures, information seminars, campaigns and awareness studies of relevant public institutions on the importance of fish meat for health. In addition, in order to increase the rate of fish consumption in every season, processed fish consumption culture should be expanded. Future studies are recommended to consider conducting longitudinal and experimental methods to address these limitations. Although the study results are limited to the Turkish adolescent of interest, they could be used as a starting point for a future study in other countries' school health education curricula. This study provides baseline data that encourages researchers to conduct intervention studies.

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Researchers' Contribution Rate Statement Summary

The authors declare that they have contributed equally to the article.

Conflict of Interest Statement

The authors declared no potential conflicts of interest with respect to the authorship and/or publication of this article. All authors have contributed significantly to the research.

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Çapakçur Havzasında (Bingöl Türkiye) Toprak Kayıplarının RUSLE Metodu ile Tahmini ve Sediment Verimi ile Karşılaştırılması

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ÖZET
Bu çalışmada yüksek erozyon riski bulunan Çapakçur havzasında toplam ve net erozyonun belirlenmesi amaçlanmıştır. Bu doğrultuda Çapakçur havzasında yıllık toprak kaybı, RUSLE modeli kullanılarak tahmin edilmiştir. Net erozyon ise Çapakçur çayının 2019 yılı boyunca aylık debi ve sediment konsantrasyonlarının belirlenmesi ile doğrudan ölçülmüştür. Çapakçur havzasında meydana gelen toprak kaybı 96916.20 ton yıl^{-1} olarak tahmin edilmiş ve Çapakçur çayından taşınan toprak miktarı ise 68656.09 ton yıl^{-1} olarak gerçekleşmiştir. Havzada, sediment iletim oranı (SDR) 0.78 olarak hesaplanmıştır. Bu oran Türkiye ortalamasının (0.23) oldukça üstündedir. Havzadaki eğim uzunluğu ve derecesinin yüksek, yağış ve vejetasyon kapalılık oranının düşük olması SDR'nin yüksek olmasının ana nedenidir. Yüksek SDR nedeniyle havzanın verimli olan üst toprak katmanı Murat Nehrine taşınmaktadır. Bu durum hem toprakların verimsizleşmesine hem de kısıtlı olan tatlı su kaynaklarının kirlenmesine neden olmaktadır. Havzada bitki örtüsü ve amenajman, uygulamalarının iyileştirilmesi için ağaçlandırma ve teras, tel kafes ve oyuntularda taş duvar gibi toprak koruma uygulamalarının arttırılması gerekmektedir.

Toprak Bilimi

Araştırma Makalesi

Makale Tarihçesi
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Anahtar Kelimeler
Havza yönetimi
RUSLE
Toprak erozyonu
Sediment taşıma oranı
Toprak ve Su Koruma

Estimation of Soil Losses in Çapakcur Watershed (Bingol, Turkey) Using RUSLE Method and Comparison of Predicted Soil Losses with Sediment Yield

ABSTRACT
The present study aimed to determine the total and net erosion in the Capakcur watershed, which has a high erosion risk. Accordingly, annual soil loss in the Capakcur watershed was estimated using RUSLE method. Net erosion was determined directly by measuring the monthly flow rate and sediment concentrations of the Capakcur stream, which originated from the Capakcur watershed and flowed into the Murat River throughout 2019. Estimated soil loss in the Capakcur watershed was 96916.20 ton yr^{-1}, and the amount of soil transported from the Capakcur stream was 68656.09 ton yr^{-1}. Sediment delivery ratio (SDR) was calculated as 0.78. This ratio was well above the average SDR of Turkey (0.23). Topographic factors such as slope length and degree, rainfall, and low vegetation cover ratio in the watershed are the main causes of the high SDR. Due to the high SDR, the fertile surface soil layers of lands in the basin are carried to the streams. This causes both decrease in fertility in soils and pollution of the limited freshwater resources. In order to improve vegetation and management practices in the watershed, soil protection practices such as afforestation and terraces, wire cages and stone walls in gullies should be increased.

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INTRODUCTION

Erosion is a global threat that plays a significant role in the degradation of important natural resources such as soil and water. Approximately 85% of land degradation in the world is related to soil erosion, which has resulted, directly or indirectly, in a 17% loss in agricultural productivity since World War II (Oldeman et al., 1990; Angima et al., 2003). Soil erosion reduces the quality of natural resources and can cause major environmental disasters (drought, floods). The negative ecological and social impacts of erosion constrains the sustainable regional development (Pan and Wen, 2014).

Soil erosion varies with the changes in the conditions of biophysical environment, including soil texture, climate, land conditions, ground cover and their interactions. Soil erosion, a dynamic, complex, and irregular process, occurs as a result of the effect of many factors. The most important factors affecting the soil erosion mechanism are land shape, slope, land area, and land use. The direction and impact of the slope play an important role in the runoff mechanism. High slope increases the runoff and reduces the amount of water infiltrating into the soil (Meral et al., 2019). Soil erosion is often the dominant cause of topsoil loss (Onori et al, 2006; Kouli et al., 2009). The soil cover (plants, organic wastes, etc.) decreases the surface area of soil susceptible to raindrop effect and also reduces the runoff velocity. Therefore, no-tillage or reduced tillage practices are considered appropriate management practices to reduce soil erosion in agricultural areas due to the residues on soil surface and a decrease in runoff (Fu et al., 2006). Rainfall (R) plays an important role as a trigger in the initial phase of erosion. The previous studies have emphasized that rainfall and rainfall intensity are the most critical factors (Mohamadi and Kavian, 2015; Katebikord et al., 2017).

Serious efforts have been carried out to develop soil erosion models around the world. Usually, a quantitative evaluation is needed to make conclusions about the extent and magnitude of soil erosion problems (Prasannakumar et al., 2012). Effective management strategies can be developed on a regional basis using the quantitative data obtained from local measurements. Erosion models can be also used to develop alternative land management scenarios using both measured and unmeasured basins and to make decisions for natural resource management (Fıstıkoğlu ve Harmancıoğlu, 2002). Various approaches and models have been developed to evaluate soil water erosion and predict soil erosion risk. Each approach or model has its characteristics and application purposes (De Vente and Poesen, 2005; Boardman, 2006). Experimental (USLE and RUSLE), conceptual (AGNPS and SWAT) and physically based models (EROSEM and CREAMS) have been used frequently for soil erosion and sediment transport (Farhan and Nawaiseh, 2015). One of the most widely used experimental models to evaluate soil and gully erosion is the Universal Soil Loss Equation (USLE) developed by Wischmeier and Smith in 1978. Initially, soil erosion estimation in cultivated lands using USLE was developed for slightly sloping topographic areas. The revised versions (RUSLE, MUSLE) have are the most commonly used and well-known and universally accepted and implemented empiri-cal soil erosion estimation model methods in the world for soil loss estimation (Wischmeier and Smith, 1978; Jha and Paudel, 2010, Shit et al., 2015; Ghosal 2020; Meral et al, 2021).

In erosion modelling, Sediment Delivery Ratio (SDR) has been treated as a constant parameter for a long time; however, there has been increasing interest in accounting for deposition and applying spatially variable or regionalised SDR (Ali and De Boer, 2010; Vigiak et al., 2012). The sediment delivery ratio (SDR) is the ratio of sediment yield to total surface erosion. The importance of the SDR and the sources for the region to be impacted by the catchment sediment, the transport system, the texture of the erosion content, the urbanization and land cover, etc., must be determined. Rainfall erosivity, soil erodibilty, land use cover, and topography are significant factors affecting sediment yield and sediment distribution. Sediment delivery to a given watercourse is increasing as catchments are progressively modified by human activities such as deforestation, agriculture expansion, construction, and urbanization (Dong et al.; Dutta, 2016; Berta et al., 2020; Joshi and Yadav, 2021). The RUSLE model was used with (SDR) to determine the life ratios in arid basins of Turkey (Saygın et al., 2014). SDR is defined as the fraction of grosserosion that is transported from a given catchment ina given time interval (Lu et al., 2006). Sediment yield can be quantified by multiplying gross erosion and sediment delivery ratio (Llena et al., 2021; Alencar et al., 2021).

Land use status and vegetation, especially precipitation and topographic factors, have an effect on the severity of erosion (Kijowska-Strugała et al., 2018; Wubie and Assen, 2020). Soil erosion generally consists of three stages: decomposition of soil aggregates, transport of decomposed material, and accumulation of this material (Foster, 1982). Soil losses estimated using empirical equations and sediment load transported from the same area are not directly related to each other. However, it gives an

idea about the production, storage, and transportation of sediment formed in the watershed in relation to environmental processes (Fayas et al., 2019). While estimating the total amount of displaced soil using soil loss estimation equations, the net erosion is determined by calculating the amount of sediment carried in the rivers (Jain and Kothyari, 2000; Thomas et al., 2018). Many methods have been developed to estimate SDR (Boyce, 1972; Williams, 1977).

The present study aimed to estimate total soil loss in the Capakcur watershed (Bingöl), where the Capakcur stream with high sediment concentration crosses, to determine the sediment yield by periodic sediment and flow measurements in the Capakcur stream, and to determine the SDR using the data obtained. Accordingly, the soil loss occurring in the Capakcur watershed was determined using the revised soil loss estimation equation (RUSLE).

MATERIAL ve METHOD

The present study was carried out in Capakcur watershed, located in the upper Fırat Basin which is a part of Eastern Anatolia Region of Turkey (Figure 1). The study area is located between 38° 51'N, 40° 16'E and 38° 53'N, 40° 28'E. The coverage of study area is 9556.87 ha and has a very rugged topographic structure. The altitude of the watershed ranges from 1150 m to 2500 m. The average altitude is 1650 m, the total annual precipitation is 949 mm, the number of snowy days is 117, and the number of days covered with snow is 76 (Figure 2).

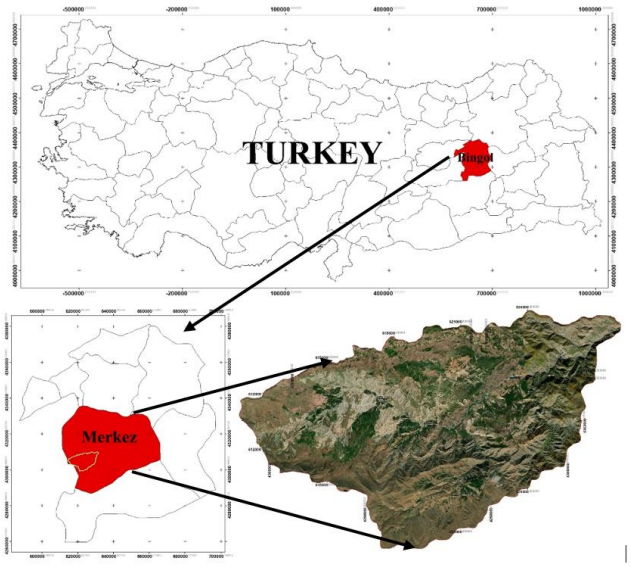

Figure 1. Location of Capakcur watershed, Southeast Turkey.
Şekil 1. Çapakçur havzasının konumu

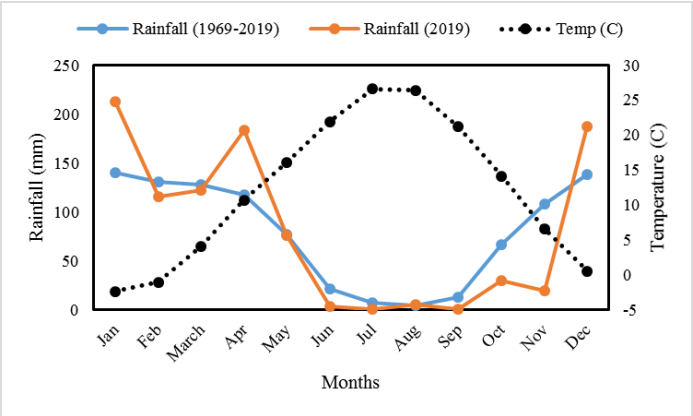

Figure 2. Climate data for period of 1969-2019
Şekil 2. 1969-2019 dönemi için iklim verileri

Data Sources

Surface soils (0-20 cm) were sampled to determine the soil erodobility factor (K) of the study area. Soil samples were collected from approximtely the corners of 500m x 500m grids (Carter and Gregorich, 2007). Total of 428 surface soil samples were collected throughout the basin. For the calculation of rainfall and runoff erosivity factor (R), long-term climate data of the meteorology station closest to the study area were used. Digital elevation model (DEM) data of the Capakcur watershed with a resolution of 15m x 15m was obtained to determine the slope length and slope degree factor (LS). DEM data was generated used ArcGIS Pro software using isohips curves.

The DEM data was passed through Fiil skins, Flow direction and Flow accumulation processes, respectively, using the ArcMap software, and the LS factor codes of the area were produced. Land use and land cover factor (C) obtained from CORINE Land Cover 2018 (URL 1) and soil protection measures factor (P) were obtained from the map and numerical data produced in the rehabilitation study conducted by the Ministry of Agriculture and Forestry, General Directorate of Combating Desertification and Erosion in 2015 (Anonymous, 2015). In the study, periodic water sampling and flow measurements were carried out from the exit point of the watershed to determine the amount of sediment carried in the Capakcur stream.

Methods

The Revised Universal Soil Loss Equation (RUSLE) model was used to estimate soil losses from the Capakcur watershed. In addition, the monthly sediment load carried in the Capakcur stream was measured to determine the amount of sediment carried from the basin.

Description of RUSLE Model

The RUSLE model was used due to the easy integration of spatial analysis with GIS (Wischmeier

and Smith, 1978). The RUSLE model is expressed by the following equation (Renard et al., 1997);

$$A=R \times K \times LS \times C \times P \quad (1)$$

In the quation; A is average soil erosion per unit surface (t/ha·year), R is rainfall and runoff erosivity factor (MJ·mm/ha·h·year), K is soil erodibility factor (t ha h/ha MJ mm), LS is slope length and slope steepness factor, C is vegetation cover, management, and culture practices factor, P is mechanical practices factor.

Rainfall has a significant impact on the occurrence and severity of erosion (Wischmeier and Smith, 1978; Fraser et al., 1999; Nearing, 2001). The R factor is a function of the diameter of each raindrop, duration of rainfall, mass, the intensity of rainfall, and the falling rate of raindrops (Renard et al., 1997). Current monthly and annual rainfall data for 58 years (1961-2019) collected from the Bingöl meteorology station (38°53'04.9"N 40°30'02.5"E), located at 1145 m altitude, were used to calculate the R factor using the a commonly used equation (Eq 2) (Wischmeier and Smith, 1978).

$$R=\sum(k=1)^12⟦1.735x⟦10⟧^((1.5log10(Pi⟧^2/P)-0.08188))⟧ \quad (2)$$

In the equation; R refers to erosion power of precipitation (MJ mm ha^{-1} $hr^{-1}y^{-1}$), Pi (mm) to average monthly rainfall, and P (mm) to total annual rainfall. For the Bingöl meteorology station, the 58 year average total rainfall was measured as 949 mm. The altitude of the study area is higher than the meteorology station. The R-value calculated according to this station does not exactly represent the Capakcur watershed. Accordingly, the R factor was calculated for each point sampled from the watershed using the following equation (Eq. 3) (Toy and Foster, 1998).

$$Rn=Rr(⟦Py/Pr)⟧^1,75 \quad (3)$$

Where; Ry is the corrected R-value for the location with the unknown R variable; Rr is the R-value for the reference station with known R variable; Py is the average annual rainfall (mm) for the location with unknown R variable, and Pr is the average annual rainfall (mm) for the reference station with known R variable. The precipitation erosion factor map for the Capakcur watershed was created by Inverse Distance Weighted (IDW) interpolation using the obtained point precipitation values with ArcGIS (Pro).

The parameters required to calculate the soil erodibility factor (K) of the Capakcur watershed were determined. Accordingly, 428 soil samples from the study area were prepared for analysis after preliminary preparations (such as drying, grinding, sieving). The particle size distribution of the soils was carried out according to the hydrometer method stated by Gee and Bauder (2002). Organic matter content (OM) of soils was determined according to Walkley and Blake metthod (1934). Permeability values of soils (disturbed samples) were determined using constant water level hydraulic permeability sets (Klute and Dirksen, 1986). The structural properties of each sample were classified as specified by Dexter (1988). Aggregate stability analysis was performed according to the wet sieving method (Kemper and Rosenau, 1986). Using these parameters, the K factor for each point was calculated using the following equation (Eq 4).

$$100 \times K=(2.1 \times ⟦10⟧^4) \times (12-OM) \times ⟦M⟧^1.14+3.25 \times (S-2)+2.5 \times (P-3)/d \quad (4)$$

In the equation; K is the soil erodibility factor to erosion, OM is the organic matter, S is the soil structure class code (1-6), P is permeability (1-4), M: Grain size distribution parameter, d: Conversion coefficient to the metric system. The following formula (Eq 5) was used to calculate the M factor in this equation (Wischmeier and Smith, 1978).

$$M=(\%Silt+\%Sand) \times (100-\%\ Clay) \quad (5)$$

The length and degree of the slope are parameters directly related to the severity of erosion (Khare et al., 2017). In a standard USLE parcel with a slope of 9% and a length of 22.1 meters, LS value is equal to 1 and LS values are not absolute values (Wischmeier and Smith, 1978). Since the study area has a very heterogeneous topography, a DEM (Digital Elevation Model) was created by digitizing contour lines from topographic maps in the ArcGIS software to estimate the LS factor (Rozos et al., 2013). LS values were calculated using the following formula (Eq 6) developed by Moore and Burch (1986) using the ArcGIS Pro software "flow direction" function.

$$LS=⟦(L/22.13)⟧^0.4 \times ⟦(0.01745xSin\theta/0.0896)⟧^1.4 \times 1.4 \quad (6)$$

Here: L is flow accumulation x cell resolution (DEM) and θ is "slope in degree". To compute LS factor using DEM in Arc MAP follow the following steps (Fig 3).

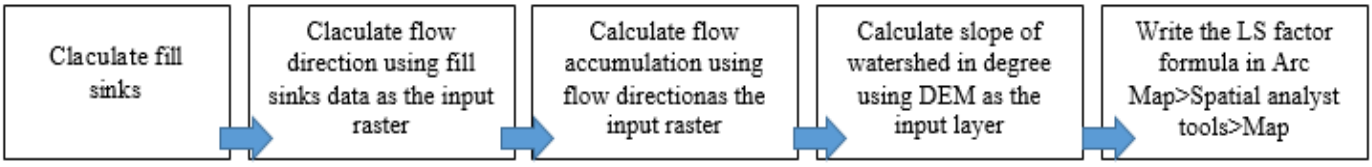

Figure 3. Methodology flow chart of Calculating LS factor using GIS
Şekil 3. CBS kullanarak LS faktörünü hesaplamanın metodoloji akış şeması

Forest stand maps of the study area produced by the General Directorate of Forestry and the CORINE 2012 data sets produced by the Department of Information Technologies of the Ministry of Agriculture and Forestry were used to obtain this data cover and management factor (C), (Panagos et al., 2015). However, in soil sampling studies, the land-use status (estimated vegetation coverage rate, vegetation type) of the sampling point was noted. Thus, C values were assigned to the area represented by each soil sampling point. C values assigned according to different land cover are given in Table 1. C values are classified according to Kayet et al. 2018 and Meral 2021.

Table 1. Land use in the Capakcur watershed and the corresponding C factor code

Çizelge 1. Çapakçur havzasında arazi kullanımı ve buna karşılık olan C faktör kodu

Land use/Land cover Type	C factor
Fallow	1
Dry fallow	1
Agricultural crop (Wheat)	0.4
Agricultural crop (corn)	0.45
Poorly managed pasture	0.25
Settlement	0.1
Dense forest	0.15
Natural shrubs	0.15
Water bodies	0.1
Fruit orchards	0.4

Support and Conservation Practices factor (P) is defined as erosion prevention applications to reduce soil loss by erosion. The measures taken to keep the free soil particles close to the source and to prevent the further transport of the particles are calculated to determine the value of the P factor,. P-value takes a value between 0 and 1, according to soil protection measures. P-value is assigned as 1 for areas where no protection application is applied (Renard et al., 1994). In the Capakcur watershed, some soil protection and erosion prevention practices were carried out by the General Directorate of Combating Desertification and Erosion in 2015 (ÇEM, 2015). Within the scope of these applications, terraces, stone walls, and wire cage structures were established in the work area. Within the scope of this study, a P factor value was assigned for each application area (Table 2).

the annual soil loss of the watershed (tons.ha^{-1}.yr^{-1}) were determined and mapped. To create the soil erosion sensitivity map of the Capakcur watershed, soil erosion severity was grouped into five classes (low, medium, high, very high, and extremely high) according to the estimated pixel-level soil loss values (Singh and Panda, 2017; Fayas et al., 2019).

Table 2. Soil protection practices in the Capakcur basin and the corresponding P factor codes

Çizelge 2. *Çapakçur havzasında Toprak koruma uygulamaları ve buna karşılık gelen P faktör kodları*

Soil conservation practices	P Value
Non practice	1.00
Contouring	0.60
Contoru strip-cropping	0.35
Terrracing	0.15

Soil erosion severity classes and mapping

After calculating the R, K, LS, C, and P factors, which are the components of the RUSLE equation for the Capakcur watershed, all the base maps were created. Considering that the maps produced had the same resolution (15 m x 15 m pixels), using the ArcGIS software and the "Raster Calculator" command (multiplying all the created maps with each other),

Sediment Yield (SY)

The Capakcur stream is the merging of many small streams in the Capakcur watershed. This water source rises at an altitude of approximately 2400 m, moves 50 km from west to east, and flows into the Murat River, the largest branch of the Fırat River (Fig 4).

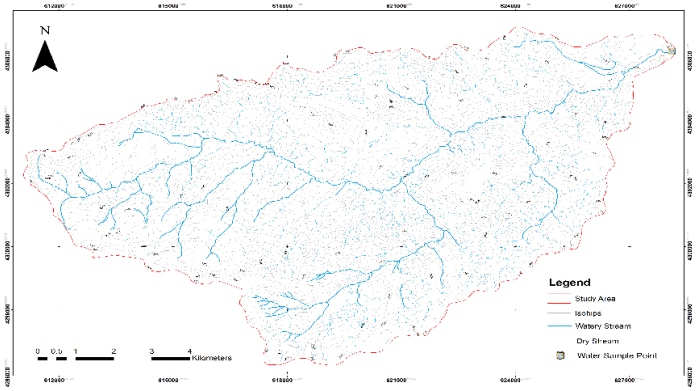

Figure 4. Water collection network of Capakcur stream in the basin

Şekil 4. Çapakçur çayının havzadaki su toplama ağı

Sediment yield (SY) is defined as the amount of sediment measured at a reference point for a certain period and is given as tons/year or spatially tons/ year/km^2, m^3/year/km^2 (Verstraeten and Poesen, 2001). The net erosion in the watershed was determined by calculating the amount of sediment carried from the Capakcur stream. In this context, water samples were collected on the Capakcur stream at the exit of the Capakcur watershed, and the amount of sediment was determined in the laboratory.

Water samples taken three times amonth (periodic sampling was done every 10 days.) rom Capakcur stream were analyzed gravimetrically by filtration, drying, and weighing processes in the laboratory (Walling, 1994). The amount of solid matter per unit volume is taken as the ratio of the dry sediment weight to the volume of the water + sediment mixture (Mermer, 1996). The following equation (Eq 7) was used to determine the amount of sediment in water samples.

SC=DSW/((wv+Sv)) (7)

where: SC: sediment concentration per unit volume (mg/L), DSW: dry sediment weight (mg), wv + Sv: volume of water sample taken from the stream (L). Flow velocity was measured simultaneously with water sampling from the Capakcur watershed. Flow velocity measurements were carried out using a digital velocity measurement device (Fig 5).

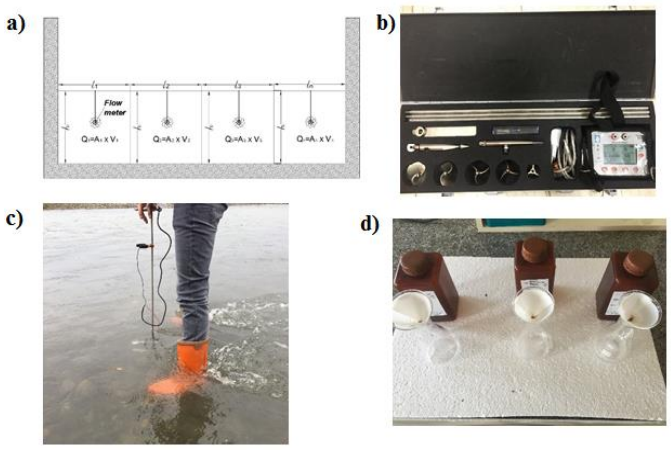

Figure 5. Flow and sediment measurement at the exit point of the watershed in the Capakcur stream. a) water bed section and definitions at the measurement point b) digital flow meter used in flow measurement, c) measurement of water velocity with digital flow meter, (the device was calibrated before measurements), d) Determination of the sediment content of water samples taken from the Capakcur stream.

Şekil 5. *Çapakçur çayında havzanın çıkış noktasında debi ve sediment ölçümü. a) ölçüm noktasındaki su yatak kesiti ve tanımlamalar b) debi ölçümünde kullanılan digital flow meter, c) digital flow meter ile su hızının ölçülmesi, (ölçümlerden önce cihazın kalibrasyonu yapılmıştır), d) Çapakçur çayından alınan su örneklerinin içeriğindeki sediment miktarının belirlenmesi*

Both water sampling and flow measurements were carried out separately for each 10-cm-depth (h1, h2,..) and each 1 m. stream width (L), and the average values were recorded. Thus, the velocity of the water flowing through a certain waterbed section was measured at the time of water sampling. Using the obtained data, the flow rate of water was measured with the help of the following equation (Eq 8).

Q=A x V (8)

Where: Q: flow rate (m^3/sec), A: streambed cross section area (m^2), V; flow velocity (m/sec). Sediment yield was calculated using the following equation (Eq 9).

SY=Q x SC x k (9)

where: SY: sediment yield (ton/year), SC: sediment concentration, k:conversion coefficient (from mg/sec to ton/year).

Sediment delivery ratio (SDR)

The sediment delivery ratio (SDR) was determined using the estimated erosion amount (SE) from the watershed using the RUSLE equation and the sediment yield (SY) measured in the Capakcur stream in 2019. Sediment delivery rate (SDR) is defined as sediment yield from an area divided by gross erosion in the same area. It is calculated using the following formula (Eq 10) (Nguyen and Chen, 2018).

SDR=SY/SE (10)

where: SDR: sediment delivery ratio, SY: sediment yield, SE: total soil erosion,

In recent years, SDR was estimated using some equations and GIS methods. Traditionally, delivery ratio estimation equations have been developed by correlating the basin characteristics with the measured sediment yield divided by the estimated gross erosion (Williams, 1977). However, in the present study, SDR was directly determined by estimating soil losses from the Capakcur watershed with RUSLE and directly measuring the sediment carried from the watershed.

Statistical evaluation

Data analysis for each of soil properties were conducted, exploratory data analysis was carried out calculating minimum, maximum, arithmetic mean, standard deviation, the coefficient of variation (CV), skewness were calculated. The effect of land use type (forest, pasture etc.) on AS was analyzed. The mean AS values of land use types were compared.The SPSS 16.0 software was used for statistical evaluations. The effect of land use type on AS was evaluated by one-way analysis of variance (ANOVA) ($P < 0.05$). The Duncan homogenity test was adopted to group statistically different mean values.

RESULTS and DISCUSSION

Prediction of Soil Loss

The R factor value of the watershed was between 400.72 and 497.93 Mj $mm.ha^{-1}.h^{-1}.yr^{-1}$ with an average

of 449.32 Mj mm.ha^{-1}.h^{-1}.yr^{-1}. These equations were solved with the assumption that every 300 m height increase will cause a 50-mm-increase in rainfall. The obtained R factor values corresponded to the values calculated by the General Directorate of Combating Desertification and Erosion (Erpul et al., 2018). The R factor values calculated for the Capakcur watershed were 427.46 and 499.26 Mj mm.ha^{-1}.h^{-1}.yr^{-1}. In regions with dominant continental climate (Li and Fang, 2016), such as the Capakcur watershed, the exposure of the soil surface to heavy rain is effective in the occurrence of water erosion and floods (Diodato et al., 2017). Previous studies indicated that low intensity long-term rainfall and short-period repetitive low-intensity rainfalls contribute significantly to total soil erosion (Baartman et al., 2013). This effect is more apparent in poor vegetation cover. R factor plays an important role in increasing the severity of erosion since a significant part of the Capakcur watershed has a bare soil surface.

Descriptive statisticsof the Capakcur watershed soils are given in Table 3. The average sand content was higher than clay and silt. Generally, areas other than some agricultural areas have a sandy texture. The average organic matter (OM) content was above 2% throughout the watershed. In a previous study in the basin, it was reported that OM content varied according to the land-use status. The highest OM content was found in forest areas, while the lowest was found in bare soils (Demir and Ersoy Mirici, 2020). The saturated hydraulic conductivity (HC) values were between 0.36 and 8.01 cm/h with an average HC of 4.23 cm/h. This value was classified as "moderately fast" (Warrick, 2003). The AS values were 2.25 and 90.90% with an average value of 40.07%. Variability of the properties in terms of coefficient of variation (CV) was classified as low (<15%), medium (15-35%) and moderate (>35%) (Mallants et al 1996). In this case, Silt showed medium variation, other parameters showed high variation.Tthe K factor of the Capakcur watershed was presented in Fig. 7.

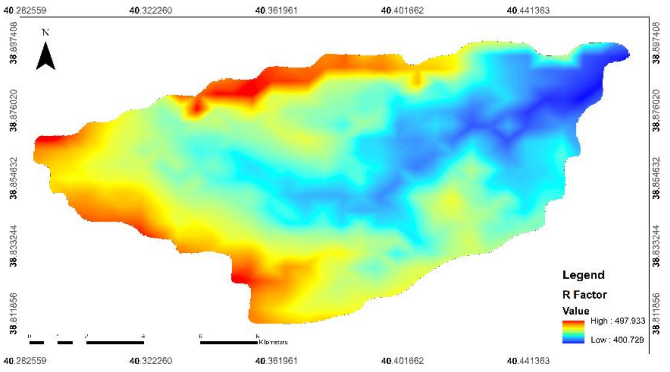

Figure 6. R factor map of Capakcur watershed
Şekil 6. Çapakçur havzası R faktörü haritası

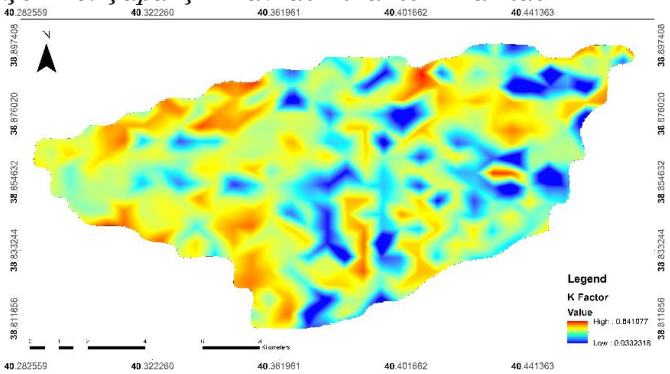

Figure 7. K factor map of Capakcur watershed
Şekil 7. Çapakçur havzası K faktörü haritası

Table 3. The area and percentage of soil loss values and classes in Capakcur watershed
Çizelge 3. Çapakçur havzasında toprak kaybı değerleri ve sınıflarının alan ve yüzdesi

Soil Properties	N	Min	Max	Mean	Std. Deviation	%Cv	Skewness
Clay (%)	428	2.00	33.36	13.14	5.54	42.16	1,26
Silt (%)	428	5.50	36.00	18.19	6.52	35.84	0,92
Sand (%)	428	36.61	89.50	68.65	9.06	13.19	-0,78
OM (%)	428	0.00	11.76	2.36	2.25	95.33	2,03
AS (%)	428	2.25	90.90	40.07	18.58	46.36	0,20
K (cm/h)	428	0.36	8.01	4.23	1.83	43.26	-0,05

OM: Organic matter, AS: Agregate stability, K:Permability. Skewness's STD. of AS values was calculated as 0.118.

K factor values of the Capakcur watershed were between 0.033 and 0.841. K factor was lower in forest areas with relatively high organic matter content and plains with high sand content. However, the K factor was higher in the west of the watershed where the silt content was high and the organic matter content was low. Liu et al. (2020) reported that the silt percentage and OM content of the soils directly affect the K factor. The slope length and steepness factor (LS) is an important due to the impact on the severity of soil loss in the Capakcur watershed. The slope in 50.36% of the watershed area was more than 40% (Table 4). This further facilitates runoff and therefore erosion on steep slopes.

The LS factor distribution map of the Capakcur watershed is given in Fig. 8. The LS values were

between 0 and 107.238. The LS factor values were high in the southern parts of the watershed with sharp and steep slopes. The interaction of L and S has an effect on the magnitude of erosion. Accordingly, the effect of L and S should always be considered together (Edwards, 1987). The increase in slope length and slope height causes higher delivery speeds and therefore higher erosion (Haan et al., 1994)..

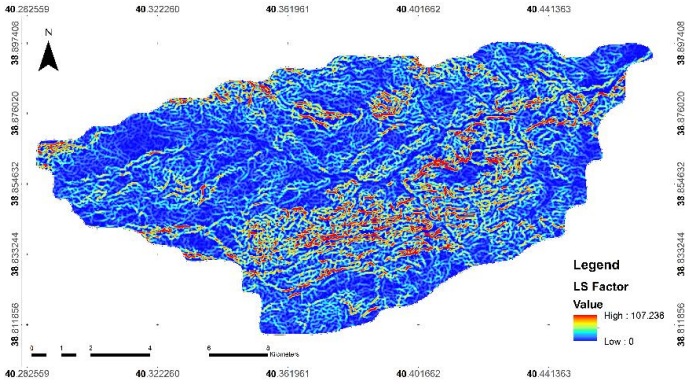

Figure 8. LS factor map of Capakcur watershed
Şekil 8. Çapakçur havzası LS faktörü haritası

Table 4. Slope degrees and the area they cover in the Capakcur watershed
Çizelge 4. Çapakçur havzasında eğim dereceleri ve kapladıkları alan

Slope (%)	Area (Ha)	Rate (%)
0-12	650.822	6.81
12-20	738.746	7.73
20-40	3354.461	35.10
40-60	3080.179	32.23
60<	1732.661	18.13
Total	9556.87	100

The type of the existing vegetation cover and the coverage area of the watershed were determined by field observations. The Capakcur watershed has a generally poor vegetation cover. The vegetation cover area in the watershed was 34.8%. This ratio was very close to the vegetation covering ratio of 31.8% determined by Demir and Ersoy Mirici (2020) using GIS. The C factor distribution map of the watershed is given in Fig 9. The C factor value in the watershed varied between 0.103 and 1. The Capakcur watershed has a very low topsoil coverage. The proportionality of forest, pasture, and agricultural lands resulted in a high average C factor (Cmean: 0.55). This average ratio was estimated to be 0.1043 in the European Union (Panagos et al., 2015). To reduce the erosion power of the C factor in the basin, it is necessary to make afforestation and soil conservation practices, especially in uncovered areas, and agricultural lands without tillage. Soil protection management practices (reduced/ no-tillage, use of cover plants, and plant residues) have been reported to reduce the C factor by an average of 19.1% in farming areas (Panagos et al., 2015).

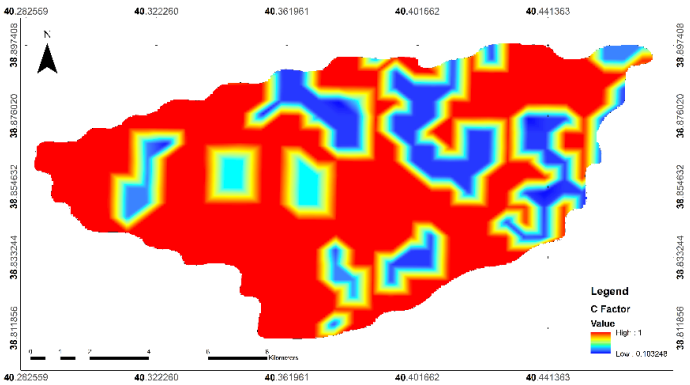

Figure 9. C factor map of Capakcur watershed
Şekil 9. Çapakçur havzası C faktörü haritası

In the Capakcur watershed, an improvement (erosion prevention and soil protection) study was carried out by GDF in 2015. In this context, soil conservation and erosion control (terrace, wire mesh) studies were carried out on a 506.9-hectare section. Accordingly, the P factor distribution map of the watershed was prepared (Fig 10). The P factor value in the Capakcur watershed varied between 0.4 and 1.

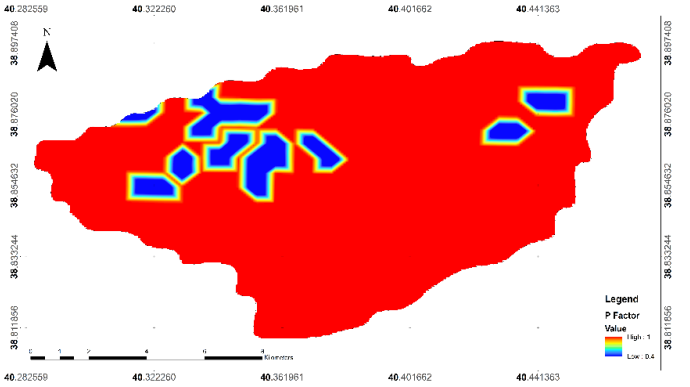

Figure 10. P factor map of Capakcur watershed
Şekil 10. Çapakçur havzası P faktörü haritası

The RUSLE soil loss (A) prediction map of the Capakcur watershed was produced by multiplying the R, K, C, LS, and P factors in the GIS environment. The distribution map of the estimated soil loss (ton.ha^{-1}.yr^{-1}) from the watershed is given in Figure 11.

The estimated soil loss in 66.14% of the Capakcur watershed was lower than 5 ton.ha^{-1}.yr^{-1} (Figure 11). This is a low value according to the erosion susceptibility classification system. The occurrence of low soil loss in approximately 2/3 of the watershed is important in terms of soil management practices. The estimated soil loss in 14.73% of the area was 5-12 ton.ha^{-1}.yr^{-1} (low). In the 7.2% area where moderate soil loss occurred, the estimated soil loss was between

12 and 25 ton.ha-1.yr-1. The estimated soil loss in an area of 682.53 ha where soil loss was classified as "high" was 25-60 ton.ha^{-1}.yr^{-1}. More than 60 tons.ha^{-1}.yr^{-1} land loss occurs in an area of 456.77 ha in the watershed where land losses are estimated as "severe" or "very high". According to these data, the total yearly soil loss from the watershed was found to be 96,915.20 tons. The average annual amount of soil loss per unit area from the watershed was determined to be 10.14 tons.ha-1. Every year, 642 million tons of soil erosion as a result of water erosion are displaced in Turkey. This value corresponds to an average of 8.24 tons of land per hectare (Erpul et al., 2018). The annual soil loss calculated in the Capakcur watershed is 18.77% higher than the average of Turkey. In the Fırat-Dicle main basin, where the Capakcur watershed is located as a sub-basin, the annual soil loss is 4.9 million tons.yr^{-1}. Thus, annual soil loss in the Capakcur watershed is higher than the loss of both Turkey and the Fırat-Dicle main basin.

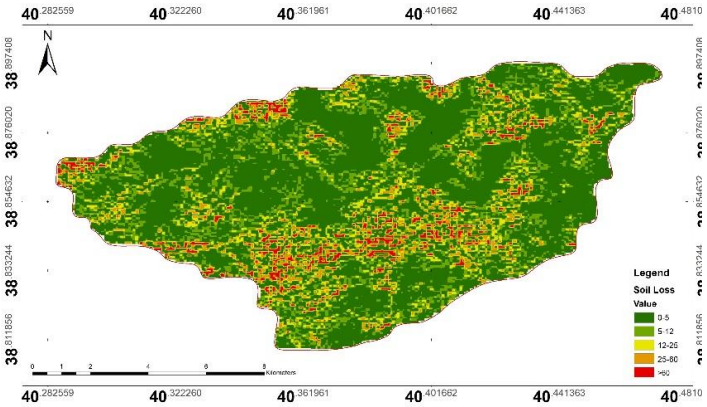

Figure 11. Erosion severity and soil loss map of Capakcur watershed

Şekil 11. Çapakçur havzasının erozyon şiddeti vetoprak kaybı haritası

Sediment Yield in Capakcur Stream

The Capakcur stream, which is formed by the combination of streams and runoff waters in the Capakcur watershed, passes through the Bingöl province center and flows into the Murat River. At the exit point of the watershed, the sediment concentration was determined periodically by sampling water from the Capakcur stream monthly during 2019. Also, the flow rate of the Capakcur stream was measured and the sediment load was determined using the equations (Fig. 12). The sediment concentration in the measurements made in Capakcur stream varied between 0.39 and 1.53 gr.L. However, the flow rate varied between 0.71 and 3.2 m^3.s. The monthly sediment amount delivered from the entire watershed varied between 769.82 and 11,540.36 tons. Total amount of soil lost due to the annual sediment carried from the watershed was 68,656.09 tons. In other words, the average amount of soil lost due to the sediment carried in the Capakcur watershed was 7.18 ton.ha.yr^{-1} (Fig. 13).

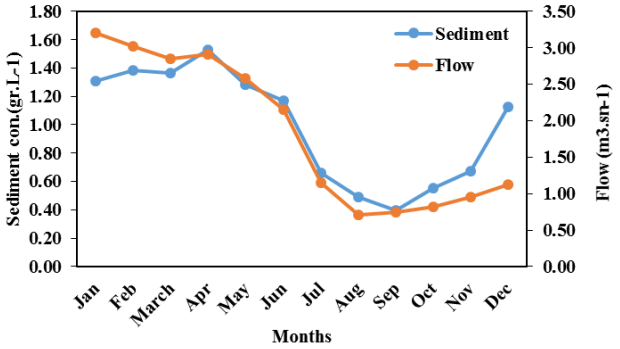

Figure 12. Monthly average sediment load and flow rate CS

Şekil 12. Çapakçur çayının aylık ortalama sediment yükü ve debi miktarı

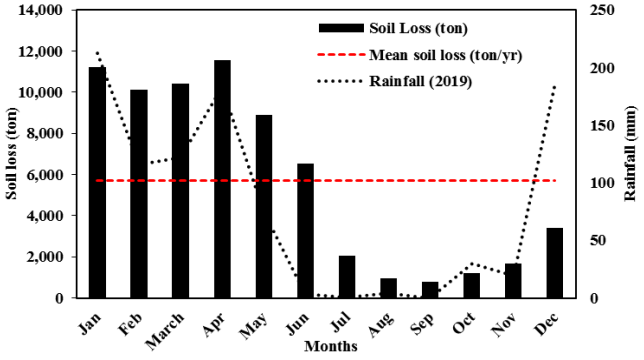

Figure 13. Amount of soil transported from Capakcur stream

Şekil 13. Çapakçur çayından taşınan toprak miktarı

In the present study, although sediment and flow rate measurements were carried out 3 times a month in the Capakcur stream, the data obtained may not reflect the actual results. On the other hand, the widely used sampling method is restrictive for spatial and temporal measurement of sediment and causes a heavy workload. These limitations and technological advances have led to methods that rely on sound or light travel in water. Therefore, measurements must be made continuously and instantaneously. Accordingly, it is necessary to use digital technologies in sediment and flow measurements in rivers in recent years (Meral et al., 2018). However, the measurements made in the present study (36 measurements/year) were predicted to be close to the actual result.

Sediment Delivery Ratio (SDR)

The sediment delivery rate (SDR) was directly calculated using the measurements made in

Capakcur stream and the estimated amount of soil loss in the watershed. The estimated amount of soil loss in the study was 96,915.20 tons.yr^{-1} while the amount of sediment carried by the Capakcur stream was 68 656.09 tons. yr^{-1}. According to these values, 70.81% of the estimated soil loss in the Capakcur watershed was realized as the net erosion. Therefore, SDR in the Capakcur watershed was determined to be 0.78. In the watershed where the summer months are arid, total annual precipitation is much lower than total evaporation. This increases the susceptibility of soils to erosion along with the wetting-drying process. The amount of moisture contained in pre-rainfall soils has an effect on the SDR variability (Santos et al., 2017). Also, it is effective on SDR in case of land use (agriculture, forest, pasture, etc.) (Pınar et al., 2020). Of the erosion in Turkey, 53.66% occurs in pastures, 38.71% in agricultural areas, and 4.17% in forest areas. It has been reported that 47.55% topography, 34.82% vegetation, 14.26% rainfall, and 3.36% soil factors had an effect on vegetation in Turkey. However, SDR has been reported to be 0.24 SDR in Turkey (Erpul et al., 2018). This ratio is considerably lower than the SDR determined in the Capakcur watershed. This requires effective planning of soil protection practices in the Capakcur watershed. The higher SDR is attributable to the above-average rainfall in certain months in 2019 in addition to the high annual rainfall in the watershed. The amount of rainfall in the Capakcur watershed in 2019 was the highest in January and April (212.6 mm and 183 mm, respectively). During these months, the flow rate and sediment concentration in the Capakcur stream increased compared to other months. This shows that the ratio of rainfall to runoff is high in this watershed. The dominance of the R factor, i.e. rainfall over the erosion severity in the watershed and the fact that a large part of the watershed has a slope of 40% or more increases the surface flow rate. Doğan Demir ve Demir (2016) have reported that there was a linear relationship between the amount of rainfall and the amount of sediment carried in the Palu watershed, which also includes the Capakcur watershed. The sediment delivery rate is affected by many highly variable physical properties of a watershed. It varies according to the drainage area, slope, relief-to-length ratio, flow-precipitation factors, land use/land cover, sediment particle size, etc. (Ouyang and Bartholic, 1997). The excess of steep slopes in the Capakcur watershed limited the accumulation of eroded soils, transmitting the vast majority to the Capakcur stream through gulleys (Figure 14).

Figure 14. The detachment area and Deposition area as a result of severe erosion in the Capakcur watershed, a large part of which is bare area, and high sediment concentration in the Capakcur stream due to the high SDR.

Şekil 14. Büyük bir kısmı çıplak alan olan Çapakçur havzasındaki şiddetli erozyon ve yüksek SDR nedeniyle Çapakçur deresinde yüksek sediment konsantrasyonu sonucu oluşan taşınma alanı ve çökelme alanı.

One of the most important reasons for the high annual soil cup and SDR in the Capakcur watershed is the current land use status of the watershed. The land-use case has significant effects on erosion (Sharma et al., 2011). Bare areas without vegetation constitute a large part of the watershed area. The vulnerability of soil aggregates in these areas to the crushing effect of rain increases the potential for erosion. Many studies have shown that land cover reduces erosion and increases the physicochemical properties of the soil (Wijitkosum, 2012; Alkharabsheh et al., 2013; Zaimes et al., 2017). In the present study, the effect of different land use on the aggregate stability of soils in the Capakcur watershed was evaluated (Table 5).

Table 5. Effect of land use-cover on agregat stability
Çizelge 5. Arazi kullanım örtüsünün agrega stabilitesi üzerindeki etkisi

Land Use	N	Mean±SD
Barren are soils (BAS)	290	40.82±18.3b
Degraded Forest Soils (DFS)	103	35.32±18.6b
Forest Soils (FS)	19	58.41±8.89a*
Agriculture and Pasture Soils (APS)	15	32.12±14.98b

*($p<0.05$)

The results showed that the land use or land cover has a statistically significant effect on the aggregate stability of soils. In soil sampling from areas with fertile forest cover (FC), the average AS was determined to be 58.41%. On the other hand, AS values of BAS, DFS, and APS areas were found in the same group and lower. It is also difficult to obtain a consistent correlation between aggregate stability and other important soil properties such as soil erosion or scaling potential. However, it affects the movement and storage of aggregates and the soil pore structure, the course of erosion, and biological activity (Amezketa, 1999). Numerous studies reported close relationships between AS and soil erosion. In these studies, it has been reported that soil erosion decreased with the increase in AS (Shainberg et al., 1992; Le Bissonnais, 1996). This indicates that land use or land cover has a direct or indirect effect on erosion. Performing afforestation activities especially in bare areas in the Capakcur watershed and increasing the vegetation rate will both improve soil properties and reduce the severity of erosion.

CONCLUSIONS AND SUGGESTIONS

The Capakcur watershed has a high erosion capacity due to its topographic structure, agricultural activities (grazing, tillage etc.), vegetation, and precipitation regime. The Capakcur watershed had a high erosion-generating capacity after the rehabilitation studies. Annual total erosion occurring in the watershed was 10.14 tons.ha^{-1} which is considerably higher than the average (8.24 tons.ha^{-1}) of Turkey and the Euphrates and Tigris basins (9.1 tons/ha). The results showed that 19.09% of the basin area was exposed to soil loss of 12 tons.ha^{-1} or higher. This value is high in spite of some soil conservation practices (terrace, wire mesh, stone wall over the gully, etc.) carried out in the watershed in 2015. The high slope and length of the slope in the watershed, the low vegetation closure ratio on the ground, and the fact that rainfall is an effective factor cause high erosion and sediment delivery ratios. Here, it is necessary to increase the ratio of the cover and management factor in the watershed within the scope of effective combat against erosion. It is difficult to stop the severity and magnitude of soil erosion. However, it can be reduced by proper land use management and adequate support practices to protect the fertile topsoil in the watershed. Institutions authorized in this regard prioritize these areas on the soil loss map in soil conservation studies. On the other hand, the equations and estimation methods used to determine the net erosion in the watershed are undoubtedly very useful. However, using technologies (acoustic and laser beam) in which sediment transported from the watershed in territorial waters can be measured instantaneously and continuously will contribute to the fight against erosion in a more effective way. Because instant data obtained by the use of these technologies allows the continuous updating of soil management strategies.

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Researchers' Contribution Rate Declaration Summary

Authors declares that the contribution of the authors is equal.

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Authors have declared no conflict of interest.

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Effects of Different Polyethylene Mulch Colors on Greenhouse Cucumber Cultivation

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ABSTRACT
In this study, effects of blue and black polyethylene mulches used together with drip irrigation on yield of Yağmur cucumber cultivar were investigated under greenhouse conditions and mulching treatments were compared with unmulched drip irrigation treatments. Experiments were conducted in a polycarbonate greenhouse in Kayseri province of Turkey in 2019. Throughout the growing season, 655 mm irrigation water was applied in drip+mulch treatments and 776 mm in unmulched drip irrigation treatments. Although 16% less water was applied in drip+mulch treatments, 37% more yield was obtained from drip+blue mulch treatments and 34% more from drip+black mulch treatments. The highest cucumber yields per decare (12296.7 and 11783.6 kg) were respectively obtained from drip+blue mulch and drip+black mulch treatments and the lowest (7771 kg) from unmulched drip irrigation treatments. The highest weed dry biomass (88.42 g m-2) was obtained from unmulched drip irrigation treatments. While the effects of mulching treatments on weed biomass were found to be significant as compared to unmulched treatments, blue and black mulches were not significantly different.

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Farklı Renklerdeki Polietilen Malçların Serada Hıyar Yetiştiriciliği Üzerine Etkileri

ÖZET
Bu çalışmada damla sulama ile kullanılan mavi ve siyah polietilen malçların Yağmur sırık hıyar çeşidinin verimi üzerine etkileri araştırılmış, malçsız damla sulama konuları ile kıyaslanmıştır. Deneme Kayseri iklim şartlarında, 2019 yılında polikarbon serada yürütülmüştür. Mevsimlik su uygulamaları malçlı ve malçsız konularda sırasıyla 655 ve 776 mm olarak ölçülmüştür. Damla sulama+malçlı konulara verilen sulama suyu miktarları malçsız damla sulama konusuna göre yaklaşık %16 daha az olmasına rağmen verim değerleri damla sulama+mavi malç konusunda %37, damla sulama+siyah malç konusunda ise %34 daha fazla bulunmuştur. En yüksek dekara hıyar meyve verimleri mavi ve siyah malç konularında sırasıyla 12296.7 ve 11783,6 kg, en az ise 7771 kg ile malçsız damla sulama konusundadır. Çalışmada en yüksek yabancı ot kuru biyoması malçsız damla sulama yapılan parsellerden 88.42 g m-2) elde edilmiştir. Malç uygulamaların yabancı ot biyomasına etkileri malçsız konuya göre istatistiki olarak önemli iken, malçlı konular kendi arasında istatistiki olarak farklı bulunmamıştır.

Biyosistem Mühendisliği

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INTRODUCTION

Vegetables play a key role in human diets. Therefore, humans should consume vegetables regularly for a healthy life. Cucumber is highly rich in iron and calcium minerals and vitamins A, B, C and K. With a rich vitamin B content, it helps to protect emotional and mental health. About 95% of cucumber is composed of water, so it helps to remove toxins from the body easily. It relieves bad breath, moisturizes the body, and meets most of the daily vitamin needs. It is beneficial against high blood pressure. It has diuretic and laxative characteristics. It regulates blood pressure. It is highly rich in fiber. It prevents indigestion by contributing to the digestive system. It is also a good tonic for liver, kidneys and gall bladder (Şalk et al., 2008).

Cucumber is among widely produced vegetables in the world and in Turkey. With about 88 million tons of annual production in 2019, cucumber was ranked as the 5th vegetable in the world (Anonymous, 2021a). In Turkey, annual cucumber production was 1 886 239 tons in 2020 and with this value, it is the third vegetable produced after tomato and watermelon (Anonymous, 2021b).

The increase in crop production is affected by three basic components: climate, soil and plant. There are other yield-increasing measures such as irrigation, good quality seeds, plant nutrition, pests and disease control and cultural practices (Delibaş, 1994). However, the role of irrigation in increasing yields is quite different as compared to others. Irrigation not only ensures yield increase, but also facilitates the effect of other agronomic practices on yield levels. For instance, expected yield increase with good quality seeds and fertilization can only be achieved if the proper irrigation was practiced (Delibaş, 1994). Today, pressurized irrigation methods are known as modern methods. Among the modern irrigation methods, drip irrigation offers both high irrigation efficiency and greater yield levels. In this method, supplementary mulch treatments are applied to increase water use efficiency, for weed control and ultimately to increase yields (Gerçek et al., 2017).

Mulch is an organic material such as straw, hay, sawdust, or an inorganic material such as plastic that covers the soil surface. Mulches prevent evaporation of irrigation water from the soil, thus increase irrigation water use efficiency and provide a constant moisture level around field capacity within the rootzone. Mulch also prevents weed development. Dark and dull-colored mulches generate a physical barrier over the soil surface, prevent intrusion of light into the soil, thus prevent germination and emergence of weeds (Jabran, 2019). Weeds often compete with the main plant and accelerate consumption of available plant nutrients and available soil water. Growers generally use agro-chemicals and hoeing for weed control. Chemical control increases production inputs and missuses often cause serious damage to environment, soil, and plant, and, therefore, to people.

Mulch covers increase soil temperature, thus have positive effects on soil microbial activities within the rootzone and plant growth. Such covers also allow irrigated farming in places with deficit water resources and offer a kind of crop insurance. Effects of plastic mulches of different colors on plants are quite different. Previous researchers indicated the effects of colors on yield. It was reported that colored plastic mulches had different effects based on season, location, and years (Csizinky et al., 1995). Black-colored mulches increased soil temperature more in spring season, white and aluminum-colored mulches in summer and autumn seasons, thus increased yield levels (Tarara, 2000). It was reported in a study conducted on tomato with black, gray, silvery, red, white mulches that different yield values were obtained in different seasons (Teasdale and Mohler, 2000).

In a previous study, effects of dark-colored paper and biodegradable plastic mulches on cucumber yield were found to be significant as compared to the control treatment without mulch and it was indicated that these mulches also prevented weed growth and development (Happala et al., 2015). Torres-Olivar et al. (2018) investigated the effects of mulches of different colors on pickling cucumber yield under field conditions and the highest yield per plant was reported as 4.88 kg for silver/black, 4.78 kg for aluminum/black, 4.51 kg for black, 3.86 kg for white/black mulches and 2.45 kg for control treatment without mulching. Researchers reported significantly greater nutrient concentration, soil temperature, yield, growth, and gas exchange values for mulching treatments than for the control treatment.

In this study, effects of blue and black mulches on yield and irrigation water use of cucumber (cv. Yağmur) grown in a greenhouse in summer season were investigated and comparisons were made with non-mulched drip irrigation method.

MATERIALS and METHODS

Study Area

Present research was carried out in 2019 in an east-west oriented, Venlo-type, anti-frost and heated polycarbonate greenhouse (9x18 m) over the experimental fields of Kayseri University Safiye Çıkrıkçıoğlu Vocational Collage.

Central Anatolian climate with cold and snowy winters and hot and dry summers is dominant in the region. Greenhouse indoor average temperature and

relative humidity values are provided in Table 1. Average temperatures varied between 13.3 - 24.7 °C and the relative humidity between 50 - 73%. Soil samples were taken from 0 - 30 cm soil profile. Soil analyses revealed that greenhouse soils were loamy in texture, unsaline, slightly alkaline in pH, moderate in organic matter and lime content and sufficient in available phosphorus and potassium (Table 2).

Table 1. Greenhouse indoor temperature and relative humidity values
Çizelge 1. Sera içi ortalama sıcaklık ve nispi nem değerleri

Months(Aylar)	March(Mart)	April(Nisan)	May(Mayıs)	June(Haziran)	July(Temmuz)
Relative humidity, %	73	67	58	55	50
Temperature, ºC	13.3	15.6	20.6	23.2	24.7

Table 2. Some physical and chemical properties of greenhouse soil
Çizelge 2. Sera toprağı bazı kimyasal ve fiziksel değerler

Depth, cm Derinlik, cm	Texture Bünye	pH	OM, %	Lime, % Kireç, %	EC (dS m^{-1})	Available, kg da^{-1}		
						K_2O	P_2O_5	N
0-30	Loamy	7.79	3.17	7.31	0.85	21.56	42.81	16.25

Om: Organic material, EC: Electrical conductivity

Plant material and method

The F1 hybrid Yağmur pole cucumber cultivar was used as the plant material of the study. Ready-to-plant seedlings with 4-5 true leaves were used in present experiments. The cultivar has a strong structure, medium-long internodes, medium-sized leaves, 2-4 fruits in mid-early node, long fruit stalk (12-14 cm) and strong root structure. Fruits have bright green color, long shelf-life, and good aroma. The cultivar is suitable for winter-early spring greenhouse cultivation. It is a variety with suitable, high yielding, strong root structure. When the seedlings had four true-leaves, they were planted on 25.03.2019 in a 90*50*50 cm double-row planting in the north-south direction with 24 plants in each plot. Along with planting, 10 mm initial water was applied to all treatments at one day intervals, and then irrigation treatments were initiated. The first harvest was performed on 30.04.2019 and the last on 26.07.2019. A total of 31 harvests were made. Cucumbers harvested from each plant were counted and the number of fruits per plant and square meter were determined. Fruits were weighed and yield per plant and decare were calculated.

Experimental treatments included unmulched drip irrigation, drip irrigation+ black polyethylene mulch and drip irrigation+blue polyethylene mulch. Experiments were conducted in randomized blocks design with three replications. In all treatments, two drip lines were placed between two rows (Figure 1).

In drip irrigation+mulch treatments, drip lines were lied over the ground and blue or black polyethylene mulches were placed on top of them. Considering the changes in soil moisture content, irrigation interval was selected as 7 days for drip irrigation + mulch and 4 days for unmulched drip irrigation treatments. Irrigation water is passed through water meters. Irrigation water use efficiency (IWUE) was calculated with the use of Equation 1.

$$IWUE = Y/I \quad (1)$$

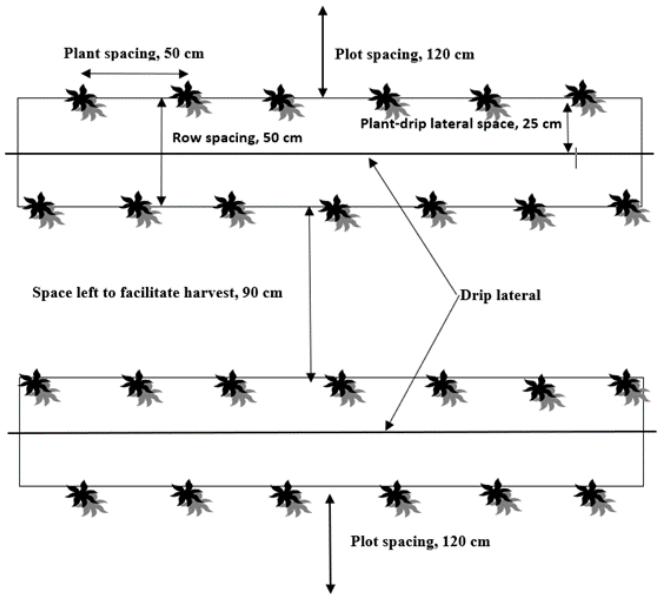

Figure 1. Experimental setup
Şekil 1. Deney düzeni

Where; Y: yield per hectare (kg), I: Amount of irrigation water applied throughout the growing season (mm).

Weed detection as performed from two different parts of each plot with the use of 0.25 m2 (0.5 m X 0.5 m) frames. Following the identification of weed coverage (%) and the number of species, the weeds in the frame were cut from the ground surface and placed into paper bags. Freshly weighed weeds were then dried in an oven at 70 ˚C for 72 hours to get dry biomass (Isik et al., 2009).

Statistical Analysis

Experimental data were subjected to analysis of variance in accordance with randomized blocks design

with the use of SPSS (10.0 for Windows) statistical software. Significant means were compared with the use of LSD test at 0.05 significance level.kale metni. Makale metni. Makale metni. Makale metni. Makale metni. Makale metni. Makale metni. Makale metni. [Century12 regular].

RESULTS AND DISCUSSION

Experimental results were provided in Table 3. The highest yields were obtained from drip irrigation + blue mulch and drip irrigation+black mulch treatments, respectively, and the lowest yields were obtained from unmulched drip irrigation treatments. In terms of yield per decare and per plant, number of fruits per plant and per square meter, significant differences were not seen between blue and black mulch, but there were significant differences between mulching and unmulched treatments.

Table 3. Yield and irrigation water use efficiency of experimental treatments (mean ± SE)
Çizelge 3. Uygulamalardaki verim ve su kullanım etkinliği değerleri (ortalama ± SE)

Treatments Uygulamalar	Yield per plant, kg Bitki başına verim, kg	Yield per unit area, kg m^{-2} Metrekareye verim, kg m^{-2}	Yield per decare, kg da^{-1} Dekara verim, Kg da^{-1}	Number of fruits per plant Bitki başına meyve sayısı	Number of fruits per unit area, fruit m^{-2} Metrekareye meyve sayısı	IWUE (kg $ha^{-1}mm^{-1}$)
Drip+blue mulch Damla+mavi malç	4.28[a*] (±0.45)	12.22[a] (±1.30)	12297[a] (±1300.3)	46.00[a] (±3.65)	131.42[a] (±13.31)	187.73
Drip+black mulch Damla+siyah malç	4.12[a] (±0.39)	11.78[a] (±1.12)	11784[a] (±1124.8)	45.16[a] (±1.811)	129.02[a] (±5.17)	179.90
Unmulched drip Malçsız damla	2.72[b] (±0.38)	7.77[b] (±1.09)	7771[b] (±1094.3)	31.33[b] (±4.12)	89.51[b] (±11.79)	100.14

*: significant at 0.05 level

Throughout the growing season, 16 irrigations were practiced in drip irrigation+mulch treatments and subjects and 29 irrigations were practiced in unmulched drip irrigation treatments. Again, throughout the growing season, 655 mm water was applied in drip irrigation+mulch treatments and 776 mm water was applied in unmulched drip irrigation treatments. The highest yield per decare (12296.7 kg) was obtained from drip irrigation+blue mulch treatments and the lowest yield (7771.0 kg) was obtained from unmulched drip irrigation treatments. The difference is 4525.7 kg, and 37% more yield was obtained from drip irrigation+blue mulch treatments. As compared to unmulched drip irrigation treatments, 34% more yield was obtained from drip irrigation+black mulch treatments. Similar findings were obtained for the other yield components (Table 3).

Irrigation water use efficiency (IWUE) indicates the amount of yield obtained per unit of irrigation water. Higher values indicate more efficient use of irrigation water and greater yield levels. The highest irrigation water usage efficiency values were obtained from drip irrigation+blue mulch treatments and the least from unmulched drip irrigation treatments. Present findings revealed that drip irrigation + mulch treatments were more effective in terms of yield and irrigation water use efficiency as compared to unmulched drip irrigation treatments in cucumber cultivation in greenhouses. It was indicated in a study (Díaz-Pérez and Batal., 2002) that yield, and water use efficiency of cucumber grown in field conditions was higher in drip irrigation+black mulch treatments than in unmulched drip irrigation treatments and yield increase was attributed to mulching treatments. In another study comparing unmulched drip irrigation and drip irrigation+mulch (black and transparent) treatments, the highest yield was obtained from drip irrigation+black mulch treatments (Spiżewski et al., 2010). Similarly, Yaghi et al. (2013) investigated the effects of different mulches on cucumber yield and obtained the greatest yield from black plastic mulch treatments. In a study carried out on eggplant under greenhouse conditions, the highest yield values (115 and 107 t ha-1) were respectively obtained from black and blue mulch treatments (Gerçek and Demirkaya, 2020). In another study investigating the effect of black and blue mulches on two different pepper varieties under greenhouse conditions, the highest yields were obtained from blue mulching treatments and there was no significant difference between blue and black mulches (Gerçek and Demirkaya, 2021). Present findings comply with those earlier ones. In general, it can be concluded that use of mulch together with drip irrigation was a measure to increase the yield of cucumbers. In addition, since black mulches prevent weeds and increase soil temperature, it can easily be used in cucumber cultivation.

Weed species encountered in experimental plots and weed densities are given in Table 4.

Table 4. Weeds encountered and densities
Çizelge 4. Yabancı otlar ve yoğunlukları

Weeds Yabancı otlar	*Density, crop m^{-2}* *Yoğunluk, bitki m^{-2}*	
	04.07.2019	23.08.2019
Portulaca oleracea L.	16.8	4.2
Poa annua L.	12.4	5.2
Tribulus terrestris L.	7.2	3.4
Digitaria sanguinalis (L.) Scop.	6.5	4.4
Amaranthus retroflexus L.	6.0	3.4
Chenopodium album L.	5.2	2.1
Polygonum aviculare L.	3.6	0.8
Seteria viridis (L.) P.Beauv	3.4	4.2
Atriplex patula L.	3.4	-
Convolvulus arvensis L.	2.6	0.3
Heliotropium europaeum L.	1.0	0.8
Solanum nigrum L.	0.8	0.6
Stellaria media (L.) Vill.	0.4	-
Lactuca serriola L.	0.3	-
Crepis macropus Boiss.	0.2	-
Toplam	69.8	29.4

The most common weed species encountered in the experimental plots were Portulaca oleracea L., Poa annua L. Tribulus terrestris L., Digitaria sanguinalis (L.) Scop. Amaranthus retroflexus L. and Chenopodium album L. All these weeds are also common in the other summer crops of the region (Özdemir and Işık 2000; Mennan et al. 2009). Especially weeds such as P. oleracea, A. retroflexus, C. album and Seteria spp were reported in previous studies on different crops cultivated in Turkey (Özdemir and Işık 2000; Mennan et al. 2009; Akça and Işık 2016).

Weed fresh and dry weights and cover ratios of experimental treatments are provided Table 5. Weed population and biomass were significantly affected by the experimental treatments. As seen in Table 5, weeds formed biomass only in unmulched drip irrigation plots. In drip irrigation+mulch treatments, quite a small amount of weed growth was seen from holes around seedling bottoms. In terms of weed biomass, there was no significant difference between black and blue mulches ($p<0.05$). Mulching prevents weed growth by both blocking light intrusion and creating a physical barrier (Jabran, 2019). Previous researchers also pointed out weed growth-inhibition effect of plastic mulches (Teasdale and Mohler 2000; Ramakrishna 2006; Rajablariani et al. 2012).

Table 5. Effects of experimental treatments on weed biomass and cover ratios (mean ± SE)
Çizelge 5. Uygulamalardaki yabancı ot ağırlıkları ve alanı kaplama oranları (ortalama ± SE)

Treatments Uygulamalar	Weed fresh weight, g m^{-2} Yabancı ot yaş ağırlığı, g m^{-2}	Weed dry weight, g m^{-2} Yabancı ot kuru ağırlığı, g m^{-2}	Cover Ratio, % Kaplama oranı, %
Unmulched drip Malçsız damla	842.54^{a*} (±20.11)	88.42^{a} (±2.69)	95
Drip+black mulch Damla+siyah malç	52.59^{b} (±6.40)	5.08^{b} (±0.13)	< 1
Drip+blue mulch Damla+mavi malç	43.04^{b} (±1.87)	3.04^{b} (±0.19)	< 1

*: significant at 0.05 level.

CONCLUSION

It was concluded based on present findings that in cucumber cultivation under greenhouse conditions, mulching treatments applied together with drip irrigation significantly prevented weed growth, thus offered significant savings of resources and labour for weed control; increased irrigation water use efficiency thus offered greater quantity of production. Therefore, blue, and black mulches could reliably be recommended for greenhouse cucumber cultivation. On the other hand, measures to be taken to increase yields in open fields are highly limited because of unexpected instantaneous changes in climate parameters frequently encountered in open fields.

However, climate and plant growth parameters are continuously monitored and controlled in greenhouse, such negative effects of instant changes are not encountered in greenhouses. Mulching treatments are recommended also for high irrigation water use efficiencies.

Author's Contributions

The contribution of the authors is equal.

Statement of Conflict of Interest

Authors have declared no conflict of interest.

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Kafkas Bal Arısı (*Apis mellifera caucasia*) Gen Merkezinin Bozulmasına Neden Olan Etmenler ve Çözüm Önerileri

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ÖZET

Bu çalışma Kafkas bal arısının saf yetiştirildiği Ardahan ve Artvin illerinde yürütülmüştür. Çalışmada arıcılığın yapısı, sosyo-ekonomik nitelikleri, arı hastalık ve zararlılarının durumu, Kafkas Arısı Gen Merkezi'ni tahrip eden sorunların belirlenmesi ve çözüm önerilerinin bulunması amaçlanmıştır. Çalışma kapsamında ana arı üretim izni olan 24 üretici ile yüz yüze görüşülerek anket çalışması yapılmıştır. Elde edilen verilere göre arıcıların yaş ortalamasının 52.5 yıl, arıcılık deneyimlerinin ortalama 24.3 yıl olduğu tespit edilmiştir. Üreticilerin %48.3'ünün lise mezunu olduğu, %35.6'sının asıl mesleğinin arıcılık olduğu, %46.1'inin arılı kovan alarak arıcılığa başladığı, %83,6'sının ana arı üretiminden memnun olduğu, %79'unun kolonilerinin damızlık ana arısını gen merkezlerinden temin ettiği ve %100'ünün Melez Kafkas Arısı ile çalıştığı tespit edilmiştir. Üreticilerin %46.1'i bölgenin izole bölge kapsamında olduğu, %72.8'i Kafkas Arılarının korunması için tedbirlerin alınmadığını, %63.3'ü kaçak olarak bölgeye gezginci arıcıların geldiğini, %76.6'sı Gen Merkezinin korunması için bölgeye kaçak girişlerin engellenmesi gerektiğini bildirmişlerdir.

Zootekni

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Anahtar Kelimeler
Kafkas arısı
Gen Merkezi
Arıcılık
Ardahan
Artvin

Caucasian Honey Bee (*Apis mellifera caucasia*) Factors Causing Deterioration of the Gene Center and Suggestions for Solutions

ABSTRACT

This study was carried out in Ardahan and Artvin cities where Caucasian honeybees were purely bred. In this study, it was aimed to determine the structure of beekeeping, socio-economic quantity, the status of bee diseases and pests, the problems that destroy the Caucasian Bees Gene Center and to offer some solutions. Within the scope of this study, a questionnaire was carried out face to face with 24 beekeepers who have permissions to produce queen bee. According to the obtained data, it has been determined that the mean age of beekeepers is 52.5, their mean experience is 24.3 years. 48.3% of the breeders were graduated from high school, the main job of 35.6% of breeders is beekeeping, 46.1% of them started to beekeeping via buying hive with honeybees, 83.6% of breeders are pleased with queen producing, 79% of them maintain the queens from gene centers and, 100% of breeders were determined to study with cross breed Caucasian Bees. 46.1% of breeders believe that the region is isolated from the other honeybee species, 72.8% of beekeepers reported that precautions were not taken to protect Caucasian bees. 63.3% of them reported that migratory beekeepers came to the region illegally, 76.6% of them reported that illegal entry to the region should be prevented to protect the Gene Center.

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Artvin

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GİRİŞ

Türkiye'de arıcılık, çok eski yıllardan beri bir gelenek olarak yapılan sosyo-ekonomik bir faaliyettir. Ülkemiz sahip olduğu uygun ekoloji, zengin bitki florası ve koloni varlığı bakımından arıcılık açısından iyi bir potansiyele sahiptir (Şahinler ve Gül, 2003). Yapılan çalışmalara göre, Avrupa'da bulunan 11.500 çiçekli bitki türünün 9.000'e yakını ülkemizde bulunmaktadır (Ceylan, 2004; Fıratlı ve ark., 2005). Sahip olduğu bu avantajlar nedeniyle arıcılık yurdumuzun her yerine yayılmış bulunmaktadır (Balcı, 1988).

Türkiye'de saf olarak Ardahan ve Artvin illerinde olmak üzere yakın çevrelerindeki diğer il ve ilçelerinde de Kafkas ana arıları üretilmekte ve üretimde kullanılmaktadır. Bu iller arasında Ardahan ve Artvin, Kafkas arıları için izole bölgeler olup yabancı arıcıların girişine kapatılmıştır. Kafkas arısı ülkemiz için önemli bir arı genotipi olup, Tarım ve Orman Bakanlığınca üretim izni verilen yerli ırklarımızdandır. Kafkasya veya Kafkasya iklimine yakın bölgelerde Kafkas arısından üstün verimler alınabilmektedir. Bu sebeple bu bölgelerde ana arının üretimi ve diğer bölgelere gönderilmesinin yanında bu alanda Kafkas ırkını korunması için de çalışmalar yapılmaktadır. Ancak büyük bir coğrafya olması sebebi ile zaman zaman sıkıntılar yaşanabilmektedir. Bölgede ana arı üretimi yanında saf ırkın korunması için yapılan çalışmalar Ardahan ve Artvin illerinde yoğunlaşmaktadır. Ardahan ve Artvin illeri, dünyada tanımlı dört verimli arı ırkından biri olan Kafkas arı ırkının gen merkezleri arasında ilk sırayı alması sebebiyle Türkiye arıcılığında özel bir yere sahiptir. Bunun yanı sıra Ardahan ili, sanayileşmeden etkilenmemiş, nüfus yoğunluğu az ve el değmemiş coğrafyasıyla verimli Kafkas arı ırkının kaliteli bal üretebilmesi için uygun bir bitki örtüsüne sahiptir.

FAO'nun 2021 verilerine göre 13 milyon adet koloni ile Hindistan ilk sırada yer alırken, 9.7 milyon adet koloni ile Çin ikinci sırada ve 8.1 milyon adet koloni ile Türkiye üçüncü sırada yer almaktadır (FAO, 2021a). FAO'nun 2021 yılı verilerine göre Çin 457.000 ton üretim ile dünyada en çok bal üreten ülke olup 1. sırada yer almaktadır. Türkiye 114.000 ton ile 2. sırada yer almakta ve Arjantin ise 79.500 ton ile 3. sırada yer almaktadır (FAO, 2021b). Kafkas Arı Irkı Gen Merkezi illerinden olan Ardahan'da 2020 yılında yaklaşık 52 bin kovandan 1.022 ton bal üretilmiş, Artvin'de ise 86 bin kovandan 1.256 ton bal üretilmiştir. Kovan başına bal verimliliği bu iki ilde 15 ve 14.6 kg ile Türkiye ortalamasının (14.04 kg) çok küçük bir farkla üzerinde gerçekleşmiştir (Çizelge 1).

Ardahan'da Arı Yetiştiricileri Birliği'ne kayıtlı 786 arıcılık işletmesi bulunmaktadır (Çizelge 2). Bu işletmelerin toplam 69.770 adet kayıtlı kovanı bulunmaktadır. Tablo 2'deki verilere bakıldığında Ardahan'da arıcılığın Merkez, Hanak ve Posof ilçelerinde yoğunlaştığı görülmektedir.

Çizelge 1. Türkiye'nin, Ardahan ve Artvin illerinin kovan sayıları ve bal üretim miktarları (TÜİK, 2020)
Table 1. Number of hives and honey production amounts of Ardahan and Artvin provinces in Türkiye (TÜİK, 2020)

	Türkiye *(Turkey)*	Ardahan *(Ardahan)*	Artvin *(Artvin)*
Kovan Sayısı (adet) *(Hive Number)*	8.456.305	52.34	86.328
Bal Üretimi (Ton) *(Honey production (Tone))*	96.344	1.022	1.256
Kovan Başına Bal Verimi (Kg) *(Honey yield per hive (kg))*	11.43	15.0	14.6

Çizelge 2. Ardahan ili ve ilçelerinde koloni ve işletme sayısı (Anonim, 2021)
Table 2. Number of colonies and businesses in Ardahan province and districts (Anonim, 2021)

İlçe *(District)*	Kovan sayısı *(Number of hives)*	İşletme sayısı *(Number of producers)*
Merkez *(City Center)*	21.087	244
Hanak	15.438	166
Posof	15.502	173
Çıldır	11.019	119
Göle	4.734	58
Damal	1.990	26
Ardahan Toplamı *(Ardahan Total)*	69.770	786

Kafkas Arısı dünyada bilinen ve ekonomik değere sahip dört arı ırkından bir tanesidir. Bu ırkın anavatanı, Orta Kafkasların yüksek vadileridir. Kafkas Arısı; "Hayvan Irklarının Tesciline İlişkin Yönetmelik" esaslarına göre hazırlanmış olan 'Yerli Hayvan Irk ve Hatlarının Tescili Hakkında Tebliğ (2004/39)' kapsamında tescil edilmiştir.

Türkiye'nin değişik yerlerinde araştırmalar yapılarak arıcıların sosyo-ekonomik durumu ve arıcılığın teknik özelliklerinin belirlenmesi amaçlanmıştır (Şahinler ve Gül, 2003; Yerlikaya, 2008; Erkan ve Bingöl, 2014; Aydın, 2014; Demen, 2015; Tabur ve Gül, 2019). Ardahan ve Artvin illeri Tarım ve Orman Bakanlığı tarafından 2000 yılında Kafkas arı ırkı için gen merkezi olarak ilan edilmiş ve bu sayede bölgelerde yetiştirilen saf Kafkas arı ırkı korumaya almıştır.

Koruma altına alınan Kafkas arı ırkının bölge arıcılığı için önemi, korunması için nasıl tedbirlerin alınması gerektiği ve gen merkezinin bozulmasına neden olabilecek etmenlerin belirlenmesi için Ardahan'da arı ve ana arı üreten işletmeler ile yüz yüze görüşülerek bölgenin yapısı, geleceği, sorunları ve bu sorunların çözümüne yönelik önlemlerin tespit edilmesi amaçlanmaktadır.

MATERYAL ve METOD

Çalışmanın materyalini Kafkas Arısı Gen Merkezi olan bölgedeki Kafkas ırkı ana arı üretim izni alan işletmeler oluşturmaktadır. Artvin ve Ardahan Tarım ve Orman Bakanlığı il müdürlükleri ve Arı yetiştiricileri birliği ile işbirliği yapılıp arıcıların listeleri temin edilerek Kafkas ırkı ana arı üretme iznine sahip olan 39 işletmenin tamamıyla anket yapılması hedeflenmiştir. Ancak, 15 üreticinin anket çalışmasının yapıldığı tarihlerde ana arı üretimi yapmadığından aktif ana arı üretimi yapan 24 üretici ile anket çalışması yapılmıştır. Anketlerin tamamı arıcılık işletmelerinde yüz yüze görüşülerek yapılmıştır. Anket formları 4 ana başlık altında 59 sorudan oluşmuştur. İlk bölümde arıcıların sosyo-ekonomik nitelikleri, ikinci bölümde arıcılığın teknik özellikleri, üçüncü bölümde arı hastalık ve zararlıların teşhisinin ve tedavisinin yapılması ve dördüncü bölümde ise Kafkas Arısı Gen Merkezinin bozulmasına neden olan etmenlerin tespiti ile ilgili sorulara yer verilmiştir.

İstatistik Analizler

Verilerin analizinde; üreticilerin sorulara vermiş olduğu cevaplara göre tanımlayıcı istatistikler (aritmetik ortalama, frekans tabloları, ve yüzde (%) hesaplamaları) kullanılmıştır.

Çalışmanın yürütülmesi için Hatay Mustafa Kemal Üniversitesi Sosyal ve Beşeri Bilimler Bilimsel Araştirma ve Yayin Etiği Kurulundan 07.04.2022 tarih ve 05/17 sayılı yazı ile etik kurul izni alınmıştır.

BULGULAR ve TARTIŞMA

Araştırma bölgesinde üreticilere dört bölümde toplam 59 adet soru yöneltilerek bölgenin arıcılık yapısı ve sosyo-ekonomik durumu, arı hastalık ve zararlı durumu ile Kafkas Arısı Gen Merkezinin bozulmasına neden olan etmenler incelenmiştir.

Sosyo-ekonomik Durum

Araştırmanın bu bölümünde bölgede ana arı üretim izni olan işletmelerin sosyo-ekonomik yapıları incelenmiştir. Bu amaçla işletmeler koloni sayılarına göre 3 gruba ayrılmıştır. Araştırmada; 1-149 adet arası koloniye sahip olan işletmeler 1. grup, 150-249 adet koloniye sahip işletmeler 2. grup, 250 adet ve üzeri sayıda koloniye sahip olan işletmeler ise 3. grup olarak belirlenmiştir.

Çalışma bölgesindeki arıcıların yaş ortalamasının 52.5 yıl ve deneyimlerinin de ortalama 24.3 yıl olduğu tespit edilmiştir (Çizelge 3). Yapılan benzer çalışmalarda; Hatay İlinde arıcıların yaş ortalamasının 44.4 yıl ve deneyimlerinin de 10.5 yıl olarak (Şahinler ve Gül, 2003), Uşak İlinde ise üreticilerin yaş ortalamasını 53.19 yıl, deneyimlerini ise 18.47 yıl olarak bildirilmiştir (Tabur ve Gül, 2019). Sonuç olarak tespit edilen yaş ortalaması Hatay ilinden yüksek iken, Uşak İli ile birbirine yakın bulunmuştur. Elde edilen deneyim süresi ise Hatay ve Uşak illerinden yüksek bulunmuştur.

Araştırma bölgesindeki üreticilerin yarısına yakınının (%48.3) lise mezunu olduğu belirlenmiştir (Çizelge 3). Sonuç itibariyle üniversite mezunlarının arıcılık mesleği tercihinin az olduğu ifade edilebilir. Van İlinde yapılan bir çalışmada arıcıların büyük çoğunluğunun (%59.15) ilkokul mezunu olduğu tespit edilmiştir (Erkan ve Bingöl, 2014). Diyarbakır İlinde yapılan başka bir çalışmada ise arıcıların %60.26'sının ilkokul mezunu olduğu belirtilmiştir (Demen, 2015).

Anket çalışmasına katılan işletmecilerin büyük çoğunluğu (%40.5) ek gelir kaynağı %19.7'si ise esas geçim kaynağı olduğu için arıcılık yaptığı saptanmıştır (Çizelge 3). Hatay İlinde işletmelerin %38,95'inin geçimini sağlamak amacıyla, %42.11'inin yan gelir sağlamak amacıyla arıcılık yaptığı bildirilmiştir (Şahinler ve Gül, 2003). Tabur ve Gül (2019), Uşak İlinde işletmelerin büyük çoğunluğunun (%55.9) ek gelir kaynağı olduğu için, %5.4'ünün ise esas geçim kaynağı olduğu için arıcılık yaptığını tespit etmiştir.

Araştırma bölgesindeki işletmelerin %46.1'inin arılı kovan ve %16.4'ünün oğul satın alarak arıcılığa başladığını, %37.5'inin de baba mesleği olduğu için arıcılığa başladığı belirlenmiştir (Çizelge 3). Tabur ve Gül (2019), Uşak İlinde üreticilerin %33.1'i yeni tip arılı kovan satın alarak, %9.3'ü eski tip arılı kovan satın alarak, %20.6'sı oğul satın alarak, %21.1'i de baba mesleği olduğu için arıcılığa başladığını belirlemiştir.

Anket çalışmasına katılan arıcıların büyük çoğunluğunun (%37.5) arıcılık hakkındaki bilgi kaynaklarını kursa giderek öğrendiği, %18.90'ının da diğer arıcılardan öğrendiği belirlenmiştir (Çizelge 3).

Demen (2015), Diyarbakır'daki üreticilerin büyük çoğunluğunun arıcılık hakkındaki bilgilerini diğer arıcılardan (%47.76) ve kurslardan (%31.34) öğrendiğini tespit etmiştir. Tabur ve Gül (2019), Uşak ilinde ise işletmecilerin %57.4'ünün arıcılık ile ilgili bilgileri dergi, kitap ve yayın organlarından, %45.2'sinin de kurs, eğitim ve seminerlerden öğrendiğini tespit etmiştir.

Çizelge 3. Arıcıların sosyo-ekonomik nitelikleri
Table 3. Socio-economic characteristics of beekeepers

		1.Grup *(1nd group)* **Koloni sayısı: 1-149 arası** *(number of colonies: 1-149)*	**2.Grup** *(2nd group)* **Koloni sayısı: 150-249 arası** (number of colonies: 150-249)	**3.Grup** *(3nd group)* **Koloni sayısı: 250 ve üzeri** *(number of colonies: >250)*	**Ortalama** (*average*)
Yaş (yıl) *(Age (year))*		18.61	35.79	45.60	52.50
Deneyim (yıl) *(experience (year))*		8.12	18.18	50.00	24.3
Arıcıların eğitim durumu (%) (*educational status of beekeepers (%)*)	İlkokul	28.57	18.18	50.00	32.25
	Ortaokul	14.29	9.09	16.67	13.35
	Lise	57.14	54.55	33.33	48.34
	Üniversite	0.00	18.18	0.00	6.06
Üreticilerin arıcılığı yapma amacı (%) (*The purpose of doing beekeeping of producers (%)*)	Ailemin uğraşı olması	28.57	9.09	0.00	12.55
	Bildiğim en kazançlı iş olması	14.29	0.00	0.00	4.76
	Faz. sermaye ve araziye ger. yok	14.29	18.18	16.67	16.38
	Esas geçim kaynağım	0.00	9.09	50.00	19.70
	Ek gelir kaynağım	42.86	45.45	33.33	40.55
	Diğer	0.00	18.18	0.00	6.06
Üreticilerin arıcılığa başlama şekli (%) (*How producers started beekeeping (%)*)	Arılı kovan satın aldım	42.86	45.45	50.00	46.10
	Oğul satın aldım	14.29	18.18	16.67	16.38
	Baba mesleği	42.86	36.36	33.33	37.52
Arıcılık hakkında bilgi kaynağı (%) (*Sources of information about beekeeping (%)*)	Aile	28.57	18.18	0.00	15.58
	Diğer arıcılardan	14.29	9.09	33.33	18.90
	Kursa giderek	42.86	36.36	33.33	37.52
	Tarım İl Müd. ve Üniversiteler	0.00	27.27	16.67	14.65
	Kitap ve broşür	14.29	9.09	16.67	13.35

Teknik Özellikler

Üreticilerin %71.6'sı kolonilerini içeride, %23.7'si de dışarıda kışlatmaktadır (Çizelge 4). Posof ilçesi hariç Ardahan ili ve ilçelerinde olumsuz iklim koşullarından dolayı üreticilerin tamamı kolonilerini içeride kışlatmaktadır. Artvin İli geneli ve Posof'ta ise ılıman bir iklime sahip olduğundan arıcılar kolonilerini dışarıda kışlatmaktadır. Soğuk iklime sahip bölgelerimizde sabit arıcıların büyük bir çoğunluğu arılarını içeride kışlatmakta gezginci arıcıların tamamı ise arılarını ılıman bölgelere naklederek kışlatma yapmaktadır (Cengiz ve Yazıcı, 2018). Hatay İli arıcılarının %88.30'u kolonileri dışarıda kışlattığını belirtmişken (Şahinler ve Gül, 2003), bu oran Tunceli ili arıcıları için %60.8'dir (Yerlikaya, 2008). Elde edilen verilere göre Ardahan ve Artvin illerinde üreticilerin büyük bir kısmının kolonilerini içeride, ülke genelinde ise büyük oranda arıcıların kolonileri dışarıda kışlattığı söylenebilir.

Anket yapılan bölgedeki işletmelerin %93.9'u erken ilkbahar ve geç sonbaharda kolonilerini şurup ile beslediği tespit edilmiştir (Çizelge 4). Bu çalışmaya benzer olarak Şahinler ve Gül (2003), Hatay İlinde arıcılardan %60'ının Şubat ayında, %38'inin Mart ayında ve %2'sinin ise daha sonraki aylarda besleme yaptıklarını tespit etmişlerdir. Tunceli İlinde arıcıların %34.2'sinin Mart ayında, %59.6'sının Nisan ayında, %6.2'sinin ise Mayıs ayında kolonilere ek besleme yaptıklarını belirlemiştir (Yerlikaya, 2008).

Çizelge 4. İşletmecilerin teknik özellikleri
Table 4. Technical characteristics of producers

		1.Grup *(1nd group)* **Koloni sayısı: 1-149 arası** *(number of colonies: 1-149)*	**2.Grup** *(2nd group)* **Koloni sayısı: 150-249 arası** *(number of colonies: 150-249)*	**3.Grup** *(3nd group)* **Koloni sayısı: 250 ve üzeri** *(number of colonies: >250)*	**Ortalama** *(average)*
Uygulanan kışlatma yöntemi (%) *(The applied wintering method (%))*	Dışarıda	28.57	9.09	33.33	23.67
	İçeride	57.14	90.91	66.67	71.57
	Sundurma altında dışarıda	14.29	0.00	0.00	4.76
Şurupla besleme zamanı (%) *(The feeding time with syrup (%))*	Erk. ilkbahar ve geç sonbaharda	100.00	81.82	100.00	93.94
	İkbaharda arıları gelişt. için	0.00	9.09	0.00	3.03
	Yaz. yeters. flora daha faz. bal alm.	0.00	9.09	0.00	3.03
Ana arı değişimi (%) *(queen exchange(%))*	Evet	100.00	10.00	100.00	100.00
	Hayır	0.00	0.00	0.00	0.00
Ana arı temini (%) (*queen supply (%)*)	Kendim yetiştiriyorum	85.71	36.36	33.33	51.80
	Arıcı üret. istas. (Gen merkezi)	14.29	63.64	50.00	42.64
	Diğer ana arı üreten işletm.	0.00	0.00	16.67	5.56
Ana arıyı değiştirme süresi (%) *(queen replacement time (%))*	2 yılda bir kez	71.43	81.82	83.33	78.86
	3 yılda bir kez	0.00	9.09	0.00	3.03
	Yılda bir	14.29	9.09	16.67	13.35
Güvenlik sorunu (%) *(security problem(%))*	Evet	42.86	45.45	66.67	51.66
	Hayır	57.14	54.55	33.33	48.34

Anket çalışmasına bakıldığında arıcıların tamamının kolonilerinin ana arılarını değiştirdiği görülmektedir (Çizelge 4). Şahinler ve Gül (2003), Hatay İlinde işletmelerin %65'inin kolonilerin ana arısını değiştirdiğini belirlemişlerdir. İşletmecilerin %51.8'i ana arıyı kendisi üretirken %42.6'sı Arıcılık Üretme İstasyonundan (Gen Merkezi) temin ettiği, %5.6'sı da diğer ana arı üretim işletmesinden aldığı tespit edilmiştir (Çizelge 4). Şahinler ve Gül (2003), Hatay İlinde arıcıların %44.21'inin ana arıyı doğal yolla kendisi üretip kullandığını, yalnız %14.74'ünün modern ana arı üretim tekniği ile yetiştiricilik yapan özel sektörden ana arı satın aldığını, %28.42'sinin kendisi kontrollü olarak ürettiğini ve %8.42'sinin de bölgesindeki arıcılardan temin ettiğini tespit etmişler.

Anket çalışmasına katılan üreticilerin %78.9'u ana arıyı iki yılda bir değiştirirken, yılda bir değiştirenlerin oranı ise %13.3 olarak tespit edilmiştir (Çizelge 4). Tabur ve Gül (2019), Uşak İlinde arıcıların %30.1'inin ana arıyı iki yılda bir değiştirdiğini, %3.3'ünün her yıl değiştirdiğini, %63.1'inin de değiştirmediği ve kendilerinin yenilediğini tespit etmişlerdir. Elde edilen verilere göre işletmelerin büyük bir kısmının (%78.9) koloni ana arısını iki yılda bir değiştiği gözlenmektedir. Buna göre çalışma bölgesindeki arıcıların ana değişimi konusunda bilinçli oldukları ifade edilebilir. Araştırma bölgesinde anket çalışmasına katılan arıcıların %48.34'ünün herhangi bir sorun yaşamadığı, %51.7'sinin ise güvenlik sorunu ile karşılaştığı ve bu sorunların can güvenliği ve ayı tehlikesi vb. olduğu tespit edilmiştir (Çizelge 4). Tunceli ilinde yapılmış benzer bir çalışmada, arıcıların

%57.3'ünün arılarını götürdükleri yerlerde güvenlik sorunu ile karşılaştıkları bildirilmiştir (Yerlikaya, 2008).

Hastalık ve Zararlıların Teşhisi ve Tedavisi

Anket çalışmasına katılan işletmelerin %92.2'sinin erken ilkbahar ve geç sonbaharda hastalık ve zararlı mücadelesi yaptığı tespit edilmiştir (Çizelge 5). Benzer olarak Van İlinde işletmelerin %97.14'ünün erken ilkbahar ve geç sonbaharda hastalık ve zararlı mücadelesini yaptığını saptanmıştır (Erkan ve Bingöl, 2014). Tabur ve Gül (2019), Uşak İlinde arıcıların %57.1'inin hastalık ve zararlı mücadelesini erken ilkbahar ve geç sonbaharda yaptığını tespit etmişlerdir. Elde edilen sonuçlara göre bölge arıcılarının hastalık ve zararlıları ile mücadele zamanı konusunda bilinçli olduğu ifade edilebilir.

Çizelge 5. Arıcıların hastalık ve zararlıları teşhis ve tedavi etmesi durumu
Table 5. Diagnosis and treatment of diseases and pests by beekeepers

		1.Grup *(1nd group)* Koloni sayısı: 1-149 arası *(number of colonies: 1-149)*	2.Grup *(2nd group)* Koloni sayısı: 150-249 arası *(number of colonies: 150-249)*	3.Grup *(3nd group)* Koloni sayısı: 250 ve üzeri *(number of colonies: >250)*	Ortalama *(average)*
Hastalık ve zararlı ile mücadele zamanı (%) *(Disease and pest control time (%))*	Erken ilkbahar ve geç sonbaharda	85.71	90.91	100.00	92.21
	Görür görmez	14.29	0.00	0.00	4.76
	Bal hasadından sonra	0.00	9.09	0.00	3.03
Varroa ile mücadele yöntemi (%) *(Method of control against Varroa mite (%))*	Parazit yokken ilaçlama yaparak	42.86	18.18	33.33	31.46
	Parazit görüldüğünde ilaçlama yap.	14.29	54.55	50.00	39.61
	Erkek arı gözlerini yok ederek	28.57	18.18	0.00	15.58
	Sürekli ilaçlama yaparak	14.29	9.09	16.67	13.35
Amerikan yavru çürüklüğü hastalığı tespiti (%) *(Detection of American foul Brood (%))*	Evet	71.43	81.82	100.00	84.42
	Hayır	28.57	18.18	0.00	15.58
Nosema hastalığının tespiti (%) *(Detection of Nosema spp. (%))*	Evet	85.71	90.91	100.00	92.21
	Hayır	14.29	9.09	0.00	7.79
Hastalık ve zararlı kontrol müracaat yeri (%) *(Disease and pest control referral place (%))*	Tecrübeli arıcılara	71.43	45.45	66.67	61.18
	Özel veterinerlere	0.00	18.18	16.67	11.62
	İlaç satıcılarına	42.86	36.36	50.00	43.07
	Üniversitelere	28.57	18.18	33.33	26.70
	Tarım il ve ilçe müdürlüklerine	28.57	9.09	33.33	23.67
	Arıcılık ile ilgili kitap, broşür vb.	0.00	9.09	0.00	3.03
	Herhangi bir yere müracaat etmiyorum	28.57	27.27	0.00	18.61

Üreticilerin %39.6'sı parazit görüldüğünde ilaçlama yaparak, %31.5'i parazit yokken ilaçlama yaparak, %15'i erkek arı gözlerini yok ederek ve %13.4'ü de sürekli ilaçlama yaparak Varroa (*Varroa destructor*) akarı ile mücadele ettiği tespit edilmiştir (Çizelge 5). Bu çalışmada anket çalışmasına katılan arıcıların %84.4 gibi büyük bir kısmı Amerikan Yavru Çürüklüğü hastalığını tespit ettiği belirlenmiştir(Çizelge 5). İşletme grupları itibariyle 1. grubun %71'i, 2. grubun %81.8'i ve 3. grubun ise %100'ünün Amerikan Yavru Çürüklüğü (*Paenibacillus larvae*) hastalığını tespit edebildiği saptanmıştır

(Çizelge 5). Elde edilen verilere göre ankete katılan bölge arıcılarının büyük bir kısmının Amerikan Yavru Çürüklüğü Hastalığını tanıdığı ifade edilebilir.

Araştırma bölgesindeki arıcıların tamamına yakınının (%92.2) Nosema (*Nosema* spp.) hastalığını tanıdığı tespit edilmiştir (Çizelge 5). Çalışma sonucu elde edilen verilere göre ankete katılan bölge arıcılarının büyük bir kısmının Nosema hastalığının teşhisi konusunda bilgi sahibi olduğu söylenebilir. Anket çalışmasına katılan arıcıların büyük bir kısmı (%61.2) arı hastalık ve zararlıları ile ilgili tecrübeli arıcılara danışırken, %23.7'si Tarım İl ve İlçe Müdürlüklerine müracaat etmiştir (Çizelge 5). Benzer bir çalışma ile Şahinler ve Gül (2003) Hatay ilinde arıcılık işletmelerinin herhangi bir sorunla karşılaştıklarında en fazla Tarım İl ve İlçe Müdürlüklerine müracaat ettiklerini tespit etmişlerdir (Şahinler ve Gül, 2003). Aydın (2014), Ardahan ilinde arıcıların %25'inin İl-İlçe Müdürlükleri, %16'sının arıcılardan, %7'sinin Veterinerlerden ve %12'sinin de diğer yerlerden yardım aldıklarını belirtmiştir.

Kafkas Arısı Gen Merkezinin Bozulmasına Neden Olan Etmenler

İşletmecilerin %46.1'i bölgenin izole olduğunu, %32.8'i izole bölge olmadığını belirtirken, %21.1'inin de tam izole olmadığını belirttiği tespit edilmiştir (Çizelge 6). Arıcıların %78'i damızlık ana arıyı Gen Merkezinden temin ederken, %13.3'ü Kafkas Ana Arı Üretim İstasyonundan ve %7.8'inin de sertifikalı üreticilerden temin ettiği tespit edilmiştir (Çizelge 6). Aydın (2014), Ardahan ilinde üreticilerin % 45'inin ana arıyı kendisinin yetiştirdiğini, % 39'unun diğer arıcılardan satın aldığını ve % 1'inin de KAGEM' den satın aldığını tespit etmiştir.

Çizelge 6. Kafkas arısı gen merkezinin bozulmasına neden olan etmenler
Table 6. Factors causing disruption of Caucasian bee gene center

		1.Grup *(1nd group)*	2.Grup *(2nd group)*	3.Grup *(3nd group)*	
		Koloni sayısı: 1-149 arası *(number of colonies: 1-149)*	**Koloni sayısı: 150-249 arası** *(number of colonies: 150-249)*	**Koloni sayısı: 250 ve üzeri** *(number of colonies: >250)*	**Ortalama** *(average)*
İzole bölge kapsamındaki konumu (%) *(Location within the isolated region (%))*	Evet	42.86	45.45	50.00	46.10
	Hayır	28.57	36.36	33.33	32.76
	Tam izole değil	28.57	18.18	16.67	21.14
Damızlık ana arı temini (%) *(Breeder queen supply(%))*	Sertifikalı üreticilerden	14.29	9.09	0.00	7.79
	Gen merkezlerinden	71.43	81.82	83.33	78.86
	Kafkas ana arısı üretim İstasyonu	14.29	9.09	16.67	13.35
Üretimi yapılan ana arının saflık derecesini belirleme metodları (%) *(Methods for determining the purity grade of the reared queen (%)*	Kendim belirliyorum	42.86	63.64	50.00	52.16
	Bakanlık yetkilileri	28.57	27.27	16.67	24.17
	Üniversiteler	0.00	0.00	16.67	5.56
	Sertifikalı üreticiler	28.57	9.09	16.67	18.11
Üretimi yapılan ana arının kontrol yapan kurum (%) (*Institution controlling the reared queen (%))*	Yapılmıyor	42.86	72.73	66.67	60.75
	Bakanlık yetkilileri	57.14	27.27	33.33	39.25
Kafkas arısının korunması için alınması gereken önlemler (%)	Tedbir alınmamaktadır	71.43	63.64	83.33	72.80
	İhbar halinde kontrol edilmektedir	14.29	18.18	16.67	16.38
	Cevap vermeyen	14.29	18.18	0.00	10.82

(Precautions that need to be taken to protect the Caucasian bee (%))					
Üreticilerin melez Kafkas arısını tespiti (%)	Evet	100.00	100.00	100.00	100.00
(Detection of hybrid caucasian bee by beekeeper (%))	Hayır	0.00	0.00	0.00	0.00
Melez Kafkas tespiti sonrası yapılması gereken (%)	Ana arıyı değiştiriyorum	100.00	100.00	100.00	100.00
(What does the beekeeper do after the hybrid queen detection? (%))	Ana arıyı değiştirmiyorum	0.00	0.00	0.00	0.00
Bölgeye kaçak gelen gezginci arıcıların tespiti (%)	Evet	42.86	63.64	83.33	63.28
(Detection of beekeepers illegally entering the isolated region (%))	Hayır	57.14	36.36	16.67	36.72
Melezleşmeye neden olan faktörler (%)	Gezginci arıcı girişleri	71.43	72.73	83.33	75.83
(Birden fazla cevap verilebilir)	Kaçak ana arı girişi	57.14	54.55	66.67	59.45
(the reasons for the hybridization of queens (%))	Melez oğul girişi	42.86	9.09	0.00	17.32
(*allowing multiple responses)*	Kontrollerin yetersizliği	57.14	45.45	50.00	50.87
Dışarıdan gelen arıların verdiği zararlar (%)	Irk bozulması	100.00	100.00	100.00	100.00
(damages caused by illegally entering bees (%))	Yağmacılık	14.29	36.36	16.67	22.44
(allowing multiple responses)	Verimin düşmesi	14.29	27.27	33.33	24.96
Gen merkez. korunm. için alınm. gerek. tedbir (%)	Bölgeye kaçak giriş engellenmelidir	57.14	72.73	100.00	76.62
(Measures that must be taken to protect the gene center (%))	Saf olmayan arılar kullanılmamalıdır	42.86	9.09	66.67	39.54
(allowing multiple responses)	Kontroller artırılmalıdır	85.71	45.45	100.00	77.06

Arıcıların %52.1'inin ana arının saflık derecesini kendisi belirlediği, %24.2'sinin bakanlık yetkililerince tespit edildiği, %18.1'inin sertifikalı üreticiler tarafından tespit edildiği, %5.5'inin de üniversiteler tarafından belirlendiği saptanmıştır (Çizelge 6). Araştırma bölgesindeki işletmelerin %60.8'i üretilen damızlık ana arıların kontrolünün yapılmadığı belirtilirken, %39.2'sinin ise bakanlık yetkilileri tarafından kontrolünün yapıldığı belirlenmiştir (Çizelge 6). Araştırma alanındaki üreticilerin %72.8'i Kafkas Arısının korunması için herhangi bir tedbir alınmadığını bildirmişlerdir (Çizelge 6).

Çalışma bölgesindeki üreticilerin tamamının (%100) melez Kafkas Arısını tanıyabildiği ve yine arıcıların tamamının (%100) melez Kafkas Arısının tespitinden

sonra ana arıyı değiştirdiği belirlenmiştir (Çizelge 6). Üreticilerin %63.3'ü gezginci arıcıların bölgeye geldiğini belirtirken, %36.7'si ise gezginci arıcıların gelmediğini belirtmiştir (Çizelge 6).

Anket bölgesindeki işletmelerin %75.8'i gezginci arıcı girişleri, %59.5'i kaçak ana arı girişi, %50.9'u yapılan kontrollerin yetersizliği ve %17.3'ünün de melez oğul girişinden dolayı Kafkas Arısının melezleştiğini ifade etmiştir (Çizelge 6). Anket bölgesine kontrolsüz olarak diğer illerden giren arıların Kafkas Arısına verdiği zararları belirlemek amacıyla sorulan soruya işletmecilerin %100'ü ırkın bozulduğunu, %25'i verimin düştüğünü ve %22.4'ünün de yağmacılığın artmasına sebep olduğunu söylemişlerdir (Çizelge 6). İşletmelerin %77.6'sı gen merkezinin korunması için kontrollerin sıklaştırılması gerektiğini, %76.6'sı bölgeye kaçak girişlerin engellenmesi gerektiğini ve %39.5'i de saf olmayan arıların kullanılmaması gerektiğini belirtmiştir (Çizelge 6).

SONUÇ ve ÖNERİLER

Mevcut çalışmanın bulguları dikkate alınarak, araştırma bölgesinde üreticilerin karşılaştıkları sorunlar ve bu sorunların çözümüne yönelik öneriler maddeler halinde aşağıda verilmiştir.

1. Bölgeye dışarıdan arı girişleri kontrolünün etkili bir şekilde yapılması, Artvin ve Ardahan girişlerindeki tüm yol güzergahlarına gerekli uyarı tabelalarının asılması gerekmektedir. Belirlenen kural ve yasaklara uymayanlar ile ilgili gerekli cezai yaptırımlar uygulanmalıdır.
2. İzole alan bölgesindeki arı yetiştiricileri Tarım ve Orman Bakanlığı tarafından teşvik edilecek şekilde, diğer illerde yapılan desteklemelerin haricinde gen kaynağının korunması projesi kapsamında tüm Ardahan ve Artvin ilinde bulunan arı yetiştiricilerine verilmesi uygun olacaktır.
3. Hastalık ve parazitler ile mücadele konusunda Tarım ve Orman Bakanlığı tarafından izole bölgeye uygun faaliyetler geliştirilmelidir.
4. Ana arı işletmeleri her yıl "yerli kaynakların saflığı" bakımından bakanlığın yetkili organları tarafından rutin olarak kontrolden geçirilmeli ve saflık değerini kaybetmiş damızlıkların imhası veya izole (gen kaynağı) alan dışarısına çıkarılması sağlanmalıdır.
5. İl, İlçe Tarım ve Orman İl Müdürlükleri, Üniversiteler, Birlikler ve Kooperatifler koordineli çalışarak arıcılığın geliştirilmesi için projeler yapılmalı, kurslar, eğitimler ve seminerler düzenleyerek, üreticiler bilinçlendirilmelidir.
6. İzole bölge içerisinde Kafkas arısının ıslahı ile ilgili ıslah projeleri hazırlanarak damızlık özellik gösteren materyaller belirlenmeli veya bu yönde ıslah çalışması başlatılmalıdır. Ayrıca Ardahan ve Artvin illerindeki tüm arılıklarda bulunan arı kolonilerinin konusunda uzman komisyon tarafından incelemeleri yapılarak, Kafkas arısı özelliklerini taşımayan kolonilerin il dışına çıkarılması gerekmektedir. Bu tespit ve kontrollerin iki yılda bir yapılması gen kaynağının korunması bakımından uygun olacaktır.
7. Ardahan, Artvin illerindeki Tarım ve Orman İl Müdürlüklerinde ve yetiştirici birliklerinde uzman teknik eleman istihdam edilmelidir.
8. Özellikle ana arı üreticilerine yönelik periyodik olarak eğitimler verilerek, üreticiler modern üretim yöntemleri, seleksiyon ve ıslah konularında bilinçlendirilmelidir.

Çalışma bölgesinde Kafkas bal arısının (*Apis mellifera caucasia*) korunması için gerekli tedbirlerin alınmasıyla birlikte hem ekonomik öneme sahip arı ırkından biri olan Kafkas Arı ırkı korunması sağlanmış olacak, hem de ülke ekonomisi katkı sağlayacaktır. Ancak bu önerilerin uygulanması konusunda resmi kurumlar, arı yetiştiricileri birlikleri ve arıcıların ortak çalışmaları kaçınılmazdır.

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Araştırmacıların Katkı Oranı Beyan Özeti

Yazarlar makaleye eşit oranda katkı sağlamış olduklarını beyan eder.

Çıkar Çatışması Beyanı

Makale yazarları aralarında herhangi bir çıkar çatışması olmadığını beyan ederler.

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Elazığ İlindeki Koyunculuk İşletmelerinde Sürü Yapısının ve Yetiştiricilik Uygulamalarının Araştırılması

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ÖZET

Bu araştırmanın amacı, Elazığ ilindeki işletmelerde koyun varlığının, sürü yapısının ve temel yetiştiricilik faaliyetlerinin durumunu belirlemektir. Araştırmada 167 adet koyunculuk işletmesinde Mayıs-Aralık 2020 tarihlerinde yetiştiricilerle yüz yüze anket gerçekleştirilmiştir. Çalışmada, işletmelerin %61.inin 7-17 baş koça sahip olması, %83.9'unda dişi tokluların damızlıkta ilk kez 12-17 aylıkken, %100'ünde erkek tokluların ilk kez 1.5-2 yaşındayken aşımda kullanılması, %71.7'sinin damızlıkları kendi işletmesinden sağlaması, %83.7'sinin koçları sürüde kızgınlık süresince tutması, %73.1'inin yeme tuz ve mineral ilavesi yapması, %77.7'sinin kadın ve erkek birlikte sağım yapması, %78.2'sinin sütü peynir yapması olumlu ve yeterli olarak belirlenmiştir. Bunun yanında, işletmelerin ancak %66.1'sında kayıtların tutulması, %90.4'ünde serbest aşım metodunun tercih edilmesi, %89.9'unda kuzulara doğal büyütme uygulanması, yetiştiricilerin %55.7'sinin koyunlarına kaba yem olarak sadece saman vermesi, %44.6'sının ek yemleme yapmaması, %78.4'ünün yemi fabrikalardan temin etmesi, %97.6'sının sağımı elle gerçekleştirmesi ve %89.3'ünün kırkımı makasla yapması ise mevcut olumsuzluklar ve yetersizlikler olarak saptanmıştır. Sonuç olarak, koyun sayısı ve yetiştiricilik imkânları bakımından önemli bir potansiyeli olan Elazığ ilinde, işletmelerdeki yeterli-olumlu yetiştiricilik faaliyetlerinin sürdürülmesi yanında, belirlenmiş yetersizliklerin ve olumsuzlukların giderilmesiyle gelir ve ekonomik kazancın artırılabileceği kanaatine varılmıştır.

Zootekni

Araştırma Makalesi

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Anahtar Kelimeler
Besleme
Çiftlik
Koyun
Verim
Yetiştiricilik

Investigation of Herd Structure and Breeding Practices in Sheep Farms in Elazig Province

ABSTRACT

The aim of this research was to determine the status of sheep existence, herd structure, and basic breeding activities in farms in Elazig province. In the research, a face-to-face survey was conducted with breeders in 167 sheep farms between May-December 2020. In the study, it was determined that 61.7% of the farms had7-17 rams, 83.9% of breeders used female yearling lamb for first breeding when they were 12-17 months old, 100% used male yearling lamb for the first insemination when they were 1.5-2 years old, 71.7% of the breeders from their own farms, 83.7% of the rams kept the herd during estrus, 73.1% used salt and mineral addition for food, 77.7% of women and men milking together, 78.2% of them evaluated milk positive and sufficient as long as it wasusable for cheese-making. In addition, only 66.1% of the farms kept records, 90.4% preferred the free-breeding method, 89.9% applied natural rearing to the lambs, 55.7% of the breeders gave only straw to their sheep as roughage, 44.6% of them supplemented feeding. The existing problems and inadequacies were determined as the fact that 78.4% of them procure

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Yield
Breeding

the feed from the factories, 97.6% of them perform the milking by hand and 89.3% of them do the shearing with shears. As a result, it has been concluded that income and economic gain can be increased by eliminating the identified deficiencies and negativities, as well as maintaining adequate-positive breeding activities in the province of Elazig, which has an important potential in terms of sheep number and breeding opportunities..

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GİRİŞ -

Türkiye'nin iklimi, mevcut arazi varlığı, yapısı ve bitki örtüsü gibi doğal koşulları, ayrıca ekonomik, tarımsal yapısı ve gelenekleri koyun yetiştiriciliğinin daha yaygın olarak yapılmasına ve hayvancılık faaliyetleri içerisinde önemli bir yer tutmasına neden olmaktadır (Karaca ve ark., 2003). Koyun yetiştiriciliği kırmızı et ve süt gibi hayvansal protein açığını kapatacak potansiyel üretim kaynaklarından birisi olması ve yapağı gibi birçok ürünün elde edildiği bir faaliyet alanı olmasından dolayı ekonomik bakımdan büyük değer taşımaktadır. Ayrıca, daha az sermaye ve yatırım gerektirmesi, potansiyel işgücünün değerlendirilmesindeki payı bakımından da oldukça önemli bir yere sahiptir (Şahinli, 2011; Karaman ve ark., 2012). Ülkemizde koyun yetiştiriciliğinin sosyo-ekonomik hayattaki yerinin vazgeçilmez olduğu, ancak daha karlı ve daha etkin şekilde sürdürülmesi gerektiği değerlendirilmektedir (Şahinli, 2011; Kaymakçı, 2013). Çünkü, Türkiye'de koyun yetiştiriciliği genellikle küçük aile işletmeleri şeklinde ve mera odaklı gerçekleştirildiğinden koyunculuktan sağlanan gelirler oldukça düşük düzeylerde olmaktadır (Kaymakçı ve Sönmez, 1996). Yine, Türkiye'de koyun yetiştiriciliğinin yoğun olarak ekstansif özelliklere sahip işletmelerde yapılması ve modern yetiştiricilik uygulamalarından yoksun şekilde gerçekleştirilmesi, verimli ve kârlı bir üretimin yapılabilmesini olumsuz yönde etkilemektedir. Koyun yetiştiriciliği sayesinde elde edilen gelirin, Türkiye ekonomisine olan katkısını daha yüksek düzeylere çıkarmak amacıyla, koyun yetiştiriciliğinin daha cazip, daha kârlı ve sürdürülebilir bir yetiştiriciliğe dönüştürülmesi gerekmektedir (Aydın ve Dellal, 2001).

Günümüzde, tarımsal üretim alanında kendine yeterlilik ve gıda güvencesi konusu Türkiye için de hala önemli bir sorun olmaya devam etmektedir. Bu sorunun çözümüne katkı sunması bakımından özellikle bölgelerin potansiyellerinin belirlenmesi ve değerlendirilmesi son derece önemlidir (Eren ve Gökten, 2018).

Elazığ ekonomisi ağırlıklı olarak tarım ve hayvancılığa dayalı olup, koyunculuk sektörü her geçen gün gelişmekte ve önem kazanmaktadır (Şeker ve Köseman, 2015). Elazığ ilinin 2020 yılındaki koyun varlığı 712.678 baş olup, aynı yıl 42.126.781 baş olan Türkiye koyun varlığının yaklaşık %1.69'unu oluşturmaktadır (Anonim, 2022a).

Koyunculuğun gelişmesi ve arzu edilen seviyeye kavuşabilmesi için mevcut durumun bilinmesi ve sorunların tespit edilmesi gerekmektedir. Bu alanda yapılacak her bilimsel çalışma bu nedenle değer taşımaktadır. Yapılan literatür incelemelerinde ise Elazığ ilindeki koyunculuk işletmelerinde yetiştiricilik uygulamalarının belirlenmesini içeren bir çalışmaya rastlanmamıştır. Bu bağlamda mevcut araştırma, Elazığ İlinin koyun varlığına ait kompozisyonu belirlemek, yetiştirme, bakım, besleme, sağım ve kırkım özellikleri bakımından bu ildeki koyunculuk yapısını ortaya koymak ve bu verilerden hareketle Elazığ koyunculuğu ile ilgili neler yapılması gerektiğini değerlendirmek amacıyla yürütülmüştür.

MATERYAL ve METOD

Materyal

Bu araştırmanın materyalini, Elazığ İli Damızlık Koyun Keçi Yetiştiriciler Birliği'ne üye olup, Elazığ ili ve ilçelerinde faaliyet yürüten koyunculuk işletmelerindeki yetiştiricilerle Mayıs-Aralık 2020 tarihlerinde gönüllülük esasına dayalı yüz yüze yapılan anket uygulamasından elde edilen veriler oluşturmuştur.

Metot

Araştırma için önceden belirlenen işletmelerin sahipleriyle ön görüşmeler yapılmış ve gönüllülük esasına dayalı yüz yüze görüşmeyi kabul edenlerin işletmeleri bir takvime bağlı olarak araştırma ekibi tarafından ziyaret edilmiştir. Bu ziyaretler sırasında yetiştiricilere anket uygulanmış, elde edilen veriler kayıt altına alınmıştır.

Bu çalışmanın yürütülmesi için gerekli olan etik onay belgesi, Fırat Üniversitesi Girişimsel Olmayan

Araştırmalar Etik Kurul'undan alınmıştır (29.4.2020 tarih ve 2020/07-24 sayılı izin).

Örnekleme Yöntemi

Araştırmada, öncelikle Elazığ ilinin en fazla hayvan sayısına ve işletmesine sahip ilçeleri Elazığ İl Tarım ve Orman Müdürlüğü'nden edilen veriler yardımıyla belirlenmiştir. Bu kapsamda Merkez, Kovancılar, Karakoçan, Palu, Sivrice, Baskil, Keban ilçeleri öne çıkmıştır. Bu ilçelerde araştırmaya dâhil edilecek işletmelerin seçiminde öncelikle Elazığ Damızlık Koyun Keçi Yetiştiriciler Birliği'ne üyeliği bulunması koşulu aranmış olup, bu koşulu sağlayan işletmeler arasından tesadüfi örnekleme metodu ile işletmeler seçilmiştir. Araştırmada kullanılan anketin güvenilirliğini ve geçerliliğini yükseltmek için deneme amaçlı olarak bazı işletmelerde ön çalışmalar yürütülmüş, bu çalışmalara göre araştırmadaki anket sorularına son şekilleri verilmiştir. Bu araştırmada sahada uygulanan ankete ait sorular benzer çalışmalardaki (Bilginturan ve Ayhan, 2009; Altınçekiç, 2014; Ayvazoğlu Demir ve ark., 2015; Ceyhan ve ark., 2015; Tüfekçi, 2020) anketlerden yararlanarak, araştırma ekibi tarafından oluşturulmuştur.

İstatistik Analizler

Benzer araştırmalarda popülasyonu en iyi düzeyde temsil edebilecek nitelikte örnek büyüklüğünün belirlenmesinde, popülasyonun %3'ü (Yamane, 2010) ila %10'unun (Cochran, 1997; Sümbüloğlu ve Sümbüloğlu, 2000) alınmasının yeterli olacağı kaydedilmiştir. Bilimsel araştırmalarda örnek büyüklüğü arttıkça ilgili popülasyonu temsil gücü de artmaktadır. Bundan dolayı mevcut çalışma sonuçlarını daha güvenli kılmak için birliğin aktif işletme sayısı olan 1669 işletmeden oluşan popülasyonun en az %10'unun örneğe dahil edilmesi kararlaştırılmıştır. Bu kapsamda 167 işletme araştırmaya dâhil edilmiştir. Anketlerdeki sorulara verilen cevaplar analiz edilmiş, SPSS istatistik paket programı kullanılarak tanımlayıcı istatistikler hesaplanmıştır (SPSS, 2015).

BULGULAR ve TARTIŞMA

Koyunculuk İşletmelerinin Hayvan Varlığı ve Sürü Yapısı

Elazığ koyunculuk işletmelerinin hayvan varlığı ve sürü yapısına ait araştırma bulguları Çizelge 1'de verilmiştir.

Araştırmada, yetiştiricilerin çoğunun (%66.1) işletme kayıtlarını tuttukları belirlenmiştir. Gaziantep'te yapılan bir araştırmada yetiştiricilerin %40.7'sinin kayıt tuttuğu tespit edilmiştir (Gül ve Örnek, 2018). Bulgulara göre Elazığ ilindeki işletmelerde kayıt tutma oranı Gaziantep'teki işletmelerden daha yüksektir. Hayvancılık işletmelerinin tümünde, kayıt tutulması yüksek verim elde edilebilmesi ve dolayısıyla kârlılığın artırılması için son derece önemlidir. Zira kayıtlar öncelikle işletmelerdeki mevcut durumun tespitine ve sonrasında da işletmelerin geleceklerini doğru şekilde planlamalarına katkı sunmaktadır (Gökçen, 2016). Elazığ'da işletmelerin çoğunda kayıt tutulduğu belirlenmiş olmasına rağmen, modern yetiştiricilik gereklilikleri kapsamında bu durumun yine de yetersiz olduğu değerlendirilmektedir. Tüm işletmelerde kayıt tutulmasının gerekli ve önemli olduğunun yetiştiricilere aktarılması, benimsetilmesi ve teşvik edilmesinin yararlı olacağı düşünülmektedir.

Yapılan çalışmaya göre işletmelerin büyük kısmı 100-299 baş koyun (%63.5) ve anaç koyun (%61.7) ile 7-17 baş (%61.7) koç varlığına sahiptirler. Bünyesinde 50 baştan az dişi kuzu (%71.4) ve 50 baştan az erkek kuzu (%62.1) ile 30 baştan az erkek toklu (%82.0) ve 60 baştan az dişi toklu (%69.2) bulunan işletmeler de en yüksek orana sahiptirler. Muğla'daki işletmelerin en yüksek anaç koyun sayıları 101-200 baş, koç sayıları ise 1-3 baş (Aydın ve Keskin, 2018), Niğde'deki sürülerin ortalama koyun sayıları 315 baş ve koç sayıları ise 12.6 baş Ceyhan ve ark., (2015), Ardahan'daki işletmelerde ortalama koyun sayısı 74 baş (Ayvazoğlu Demir ve ark., 2015) olarak bildirilmiştir. Buna göre Elazığ'daki sürü büyüklüklerinin Niğde ilindekilerden daha küçük, Muğla ve Ardahan ilindekilerden ise daha büyük olduğu söylenebilir. Şeker ve ark., (2021) tarafından Elazığ'a komşu ve benzer çevresel koşullara sahip olan Malatya ilinde yapılan bir araştırmada, 100-299 baş koyun ve anaç koyun, 6 ve daha az sayıda koç, 50 baştan az dişi ve erkek kuzu, 30 baştan az erkek toklu ve 60 baştan az dişi toklu sahibi olanlar en yüksek oranlara sahip işletmeler olarak tespit edilmiş, bu işletmelerin oranları aynı sırayla %65.11, %60.11, %65.17, %83.15, %87.07, %81.46 ve %57.87 olarak tespit edilmiştir. Araştırmada belirlenen, 100-299 baş koyun ve anaç koyun ile 30 baştan az erkek toklu oranları, Şeker ve ark. (2021) tarafından saptanan oranlarla benzerlik göstermektedir. Buna karşın, 60 baştan az dişi toklu sahibi olanlar Malatya'daki işletmelerden daha yüksek orana sahiptirler. Koç varlığı bakımından ise Elazığ'daki işletmelerde daha fazla sayıda ve daha yüksek oranda olmak üzere her iki il arasında farklılık bulunmaktadır. İşletmelerde her yetiştirme döneminde sürünün ortalama %20-25'inin yenilenmesi söz konusu olduğundan, sürüdeki anaç koyun sayısı ve dişi toklu sayısı ve oranı sürünün mevcut büyüklüğünün korunması ve gerektiğinde sürü büyüklüğünün artırılması için son derece önemlidir. Özellikle işletmelerin ihtiyaç duyulan damızlık hayvanları kendi sürülerinden temin

edilebilmesi, karlılığı ve başarıyı artırmaktadır. Elazığ ilindeki işletmeler için tespit edilen koyun varlığı ve sürü yapısının bu çerçevede değerlendirilmesinin daha doğru ve anlamlı olacağı düşünülmektedir.

Çizelge 1. Elazığ koyunculuk işletmelerinin hayvan varlığı ve sürü yapısı
Table 1. Animal stock and herd structure of Elazig sheep farms

		n	%
İşletme kayıtlarının tutulması			
Tutuluyor		109	66.1
Tutulmuyor		56	33.9
	Toplam	165	100.0
Sürü büyüklüğü			
100 baştan az		1	0.6
100 – 299 baş		106	63.5
300 - 499 baş		38	22.7
500 baş ve daha fazla		22	13.2
	Toplam	167	100.0
Anaç koyun sayısı			
100 baştan az		26	19,5
100 – 299 baş		82	61.7
300 - 499 baş		18	13.5
500 baş ve daha fazla		7	5.3
	Toplam	133	100.0
Koç sayısı			
6 baş ve daha az		28	21.9
7-15 baş		79	61.7
16 baş ve daha fazla		21	16.4
	Toplam	128	100.0
Dişi kuzu sayısı			
50 baştan az		60	71.4
50 baş ve daha fazla		24	28.6
	Toplam	84	100.0
Erkek kuzu sayısı			
50 baştan az		41	62.1
50 baş ve daha fazla		25	37.9
	Toplam	66	100.0
Erkek toklu sayısı			
30 baştan az		105	82.0
30 baş ve daha fazla		23	18.0
	Toplam	128	100.0
Dişi toklu sayısı			
60 baştan az		90	69.2
60-100 baş		32	24.6
100 baştan fazla		8	6.2
	Toplam	130	100.0

Koyunculuk İşletmelerinde Hayvan Yetiştirme-Üretim Uygulamaları

Elazığ koyunculuk işletmelerinin hayvan yetiştirme-üretim uygulamalarına ait araştırma bulguları Çizelge 2'de verilmiştir.

Araştırma bulgularına göre işletmelerin çok büyük bir kısmında yetiştirmede serbest aşım tercih edilmekte (%90.4), koçlar sürüde en fazla kızgınlık süresince (%83.7) bırakılmaktadır. Burdur'daki koyunculuk işletmelerinde de (%99.5) serbest aşım yapılmaktadır (Bilginturan ve Ayhan, 2009). Bu araştırmada tespit edilen, işletmelerde serbest aşım yönteminin çok yüksek oranda tercih edilmesi durumu, Elazığ ve Burdur'daki işletmeler bakımından benzerlik içermektedir. Serbest aşım yönteminin tercih edilmesinin işletmeler açısından olumsuz yönleri fazla olmaktadır. Bu yöntemde kızgınlık gösteren koyunlar herhangi bir koç tarafından aşılır. Her koçun aştığı hayvan sayısı da değişiktir. Koçlardan birisi önder durumuna geçer ve daha fazla koyun aşar. Diğer koçlarda önder koyunun

aşamadığı koyunları aşarlar. Koçlar başlangıçta çok aşım yaptıkları için koç katım süresi sonlarına doğru kızgınlık gösteren koyunlarda döl tutmama olabilir. Bu nedenle serbest aşımda diğer yöntemlere göre daha fazla koça ihtiyaç vardır. Bu dezavantajlar nedeniyle uygun sürülerde daha nitelikli diğer aşım yöntemlerinin tercih edilmesi önerilmektedir. Bir araştırmada işletmedeki koçların %61.5'nin yalnızca aşım sezonu boyunca sürüde bırakıldığı kaydedilmiştir (Tüfekçi, 2020). Yetiştiricilikte sürüdeki koçların aşım sezonu dışındaki zamanlarda da sürekli koyunlarla bir arada tutulması uygun değildir. Çünkü, koçların koyunlar rahatsız etmeleri ve zarar vermeleri durumu söz konusu olabilmektedir. Elazığ'daki işletmelerde koçların yıl boyunca sürüde tutulmasına ait oran Yozgat'tan daha düşüktür. Bu durum olumlu olarak değerlendirilmektedir.

Çizelge 2. Elazığ koyunculuk işletmelerinde hayvan yetiştirme uygulamaları
Table 2. Animal breeding practices in Elazig sheep farms

		n	%
Koç katım şekli			
Serbest		151	90.4
Sınıf usulü		7	4.2
Elde		9	5.4
	Toplam	167	100.0
Koçları sürüde tutma süresi			
Koyunların kızgınlığı süresince		139	83.7
Devamlı-tüm yıl		27	16.3
	Toplam	166	100.0
Koçları damızlıkta kullanma süresi			
4 yıl ve daha az		33	20.0
5-6 yıl		90	54.5
7 yıl ve daha fazla		42	25.5
	Toplam	165	100.0
Sürü dışından koç kullanılması			
Kullanılıyor		23	13.8
Kullanılmıyor		144	86.2
	Toplam	167	100.0
Koyunları damızlıkta kullanma süresi			
6 yıla kadar		93	56.3
7-8 yıl		58	35.2
9 yıl ve daha fazla		14	8.5
	Toplam	165	100.0
Damızlık fazlası koyunların değerlendirilme şekli			
Besi amaçlı satış		132	79.0
Damızlık olarak satış		9	5.4
Kendisi besiye alma		26	15.6
	Toplam	167	100.0
Damızlıkların temin yeri			
Kendi işletmesi		119	71.7
Komşu işletmeler		41	24.7
Devlet işletmeleri		2	1.2
Çevre pazarlar		4	2.4
	Toplam	166	100.0

Mevcut araştırmaya göre koçlar en fazla 5-6 yıl (%54.5), koyunlar ise en fazla 6 yıla kadar (%56.3) damızlıkta kullanılmaktadır. Yetiştiricilerin çoğu ihtiyaç fazlası damızlık koyunları ya besi için satmakta (%79.0) ya da bu hayvanlarla kendileri besicilik yapmaktadır (%15.6). Yozgat ilindeki işletmelerde koyunları damızlıkta kullanma süresinin dişilerde ortalama 4-6 yıl ve erkeklerde 2-3 yıl olduğu bildirilmiştir (Tüfekçi, 2020). Ceyhan ve ark. (2015) Niğde ilindeki koyunculuk işletmelerinde dişileri ortalama 6 yıl ve erkekleri ise 4 yıl kadar damızlıkta kullandıkları tespit edilmiştir. Damızlık hayvanların işletmede kullanım süreleri de yetiştiricilikte önemli bir husustur. Koyun yetiştiriciliğinde, genel olarak etçi koyun ırklarının 5-6, sütçü koyun ırklarının ise 7-8 yaşına kadar damızlıkta kullanıldığı bildirilmektedir (Akçapınar, 2000). Bu bilgiler doğrultusunda mevcut araştırmada tespit edildiği

üzere, Elazığ'daki işletmelerin çoğunda damızlık dişi ve erkek hayvanların uygun sürelerde sürülerde tutuldukları anlaşılmaktadır. Niğde ve Yozgat'taki işletmelerde damızlıkların sürüde tutulma süreleri ise Elazığ'daki işletmelerden daha kısadır.

Yapılan araştırmada, kendi işletmesinden damızlık koç sağlayanların oranı (%86.2, Şeker ve ark. (2021) tarafından Malatya'da yapılan çalışmada belirlenenden (%74.3) daha yüksek, ancak Aydın ve Keskin (2018) tarafından Muğla'da yapılan araştırmada tespit edilenden ise (%96.0) daha düşüktür. Karaca ve ark. (1993) Doğu Anadolu Bölgesinde çoğunlukla her yetiştiricinin kendi koçunu kullanma eğiliminde olduğunu belirtmişlerdir. İşletmelerin dışarıdan damızlık sağlaması gereken durumlarda öncelikle damızlık işletmelerinden veya iyi damızlıklara sahip kayıtlı ve güvenilir işletmelerden temin edilmelidir. Ancak asıl yapılması gereken işletmelerin dişi ve erkek damızlık ihtiyaçlarını kendi sürülerinden karşılamalarıdır. Bu nedenle Elazığ'da işletmelerin bu konuda genel olarak uygun bir yönetim sergiledikleri düşünülmektedir.

Koyunculuk İşletmelerinin Kuzu ve Toklularla İlgili Faaliyetleri

Elazığ koyunculuk işletmelerinin kuzu ve toklularla ilgili sürü yönetim faaliyetlerine ait araştırma bulguları Çizelge 3'te verilmiştir.

Bu çalışmada, Elazığ ilindeki işletmelerin çok büyük bir kısmında kuzulara doğal büyütme uygulandığı (%89.9) ve kuzuların en fazla 5 aylıkken (%32.3) ya da 6 aylıkken (%29.8) sütten kesildiği belirlenmiştir. Malatya'daki koyunculuk işletmelerinde yapılan araştırmada ise kuzulara %96.8 oranında doğal büyütme uygulandığı, kuzuların en fazla %48.9 oranında 3 aylıkken ve %19.2 oranında 4 aylıkken sütten kesildiği bildirilmiştir (Şeker ve ark., 2021). Elazığ'daki işletmelerde kuzulara en fazla doğal büyütme yöntemi uygulandığına ait tespit edilen bulgu, Türkiye'nin bir çok ilinde geleneksel yetiştiriciliğin daha fazla öne çıktığını bildiren diğer araştırma bulgularıyla (Şeker ve ark., 2021; Bilginturan ve Ayhan, 2009) benzerlik göstermektedir. Ancak sürü özelliklerine göre oluşturulacak diğer kuzu büyütme yöntemlerinin de tercih edilmesi daha nitelikli bir koyunculuk faaliyeti için gerekmektedir. Kuzuları sütten kesim zamanı bakımından ise Elazığ ve Malatya'daki işletmeler arasında farklılık görülmektedir. Araştırma bulgularına göre, Elazığ'daki işletmelerde kuzular Malatya'daki işletmelere göre daha geç sütten kesilmektedirler. Bu durum, Elazığ'daki işletmeleri kuzuların ana sütünden daha fazla yararlanması bakımından öne çıkartmakla birlikte, tüketicilerin tercihleri açısından çok önemli bir yeri olan peynir ve yoğurt üretiminde kullanılan koyun sütünün ekonomik getirileri noktasında, Malatya'daki işletmeler gibi kuzuları daha erken dönemde sütten kesen işletmelere kıyasla daha dezavantajlı hale getirmektedir.

Çizelge 3. Elazığ koyunculuk işletmelerinde kuzu ve toklularla ilgili sürü yönetim faaliyetleri
Table 3. Herd management activities related to lambs and yearling lambs in Elazig sheep farms

		n	%
Kuzu büyütme yöntemi			
Doğal		134	89.9
Erken sütten kesme		15	10.1
	Toplam	149	100.0
Kuzuları sütten kesim yaşı (ay)			
2		12	7.5
3		31	19.2
4		18	11.2
5		52	32.3
6		48	29.8
	Toplam	161	100.0
Dişi tokluları ilk tohumlatma yaşı			
12-17 ay		135	83.9
18 ay ve daha fazla		26	16.1
	Toplam	161	100.0
Erkek tokluları damızlıkta ilk kullanma yaşı			
1.5-2 yıl		162	100.0
2 yıldan daha fazla		0	0.0
	Toplam	162	100.0

Araştırmaya göre dişi toklular ilk kez 12-17 aylık yaşta (%83.9) tohumlatılmakta, erkek toklular ise ilk kez 1,5-2 yaşındayken (%100) damızlıkta kullanılmaktadır. Malatya'daki işletmelerde dişi

tokluların ilk tohumlatılması en fazla, 24 ay ve üzeri yaşta (%48.64), erkek tokluların damızlıkta ilk kullanılması ise en fazla, 2 yaşındayken (%80.75) gerçekleştirilmektedir (Şeker ve ark., 2021). Niğde ilindeki işletmelerde koyunları damızlıkta ilk kullanma yaşı dişilerde 17.8 ay iken erkeklerde 18.2 ay olarak tespit edilmiştir (Ceyhan ve ark., 2015). Sürüdeki damızlık adayı hayvanların normalden daha geç veya erken damızlığa alınması sürü yönetim problemlerine neden olduğu gibi ekonomik kayıplara da yol açmaktadır. Elazığ'daki işletmelerde dişi ve erkek tokluların damızlıkta ilk kullanılma yaşı Malatya'daki ve Niğde'de deki işletmelerden daha düşüktür. Elazığ'daki işletmelerde dişi ve erkeklerde damızlıkta ilk kullanım yaşlarının uygun sınırlarda olduğu düşünülmektedir.

Koyunculuk İşletmelerinde Hayvan Besleme Uygulamaları

Mevcut çalışmada, Elazığ koyunculuk işletmelerinin hayvan besleme uygulamalarına ait bulgular Çizelge 4'te verilmiştir.

Çizelge 4. Elazığ koyunculuk işletmelerinde koyunların beslenmesine ait uygulamalar
Table 4. Applications of sheep feeding in Elazig sheep farms

		n	%
Çobanlık hizmetinin kaynağı			
Kendimiz çobanlık yapıyoruz		110	65.9
Çoban tutuyoruz		57	34.1
	Toplam	167	100.0
Koyunların yıl içinde beslenme yerleri ve süreleri			
Köy (12 ay)		14	9.2
Köy - mera (6 ay-6 ay)		51	33.3
Köy - yayla (7 ay-5 ay)		88	57.5
	Toplam	153	100.0
Koyunlara en çok yedirilen kesif yem			
Arpa		101	60.8
Karma yem		65	39.2
	Toplam	166	100.0
Koyunlara en çok yedirilen kaba yem			
Saman		93	55.7
Kuru ot		47	28.1
Yonca		27	16.2
	Toplam	167	100.0
Yeme tuz ve mineral ilavesi			
Yapılıyor		122	73.1
Yapılmıyor		45	26.9
	Toplam	167	100.0
Koyunlara merada ek yemleme yapılması			
Yapılıyor		74	44.6
Yapılmıyor		92	55.4
	Toplam	166	100.0
Yemi temin yeri			
İşletmenin kendi imkânlarından		22	13.2
Fabrikalardan		131	78.4
Çevre işletmelerden		14	8.4
	Toplam	167	100.0
Kaba ve kesif yemliklerin ayrı veya birlikte kullanılması			
Ayrı yemlikler kullanılıyor		65	39.2
Aynı yemlik kullanılıyor		101	60.8
	Toplam	166	100.0

Araştırmada tespit edilen sürü yönetim işini yani çobanlığı kendisi yapan yetiştiricilerin oranı, Malatya'daki (%84.97) (Şeker ve ark., 2021) ve Isparta'daki işletmelerde (%93.94) (Acar ve Ayhan, 2012) tespit oranlardan daha düşüktür.. Türkiye genelinde çoban bulmadaki zorluklar ve yüksek çoban ücretleri dikkate alındığında, Elazığ'da çobanlık hizmetinin büyük oranda bizzat yetiştiricilerin kendileri tarafından gerçekleştiriliyor olması, olumlu bir durum olarak değerlendirilmektedir.

Çalışmada yetiştiricilerin ortalama olarak 7 ay köyde - 5 ay yaylada (%57.5) ve ortalama olarak 6 ay köyde 6 ay merada (%33.3) olacak biçimde faaliyet yaptıkları belirlenmiştir, Ceyhan ve ark. (2015) tarafından Niğde ilinde yapılan bir araştırmada yerleşik ve yayla koyunculuğu yapanların oranı %19.8, Çınar ve Ceyhan (2021) tarafından aynı ilde yapılan başka bir çalışmada kışlak-yaylak şeklinde koyunculuk yapanların oranı ise %26.4 olarak belirlenmiştir. Elazığ'da yerleşik ve yayla koyunculuğu yapanların oranı Niğde ilinde yerleşik ve yayla koyunculuğu yapanlardan daha yüksektir. Daha düşüklü rakımlı olan yerleşkelerde havaların ısınması ve sıcak mevsimlerde yayla koşullarının koyunlar için daha elverişli olması nedeniyle yetiştiriciler koyunlarıyla birlikte yaylalara göç etmektedirler. Elazığ ilinde toplam alanının çoğunu platolar oluşturmaktadır. Platolara Elazığ'ın kuzeyinde Harput çevresinde, Murat Nehrinin kuzey kesimlerinde ve Ağın yöresinde rastlanmaktadır. Hayvancılık faaliyetlerinin yoğunluk kazandığı alanlar, İlin doğusunda Bingöl ile sınır oluşturan Karaboğa Dağlarında Gökdere ve Akdağ üzerindedir. Urfa yöresinde kışlayan göçerler, mayıs sonu ve haziran ayı başlarında Siverek ve Ergani üzerinden Palu çevresine gelirler. Bir bölümü yöredeki yaylalarda kalır, bir bölümü ise Bingöl dağlarındaki yaylalara göçerler (Anonim, 2022b). Ağın ilçesindeki yetiştiriciler Sivas'ın Divriği ilçesinde bulunan yaylalara (Sarıçiçek Yaylası) gitmektedirler. Havalar soğumaya başladığında ise tekrar köylere dönüş yapılmaktadır.

Bu araştırma bulgularına göre işletmelerin büyük bir kısmında koyunlara kesif yem olarak arpa (%60.8), kaba yem olarak ise en fazla saman (%55.7) yedirilmekte, yeme tuz ve mineral ilavesi yapılmaktadır (%73.1). İşletmelerin yarıdan fazlasında (%55.4) ise merada koyunlara ek yemleme yapılmamaktadır, Malatya'da ise koyunların beslenmesinde arpa (%79.5) ve saman (%80.4) tercih edilmekte, yeme tuz ve mineral ilavesi yüksek oranda (%90.9), ek yemlemenin ise düşük oranda (%9.1) yapıldığı bildirilmiştir (Şeker ve ark., 2021). Ardahan'da mera döneminde mera dışında ek besleme yapanların oranı %48.5 olarak tespit edilmiştir (Ayvazoğlu Demir ve ark., 2015). Niğde'de yetiştiricilerin %75.0'i koç katım öncesinde koçlara, %89.6'u ise gebelik döneminde dişilere ek yemleme yapmaktadır (Ceyhan ve ark., 2015). Elazığ'daki işletmelerde koyunlara daha fazla karma yem, kuru ot ve yonca yedirilmesi yanında daha fazla ek yemleme yapılması olumlu bir uygulama olarak değerlendirilmektedir. Ancak yeme tuz ve mineral katılmasına ait uygulamaların Elazığ'daki işletmelerde geliştirilmesine ihtiyaç vardır. Merada koyunlara ek yemleme yapanların oranı ise Ardahan'daki işletmelerle benzer olup, Niğde'deki işletmelerden daha düşüktür. Ek yemlemenin maliyet artışı getirmesi söz konusu olmakla birlikte, koyunlardan hem mevcut yetiştirme döneminde hem de gelecek yıllardaki yetiştirme dönemlerinde daha iyi verim ve kazanç elde edilebilmesi için bu uygulamanın yapılması tavsiye edilmektedir. Bu hususta yetiştiricilerin teşvik edilmesi ve bilinçlendirilmesinin yararlı olacağı düşünülmektedir.

Araştırmaya göre, işletmeler ihtiyaç duydukları yemi çoğunlukla fabrikalardan (%78.4) ve kısmen kendi imkânlarından (%13.2) sağlamaktadırlar. Yozgat'taki yetiştiriciler kaba yemi kendi işletmelerinden (%76.5), kesif yemi ise kendi işletmeleri ya da yem fabrikalarından (%77.5) (Tüfekçi, 2020), Malatya'daki işletmelerin yarısına yakını (%45.70) yemi kendi işletme imkânlarından sağlamaktadır (Şeker ve ark., 2021). Elazığ'da, işletmelerde ihtiyaç duyulan yemi fabrikalardan karşılayanların oranı Yozgat'taki işletmelerle benzer, ancak Malatya'daki işletmelerden yüksektir. Ekonomik bir yetiştiricilik yapılabilmesi için Elazığ'daki işletmelerin yemi daha yüksek oranda kendi işletme imkânlarından karşılamalarının gerektiği düşünülmektedir.

Koyunculuk İşletmelerinde Sağım ve Kırkım Uygulamaları

Elazığ koyunculuk işletmelerinin sağım ve kırkım uygulamalarına ait bulgular Çizelge 5'te verilmiştir.

Yapılan araştırmada, sağımın en fazla kadın ve erkek sağımcılar tarafından birlikte (%77.7), elle (%97.6) ve avluda (%74.9) yapıldığı belirlenen işletmelerde, sağılan sütler en fazla evde veya süt odası olarak kullanılan ayrı bir yerde muhafaza edilmekte (%98.2) ve elde edilen süt çoğunlukla peynir yapımında (%78.2) kullanılmaktadır.

Kadın ve erkekler tarafından sağımın birlikte yapılmasına ait Elazığ'da tespit edilen oran, Malatya'daki (%47.85) (Şeker ve ark., 2021) ve Niğde'deki (%43.4) (Ceyhan ve ark., 2015) araştırmalarda saptananlardan daha yüksektir. Elle yapılan sağıma ait Elazığ'da saptanan oran ise Niğde (%92.0) (Çınar ve Ceyhan, 2021) koyunculuk işletmelerinde tespit edilenlere yakın, Çanakkale'nin Gökçeada ilçesindeki (%52.8) (Özsayın ve Everest, 2019) işletmelerden daha yüksektir. Elazığ'da sağım yapılacak koyunların mera ve yayla şartlarında sağılmalarından, Niğde ve Konya illerindeki işletmelerde ise sağım yapılan koyunların oranı düşük olduğundan (Niğde; %16.6, Konya; %30.0) elle sağımın makinalı sağıma göre daha yüksek oranda gerçekleştiği düşünülmektedir. Teknolojik alt yapının yetersizliği yanında kültürel olarak makinalı sağımın özümsenememesi de bunda etkili olabilir. Sağılan sütlerin muhafaza ve değerlendirme şeklinin de kültür ve teknolojik alt yapı imkânları ile ilişkili olduğu düşünülmektedir. Elazığ ilindeki yetiştiriciler,

doğumdan itibaren 2-6 ay kuzu beslenmesinde yararlandıkları koyun sütlerinden genel olarak yöresel peynir yapmaktadırlar. Yörede en fazla üretilen peynir çeşidi ise tulum peyniridir. Ağırlıklı olarak köy ve yayla koyunculuğu yapılan Elazığ ilinde, köyde olunan dönemdeki sütler genel olarak kuzuların beslenmesi için kullanıldığından sağılmamaktadır. Yaylaya yapılan göçten ve kuzular sütten kesildikten sonra elde edilen sütler ise sabah ve akşam sağılarak belli bir miktara ulaşınca teleme yapılmaktadır. Aynı veya yakın ortamdaki yetiştiriciler ise sütün uygun olmayan koşullarda bekletilmesinden kaynaklı olumsuzluklardan korunması için sağılan sütlerini birleştirerek ortak ürün yapabilmektedirler. Yayla ve meralarla ilgili yol, su, elektrik ve barınak gibi temel sorunların giderilmesi, yetiştiricilerin eğitim, kültür ve bilgi alt yapısının geliştirilmesi sayesinde üretimin daha hijyenik ve güvenilir hale gelebileceği söylenebilir. Bu konunun sağlıklı ve kaliteli ürün üretimi ve tüketimi açısından çok önemli olduğu bilinmeli ve bu konuda ilgililerin üzerine düşen sorumlulukları yerine getirmeleri sağlanmalıdır.

Çizelge 5. Elazığ koyunculuk işletmelerinde sağım ve kırkım uygulamaları
Table 5. Milking and shearing practices in Elazig sheep farms

		n	%
Sağımı yapan			
Erkek		20	12.0
Kadın		17	10.3
Kadın- erkek birlikte		129	77.7
	Toplam	166	100.0
Sağım yöntemi			
Elle		162	97.6
Makine ile		4	2.4
	Toplam	166	100.0
Sağım yapılan yer			
Ağıl içinde		42	25.1
Avluda		125	74.9
	Toplam	167	100.0
Sağılan sütü muhafaza yeri			
Evde veya süt odası olarak kullanılan ayrı bir yerde		162	98.2
Soğutucu özellikli tank içinde		3	1.8
	Toplam	165	100.0
Sağılan sütü değerlendirilme şekli			
Peynir yapımı		129	78.2
Yoğurt yapımı		13	7.9
Süt olarak satış		23	13.9
	Toplam	165	100.0
Kırkım zamanı			
Mayıs		33	19.8
Haziran-Temmuz		128	76.6
Ağustos		6	3.6
	Toplam	167	100.0
Kırkım şekli			
Makasla		133	89.3
Makine ile		16	10.7
	Toplam	149	100.0

Yapılan araştırmaya göre, koyunlar en fazla haziran-temmuz aylarında (%76.6), makasla (%89.3) kırkılmaktadır. Niğde ilinde yapılan bir çalışmada, kırkım zamanının genellikle mayıs- haziran aylarında (%69.3) yapıldığı saptanmıştır (Çınar ve Ceyhan, 2021). İller ve bölgelerin kırkım zamanı farklılıkları üzerinde, iklim koşulları ile yetiştirilen ırklar ve yetiştirici kültürünün, kırkım şeklinin farklılığı üzerinde ise kültür ve teknolojik alt yapı imkânlarının etkili olabileceği düşünülmektedir.

Yapağı kalitesini artırmak ve koyunlarda makaslı kırkımdan kaynaklanabilecek enfeksiyon ve yaralanmaları azaltmak amacıyla, Elazığ İl Tarım ve Orman Müdürlüğü tarafından 9.900 ABD Doları bütçeli 2020 yılında bir proje yapılmış, proje kapsamında Elazığ Merkez ilçedeki 257 yetiştiricinin faydalanacağı 3 adet jeneratör ve 12 adet koyun kırkım makinası ve bıçakları ile satın alınmıştır

(Anonim, 2020). Bu tip projelerin ve desteklemelerin Elazığ Koyun ve Keçi Yetiştiricileri Birliğinin tüm üyelerini kapsayacak biçimde yaygınlaştırılması tavsiye edilmektedir.

SONUÇ ve ÖNERİLER

Elazığ ili, koyun varlığı ve yetiştiricilik imkânları bakımından önemli bir potansiyele sahiptir. Yetiştiricilik uygulamaları bakımından ise Türkiye hayvancılığının küçük bir modeli niteliğindedir. İlin güçlü noktaları öne çıkartılıp, eksik ve zayıf noktaları da iyileştirilerek koyun yetiştiriciliğinden elde edilen gelir, dolayısıyla kazanç artırılabilir. Bu kapsamda aşağıdaki öneriler sunulmuştur.

- Verimlilik ve kârlılık için gerekli bir unsur olan kayıt tutma alışkanlığının geliştirilmesi,
- Daha uygun ya da elit sürülerde serbest aşım dışındaki daha nitelikli diğer aşım yöntemlerinin yaygınlaştırılması,
- Koyun sütünden daha fazla yararlanılması için şartlara ve ırklara bağlı olarak kuzuların 5-6 aylıktan daha erken sütten kesilmesinin sağlanması,
- Kaba yem olarak koyunlara saman yerine kuru ot ve yonca gibi yemlerin yedirilmesinin teşvik edilmesi,
- Ekonomik bir yetiştiricilik yapılabilmesi için işletmelerin yemi kendi imkânlarından karşılaması,
- Sağılan sütün daha iyi koşullarda saklanması ve sonrasında daha sağlıklı ve kaliteli ürün haline dönüştürülebilmesi için özellikle yayla ve meralarda uygun koşulların oluşturulması,
- Makineli kırkımın yaygınlaştırılması için gerekli alet ve ekipman desteğinin sağlanması,
- Mera ve yaylaların yol, su, elektrik ve barınma alt yapısının oluşturulması ve yetiştiricilerin sosyal ihtiyaçları doğrultusunda mevcut imkânların iyileştirilmesi için bu konudaki mevzuatın günün koşullarına göre yeniden ele alınması.

Sonuç olarak, yukarıda sunulan çözüm önerilerinin, ilgili sektörün öncelikle Elazığ ili özelinde olmak üzere, ülke ihtiyaçlarına daha fazla katkı sunabilmesi ve daha güçlü bir yapıya kavuşabilmesi için ivedilikle pratiğe aktarılması ve yaygınlaştırılması önerilmektedir.

Araştırmacıların Katkı Oranı Beyan Özeti

Yazarlar makaleye eşit oranda katkı sağlamış olduklarını beyan eder.

Çıkar Çatışması Beyanı

Makale yazarları aralarında herhangi bir çıkar çatışması olmadığını beyan ederler.

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A Study on Structural Characteristics of the Cattle Barns in İspir County of Erzurum Province

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ABSTRACT
This study was carried out to determine the structural characteristics of the barns and related problems in the cattle enterprises in İspir county of Erzurum province. For this purpose, a face-to-face survey was conducted with 325 randomly selected enterprise owners. It was determined that 94.7% of the barns in the enterprises in the county consisted of tied free-stall barns. Furthermore, 33% of the barns were between 16 and 20 years old and 31.2% of them were older than 21 years. Stone (95.4%) and brick (66.2%) were commonly used as building materials in the construction of the barn walls, and mainly galvanized sheet metal (77.4%) was used for the roof. The barn floor was also determined to be mostly concrete (61.9%) or stone (48.2%). Some of the standard barn elements were available in almost all of the enterprises in the county, such as feeder (100.0%), urinary canal (85.3%), window (96.7%), however other elements such as the feeding alley (6.6%), automatic waterer (1.3%) and ventilation holes (22.8%) were available in a small number of enterprises. The relationship between the number of windows in the barns, the level of farm size and the level of education of the breeders was found to be statistically significant (P<0.01). The percentage of enterprises with two windows in their barns was the highest (48.2%) in the county, and this was followed by enterprises with three, four, one and five windows in the barn, respectively.

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Erzurum İli İspir İlçesindeki Sığır Barınaklarının Yapısal Özellikleri Üzerine Bir Araştırma

ÖZET
Bu çalışma, Erzurum ili İspir ilçesinde sığırcılık işletmelerinde bulunan barınakların yapısal özelliklerini ve bunlarla ilgili sorunları belirlemek amacıyla yürütülmüştür. Bu amaçla, şansa bağlı olarak seçilen 325 işletme sahibi ile yüz yüze anket yapılmıştır. İlçedeki işletmelerde mevcut sığır barınaklarının %94,7'sinin bağlı duraksız kapalı ahırlardan oluştuğu belirlenmiştir. Ahırların %33'ünün 16-20 yaşında olduğu, %31,2'sinin ise 21 yıldan daha fazla kullanıldığı tespit edilmiştir. Ahır duvarları inşasında yapı malzemesi olarak genellikle taş (%95,4) ve tuğladan (%66,2) yararlanıldığı, çatısında ise çoğunlukla galvaniz sac (%77,4) kullanıldığı saptanmıştır. Ahır zemininin ise büyük oranda beton (%61,9) veya taş (%48,2) olduğu tespit edilmiştir. Yemlik (%100,0), idrar yolu (%85,3), pencere (%96,7) gibi standart barınak elemanlarının işletmelerin büyük çoğunluğunda bulunduğu ancak yemlik yolu (%6,6), otomatik suluk (%1,3) ve havalandırma deliklerinin (22,8%) az sayıda işletmede bulunduğu tespit edilmiştir. Barınaklardaki pencere sayıları ile işletme büyüklüğü ve yetiştiricilerin öğrenim durumları arasındaki ilişki istatiksel olarak önemli (P<0,01) bulunmuştur. Ahırında iki pencere bulunan işletmelerin oranı ilçede en yüksek olup (%48,2) bunu ahırında sırasıyla üç, dört, bir ve beş pencere bulunan işletmeler takip etmiştir.

Zootekni

Araştırma Makalesi

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Anahtar Kelimeler
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INTRODUCTION

Although world population has been increasing geometrically in recent years, food producing has gone up arithmetically in the world. As a result of this, the world population grew faster than food production and tended to exceed it in a short time. Due to this fact, food shortages in many countries have become a significant threat to human beings (Özsağlıcak and Yanar, 2021). Cattle are one of the most significant farm animals that produce animal-sourced foods such as milk and meat. The main purpose of cattle raising is to obtain the highest possible yield at the minimum cost. This can only be possible if the animals in the farm are fed adequately and have the high genetic capacity. Another requirement for profitable cattle farming is to provide appropriate environmental conditions in the barn. The terms of environmental conditions cover all factors affecting the growth, development and yield of animals. These ones can be classified as climatic, structural, social and other factors. In barns, temperature, relative humidity, air movements, and lighting are climatic factors, and ventilation, insulation status of the barn, and equipment are called structural factors. While animal density, water supply and feeding practices are social factors and odor, atmospheric pressure, dust, presence of pathogenic microorganisms is considered as other factors (Avci, 2015).

The environmental requirements of cattle are determined and applied mainly on the basis of human needs when cattle breeders do not have enough information about animal physiology (Akman, 2003). Providing the environmental conditions suitable for humans (especially temperature) in the barn for cattle often leads to detrimental consequences. For this reason, it is highly important to reveal the structural conditions and environmental features of the cattle barns and to determine the deficiencies and malpractices in these barns. The most suitable barn types that can provide the optimum environmental conditions for the animals can be determined by studies to be carried out in different geographical regions of Türkiye. Therefore, some studies were conducted to reveal the characteristics of the barn in different regions of the country (Mundan et al., 2018; Ünlü, 2018; Alkan and Güney, 2019; Bakır and Kibar, 2019; Bakır and Kibar, 2020; Kılıç et al., 2020; Öcal, 2020; Yılmaz et al., 2020; Kaygısız and Özkan, 2021).

Erzurum province has an important place in terms of cattle breeding in Türkiye. The number of cattle in the province was 827,806 heads and 937847 tons of milk was produced from 315594 dairy cows in 2019 (TUİK, 2021). İspir county which is one of the 20 counties of Erzurum province, is located 143 km northwest of Erzurum city center at the intersection of North East Anatolia and Eastern Black Sea Region. The county is surrounded by high mountains from the north and south. Within the boundaries of the county, there are many mountains having altitudes between 2400 and 3900 meters. İspir county is located in a transition zone and the climate that prevails in the county is a transition climate between the continental climate and the maritime climate (Koçyiğit et al., 2022). Compared to other counties of Erzurum, the winter season is milder. However, the temperature differences between winter and summer and the day and night are quite high. Climatic characteristics show further differences along the Çoruh River, which passes through the borders of the county by forming a valley and a basin. The county is quiet suitable for animal husbandry due to its natural and geographical conditions. According to statistics data, the total number of cattle available in 2019 in the county was 21924, the number of dairy cattle was reported as 8263 and the total amount of milk produced was 24483 tons (TUIK, 2021).

Although studies were conducted to determine the structural characteristics of barns in cattle enterprises in Yakutiye (Çapadağ, 2016), Hınıs (Diler et al., 2016) and Narman (Diler et al., 2018) counties of Erzurum, no study was carried out in İspir county. Therefore, the present study was conducted in this region, which differs significantly from the central and southern counties of Erzurum in terms of climatic conditions to reveal deficiencies in terms of equipment and structural features of cattle barns and reveal concerning problems to suggest solutions.

MATERIAL and METHOD

The study has been approved by Atatürk University Faculty of Agriculture Ethics Committee Chairmanship and then was conducted on the owners of randomly selected dairy cattle enterprises in İspir county of Erzurum province. A face-to-face survey was conducted with 394 individuals, and data obtained from a questionnaire consisted of the material of the current research. After visiting the cattle enterprises, their current situation was investigated by observation along with survey questions. Since the population is limited in addition to the variance being unknown, there are qualitative variables dependent on probability, the formula given below was used to determine the sample size of the study as suggested by

Arıkan (2007).

$$n = \frac{N.t^2.p.q}{(N-1).D^2 + t^2.p.q}$$

In this formula;

n=Minimum number of samples, N=Population size, D=Acceptable or desired sampling error (5%), t=Table value (t=1.96 for α= 0.05), p=The rate to be calculated (0.5), q=1-p.

$$n = \frac{2107.(1.96)^2.0.5.(1-0.5)}{(2107-1).(0.05)^2+(1.96)^2.0.5.(1-0.5)} = 325$$

The estimated minimum sample size was found to be 325 using the formula given above. After calculating the minimum sample counts, the number of surveys increased by 21.23%. The final number of surveys to be carried out in the villages of the İspir county of Erzurum province was determined as 394. The data obtained from the survey work were transferred to Excel 2010 computer program prior to statistical analysis. Number of cattle in the farms were grouped as less than 11, 11-20, 21-30, 31-40 and more than 40 heads. Additionally, the educational status of the cattle breeders was grouped as illiterate, literate, primary school graduate, secondary school graduate and high school graduate. Chi-Square analysis available in SPSS statistics program were utilized to determine effects of the number of cattle in the farms and the educational status of the owners of the enterprises on the structural characteristics of barns in these enterprises (SPSS, 2011).

RESULTS and DISCUSSION

Barn Types

In the present study, it was revealed that 94.7% of the enterprises in İspir county have tied free-stall closed barns and 4.3% of them have tied-stall closed barns. Additionally, the percentages of open, semi-open and free-stall closed barns in the county were also very low (Figure 1). Similarly, results of the studies conducted in Central Anatolia and Eastern Anatolia (Uğurlu and Şahin, 2010; Şeker et al., 2012; Tilki et al., 2013; Bakan, 2014) agree with the findings of the present study. However, the percentage of closed barns with tied-stall in İspir county (4.3%) was found to be lower than the findings of studies carried out in both the Black Sea Region (Tugay and Bakır, 2006; Yenice and Savaş 2016) and the West Anatolia Region (Demirhan and Yenilmez, 2019; Kılıç et al., 2020). On the other hand, Yener et al. (2013) reported that 17.5% of barns are closed barns and 82.5% are semi-open barns in the South East Anatolia Region.

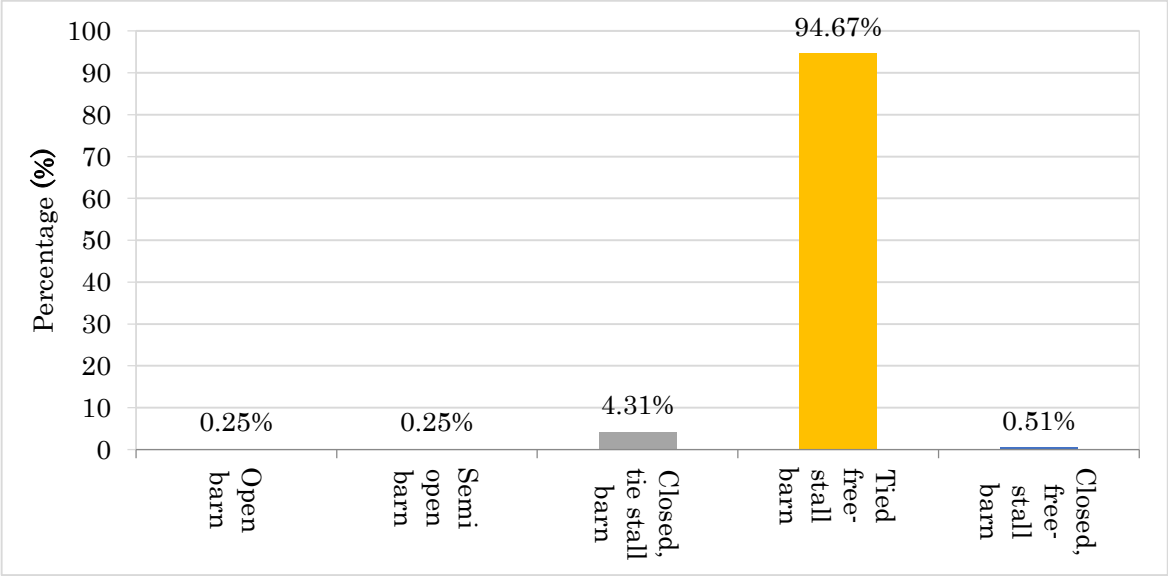

Figure1. Barn types
Şekil 1. Ahır tipleri

Dou et al. (2001) reported that 68% of the cattle enterprises had tied-stall barns in the state of Pennsylvania. Moreover, Sheppard et al. (2011) determined that less than 31% in Western Canada and 80% of the cattle barns in St. Lawrence Plains were tied-stall barn. In these types of barn workers mostly experience tedious difficulties and inadequacies in the application of the most important tasks such as feeding, manure extraction, milking, and irrigation. Therefore, younger generations, especially women, do not want to be employed in animal farming work (Anonymous, 2018). Additionally, Valde et al. (1997) noted that tied-stall barns have a higher incidence of clinical mastitis and suggested that free-stall barns should be preferred for lower disease incidence and higher fertility status. Furthermore, Gökalp (2019) stated that free-stall barns are the most widely used housing system in dairy cattle breeding, but these barns are only profitable in enterprises with 60 or more dairy cattle.

Thoughts of the Cattle Breeders Concerning the Effect of Their Barns on Both Health of Humans and Animals

Majority of the participants stated that their barns had no adverse influence on the human's health (81.5%), animals' growth and development (83.2%) and milk yield (83.5%) (Figure 2a, b, c).

Contrary to the findings of the present study, 48.79% of the owners of the enterprises in Kars province stated that their health was adversely affected due to the structural characteristics of the barns. Furthermore, in the same study in more than half of the enterprises, milk production (57.04%) and cattle growth (57.04%) were reported to be negatively affected due to inadequate barn structure (Tilki et al., 2013).

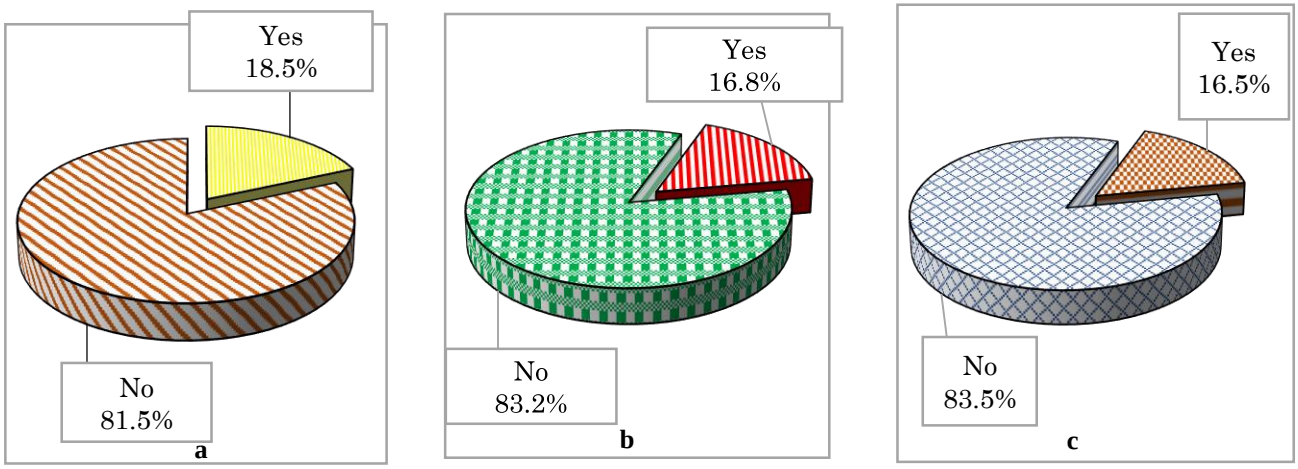

Figure 2. Does the barn environment adversely affect the health of the breeders (a), the growth and development of the animals (b), and the milk production of cows (c).

Şekil 2. Ahır ortamı yetiştiricilerin sağlığını (a), hayvanların büyüme ve gelişmelerini (b), ineklerin süt verimini olumsuz yönde etkiliyor mu?

Similarly, Aydın et al. (2016) also indicated that in the majority of the enterprises in Hınıs county of Erzurum province barn structure adversely affected the health of enterprise owners (88.8%), milk yield (88.6%), and growth and development of animals (81.0%). It could be seen that the results determined in Ispir county were worse compared to other regions of Türkiye. The findings could be attributed to low level of awareness and the lack of information of the breeders about the negative effects of barn conditions on yield and health in İspir county. It was also determined that there were significant ($P<0.05$) relation between education level of the breeders and their thoughts about influence of their barns on the health as well as milk yield.

Age of the Barn Facilities

The age of the barns in the İspir county was determined to be generally over 15 years (64.2%) and the number of newly built barns is quite low (3.0%). The percentages of the barns aged less than 5 years were determined as 3.0%, between 6-10 years 14.0%, between 11-15 years 18.8%, between 16-20 years 33.0% and for 21 years and above 31.2% in the county (Figure 3). The findings of the present study are in accordance with the findings of Aydın et al. (2016) in terms of the age of barns buildings which were younger than 10 years of age (17.0%), however lower than the reports of Güler et al. (2017) for the same building age groups (40.4%). Additionally, the findings of the current study are in harmony with the results of Tilki ve ark. (2013) in central county of Kars as well as Bakan (2014) in Ağrı Province.

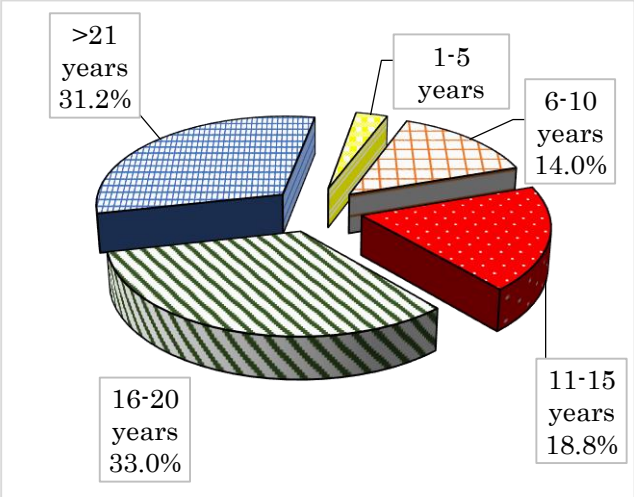

Figure 3. Age of the barns

Şekil 3. Ahırların yaşları

Location of Barns

In dairy cattle enterprises, barns are recommended to be detached in terms of animal health, welfare, and productivity. In İspir county 80.5% of the barns were determined to be detached from the house building (Figure 4). The percentages of detached barn building were reported as 63%, 77%, 70.7% and 75% in

Kahramanmaraş (Kaygısız and Tümer 2009), in Muş (Şeker et al., 2012), in Hınıs (Aydın et al., 2016) and Narman counties of Erzurum province (Güler et al., 2017), respectively. The findings of the present study were determined to be higher than results of these reports. However, the results of the current study were lower than those that were reported for Ergani county of Diyarbakır province (90.4%), Malatya (91.9%), and Muş (85.8%) (Han and Bakır, 2010; Köseman and Şeker, 2016; Bakır and Kibar 2020). Furthermore, it was determined that the relationship between the location of the barns and the educational levels of the owners of the enterprises was statistically significant ($P<0.05$).

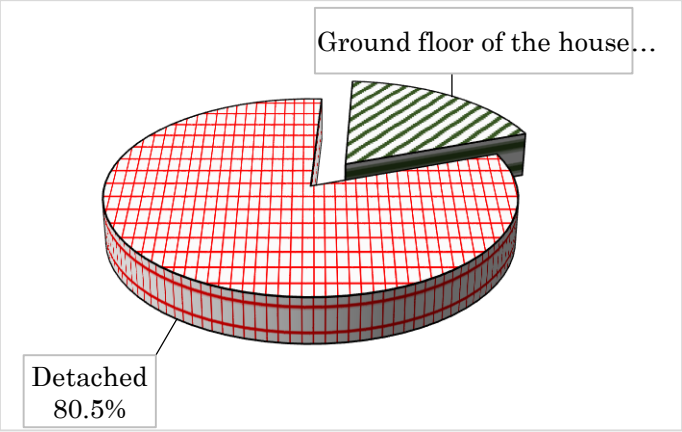

Figure 4. Location of the barns
Şekil 4. Ahırların lokasyonları

Building Materials Utilized for the Construction of Barn Walls

Stone (95.4%) and bricks (66.2%) were determined to be commonly used building material on the construction of the barns' walls in İspir county (Figure 5). Stone is a sturdy, readily available and free construction material, as well as a traditional construction material used by farmers in the İspir county. Therefore, it may be possibly the main reason for the use of stone commonly in barn buildings of İspir county. Similarly, Şeker et al. (2012) reported that stone (42.1%), briquette (39.7%) and mudbrick (18.2%) were widely used in barn construction in Muş province. Furthermore, in Narman county of Erzurum province stone (55.3%), brick (32.7%) and mudbrick (12.0%) were reported to be the most widely used building materials (Güler et al., 2017). On the other hand, Aydın et al. (2016) reported that in Hınıs which is another county of Erzurum province, mudbrick was used most commonly with 45.6% in barn's walls, and it was followed by brick (25.8%), stone (25.5%), wood (2.2%) and briquette (0.8%). Furthermore, the wall building material in 69.56% of the dairy cattle barns was reported to be brick, 13.04% was stone and 8.70% was briquette in the Çankırı province (Yıldız, 2013). Similarly, Kurç and Kocaman (2016) determined that brick, concrete, briquette, sheet metal along with brick and sheet metal were used as wall materials in barns in 80.65%, 3.23%, 3.23%, 3.23% and 8.06% of the enterprises respectively in Malkara county of Tekirdağ province. In contrast, oak, a wood material, was used as wall construction material in most (79.17%) of the cattle barns in Şenpazar county of Kastamonu province (Şahin, 2016). In addition, while stone was used together with wood material in the construction of barn walls in 4.16% of the enterprises, only brick was used in 16.67% of the barns.

Furthermore, Bardakcioglu et al. (2004) reported that 62.6% of the barn walls were made of bricks, 34.4% of perforated bricks, and 3% of other materials (briquettes, mudbrick, etc.) in Aydın province. In Kahramanmaraş province, it was also reported by Kaygısız and Tümer (2009), walls of the cattle barns in Kahramanmaraş province were constructed by using stone (33%), mudbrick (% 26), Briquette (% 40) and lumber (1%) materials.

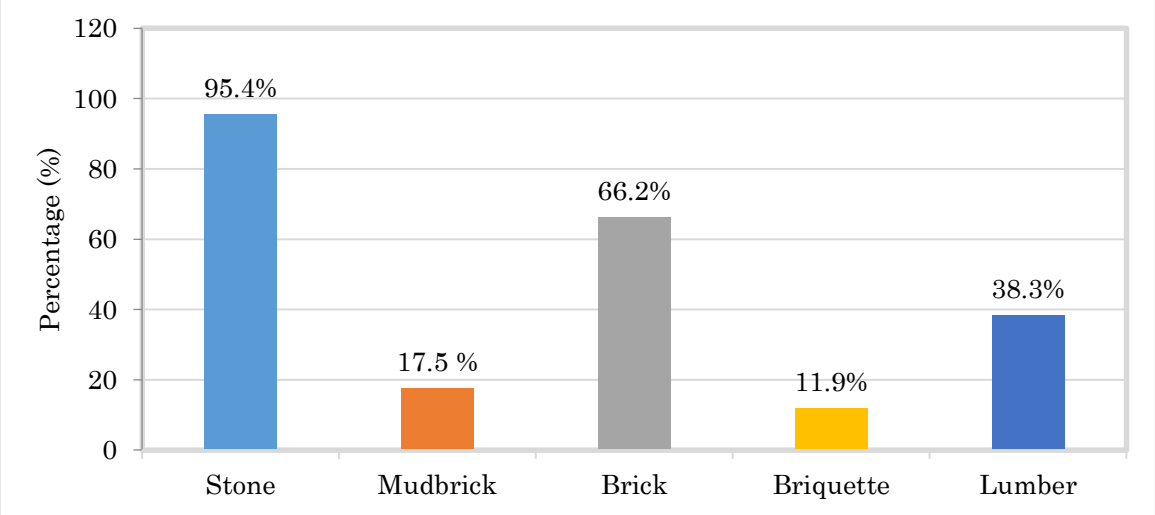

Figure 5. Building materials used for the walls of the barns
Şekil 5. Ahır duvarları için kullanılan yapı malzemeleri

Structural Materials Used for Barn Roofs

In İspir county, galvanized sheet metal was mostly preferred for the construction of barn roofs in the county (65.4%), followed by concrete (15.0%), soil (mud) (10.8%) and lumber (8.8%) (Figure 6). Similarly, galvanized sheet metal and concrete were reported to have been used for the construction of the barn roof in 48.1% and 22.6% of the enterprises respectively in Narman county of Erzurum province (Güler et al., 2017). However, in Yakutiye county of Erzurum, the percentage of enterprises that preferred galvanized sheet metal for roof construction was reported as 84.2% (Çapadağ, 2016). Furthermore, the sheet metal (56.5%) was determined to be widely used among the roofing materials in dairy cattle enterprises in Aydın province, while different construction materials such as eternite (25.3%), tile (13.1%) and thatch or nylon (5.1%) were also used in these enterprises (Bardakçıoğlu et al., 2004).

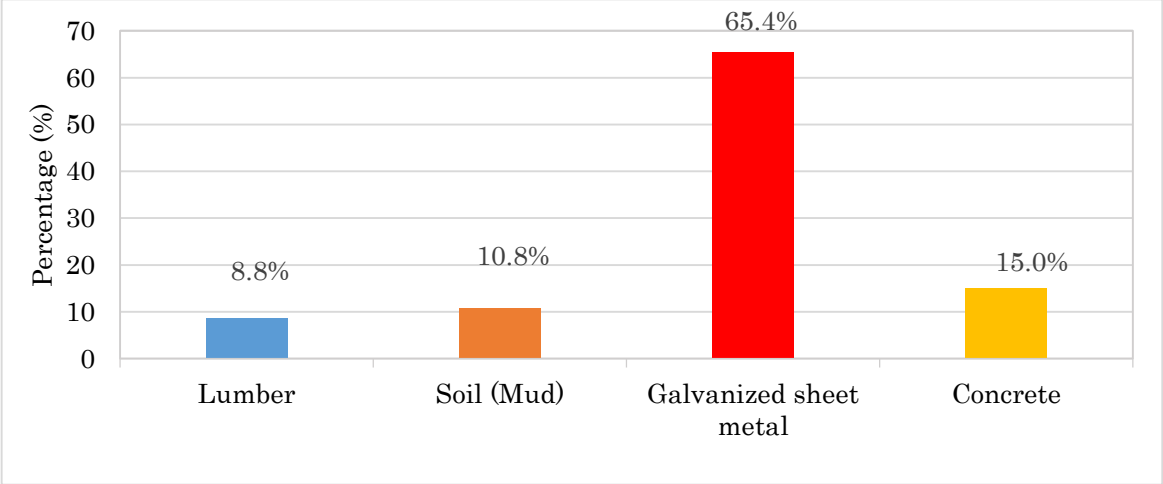

Figure 6. Building materials used for the constraction of barn roofs
Şekil 6. Ahır çatılarının yapımında kullanılan yapı malzemeleri

Building Materials Utilized for Constructing of Barns' Floors

While only one structural material was used for construction of the barn floors, it was observed that more than one type of material was used in some of the barns. The building material used for construction of the barns' floors in the majority (61.9%) of the cattle farms in the İspir county is concrete. However, stone (22.0%), compacted soil (28.2%), and wood (27.9%) are the other materials preferred by the owners of the enterprise (Figure 7). It is recommended that the floor material of cattle barns should be stable, durable, impermeable, resistant to chemicals and urine and easy to clean. Even though the most economical floor material is compacted soil, the floor should be concrete for better animal cleanliness and easier manure cleaning (Özhan et al., 2009; Yıldız, 2013). In many studies conducted in different provinces and counties in Türkiye, it was reported that the use of concrete in the construction of barn floors was quite common (Yener et al., 2013; Bakan, 2014; Köseman and Şeker 2016; Mundan et al., 2018; Demirhan and Yenilmez, 2019; Bakır and Kibar 2020). Moreover, Vasseur et al. (2010) reported that concrete (74.4%) was the most commonly preferred flooring material in the barns of cattle farms in Pennsylvania state of the USA.

Structural Materials Utilized for Building Feeders in Cattle Barns

Lumber (70.8%) and concrete (29.2%) are the most widely preferred for constructing of the feeders in the enterprises in İspir county of Erzurum province (Figure 8). Similarly, Aydın et al. (2016) reported that 61.2% of the enterprises used concrete and 38.8% used wooden materials for the construction of the feeders in Hınıs county. On the other hand, Güler et al. (2017) reported that in the Narman county of Erzurum province, in 48.1% of the enterprises, feeders were made of lumber materials and in 35.6% of them they were made of concrete. However, in contrast to the present study, they also reported that galvanized sheet metal was another material used for feeder construction (16.3%).

It was also stated in several other studies that the use of concrete is more common in the making of feeders, in 98.4% of the enterprises in Ankara, 89.4% Aksaray (Tatar, 2007), and in all of the enterprises in the Ahlat county of Bitlis (Bayraktar et al., 2010) feeders were made of concrete. The possible reasons for the widespread usage of concrete feeders in barns could be easy to clean, as that well as they can be filled with water for the watering of the animals.

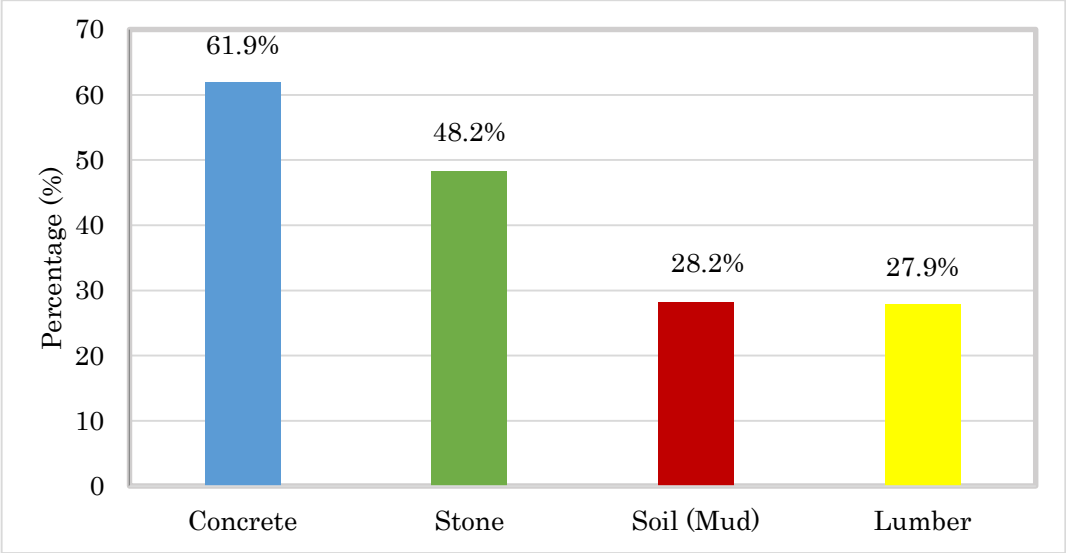

Figure 7. Building materials used for the construction of the floors of the barns
Şekil 7. Ahırların zeminlerinin yapımında kullanılan yapı malzemeleri

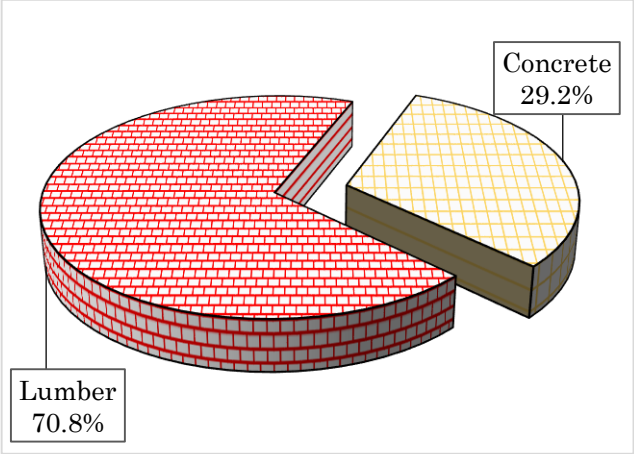

Figure 8. Building materials used to make the feeder
Şekil 8. Yemlik yapımında kullanılan yapı malzemeleri

Interior Design of the Cattle Barns

In the construction of cattle barns, design, types, locations, and sizes of the structural elements such as feeders, waterers, stalls, urinary channels, tying arrangements and feed alleys, etc. should be planned taking into account the ease of the working of workers as well as health and welfare of the animals. Some of the standard barn structural elements were available in almost all of the enterprises in the county such as feeder (100.0%), urinary canal (85.3%), window (96.7%). However, other elements such as the feeding alley (6.6%), the automatic watering system (1.3%), and the ventilation holes (22.8%) were available in a small number of enterprises (Figure 9). Results of a study conducted by Aydın et al., (2016) indicated that in the enterprises not having feeding alley where feeders were built in a position adjacent to the barn walls, breeders have to get too close to cows to feed them. In addition to this, they noted that breeders might encounter high injury risk as a consequence of this practice. Güler et al. (2017) found that only 6.3% and 6.7% of the enterprises, respectively, in Narman county of Erzurum had feeding alley and automatic waterers in their barns. Additionally, it was reported by Bayraktar et al. (2010) that only 30.43% of the enterprises in Adilcevaz county of Bitlis province had feeding alley in their barns.

Daytime Lighting of the Cattle Barns

Having enough light during the daytime enables better observation of cow signals and allows breeders to detect signs of heat, lameness, blood, and discharge. Additionally, better lighting barn also improves workers' efficiency, comfort and safety. In the planning of the barns, windows are important in terms of ventilation and lighting. In barns with insufficient lighting, it is difficult to perform routine work inside the barn such as feeding and cleaning, the probability of accidents increases during the entrance and exit to the barn, and the animals cannot benefit from natural light (Özhan et al., 2009). It was determined for the daytime lighting of the barn most of the breeders (92.9%) in İspir county used windows and electric bulbs together (Figure 10). Although the percentage of lighting through windows was 6.6%, the percentage of enterprises that used only electricity for barn lighting was determined as 0.5%. Since lighting with electricity is an additional cost to the company, the use of windows for this purpose is recommended for profitable cattle farming (Özdemir and Karaman, 2008). However, as a result of not giving the necessary importance to cleanliness in most of the barns in the county, it was observed that the glasses of windows were extremely dirty and dusty and this situation

prevented the animals housed in these barns from benefiting from sunlight sufficiently.

Tugay and Bakır (2006) reported that in 52% of the dairy cattle farms in Giresun province, the barns had sufficient lighting and these enterprises provided the lighting through the windows. In the Hınıs county of Erzurum province, 63.5% and 36.5% of the enterprises were reported to provide natural lighting and electricity for the interior lighting of the barns respectively (Aydın et al., 2016). Daş et al. (2014) determined that almost in all barns in Bingöl province, interior lightning of the barn was provided by electricity. Therefore, the number of barns that provided lighting by using sunlight was reported as quite low.

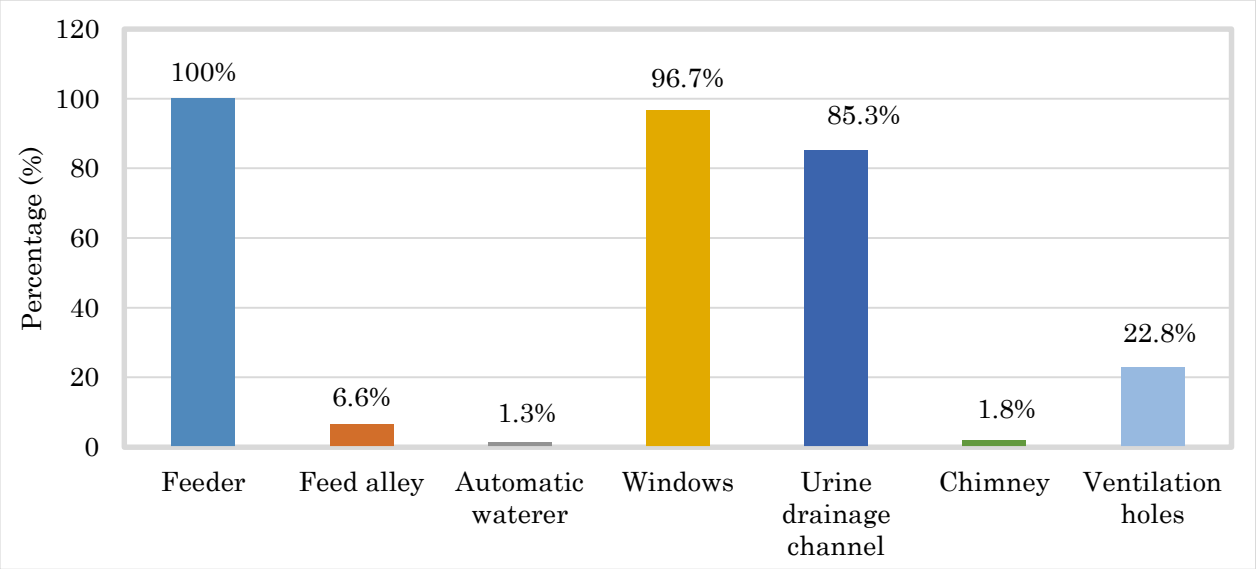

Figure 9. Structural elements existing in the barns
Şekil 9. Ahırlarda mevcut yapısal elementler

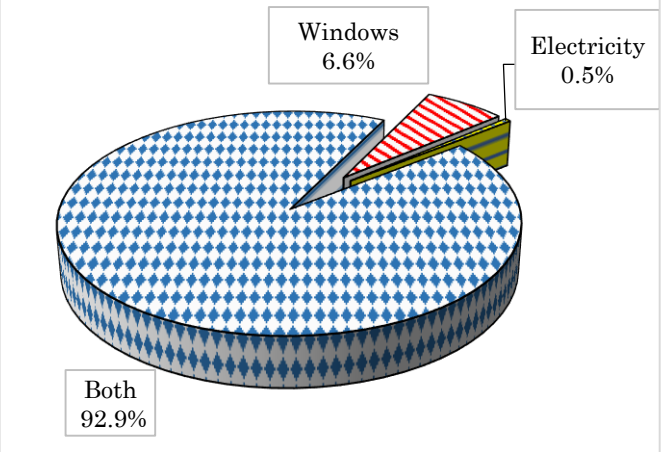

Figure 10. Techniques for daytime lighting of barns
Şekil 10. Ahırların gündüz aydınlatma teknikleri

The Number of Windows in the Barns

The number of windows in the barns is highly important for a sufficient lighting. Although the percentage of enterprises with 2 windows in their barns was the highest (48.2%) in the county, this was followed by enterprises with 3, 4, 1 and 5 windows in the barn, respectively (Figure 11). Similarly, Güler et al. (2017) reported that the barns with 2 windows (47.5%) were quite common in Narman county, followed by the barns with 4-5 windows. Furthermore, Aydın et al. (2016) indicated that barns with 3 (36.3%) and 4 (40.0%) windows were common in most of the enterprises in Hınıs county. It was also determined that there was a significant relationship ($P<0.0.1$) between the number of barn windows in the barns in the county and the size of the enterprise along with the educational status of the owners of the enterprises.

The Number of Ventilation Chimneys of the Barns

The number of ventilation chimneys in the barn is important for removing the warm air, humidity, bad odors, and gases in the barn. The number of barn chimneys in the enterprises also differs in a similar way as the number of windows in the enterprises. It was determined that 77.4% of the enterprises in the county did not have a ventilation chimney, and the enterprises with a chimney generally had 1 or 2 chimneys (Figure 12). In this case, the discharge of dirty air from the barn is only provided through windows or doors. Similarly, Kılıç et al. (2020) reported that in 58% of dairy farms in Kütahya province dirty barn air was discharged by keeping the windows open. In addition, it was determined that the number of air discharge chimneys in the barns was significantly ($P<0.01$) related to the size of the enterprise in the county.

Tilki et al. (2013) stated that 6.3% of cattle enterprises in Kars province did not have barn ventilation chimneys, while 3.6 of them had one ventilation

chimney. Unalan et al. (2013) reported that 78.1% of the enterprises in Niğde province did not have ventilation chimneys in the barns, while Kılıç et al. (2020) pointed out that only 2% of the barns in cattle farms in Kütahya province did not have barn chimneys. It was also reported that 8.5% of the animal barns in the Tokat province enterprises did not have ventilation chimneys, and doors and windows are used for air discharge (Özdemir, 2007). Aydın et al. (2016) noted that the number of barns with 2, 3 and 4 chimneys in cattle farms was quite common in Hınıs county of Erzurum province. Similarly, most of the cattle enterprises in Muş province had barn ventilation chimneys and percentage of cattle barns having ventilation chimney was 90.8% (Bakır and Kibar, 2020). On the other hand, Öztürk (2009) noted that ventilation chimneys existed in 55.17% of the enterprises in Mardin province. Likewise, most of the enterprises in Narman county of Erzurum have 1 or 2 chimneys (45.7% and 40.0%) (Güler et al., 2017).

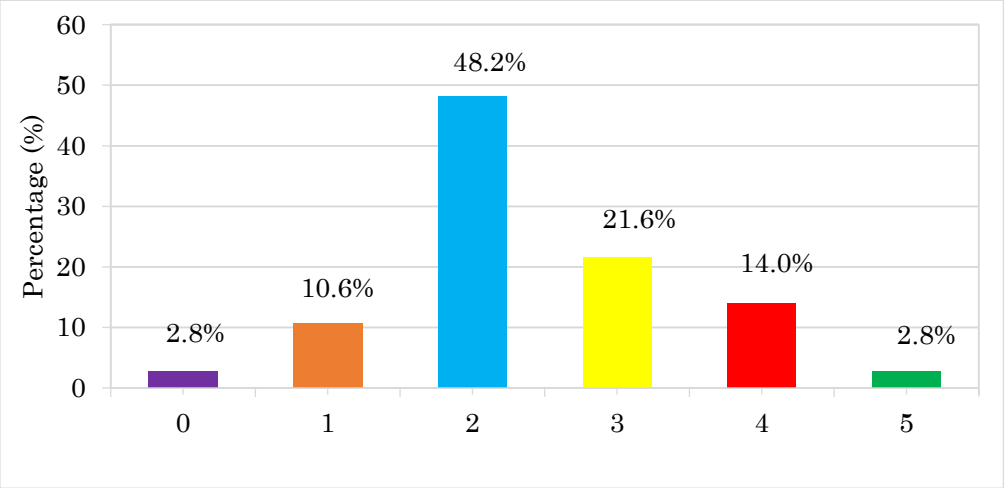

Figure 11. Number of windows in the barn
Şekil 11. Ahırdaki pencere sayısı

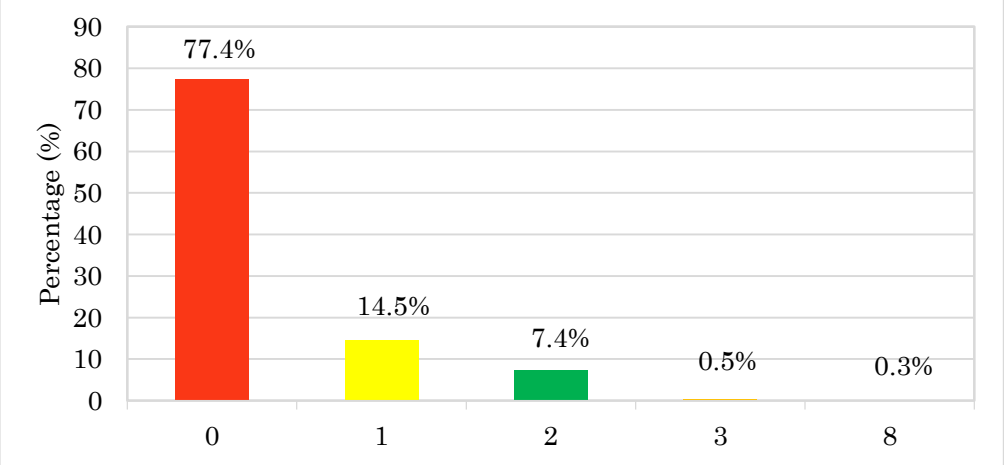

Figure 12. Number of ventilation chimneys of the barns
Şekil 12. Ahırlardaki havalandırma bacası sayısı

CONCLUSION and SUGGESTIONS

This study was carried out to determine the structural characteristics of barns and related problems in cattle enterprises in İspir county of Erzurum province. The findings revealed that the required standards were not followed in the planning and construction of the barns in the county. Most barns were not planned according to animal welfare and conditions that will provide better atmospheric conditions inside the barn. A big majority of the barns were determined to be in a tied free-stall closed barn plan, and the air discharge chimneys and windows in the barns were insufficient. For these reasons, it is difficult to provide the optimum temperature, humidity, and quality air for cattle. In almost all of the enterprises in the county barns were determined to be detached as recommended to provide better welfare to the cattle. The barn walls were commonly made of stone and brick, galvanized sheet metal material was widely utilized for the barns' roofs, concrete was used on the barn floor. These practices look promising for the cattle farming of the county. It would be appropriate to increase usage automatic cow drinkers to provide continuous water supply to the animals and to install a water system inside the barn

for this purpose. The information support and investment incentives to be given to the enterprises in the region, it would be possible to modernize the barns and ensure the barns that will be built in the future to be sufficient to provide the optimum welfare for the cattle. In this way, a more profitable cattle farming may be achieved for the breeders of the county.

Statement of Conflict of Interest

Authors have declared no conflict of interest.

Author's Contributions

Authors declares the contribution of the authors is equal.

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Gladiçya (*Gleditsia triacanthos*) Ağaç Yapraklarının Potansiyel Yem Değerinin Saptanması

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ÖZET

Bu çalışma da farklı aylarda toplanan gladiçya (*Gleditsia triacanthos*) ağaç yapraklarının kimyasal bileşimi, kondanse tanen (KT) içeriği, *in vitro* toplam gaz (TG) üretimi, metabolik enerji (ME) ve organik madde sindirim derecesi (OMSD)'nin saptanması amaçlanmıştır. Aylara göre gladiçya ağaç yapraklarının kimyasal bileşimi, kondanse tanen (KT), *in vitro* toplam gaz (TG) üretimi, metabolik enerji (ME) ve organik madde sindirim derecesi (OMSD) arasındaki farklılıklar önemli bulunmuştur (P<0.05). Gladiçya ağaç yaprakları ham protein (HP) %5.59-19.48, ham yağ (HY) %3.64-9.71, asit deterjan lif (ADF) %23.54-34.52, nötr deterjan lif (NDF) %38.58-49.00, KT %13.86-19.35, *in vitro* TG üretimleri 27.41-41.43 ml^{-1}200 mg KM, ME değerleri 6.52-8.99 Mj kg^{-1} KM ve OMSD %49.43-64.50, aralığında değişmiş ve aralarındaki farklılıklar istatistiki olarak önemli bulunmuştur (P<0.05). Sonuç olarak ham besin madde bilşimine bakıldığında gladiçya yapraklarının alternatif kaba yem kaynağı olabileceği, yüksek tanen içeriği nedeniyle kullanımına dikkat edilmesi gerektiği söylenebilir.

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Gladiçya (Gleditsia triacanthos)
Yem değeri
Hasat zamanı

Determination of Potential Forage Value of Honey Locust (*Gleditsia triacanthos*) Tree Leaves

ABSTRACT

In this study, was purposed to determine the chemical composition, condensed tannin (CT) content, in vitro total gas (TG) production, metabolic energy (ME) and organic matter digestibility (OMD) of honey locust (*Gleditsia triacanthos*) tree leaves collected in different months. The differences between the chemical composition, condensed tannin (CT), in vitro total gas production (TG), metabolic energy (ME) and organic matter digestibility (OMD) of honey locust (Gleditsia triacanthos) leaves by month were found to be significant (P<0.05). Honey locust (*Gleditsia triacanthos*) tree leaves crude protein (CP) 5.59%-19.48%, crude fat 3.64-9.71%, acid detergent fiber (ADF) 23.54-34.52%, neutral detergent fiber (NDF) 38.58%-49.00, CT 13.86-19.35% , in vitro TG productions varied between 27.41-41.43 ml-1200 mg DM, ME values 6.52-8.99 Mj kg-1 DM and OMD 49.43-64.50%, and differences between them were found to be statistically significant (P<0.05). As a result, considering the crude nutrient composition, it can be said that leaves of honey locust can be an alternative source of roughage, and attention should be paid to their use due to their high tannin content.

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GİRİŞ

Hayvancılık işletmelerinde toplam üretim masraflarının %60-70'ını yem giderleri oluşturmaktadır (Alçiçek ve ark., 2010). Karlı bir hayvancılığın yapılmasında en etkili unsur ucuz yem kaynağının sağlanmasıdır. Bu kaynakların başında çayır meralar ile kültürü yapılan yem bitkileri gelmektedir. Ancak bu kaynaklardan gelen yemler ihtiyaçları gerektiği kadar karşılayamamaktadır. Bu durum dikkate alınarak yeni yem kaynaklarının araştırılması gerekmektedir. Bu yem kaynaklarından bir grubunu ağaç dal ve yaprakları oluşturmaktadır

(Paterson ve ark., 1998; Temel ve Kır, 2015; Özyazıcı ve Açıkbaş, 2020; Boğa ve ark., 2022). Ağaç dal ve yaprakları sığır, keçi, koyun ve geyik gibi ruminant hayvanlar tarafından doğal olarak kullanılmaktadır (Papachristou ve ark., 1996; Boğa ve ark., 2022). Ağaç dal ve yaprakları ani iklim değişikliklerinden sınırlı düzeyde etkilenmeleri ve kurak mevsimde kaba yem sağlamaları bakımından öne çıkmaktadır (Özelçam ve ark., 2019). Borens ve Poppi (1990), kurak koşullarda ağaç ve çalı türlerinin, diğer buğdaygil ve baklagil otlarına göre daha uzun süre (yaklaşık 3- 5 ay) yeşil kaldıklarını vurgulamışlardır. Afrika'da yeşil yem olarak ağaç yapraklarının kullanıldığı da bildirilmektedir (Brewbaker, 1986). Kurak koşullarda ruminant beslemede ağaç yaprakları kullanımının avantajları yanı sıra, hayvan beslemede kullanımını kısıtlayan bazı dezavantajları bulunmaktadır. Bunların başında anti-besinsel (tanen ve diğer fenolik bileşikler) içeriklere sahip olmaları gelmektedir (Tolera ve ark., 1997). Ağaç yapraklarında bulunan yüksek tanenin, yem tüketimini olumsuz etkileme dışında, yemlerin sindiriminde ve vücutta azot depolama kapasitesinde düşüşe neden olduğu bildirilmektedir (Boğa ve ark., 2021). Bu araştırma bir baklagil ağaç türü olan gladiçya (Gleditsia triacanthos) yapraklarının kimyasal bileşim, in vitro TG, ME, OMSD ve KT içereklerinin saptanması amacıyla düzenlenmiştir.

MATERYAL ve METOD

Yem Materyali

Araştırmanın yem materyalini 2020 yılında Muş (1350 m) ili merkez ilçesinde yetişen on farklı Gladiçya (Gleditsia triachantos) ağaçlarından 8 ay süresince elle toplanan yapraklar oluşturmuştur. Çalışmanın yapılabilmesi için Kahramanmaraş Sütçü İmam Üniversitesi, Ziraat Fakültesi, Yerel Etik kurulundan onay alınmıştır (29.01.2021 tarih ve 2021/01 sayılı karar).

Laboratuvar analizleri

Laboratuvara getirilen yapraklar kurutulmuş ve sonrasında 1 mm elekten geçecek şekilde öğütülerek kuru madde (KM), ham kül (HK), ham protein (HP), ham yağ (HY) içerikleri AOAC (1990)'a göre, kondense tanen (KT) içerikleri Makkar ve ark. (1995)'nın bildirdikleri yöntemlere göre yapılmıştır. Yemleri asit deterjan lif (ADF) ve nötr deterjan lif (NDF) Van Soest (1991)'in, *in vitro* gaz üretimleri ise Menke ve ark. (1979)'nın bildirdikleri yöntemlere göre yapılmıştır. Gladiçya yapraklarının ME ve OMSD değerleri aşağıdaki eşitliklerle hesaplanmış olup, eşitliklerde kullanılan HP, HY, HK içerikleri % olarak alınmıştır (Menke ve Steingass, 1988).

$$ME\ (MJ\ kg^{-1}\ KM) = 2.2 + 0.1357 \times GÜ + 0.057 \times HP + 0.002859 \times HY^2 \quad (1)$$

GÜ: 24 saatlik *in vitro* gaz üretimi (ml 200 mg^{-1})

$$OMSD\ (\%) = 14.88 + 0.8893 \times GÜ + 0.448 \times HP + 0.651 \times HK \quad (2)$$

İstatistik Analizler

Çalışmada elde edilen veriler tek yönlü varyans analizi (One-way ANOVA) kullanılarak istatistiki olarak (Tukey %5) değerlendirilmiştir.

BULGULAR ve TARTIŞMA

Farklı aylarda toplanmış gladiçya ağacı yapraklarının kimyasal bileşimi saptanmış ve Çizelge 1'de verilmiştir. Gladiçya yapraklarının HP içerikleri %5.59-19.48 aralığında değişmiş olup en düşük Aralık (%5.59) ayında en yüksek Mayıs (%19.48) ayında tespit edilmiş ve HP içeriği gelişme dönemi ilerledikçe önemli düzeyde azalmıştır ($P<0.05$). Genellikle ilkbaharda bitkiler büyümeye başladıkça, protein sentezi uyarılarak genç hücre sayısı ile birlikte protein sentezinin arttığı söylenmektedir (Kaçar ve ark., 2006). Tersi olarak da büyüme dönemi ilerledikçe HP içeriğinin azaldığı rapor edilmiştir (Mountousis ve ark., 2008; Ataşoğlu ve ark., 2010). Benzer literatür bildirişlerinde farklı ekolojilerde yapılan çalışmalarda çalı ve ağaç türlerinde de aynı durumun gerçekleştiği bildirilmiştir (Tolunay ve ark., 2009; Kamalak ve ark., 2010; Parlak ve ark., 2011; Foroughbakhch ve ark., 2012; Alatürk ve ark., 2014; Oktay ve Temel, 2015; Demirkol, 2019). Benzer bir çalışmada Eylül ayında elde edilen gladiçya yaprağının HP içeriği %15.7, Haziran ayında %20.1 olarak bulunmuştur (Luginbuhl ve Mueller, 2000). Farklı illerden toplanan gladiçya yapraklarının HP içerikleri %6.7-11.9 (Kamalak ve ark., 2012), Kasım ayında gladiçya yaprağının HP içeriği %8.02 (Kaya ve ark., 2016), kurak dönem boyunca toplanan gladiçya yapraklarının HP içeriği %12.1 (Medjekal ve ark., 2018), Temmuz ayında toplanan gladiçya yapraklarının HP içeriği %8.43 (Başer ve Kamalak, 2020) olarak tespit edilmiştir. Oluşan bu farklılıkların hasat zamanı, iklim, toprak ve bitki genetik yapısından kaynaklandığı söylenebilir. Laktasyondaki koyunların yaşama payını karşılamak için ham protein oranının en az %7-9, verim payı için en az %10-12 arasında olması gerektiği vurgulanmıştır (El-Shatnawi ve Mohawesh, 2000). Bu çalışmada gladiçya yapraklarının HP içeriğinin Kasım ve Aralık ayları dışında koyunların hem verim hem de yaşama payı ihtiyaçlarını karşılayacak düzeyde olduğu söylenebilir. Kasım ve Aralık aylarında HP içeriği rumendeki mikroorganizmaların faaliyeti için yeterli olmadığı, bunun için bu aylarda yetiştiriciler tarafından kullanılması durumunda yeme mutlaka bir ek protein takviyesinin yapılması önerilebilir (Tablo 1, El-Shatnawi ve Mohawesh, 2000). Gladiçya yapraklarının aylara göre HY içerikleri %3.64-9.71 arasında değişiklik göstermiştir. En yüksek HY Aralık (%9.71) ayında, en düşük HY Mayıs (%3.64) ayında toplanan yapraklarda tespit edilmiştir (Çizelge 1).

Çizelge 1 Farklı aylarda hasat edilen gladiçya yapraklarının kimyasal bileşimi.
Table 1. Chemical composition values of honey locust leaves harvested in different months.

Aylar	KM	HP	HK	HY	ADF	NDF	KT
	$\bar{x}$±Sx	$\bar{x}$±Sx	$\bar{x}$±Sx	$\bar{x}$±Sx	$\bar{x}$±Sx	$\bar{x}$±Sx	$\bar{x}$±Sx
Mayıs	93.86^{b}±0.74	19.48^{a}±0.63	6.21^{f}±0.12	3.64^{f}±0.57	23.54^{e}±1.16	38.58^{c}±0.27	13.86^{b}±0.37
Haziran	94.40^{ab}±0.35	17.49^{b}±0.05	8.69^{e}±0.26	4.05^{ef}±0.25	26.12de±1.13	39.68^{c}±1.12	15.55^{b}±1.53
Temmuz	94.61^{ab}±0.18	16.06^{c}±0.09	9.77^{d}±0.57	5.42^{de}±0.28	27.09^{d}±0.54	40.35^{c}±1.89	16.71^{ab}±1.37
Ağustos	94.61ab±0.56	15.43^{c}±0.08	9.94^{cd}±0.12	6.35^{cd}±0.43	28.51cd±1.15	41.92^{bc}±1.44	16.78^{ab}±1.26
Eylül	94.47ab±0.19	13.40^{d}±0.14	10.29^{bcd}±0.22	7.55^{bc}±0.47	30.65^{bc}±1.57	42.16^{bc}±1.03	16.84^{ab}±1.35
Ekim	94.24^{ab}±0.23	10.18^{e}±0.04	10.61^{bc}±0.11	8.66^{ab}±0.37	31.81^{ab}±1.82	46.18^{ab}±2.66	16.91^{ab}±1.54
Kasım	94.95^{ab}±0.94	6.73^{f}±0.11	10.69^{b}±0.06	8.86^{ab}±0.95	32.49^{ab}±0.48	48.22^{a}±2.58	17.20^{ab}±0.89
Aralık (Senesens)	95.40^{a}±0.12	5.59^{g}±0.06	11.78^{a}±0.19	9.71^{a}±0.55	34.52^{a}±0.22	49.00^{a}±3.20	19.35^{a}±1.02
SHO	0.083	0.019	0.022	0.092	0.431	1.332	0.498
Ö.D.	*	**	**	**	**	**	*

a-g: Aynı sütunda yer alan farklı simgeye sahip ortalamalar birbirinden farklıdır.KM: Kuru madde, HP: Ham protein, HK: Ham kül, HY: Ham yağ, ADF :Asit çözücülerde çözünmeyen lifli bileşikler, NDF: Nötr çözücülerde çözünmeyen lifli bileşikler, KT: Kondanse tanen, Ö.D: Önem derecesi, SHO: Standart hata ortalaması, **: P≤0.01 *: P≤0.05

Çalışmada incelenen gladiçya yapraklarının HY içeriklerinin aylara göre değişiklik göstermesinde bitkilerin gelişme dönemlerinin oldukça etkili olduğu görülmüştür (P<0.05). Yapılan benzer çalışmalarda da, büyümenin başlangıç dönemlerinde ağaç ve çalıların HY içerikleri düşük olurken, olgunlaşmanın ilerlemesi ile yağ oranlarında artışlar görüldüğü bildirilmiştir (Kamalak ve ark., 2005; Singh ve Todaria, 2012; Tatlıyer ve ark., 2019). Konu ile ilgili yapılan benzer çalışmalarda Kasım ayında gladiçya yapraklarının HY oranı %9.87 (Kaya ve ark., 2016), Temmuz ayında gladiçya yapraklarının HY oranı %3.74 (Başer ve Kamalak, 2020) olarak tespit edilmiş olup, farklılıkların hasat zamanı, iklim ve bitki genetik yapısından kaynaklanabileceği düşünülmektedir. Genellikle yem materyali olarak kullanılan türler gelişmenin ilerleyen dönemlerinde daha yüksek oranda hücre duvarı bileşenlerine (NDF ve ADF) sahiptirler (Tablo 1). Asit deterjan lif (ADF) ve NDF içeriği arttıkça, kaba yemin sindirilebilirliği genellikle azaldığı için, kaba yemlerde yem kalitesi açısından ADF ve NDF oranının düşük olması istenmektedir (Van Soest, 1994). Aylara göre gladiçya yapraklarının ADF ve NDF içeriğine bakıldığında önemli değişimler göstermiş olduğu saptanmıştır. Gladiçya yapraklarının ADF içerikleri %23.54-%34.52 arasında değişmiştir. Yaprakların ADF içerikleri aylara göre artış göstermiş, en düşük Mayıs (%23.54) ayında, en yüksek ise Aralık (%34.5) ayında tespit edilmiştir. Diğer yem bitkilerinde olduğu gibi çalı ve ağaç türlerinde de olgunlaşma düzeyiyle beraber ADF içeriğinin arttığı tespit edilmiştir (Bouazza ve ark., 2012; Kökten ve ark., 2012; Tatlıyer ve ark., 2019). Gladiçya yapraklarının değerlendirildiği bir çalışmada Temmuz ayında gladiçya yapraklarının ADF içeriği %31.28 (Canbolat, 2012), dönemsel olarak gladiçya yapraklarının toplandığı çalışmada yaprakların ADF içeriği %19.0-21.0 (Foroughbakhch ve ark., 2012), farklı illerden toplanan gladiçya yapraklarının ADF içeriği ise %19.5-26.2 olarak bulunmuştur (Kamalak ve ark., 2012). Kasım ayında yaprakların ADF içeriği %30.6 (Kaya ve ark., 2016), Temmuz ayında gladiçya yapraklarının ADF içeriği %32.56 (Başer ve Kamalak, 2020) olarak tespit edilmiştir. Gladiçya yapraklarının NDF içerikleri %38.58-49.00 arasında değişmiş ve en düşük NDF içeriği Mayıs (%38.58) ayında toplanan yapraklarda, en yüksek NDF içeriği ise Aralık (%49.00) ayında toplanan yapraklarda saptanmıştır (P<0.05). Olgunlaşmanın gladiçya yapraklarının NDF içeriğini arttığına dair benzer bulgular yapılan birçok çalışmada da ortaya konmuştur (Ventura ve ark., 2004; Pecetti ve ark., 2007; Frost ve ark., 2008; Bouazza ve ark., 2012; Kökten ve ark., 2012; Tatlıyer ve ark., 2019). Gladiçya yapraklarıyla yapılan bazı çalışmalarda da NDF içerikleri %32.0-38.0 (Foroughbakhch ve ark., 2012), Temmuz ayında NDF içeriği %41.55 (Canbolat, 2012), farklı illerden toplanan gladiçya yapraklarının NDF içeriğini %29.9-41.4 (Kamalak ve ark., 2012), Kasım ayında NDF içeriği %46.1 (Kaya ve ark., 2016), Temmuz ayında NDF içeriği %48.82 (Başer ve Kamalak, 2020) olarak tespit edilmiş olup farklılıkların ekolojik ve genetik faktörler ile hasat zamanı ile ilişkili olabileceği düşünülmektedir. Araştırmada olgunlaşma

döneminin gecikmesi ile ADF ve NDF içeriklerinde artışlar olduğu ve yem kalitesinin düştüğü söylenebilir. Gladiçya yapraklarının KT içerikleri %13.86-19.35 arasında değişmiş olup aylara göre değişim düzeyi önemli bulunmuştur (P<0.05). En yüksek KT içeriği Aralık (%19.35) ayında, en düşük ise Mayıs (%13.35) ve Haziran (%15.55) ayında toplanan yapraklarda bulunmuştur. Gladiçya yaprakları ile yapılan benzer çalışmalarda Temmuz ayında %16.11 (Canbolat, 2012), Kasım ayındaki KT içeriği %15.40 (Kaya ve ark., 2016), Temmuz ayında yapılan bir başka çalışmada ise %8.25 (Başer ve Kamalak, 2020) olarak tespit edilmiştir. Tanen içeriğinin mevsime ve bitkinin yetiştiği bölgeye göre değişebileceği bildirilmektedir (Salem ve ark., 2002). Tanen seviyesi olgunlaşma dönemi ilerledikçe değişiklik göstermektedir (Hagerman, 1988). Mevcut çalışmada da olgunlaşma dönemi ilerledikçe KT miktarında artışlar saptanmıştır. Acacia saligna ağacının kullanıldığı bir çalışmada yaşlı yaprakların genç yapraklara oranla toplam tanen miktarının 1.5 kat daha fazla bulunduğu bildirilmiş (Degen ve ark., 1997) ve elde edilen bulguları desteklemektedir. Bilindiği gibi kondense tanen miktarının rasyonda bulunması gereken miktardan (kuru madde de %5-11) fazla olması halinde hayvan beslemede ciddi sorunların ortaya çıkabileceği bildirilmektedir (Budağ, 2009). Bu yüzden gladiçya ağaç yapraklarının kullanılırken kondense tanenin protein üzerindeki olumsuz etkisini azaltmak için ekonomik olması halinde polietilen glikol (PEG) kullanılması tavsiye edilebileceği belirtilmektedir (Şimşek ve Kamalak, 2019). Nitekim Canbolat, (2012) gladiçya ağaç yapraklarının önemli miktarda KT ihtiva etmesinden dolayı, tanenin negatif etkisini gidermek için PEG kullanımı tavsiye etmiştir. Yemlere PEG ilavesinin, yem tüketimi ve yemin sindirim derecesini önemli ölçüde etkilediği ancak PEG fiyatının yüksek olması nedeniyle kullanımının kısıtlandığı, bunun yerine odun külü ile muamelenin alternatif bir metot olarak tavsiye edilebileceği de bildirilmektedir (Kamalak, 2007).

Gladiçya yapraklarının 24 saatlik inkübasyon süresinde ürettiği toplam in vitro gaz miktarları aylara göre değerlendirildiğinde 41.43-27.41 ml arasında değişmiştir. En yüksek gaz üretimi Mayıs (41.43 ml) ayında en düşük gaz üretimi ise Aralık (27.41 ml) ayında toplanan yapraklardan saptanmıştır (P<0.05). Toplam in vitro gaz üretimi hasat zamanının gecikmesine bağlı olarak düşmüştür (P<0.05) aylara göre azalış gösterdiği belirlenmiştir (Çizelge 2.

Çizelge 2. Farklı aylarda hasat edilen gladiçya yapraklarının toplam in vitro gaz üretimi, metabolik enerji ve organik madde sindirim derecesi

Table 2. The total gas, metabolic energy and organic matter digestibility of honey locust leaves harvested in different months.

Aylar	TG	ME	OMSD
	$\bar{x}$±Sx	$\bar{x}$±Sx	$\bar{x}$±Sx
Mayıs	41.43^{a}±1.25	8.99^{a}±0.20	64.50^{a}±1.24
Haziran	33.16^{b}±1.08	7.75^{b}±0.14	57.87^{b}±0.82
Temmuz	33.16^{b}±1.08	7.66^{b}±0.24	57.44^{bc}±1.56
Ağustos	32.80^{b}±1.65	7.52^{b}±0.13	56.33^{bc}±0.55
Eylül	32.08^{bc}±2.85	7.47^{b}±0.09	55.84^{bc}±0.88
Ekim	31.36^{bc}±0.62	7.15^{bc}±0.23	53.56^{cd}±1.46
Kasım	29.21^{bc}±1.65	7.11^{bc}±0.37	53.39^{cd}±2.51
Aralık (Senesens)	27.41^{c}±2.25	6.52^{c}±0.34	49.43^{d}±1.95
SHO	0.953	0.018	0.749
Ö.D.	**	**	**

a,b,c,d: Aynı sütunda yer alan farklı simgeye sahip ortalamalar birbirinden farklıdır. TG: Toplam gaz, ME: Metabolik enerji, OMSD: Organik madde sindirim derecesi, Ö.D: Önem derecesi, SHO: Standart hata ortalaması, **: P≤0,01 *: P≤0,05

Bu durumun yaprakların bileşiminde hasat zamanının gecikmesine bağlı olarak hücre duvarı bileşenlerinin (ADF ve NDF) artmasından kaynaklandığı söylenebilir (Abdulrazak ve ark., 2000). Ayrıca yapısında bulunan ve hasat zamanı ile birlikte artan tanen içerikleriğininde gaz üretimini düşürdüğü bildirilmiştir (Khazaal ve ark., 1994). Mevcut çalışmada da gladiçya yapraklarının aylara göre KT içeriklerinin artması in vitro gaz üretimini düşürmüştür. Benzer çalışmalarda Kaya ve ark. (2016) Kasım ayında topladıkları gladiçya yapraklarının in vitro gaz üretimini 33.1 ml, Başer ve

Kamalak, (2020) Temmuz ayında topladıkları gladiçya yapraklarının gaz üretimini 66.60 ml olarak belirlemişlerdir. Gladiçya yapraklarının ME içeriğinin aylara göre farklılık göstermiş olup (P<0.05) en yüksek Mayıs ayında (8.99 Mj kg-1 KM) en düşük ise Aralık ayında (6.52 Mj kg-1 KM) bulunmuştur. Benzer çalışmalarda Temmuz ayında gladiçya yapraklarının ME içeriği 9.49 Mj kg-1 KM (Canbolat, 2012), farklı illerden Aralık ayında toplanan gladiçya yapraklarının ME içeriklerini 8.85-9.92 Mj kg-1 KM (Kamalak ve ark., 2012), gladiçya yapraklarının Temmuz ayında ME içerikleri 7.11 Mj kg-1 KM (Başer ve Kamalak, 2020), olarak belirlemişlerdir. Bu çalışmalar arasındaki farklılıkların sebebi; ağaç yapraklarının farklı toprak yapısı, farklı vejetasyon süreleri, farklı sürelerde hasat edilmesinden kaynaklandığı düşünülmektedir. Gladiçya yapraklarının OMSD aylara göre farklılık göstermiş ve en yüksek Mayıs ayında (%64.50), en düşük Aralık ayında (%49.43) tespit edilmiştir. Hasat zamanının gecikmesi OMSD'ni düşürmüştür. Gladiçya yapraklarının hasat zamanının gecikmesi ADF ve NDF içeriğini artırarak yaprakların mikrobial fermentasyonu sınırlaması ile açıklanabilir (Canbolat ve Karaman, 2009). Mevcut çalışmada da, NDF ve ADF içeriklerinin, mikrobial fermantasyonu sınırlayarak OMSD'ni düşürmüştür. Benzer çalışmalara bakıldığında Canbolat (2012), Temmuz ayında gladiçya yapraklarının OMSD %64.42, Kamalak ve ark. (2012)'ı farklı illerden topladıkları gladiçya yapraklarının OMSD %58.81-65.86 olarak belirlemişlerdir. Bu çalışmalar arasındaki farklılıkların ağaç yapraklarının farklı toprak yapısı, farklı vejetasyon süreleri, farklı sürelerde hasat edilmesinden kaynaklandığı söylenebilir.

SONUÇ ve ÖNERİLER

Gladiçya ağaç yapraklarının kimyasal bileşimi, toplam in vitro gaz üretimi, metabolik enerji ve organik madde sindirim derecesi olgunlaşmaya bağlı olarak önemli düzeyde değişiklik göstermiştir. Yaprakların ham protein ve diğer besin madde bileşimleri değerlendirildiğinde ruminant beslemede aşırıya kaçmayacak şekilde kullanılabileceği söylenebilir. Ruminant hayvan beslemede gladiçya ağaç yapraklarının kullanım durumlarını daha net belirleyebilmek için in vitro çalışmalarla birlikte in vivo çalışmaların da yürütülmesine ihtiyaç vardır.

Araştırmacıların Katkı Oranı Beyan Özeti

Yazarlar makaleye eşit oranda katkı sağlamış olduklarını beyan eder.

Çıkar Çatışması Beyanı

Makale yazarları aralarında herhangi bir çıkar çatışması olmadığını beyan ederler.

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Kuzu Rasyonlarına Meşe Palamudu İlavesinin Sindirim Derecesine, Metabolik Enerjisine ve Metan Üretimine Etkisinin *İn Vitro* Gaz Üretim Tekniği ile Belirlenmesi

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ÖZET

Bu çalışmanın amacı, kuzu besi rasyonlarına meşe palamudu ilavesinin, *in vitro* gaz ve metan üretimi ile sindirim derecesi, mikrobiyal protein üretimi ve mikrobiyal protein sentezleme etkinliğine olan etkisini *in vitro* gaz üretim tekniğiyle belirlemektir. Bu proje kapsamında meşe palamudu %0, 10, 20 ve 30 oranında kuzu rasyonuna katılmıştır. Kuzu besi rasyonlarına meşe palamudu ilavesi *in vitro* gaz üretimi, metan üretimi ve sindirim derecesini azaltırken, taksimat faktörünü, mikrobiyal protein üretimi ve mikrobiyal protein sentez etkinliğini artırmıştır. Gaz üretimi ve metan üretim değerleri sırasıyla 89.93 ile 129.22 ml, 10.43 ile 19.65 ml arasında değişmiştir. Metan üretim yüzdesi ise %11.53 ile 15.22 arasında değişmiştir. Sindirim derecesi ise %61.63 ile 72.90 arasında değişmiştir. Taksimat faktörü ve mikrobiyal protein üretim değerleri sırasıyla; 3.38 ile 4.10 ve 128.57 ile 143.91 mg arasında değişmiştir. Mikrobiyal protein sentezleme etkinliği ise %34.80 ile 46.31 arasında değişmiştir. Meşe palamudu antimetanojenik özellik taşımasına rağmen, rasyonun sindirim derecesini düşürmektedir. Mevcut çalışmanın sonuçları baz alınarak meşe palamudunun kuzu rasyonlarında kullanımı için uygun dozun belirlenmesi oldukça zor görülmektedir. Bunun için meşe palamudunun kuzu performanslarına etkilerini belirlemek için *in vivo* denemelerine ihtiyaç vardır.

Zootekni

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 17.05.2022
Kabul Tarihi : 30.06.2022

Anahtar Kelimeler
Meşe palamudu
Kuzu rasyonu
Mikrobiyal protein
Taksimat faktörü
Metan emisyonu

Determination of Effect of Supplementation of Acorn to Lamb Ration on Digestibility, Metabolisable Energy and Methane Production Using *İn Vitro* Gas Production

ABSTRACT

The aim of the current experiment was to determine the effect of inclusion of oak acorn on the gas production, methane production, digestibility, partitioning factor, microbial protein production, and efficiency of microbial protein protein production of lamb diets. In this project, oak acorn was included into lamb diets at the ratio of 0, 10, 20 and 30%. The inclusion of oak acorn decreased the gas production, methane production, and digestibility whereas inclusion of oak acorn increased partitioning factor, microbial protein and efficiency of microbial protein production. Gas and methane production ranged from 89.93 to 129.22 mL and 10.43 to 19.65 mL. Percentage of methane production ranged from %11.53 to 15.22. Digestibility ranged from 61.63 to 72.90%. Partitioning factor and microbial protein production ranged from 3.38 to 4.10 and 128.57 to 143.91 m respectively. Efficiency of microbial protein production ranged from 34.80 to 46.31%. Although oak acorn had an anti-methanogenic potential, oak acorn decreased the digestibility. It seems to be very difficult to determine the proper inclusion dose of oak acorn. Therefore in vivo digestibility studies are required to determine the proper inclusion dose of oak acorn to lamb diets.

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GİRİŞ

Çiftlik hayvanların besin madde ihtiyaçlarını karşılarken kaba ve kesif yemler kullanılmaktadır. Hayvan beslemede kullanılan yemlerde hayvancılık endüstrisinde büyük bir yer tutmaktadır. Üreticiler hayvan beslemede kullandıkları yemlerin fiyatlarında artışların olması ve iklim koşullarını da göz önünde bulundurarak yemleri temin etmede sıkıntılar yaşamaktadır. Bunun içinde üreticiler işletmelerde yem maliyetlerini azaltmak için alternatif yem kaynakları arayışı içerisine girmektedir (Boğa ve ark., 2020). Türkiye'nin üçte ikisi ormanlıktır ve bu ormanlık alanlarının da büyük bir çoğunluğu da meşe olup, bu ağaçların Türkiye'nin yedi bölgesinde de yetişebilmektedir (Karaca, 1999). Türkiye'de mevcut ormanlıklardaki meşe palamudu üretiminin yıllık 20 milyon ton buğday üretimine, yıllık 6,7 milyon ton arpa üretimine denk geldiği rapor edilmiştir (TÜİK, 2016). Araştırmalara göre bildirilen bu meşe miktarları Türkiye hayvancılığının gelişmesinde önemli rol oynayacağı düşünülmektedir. Geviş getiren hayvanların üretmiş oldukları enterik metan gazı, küresel ısınmaya neden olan karbondioksitten sonra en önemli sera gazıdır (Van Nevel and Demeyer, 1996). Enterik metan üretimi sera gazı emisyonunu artırmasının yanında, geviş getiren hayvanlarda önemli düzeyde enerji kaybına neden olduğu da bildirilmektedir (Jonhson ve Jonhson 1995). Rasyonlara tanen ve saponin yem ham maddelerinin geviş getiren hayvanlarda enterik metan üretimini azaltmaya yönelik araştırmalar artmıştır (Wina ve ark. 2005; Temizkan ve ark. 2011; Jayanegara ve ark. 2015; Zhou ve ark. 2020).

Bu çalışmanın amacı, meşe palamudunun kuzu rasyonlarına ilavesinin sindirim, metabolik enerji ve metan üretimine etkisinin *in vitro* gaz tekniği ile belirlemek ve kuzu beslemede potansiyel yem değeri olup, olmadığını tespit etmektir.

MATERYAL ve METOD

Yem ve Rumen Sıvısı Materyali

Bu araştırmada meşe palamutları (*Quercus ithaburensis*) Kahramanmaraş Dulkadiroğlu ilçesi Dereli Mahallesi Alıçseki mevkiinde bulunan ormanlık alanlardan Ekim-Kasım aylarında 5 farklı ağaçtan toplanarak Kahramanmaraş Sütçü İmam Üniversitesi Ziraat Fakültesi Zootekni Bölümü Yemler ve Hayvan Besleme laboratuvarına getirilmiştir. Meşe Palamutları 1 mm elek çaplı değirmende öğütülmüştür. Rumen sıvısı rumen kanüllü 3 baş koçtan alınmış ve koçların bakım ve beslenmesinde etik ilkelere uyulmuştur. Çalışmanın yapılabilmesi için KSÜ Hayvan Deneyleri Yerel Etik Kurulundan (2021/03-4 sayılı toplantı) izin alınmıştır.

Rasyonun Hazırlanması

Rasyonu oluşturan yemlerin metabolik enerji ve ham protein içerikleri Çizelge 1'de verilmişitir. Rasyonlar izokalorik ve izonitrojenik olacak şekilde hazırlanmıştır.

Çizelge 1.Çalışmada kullanılan yem örneklerinin metabolik enerji (kcal) ve ham protein değerleri (%).
Table 1. Metabolic energy (kcal) and crude protein values of the feed samples used in the study.

Yemler	ME (kcal)	Ham Protein (%)
Yonca	2369	17.6
Mısır	3098	8.2
Ayçiçek Tohum Küspesi	2147	36.11
Meşe Palamudu	2398	3.11

Kuzu besisinde kullanılan 4 farklı rasyonların yapısı Çizelge 2'de verilmiş ve rasyonların metabolik enerji içerikleri 2650 kcal/kg KM ve ham protein içeriği ise %17 olarak ayarlanmıştır.

Çizelge 2. Kuzu besisi rasyonlarının komposizyonu (g)
Table 2. Compositions of the rations used in the study

Yemler	Rasyon I	Rasyon II	Rasyon III	Rasyon IV
Mısır	46.2	35.8	25.8	15
Meşe Palamudu	0	10	20	30
Yağ	0	1.2	2.3	3.6
ATK	22.9	26.3	30.1	33.6
Yonca	28.3	24.1	19.2	15.2
Tuz	1.0	1	1	1
Kireç taşı	1.5	1.5	1.5	1.5
Min-vit*	0.1	0.1	0.1	0.1
	100	100	100	100

In Vitro Gaz ve Metan Üretiminin Belirlenmesi

Bu çalışmadaki rasyonun *in vitro* gaz üretimi Menke ve ark. (1979)'nın uyguladıkları tekniğe göre yapılmıştır. Rasyondan 0.5 g KM 100 ml'lik cam şırıngalara konulmuştur. Üzerine 40 ml tamponlanmış rumen sıvısı ilave edilerek 39 ^{0}C'de 24 saatlik inkubasyona tabii tutulmuştur. Rumen sıvısı 2-2.5 yaşlarında rumen kanülü takılmış 55-60 kg ağırlığındaki 3 baş koçtan alınmıştır. Alınan rumen sıvısı tampon çözeltisiyle 1:2 oranında hazırlanmıştır. İnkubasyon sonunda cam şırıngalarda oluşan gazın metan içerikleri Infrared metan cihazı kullanılarak

belirlenmiştir (Goel ve ark., 2008). Gaz ölçümlerinden sonra şırıngalarda kalan numüne behere koyulup üzerine 70 ml NDF eklenilerek 1 saat kaynatılmıştır. Kaynama işleminden sonra gooch por 1 krozelerden geçirilerek süzme işlemi yapılmıştır (Blümmel ve ark., 1997). Rasyonun gerçek sindirlebilebilir kuru madde (g) (GSKM), gerçek sindirim derecesi (GSD), taksimat faktörü (TF), mikrobiyal protein (MP), mikrobiyal sentezleme etkinliği (MPSE), değerleri Blümmel ve ark., (1997)'nın bildirdiği formüllere göre hesaplanmıştır.

GSKM (mg) = İnkübe edilen KM (mg) – Kalan KM (mg)
GSD (%) = (GSKM / İnkübe edilen KM)
TF = GSKM / GÜ
MP (mg/g KM) = GSKM – (GÜ X 2.2 mg/ml),
MPSE = (GSKM – (GÜ X 2.2 mg/ml))/GSKM.

Kimyasal Analizler

Bu araştırmada meşe palamutlarının kimyasal kompozisyonları kuru madde (KM), ham kül (HK), ham protein (HP), ham yağ (HY) AOAC (1990)'a göre belirlenmiştir.

İstatistik Analizler

Çalışmadan elde edilen veriler SPSS 20,0 (2011) paket programında tek yönlü varyans (ANOVA) analizine tabi tutulmuştur. Ortalamalar arasındaki farklar Duncan çoklu karşılaştırma testiyle belirlenmiştir (Duncan, 1955).

BULGULAR ve TARTIŞMA

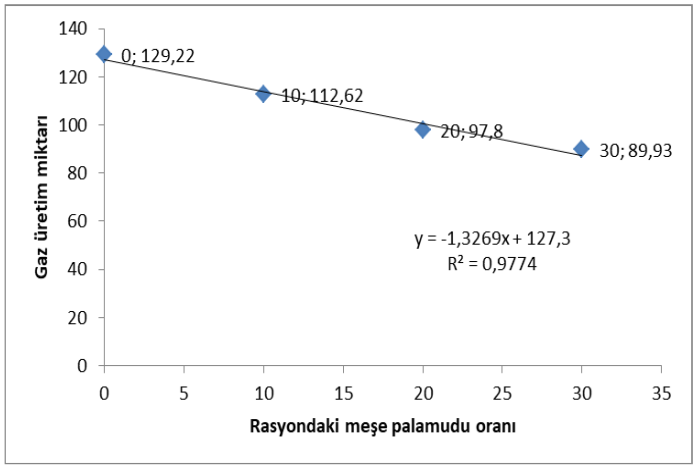
Kuzu rasyonlarına Meşe palamudu ilavesinin *in vitro* gaz üretimi ve metan üretimi ile sindirim derecesine ve mikrobiyel protein üretimine etkisi saptanmış ve Çizelge 3'de verilmiştir. Meşe palamudunun kuzu rasyonlarına ilavesi *in vitro* gaz üretimini, metan üretimi, sindirim derecesi ve mikrobiyal protein üretimine etkisi önemli bulunmuştur (P<0.001).

Çizelge 3. Kuzu besi rasyonlarına meşe palamudu ilavesinin *in vitro* gaz üretimi, metan üretimi, sindirim derecesine ve mikrobiyal protein üretimine etkisi.

Table 3. The effect of adding acorns to lamb rations on gas production, methane production, digestion degree and microbial protein production.

Parametreler	Rasyonlar				SEM	Sig.
	I	II	III	IV		
Gaz	129.22^{a}±5.93	112.62^{b}±4.05	97.80^{c}±1.68	89.93^{d}±4.48	3.195	***
Metan (ml)	19.65^{a}±1.01	16.60^{b}±1.08	13.42^{c}±0.64	10.43^{d}±1.30	0.756	***
Metan (%)	15.22^{a}±0.11	14.75^{ab}±0.91	13.72^{b}±0.49	11.53^{c}±0.96	0.486	***
TSM	368.37^{a}±22.35	354.65^{a}±14.82	345.62^{a}±9.21	310.73^{b}±13.92	11.274	***
SD	72.90^{a}±4.32	70.62^{a}±2.93	68.82^{a}±2.02	61.63^{b}±2.67	2.202	***
TF	3.38^{c}±0.17	3.73^{b}±0.21	4.19^{a}±0.13	4.10^{a}±0.17	0.121	***
MP	128.57^{b}±18.30	145.65^{ab}±17.13	164.40^{a}±9.66	143.91^{ab}±9.45	10.321	***
MPSE(%)	34.80^{c}±3.31	40.98^{b}±3.43	47.50^{a}±1.62	46.31^{ab}±2.26	1.980	***

^{abc}Aynı simgeye sahip ve aynı satırda yer alan ortalamalar arasında fark yoktur (P>0,05), Ö.D: Önemli düzeyi, TSM: Toplam sindirilebilir madde (mg), SD: Sindirim derecesi (%), TF: Taksimat faktörü, MP: Mikrobiyal protein üretimi (mg), MPSE: Mikrobiyal protein sentezleme etkinliği (%), *** P<0.001.



Şekil 1. Rasyonlara meşe palamudunun katılma oranıyla *in vitro* gaz üretimi arasındaki ilişki

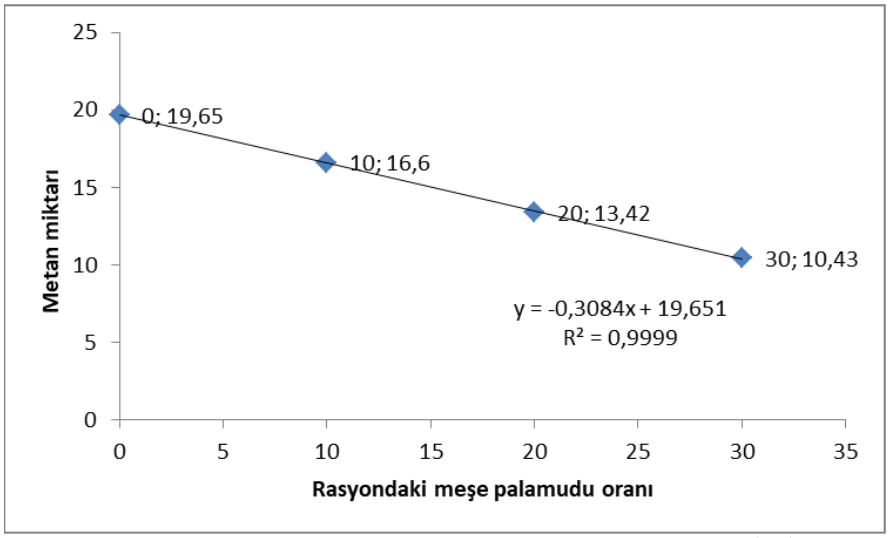
Figure 1. The relationship between the ratio of acorns in the ration and gas production

Meşe palamudunun rasyona ilavesiyle birlikte gaz üretimi önemli derecede azalmış olup gaz üretim değeri 89.93 ile 129.22 ml arasında değişmiştir. En yüksek gaz üretim değeri kontrol grubunda olurken en düşük gaz üretimi IV nolu rasyondan elde edilmiştir. Rasyona artan miktarlarda meşe palamudu ilavesi gaz üretiminin azalmasında % 97 rol oynamıştır. Geriye kalan % 3'lük kısım diğer sebeplerden gerçekleştiği söylenebilir.

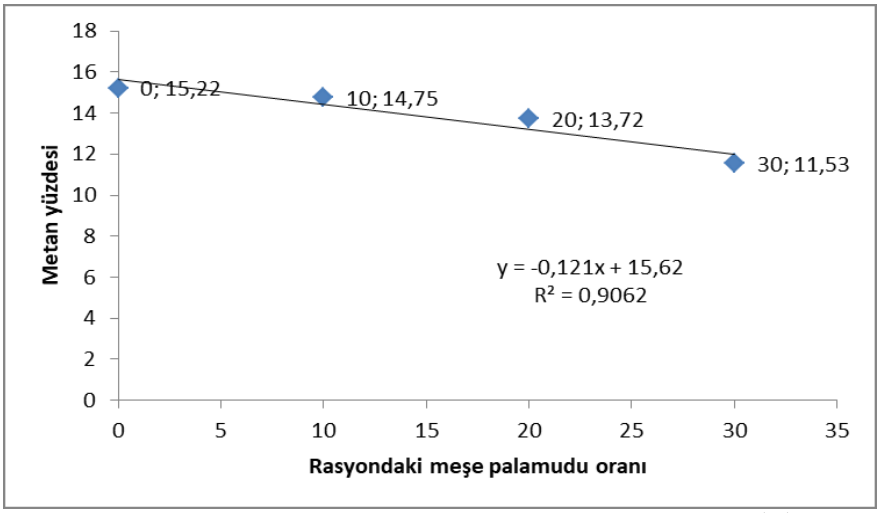
Rasyonlara meşe palamudunun katılma oranıyla gaz üretimi arasındaki ilişki saptanmış ve Şekil 1'de verilmiştir.

Rasyona 1 birim meşe palamudu ilave edildiğinde gaz üretiminde 1.3269 ml azalma meydana gelmiştir. Gaz üretim miktarlarında meydana gelen azalmanın meşe palamudunda bulunan tanen miktarının yüksek olmasından kaynaklandığı yapılan araştırmalarla bildirilmiştir (Patra ve Saxena,2009). Meşe palamudunun kuzu rasyonlarına ilavesiyle birlikte

metan miktarlarında azalma meydana gelmiştir.
Metan miktarları 10.43 ile 19.65 arasında değişmiş olup, en yüksek kontrol grubunda, en düşük ise IV nolu rasyondan elde edilmiştir.
Rasyonlara meşe palamudunun katılma oranıyla metan gazı üretimi (ml) arasındaki ilişki saptanmış ve Şekil 2'de verilmiştir. Kuzu besi rasyonlarına meşe palamudu katılma oranıyla metan miktarı (ml) arasındaki ilişki Şekil 2'de verilmiştir. Rasyona 1 birim meşe palamudu ilave edildiğinde metan miktarında 0.3084 ml azalma meydana gelmiştir. Yapılan bir çalışmada rumende metan gazı üretiminin azalması tanenlerin metanojenik mikroorganizmaların yapısında bulunan proteinlere bağlanması sonucu ortaya çıkan bakterisid veya bakteriostatik etkilerinden kaynaklandığı bildirilmiştir (Tavendale ve ark., 2005). Meşe palamudunun rasyona ilavesiyle birlikte metan içeriğinde azalmalar meydana gelmiştir. Meşe palamudunun metan içeriği %11.53 ile 15.22 arasında değişmiş olup, en yüksek değer kontrol grubundan en düşük değer ise IV nolu rasyondan elde edilmiştir.
Rasyonlara meşe palamudunun katılma oranıyla metan gazı (%)'desi arasındaki ilişki saptanmış ve Şekil 3'de verilmiştir.



Şekil 2. Rasyonlara meşe palamudunun rasyona katılma oranıyla metan miktarı (ml) arasındaki ilişki.
Figure 2. The relationship between the ratio of acorns in the ration and the amount of methane (ml) in the ration.

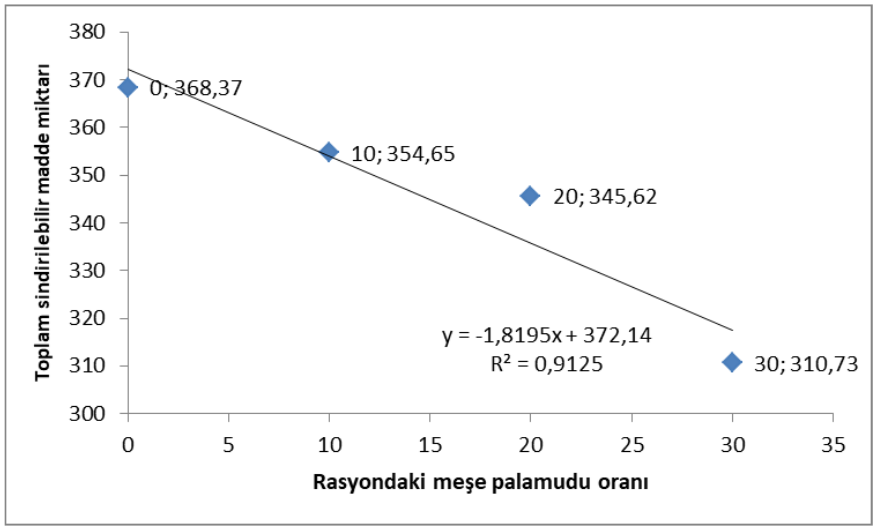


Şekil 3. Rasyonda meşe palamudunun rasyona katılma oranıyla metan (%) arasındaki ilişki
Figure 3. The relationship between the ratio of acorns in the ration and methane (%)

Rasyona 1 birim meşe palamudu ilave edildiğinde metanda %0.121 azalma meydana gelmiştir. Lopez ve ark. (2010)'nın yapmış olduğu çalışmada metan içeriklerinin $11<CH_4\leq14$ arasında olması düşük seviyede anti-metanojenik özelliğe sahip olduğunu bildirmiştir. Yapılan çalışmada meşe palamudunun metanın anti-metanojenik özelliğe ait olduğunu söyleyebiliriz. Meşe palamudunun rasyona ilavesiyle birlikte TSM miktarında azalmalar meydana gelmiştir. Meşe palamudunun TSM değeri 310.73 ile 368.37 mg arasında değişmiş olup, en yüksek değer kontrol grubunda en düşük değer ise IV nolu

rasyondan elde edilmiştir.

Rasyonlara meşe palamudunun katılma oranıyla toplam sindirilebilir besin madde miktarı arasındaki ilişki saptanmış ve Şekil 4'de verilmiştir.

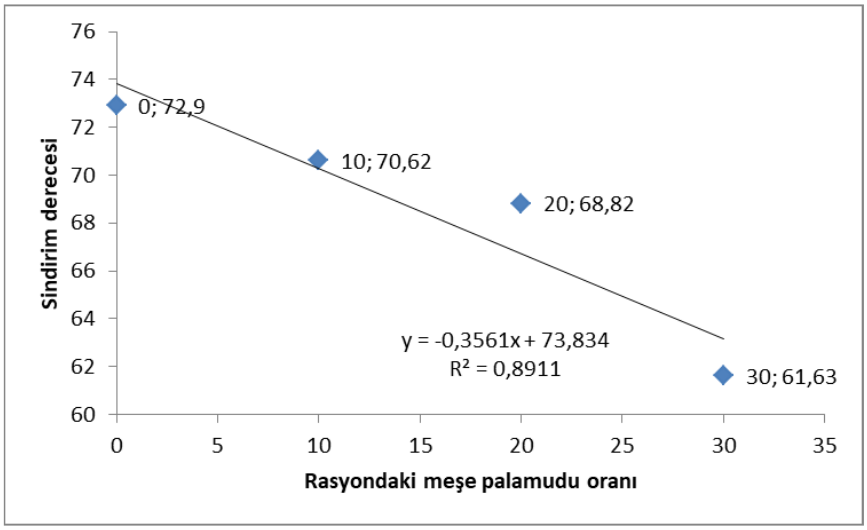


Şekil 4. Rasyonlara meşe palamudunun katılma oranıyla toplam sindirilebilir besin madde miktarı arasındaki ilişki.

Figure 4. The relationship between the ratio of acorns in the diet and the total amount of digestible matter.

Rasyona 1 birim meşe palamudu ilave edildiğinde toplam sindirilebilir besin madde miktarında 1.8195 azalma meydana gelmiştir. Meşe palamudunun rasyona ilavesiyle sindirim derecesi önemli derecede azalmıştır. Sindirim dercesi değerleri %61.63 ile 72.90 arasında değişmiş olup en yüksek değerin kontrol grubunda, en düşük değerin ise IV nolu rasyonda elde edilmiştir.

Rasyonlara meşe palamudunun katılma oranıyla sindirilme derecesi arasındaki ilişki saptanmış ve Şekil 5'de verilmiştir.



Şekil 5. Rasyonlara meşe palamudunun katılma oranıyla sindirilme derecesi arasındaki ilişki.

Figure 5. The relationship between the rate of inclusion of acorns in the ration and the degree of digestion.

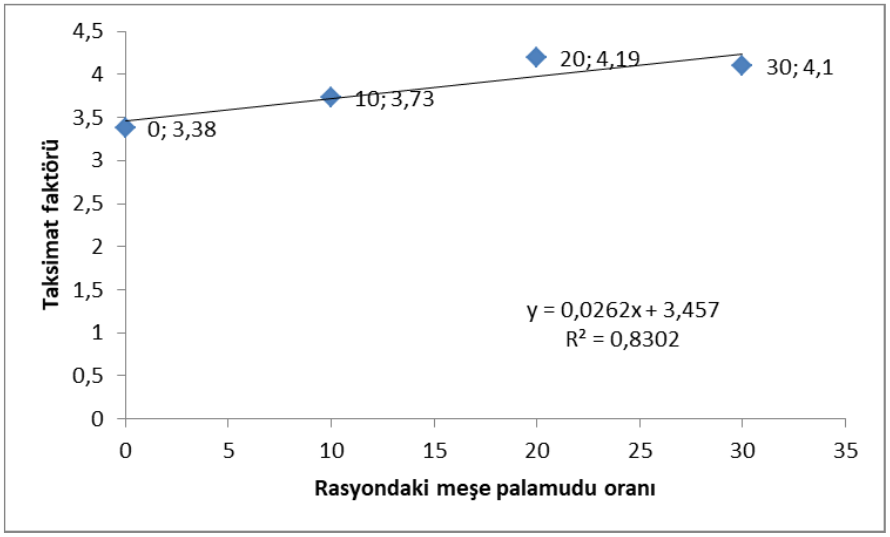
Rasyona 1 birim meşe palamudu ilave edildiğinde sindirim derecesinde 0.3561 azalma meydana gelmiştir. Kamalak ve ark., (2005)'nın yaptıkları çalışmada yemlerde farklı miktarlarda bulunan tanenlerin hayvanlar üzerine olumlu ve olumsuz etkilerinin olduğu ve tanenin fazla miktarda olması sindirim derecesini azaltacağı bildirilmiştir. Meşe palamudunun rasyona ilavesiyle birlikte TF miktarında artış meydana gelmiştir. Meşe palamudunun TF değerleri 3.38 ile 4.10 arasında değişmiş, en yüksek değer IV nolu rasyonda en düşük değer ise kontrol grubundan elde edilmiştir.

Rasyonlara meşe palamudunun katılma oranıyla taksimat faktörü arasındaki ilişki saptanmış ve Şekil 6'da verilmiştir.

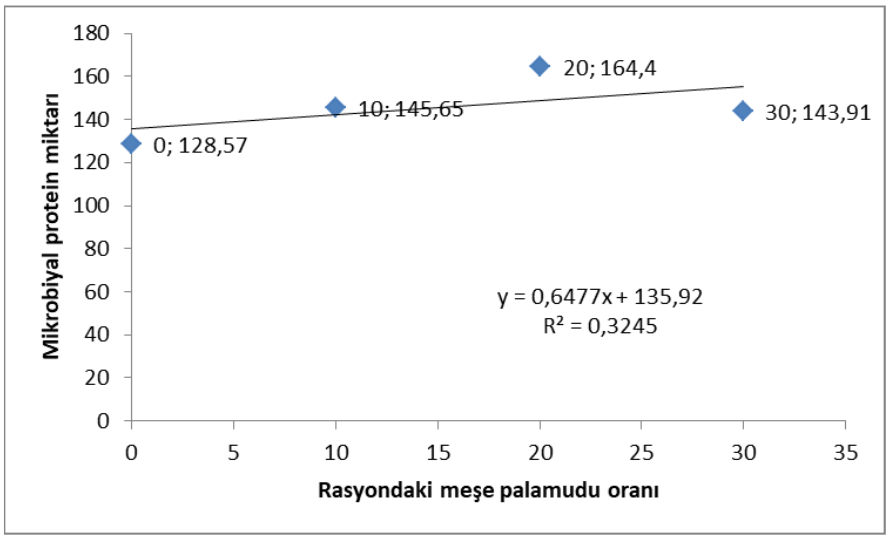
Rasyona 1 birim meşe palamudu ilave edildiğinde taksimat faktöründe 0.262 artış meydana gelmiştir. Yapılan araştırmada mikrobiyel protein sentezleme

etkinliğinin önemli parçası olan TF'nin en uygun değerininin 2.75 ile 4.41 arasında olduğu bildirilmiştir. (Blümmel ve ark., 1997; Blümmel ve Lebzien, 2001). Meşe palamudunun rasyonlara ilavesiyle birlikte mikrobiyel protein miktarında artış meydana gelmiştir. Meşe palamudunun mikrobiyal protein değeri 128.57 ile 143.91 mg arasında değişmiş olup, en yüksek değer IV nolu rasyonda, en düşük değer ise kontrol grubundan elde edilmiştir.

Rasyonlara meşe palamudunun katılma oranıyla mikrobiyal protein arasındaki ilişki saptanmış ve Şekil 7'de verilmiştir.



Şekil 6. Rasyonlara meşe palamudunun katılma oranıyla taksimat faktörü arasındaki ilişki.
Figure 6. The relationship between the proportion of acorns in the ration and the division factor.



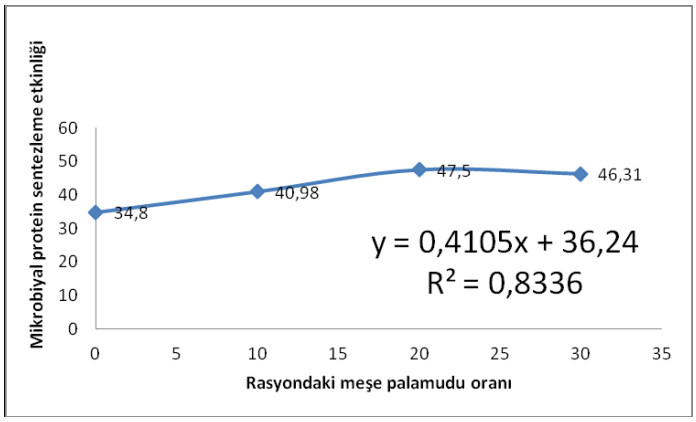
Şekil 7. Rasyonlara meşe palamudunun katılma oranıyla mikrobiyal protein miktarı arasındaki ilişki.
Figure 7. The relationship between the ratio of acorns in the diet and the amount of microbial protein.

Rasyona 1 birim meşe palamudu ilave edildiğinde mikrobiyel protein miktarında 0.6477 artış meydana gelmiştir. Yapılan bir çalışmada mikrobiyel protein deki artışın nedeni olarak yemlerdeki tanen miktarının yüksekli bildirilmiştir (Kamalak ve ark., 2005).

Meşe palamudunun rasyona ilavesiyle birlikte mikrobiyal protein sentezleme etkinliğinde önemli derecede artış meydana gelmiştir. Meşe palamudunun mikrobiyal protein sentezleme etkinliğinin değeri 34.80 ile 46.31 arasında değişmiş, en yüksek değerin IV nolu rasyonda en düşük değer ise kontrol grubundan elde edilmiştir.

Rasyonlara meşe palamudunun katılma oranıyla mikrobiyal protein sentezi etkinliği arasındaki ilişki saptanmış ve Şekil 8'de verilmiştir.

Rasyona 1 birim meşe palamudu ilave edildiğinde MPSE'de %0.4105'lik artış meydana gelmiştir. Çizelge 3'de görüldüğü gibi TF değerlerinin yüksek olduğu rasyonlarda MPSE'nin de yüksek olduğu görülmektedir. Yapılan araştırmalarda mikrobiyel sentezleme etkinliği için önemli olan TF'nin yemlerde yüksek miktarda olması, mikrobiyel protein sentezleme etkinliğini artıracağı bildirilmiştir (Blümmel ve ark., 1997; Blümmel ve Lebzien, 2001).



Şekil 8. Rasyonlara meşe palamudunun katılma oranıyla mikrobiyal protein sentezi etkinliği arasındaki ilişki

Figure 8. The relationship between the rate of inclusion of acorn in the diet and the efficiency of microbial protein synthesis.

SONUÇ ve ÖNERİLER

Çalışmada elde edilen bulgulara göre; kuzu rasyonlarına meşe palamudunun ilave edilmesi gaz üretimini, metan üretimini, toplam sindirilebilir madde miktarını ve sindirim derecesini azaltmış olup mikrobiyel protein miktarı ve mikrobiyel protein sentezleme etkinliği arttırmıştır. Meşe palamudunun kuzu rasyonlarında düşük seviyede anti-metanojenik etkiye sahip olduğu bulunmuştur. Yapılan üç farklı rasyonlarda kontol grubuna oranla gaz üretimi, metan üretimi ve yüzde metan oranını düşürdüğü gözlemlenmiştir.

Ayrıca bundan sonra yapılacak çalışmalarda meşe palamudunun kuzuların performansına, kuzuların yem tüketimine ve sindirim derecesi üzerine etkilerini belirlemek için *in vitro* çalışmalarda elde edilen sonuçların *in vivo* çalışmalar ile test edilmesine ihtiyaç vardır.

TEŞEKKÜR

Bu çalışma KSÜ Bilimsel Araştırma Birimi (BAP Proje No: 2021/6-9 YLS) tarafından desteklenmiştir.

Araştırmacıların Katkı Oranı Beyan Özeti

Yazarlar makaleye eşit oranda katkı sağlamış olduklarını beyan eder.

Çıkar Çatışması Beyanı

Yazarlar makaleye eşit oranda katkı sağlamış olduklarını beyan eder.

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Bal Arısı Sperma Sulandırıcısına Katılan Shilajitin Spermanın Dondurulması Üzerine Etkileri

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ÖZET

Bu çalışmanın amacı, bal arısı (*Apis melifera*) sperma sulandırıcısına katılan farklı dozlardaki (%0, 5, 10, 15, 20) shilajitin (S) motilite, plazma membran bütünlüğü ve akrozom bütünlüğü parametreleri üzerine etkilerini belirlemektir. Çalışmada, 5 grup ve her bir grupta 5'er adet koloni olmak üzere toplam 25 adet koloni kullanıldı. Kontrol grubu (S-0), bal arısı sulandırıcısına shilajit ilavesi yapılmayan grubu oluşturmaktadır. S-1, S-2, S-3 ve S-4 grupları ise bal arısı sulandırıcısına sırasıyla %5, 10, 15 ve 20 shilajit ilaveli gruplar olarak oluşturuldu. Gün aşırı beslenen yaklaşık 25 adet koloni içerisinde 14-21 günlük yaşta bulunan yaklaşık 400 erkek arıdan sperma toplandı ve sıvı azot buharında dondurularak, -196°C sıvı azot içerisinde yaklaşık 7 ay süre ile saklandı. Bu süre sonunda dondurulan numuneler 37°C' de çözdürülerek ilgili parametreler yönünden incelendi. Diğer gruplar ile karşılaştırıldığında, S-3 ve S-4 gruplarının sperma motilite değerini arttırdığı ($p<0.05$); S-2, S-3 ve S-4 gruplarının plazma membran bütünlüğünü arttırdığı tespit edildi. Sulandırıcıya farklı dozlarda shilajit ilave edilen gruplarda, kontrol grubuna kıyasla akrozom bütünlüğününün önemli derecede korunduğu belirlendi ($p<0.01$). Sonuç olarak, bal arısı sulandırıcısına farklı dozlardaki shilajitin spermatolojik parametreler üzerine olumlu etkileri olduğu saptandı.

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Anahtar Kelimeler
Bal arısı
Dondurma
Shilajit
Sperma

Effects of Shilajit Added to Honeybee Sperm Extender on Sperm Freezing

ABSTRACT

The aim of this study was to determine the effects of shilajit (S) at different doses (0, 5, 10, 15, 20%) added to honey bee (Apis melilifera) semen extender on motility, plasma membrane integrity and acrosome integrity parameters. In the study, a total of 25 colonies, 5 groups and 5 colonies in each group, were used. The control group (S-0) constitutes the group where shilajit was not added to the honeybee extender. S-1, S-2, S-3 and S-4 groups were formed as groups with 5, 10, 15 and 20% shilajit added to the honey bee extender, respectively. Sperm were collected from approximately 400 drones aged 14-21 days in approximately 25 colonies that were fed every other day. Then they were frozen in liquid nitrogen vapor and stored in liquid nitrogen at -196°C for approximately 7 months. At the end of this period, the frozen samples were thawed at 37°C and examined in terms of relevant parameters. Compared with other groups, S-3 and S-4 groups increased sperm motility ($p<0.05$); It was determined that S-2, S-3 and S-4 groups increased plasma membrane integrity while the acrosome integrity was significantly preserved in the groups with different doses of shilajit added to the diluent compared to the control group ($p<0.01$). As a result, it was concluded that different doses of shilajit to honey bee extender had positive effects on spermatological parameters.

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INTRODUCTION

Storage of honeybee semen is important for preserving racial characteristics and increasing yield characteristics. With the widespread use of artificial insemination in bees, studies on the storage of bee semen have been carried out. Honeybee semen can be stored at 15-28°C or 11 -13°C for a short time, protected from light (Collins, 2000). Drone semen is suitable for storage at temperatures from 13°C to 25°C (Harbo and Williams, 1987). It has been stated that the most suitable semen storage temperatures are between 12°C and 16°C. Moreover, it has been reported that the semen viability rate was above 70% when kept at 12°C for 6 weeks (Locke and Peng, 1993; Collins 2000). Fresh bee semen can be stored in a capillary glass tube. A small amount of diluent on both sides of the tube, diluted semen in the middle and the two open ends of the tube can be sealed and stored (Burley et al., 2008). Such capillary tubes can be stored in special incubators at 16 ±°C, protected from light (Paillard et al., 2017). For long-term storage, various cryoprotectants (DMSO (Dimethyl sulfoxide), glycerol) can be added to the semen diluent and stored in a liquid nitrogen tank (Harbo, 1979). It has been reported that DMSO is the cryoprotectant agent that causes the least harm among other cryoprotectants (Hopkins and Herr, 2010). It was determined that the rate of male eggs, that is, unfertilized eggs, increased in queen bees artificially inseminated with long-term stored honeybee semen. It is also thought that genetic damage may occur during the freezing of honeybee semen (Harbo, 1981).

Shilajit is a brown-blackish substance extracted from the rock layer of the mountainous region of the world, especially from some parts of India (Agarwal et al., 2007). Shilajit contains antioxidant-rich compounds in its structure (Rege et al., 2012). In particular, it is a substance rich in humic and fulvic acids (Ghosal et al., 1990). It is known that shilajit is very effective against the damage of free radicals thanks to the antioxidants it contains (Bhattacharya et al., 1995). It has been reported that shilajit shows strong antioxidant properties, as well as increasing the effectiveness of vitamin C, which has antioxidant properties (Agarwal et al., 2007). In a study on rats, it was stated that shilajit decreased the symptoms of fatigue by increasing mitochondrial activity (Surapaneni et al., 2012). It is also known that shilajit, which has more than 200 mineral substances in its structure, is effective in eliminating reproductive problems in many Asian countries. In studies on rats and humans, it has been reported that shilajit increases semen density and serum testosterone ratio (Zubair, 2016). In a study that was conducted on humans, shilajit was found to significantly reduce the harmful effects of oxidative stress. It has also been stated that shilajit contributes to increasing the motility, semen density and serum testosterone level, which are important semen parameters (Biswas et al., 2010).

The aim of this study was to determine the effects of shilajit, which is known to have significant positive effects on semen, on long-term storage by adding it to honeybee semen diluent.

MATERIAL and METHODS

Preparation of diluents and freezing of semen

All colonies used in the study were fed with syrups prepared with sugar at a ratio of 1:1 every other day. A modified Kiev diluent (D+-glucose 0.3 g, potassium chloride 0.41 g, 2.43 g disodium citrate dihydrate per 100 ml) and 10% DMSO was used as semen diluent (Rosseau and Giovenazzo, 2016). While shilajit was not added to the diluent prepared for the control group (S-0) in the colonies used in the study, semen diluents were prepared by adding 5, 10, 15, and 20% shilajit to the S-1, S-2, S-3, and S-4 groups, respectively. Sperm was collected from approximately 400 drones aged 14-21 days in 25 colonies. Fresh semen collected from drones that have reached sufficient maturity were examined in terms of motility, and it was observed that the average motile spermatozoa rate was above 80%. Also, sperm with a motility evaluation of less than 80% in fresh semen were not included in the study. Each group was separetely diluted to final concentration of approximately 100 $x10^6$ (spermatozoa/mL) with control or shilajit supplemented extenders. After the diluted semen was transferred into 0.25 ml straws in accordance with the procedure (diluent-air-space-semen-air-space-diluent), the openend of the straw was pressed and closed. Straws were kept at 4 ºC for 2 hours for equilibration. Then, it was kept in liquid nitrogen vapor 5 cm below the straws on the special wire grid in the liquid nitrogen setup for 10 minutes, and after freezing, it was stored in liquid nitrogen. Straws stored by freezing were thawed at 37 ºC for 30 seconds and evaluated.

Evaluation of semen

Motility

Semen straws were thawed at 35-36°C for 30 seconds. Afterwards, 5 µl was taken from it and placed on the slide and covered with a coverslip. Motility evaluation was done with a computer-assisted semen analyzer

(CASA) (SCA®, Microptic, Barcelona, Spain). The results were evaluated as 20%, 40%, 60%, 80% and 95% and above, in particular for honey bee semen (Taylor et al., 2009).

Plasma membrane integrity

To evaluate the integrity of the plasma membrane 100 µl of 100 mOsm HOST (Hypo-Osmotic Swelling Test) solution was used. After 10 µl of thawed semen was left at 37 °C for 30 minutes, 200 spermatozoa were counted. Coiled and bent-tailed spermatozoa were detected (Kumar et al., 2018).

Acrosome integrity

In the study, 100 ml of phosphate buffer solution (PBS) was added to 10 µl of semen. After centrifugation at 100 RCF for 5 minutes, semen was separated from PBS. After the part was completed by adding 100 ml of PBS, it was centrifuged again for the second time. After removing the supernatant PBS, the smear was taken. After staining with PSA-FITC (L0770) solution in the darkroom, it was kept in the dark at 37°C for 1 hour. At least 200 spermatozoa were evaluated under fluorescence microscopy and stained ones were expressed as a percentage (Alçay et al., 2019).

Statistical analysis

The statistical significance level for each group used in the study was made with 'One-Way Analysis of Variance'. Duncan's multiple range test was used to investigate the differences between group means. The effects of the groups were evaluated as significant at the at the P<0.05 level. SPSS 20.0 (IBM Corp., 2011) package program was used for variance analysis of the data obtained from the study.

RESULTS and DISCUSSION

In the study investigating the effects of different levels of shilajit (0, 5, 10, 15, 20 %) added to honey bee semen diluent on long-term storage of semen, it was determined that there was a significant difference between the groups in terms of motility, HOST and acrosome integrity parameters (Table 1).

Table 1. Spermatological parameter values between groups
Çizelge 1. Gruplar arasındaki spermatolojik parametre değerleri

Groups	Total Motility (%)	HOST (%)	Acrosome Integrity (%)
S0 (Control)	28.01±4.90^{c}	56.20±2.15^{c}	87.00±0.32^{c}
S1 (%5 Shilajit)	36.06±7.48^{bc}	61.40±3.49^{bc}	88.00±0.00^{b}
S2 (%10 Shilajit)	32.00±4.90^{bc}	64.00±2.59^{ab}	87.80±0.20^{b}
S3 (%15 Shilajit)	48.71±4.90^{ab}	69.20±2.46^{a}	88.40±0.25^{b}
S4 (%20 Shilajit)	56.04±4.00^{a}	69.80±1.39^{a}	89.00±0.00^{a}
P	0.008	0.005	0.000

Data is presented in Mean ± S.D.
^{a,b,c} Values Differences between means with different letters in the same row are significant.

In our study, which investigated the effects of adding different levels of shilajit to honey bee semen on the cryopreservation of bee semen, mean motility values were determined as 28,01 ±4.90%, 36,06 ±7.48%, 32,00 ±4.90%, 48,71 ±4.90% and 56,04 ±4.00% in the groups (Control, S1, S2, S3, S4), respectively (Figure 1).

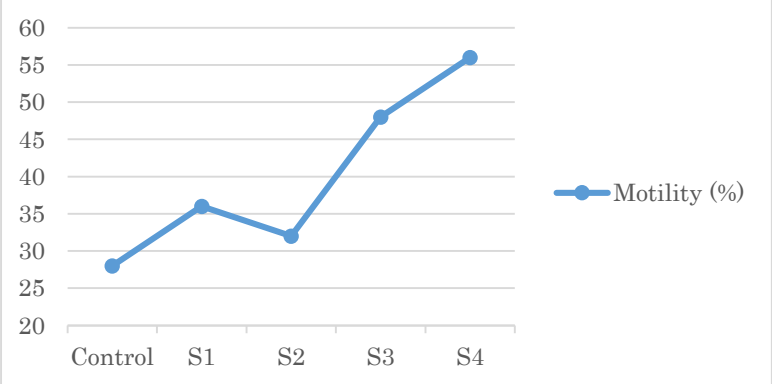

Figure 1. Semen motility distribution between groups
Şekil 1. Gruplar arasındaki sperma motilite dağılımı

There was a statistically significant difference between the groups in terms of motility values (P<0.05). Particularly, the percentage of motility was found to be the highest statistically in the S4 group,

in which shilajit added 20% to the semen diluent, compared to the other groups (P<0.05).

In the study of Ikram-ul-Haq et al., (2016) on semen parameters of shilajit in rams, 3 different groups were formed with 4 rams in each group. In the study, it was observed that shilajit administered orally at different doses for 9 weeks significantly increased the semen volume and motility values. However, it is seen that the use of shilajit is not similar to our study. In another study, the effects of shilajit treatment were observed in mice with infertility by administering cadmium, and it was reported that shilajit had a significant effect on eliminating the negative effects on semen parameters (Mishra et al., 2018). A study invested the effects of fulvic acid on semen parameters in goats observed that fulvic acid, which is found in a significant amount in the structure of shilajit applied at different doses, had a positive effect on semen motility values (Xiao et al., 2018). Fulvic acid was added to Tris-egg yolk diluent at different doses and stored in liquid nitrogen for about 2 weeks after being frozen and thawed under appropriate conditions, and its effects on goat semen parameters were observed. The presence of fulvic acid used in the study in the structure of shilajit, the addition of fulvic acid used in the study to the diluent at different doses, and the examination of frozen and thawed semen in terms of motility are in line with our study. While there was no significant difference between the different doses in the study, it was observed that fulvic acid significantly increased the percentage of motility when compared with the control group.

The mean HOST positive percentages of the groups in our study (Control, S1, S2, S3, S4) were determined as 56.20 ±2.15%, 61.40 ±3.49%, 64.00 ±2.59%, 69.20 ±2.46% and 69.80 ±1.39%, respectively (Figure 2).

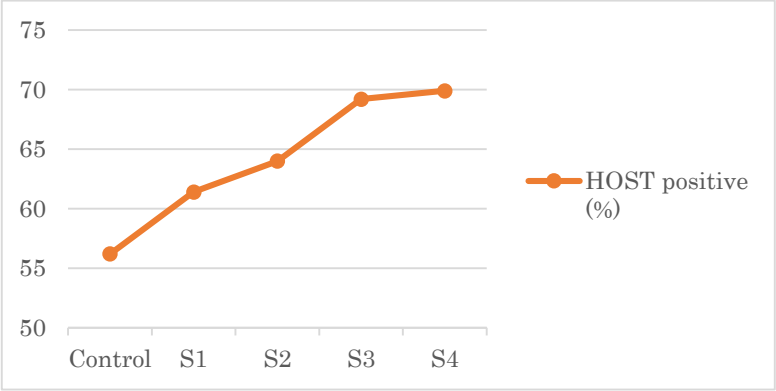

Figure 2. Distribution of HOS test positive (%) between groups
Şekil 2. Gruplar arasındaki HOS testi pozitif (%) dağılımı

It was determined that the addition of shilajit to the semen diluent significantly increased the HOST positive percentage (P<0.05). When the statistical average values were examined, it was found significant that the percentage of membrane permeability increased proportionally with the increase in the amount of shilajit used in general. The increase in the positive rate of plasma membrane integrity indicated that the membrane structure of the semen was preserved. Sultan et al., (2021), in a study in which they examined the effects of shilajit added to buffalo semen diluent at different doses, determined that 3% shilajit was more successful than other study groups. A decrease in plasma membrane integrity was found to be significant with an increase in the shilajit ratio above 3%. In a study where a special mixture containing shilajit was applied orally on buffaloes, the semen collected was diluted and frozen. In the study, a significant increase was observed in the acrosome integrity as well as the plasma membrane integrity (Kumar et al., 2018). Although the positive effect of shilajit was observed in our study, the oral administration of the application is not similar to our study. In our study, it was determined that the addition of shilajit at different doses increased acrosome integrity in the control group (P<0.01) (Table 1).

There was a significant difference between the groups in terms of positive values of acrosome integrity (P<0.05). As can be seen from the mean values (%) of control (87.00 ±0.32), S1 (88.00), S2 (87.80 ±0.20), S3 (88.40 ±0.25) and S4 (89.00), it was observed that the positive rate of acrosome integrity gradually increased as the amount of shilajit added to the semen diluent increased (Figure 3).

It was observed that the addition of shilajit statistically significantly increased the percentage of acrosome integrity (P<0.05). Acrosome integrity was found to be the highest in the S4 group, in which 20% shilajit was added to the semen diluent (P<0.05). Alçay et al., (2019) reported that the diluent had positive effects on acrosome integrity in their study on the dilution of honeybee semen with TL-Hepes based and with BSA (bovine serum albumin) added

diluent. The study is significant for determining the effectiveness of the substance added to the honeybee diluent. Although the result of the study is positive, it is not similar to our study because the content of the substance used is different. According to Tripathi et al. (1996), shilajit plays an important role in preventing lipid peroxidation, which plays an important role in the disruption of spermatozoa membrane integrity.

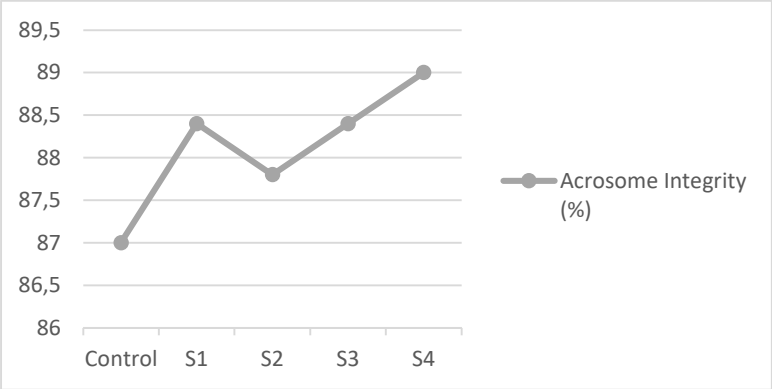

Figure 3. Distribution of acrosome integrity (%) between groups
Şekil 3. Gruplar arasındaki akrozom bütünlüğü (%) dağılımı

Regarding the storage of honeybee semen, shilajit seems to affect the storage conditions positively. As a result of the study, it was observed that the addition of shilajit to the honeybee diluent contributes to the long-term storage of honeybee semen and has a positive effect on motility, plasma membrane integrity and acrosome integrity. The results of the study reveal that the use of shilajit in semen diluent will provide long-term storage conditions in honeybee semen. It is aimed to improve long-term storage conditions with such studies on honeybee semen. As a result, it is understood that shilajit significantly improves the storage conditions by making a significant contribution to the preservation of motility, plasma membrane integrity and acrosome integrity in the long-term storage of honeybee semen.

Author's Contributions

The contribution of the athors is equal.

Conflict of Interest

The authors are declared that there is no conflict of interest.

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