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# TARIM ve DOĞA DERGİSİ

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## Unveiling Anticancer Potential in the Interactions of Melittin Peptides with CD147 Receptor: A Structural and Functional Analysis of Ligand-Target Interactions

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### ABSTRACT

In this study, the anticancer potential of melittin (MLT) peptides interacting with the CD147 receptor was investigated through *in silico* structural and functional analyses. The interaction between the transmembrane glycoprotein CD147 and cyclophilin A (CypA) activates signaling pathways crucial in cancer pathology. This study focused on the potential of melittin peptides to inhibit this interaction. Structures of the CD147 receptor and melittin peptides were obtained from the Protein Data Bank (PDB), including the three-dimensional structure of the Ig1 domain of CD147 (PDB ID: 5XF0) and melittin structures (PDB IDs: 2MLT, 6O4M, 3QRX, 8AHT, and 8AHS). Validated ligand structures were acquired through X-ray crystallography. Receptor-ligand interactions and anticancer activity were evaluated using the ClusPro2.0 molecular docking server, AncicP2.0 and ENNACT anticancer analysis servers, ProtScale hydrophobicity analysis, PDBSum amino acid interaction analysis, and PRODIGY thermodynamic stability analysis tools. Molecular docking simulations analyzed receptor-ligand interactions, emphasizing the role of hydrophobic interactions. Structural analysis revealed variability in peptide quality, with 2MLT demonstrating favorable attributes while 3QRX exhibited weak integrity. Anticancer analysis servers indicated that 2MLT and 3QRX, exhibiting similar binding patterns with 5XF0 and CD147/CypA, may demonstrate potential anticancer activity. Specifically, non-bonded interactions involving Gly181 and Arg201 in the 5XF0-2MLT complex and non-bonded interactions involving Pro180, Gly181, and Arg201 in the 5XF0-3QRX complex were highlighted, resembling the interaction pattern of CD147/CypA. Therefore, the importance of understanding molecular interactions and guiding drug discovery through structural examinations and computational analyses was emphasized, providing insights into the anticancer effects and drug design implications of these complexes; moreover, further research into their structural determinants and therapeutic potentials is critically essential for biomedical applications.

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## Melittin Peptitlerinin CD147 Reseptörü ile Etkileşimindeki Antikanser Potansiyeli: Ligand-Reseptör Etkileşiminin Yapısal ve Fonksiyonel Analizi

### ÖZET

Bu çalışmada, antikanser etkileriyle bilinen melittin (MLT) peptitlerinin CD147 reseptörüyle etkileşimlerinin antikanser potansiyeli *in silico* yapısal ve işlevsel analizlerle araştırılmıştır. CD147 transmembran glikoproteini ve siklofilinA (CypA) etkileşimi, kanser patolojisinde önemli olan sinyal yollarını aktive eder. Bu çalışmada, melittin peptitlerinin bu etkileşimi engelleme potansiyeli üzerinde durulmuştur. CD147 reseptör yapısı ve melittin peptit yapıları Protein Veri Bankası'ndan (PDB) temin edilmiş olup; bunlar arasında CD147'nin Ig1 alanının üç boyutlu yapısı (PDB No: 5XF0) ve melittin yapıları (PDB No: 2MLT, 6O4M, 3QRX, 8AHT ve 8AHS) bulunmaktadır. PDB tarafından onaylanmış ligand yapıları X-ışını

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Antikanser peptitler

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kırınımı ile elde edilmiştir. ClusPro2.0 moleküler bağlanma sunucusu, AncicP2.0 ve ENNAACT antikanser analiz sunucuları, ProtScale hidrofobisite analiz, PDBSum aminoasit etkileşim analiz ve PRODIGY termodinamik stabilite analiz araçları kullanılarak reseptör-ligand etkileşimleri ve antikanser aktivite değerlendirildi. Moleküler bağlanma simülasyonları reseptör-ligand etkileşimlerini analiz etmiş ve hidrofobik etkileşimlerin rolünü vurgulamıştır. Yapısal analiz, peptit kalitesinde değişkenlikleri göstermiş; 2MLT olumlu özellikler sergilerken, 3QRX'in zayıf bütünlük gösterdiği tespit edilmiştir. Antikanser analiz sunucuları 5XF0 ve CD147/CypA ile benzer bağlanma desenleri sergileyen 2MLT ve 3QRX'in her ikisinin de potansiyel antikanser aktivitesi gösterebileceğini ortaya koymuştur. Özellikle 5XF0-2MLT kompleksindeki Gly181 ve Arg201 ile bağımsız etkileşimler, 5XF0-3QRX kompleksindeki Pro180, Gly181 ve Arg201 ile bağımsız etkileşimler çalışmamızda ortaya konularak CD147/CypA etkileşim şekline benzerliğine dikkat çekilmiştir. Bu nedenle, moleküler etkileşimlerin anlaşılması ve ilaç keşfini yönlendirmenin önemi, yapısal incelemeler ve hesaplamalı analizlerin vurgulanarak, bu komplekslerin antikanser etkileri ve ilaç tasarımı üzerindeki etkilerine dair bilgiler sunulmuştur; ayrıca, bu yapısal belirleyicilerin ve terapötik potansiyellerinin ileri araştırmaları, biyomedikal uygulamalar için kritik öneme sahiptir.

Moleküler bağlanma  
Siklofilin A

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## INTRODUCTION

Cancer remains a formidable challenge in medical science, with treatment strategies continuously evolving to improve patient outcomes. Despite advancements in cancer therapy, treatment failures still occur due to factors like tumor heterogeneity and resistance mechanisms. Researchers are exploring innovative approaches to combat these challenges, including animal venoms. Venoms, such as those from snakes, bees, and scorpions, contain bioactive compounds that have shown potential in targeting cancer cells with precision. These venom-derived agents can inhibit protein synthesis, induce apoptosis, and disrupt angiogenesis, offering a complementary strategy to conventional treatments. The anticancer effectiveness of animal venoms is attributed to their ability to target cancer cells while minimizing toxicity to normal tissues selectively, thus presenting a promising avenue for enhancing the efficacy of cancer therapeutics (Chaisakul et al., 2016; Gomes et al., 2010; Li et al., 2018; Roy & Bharadvaja, 2021; Sjakste & Gajski, 2023).

Melittin is a potent peptide that is the main component of honeybee (*Apis mellifera*) venom, constituting about 40–60% of its dry weight (Memariani et al., 2019). It is a cationic amphiphilic peptide known for its diverse biological activities, including antimicrobial, antiviral, and anti-inflammatory effects (Tiwari et al., 2022). Notably,

melittin has garnered attention for its anticancer properties. It has been shown to exert antitumor effects by disrupting cell membranes, inhibiting cell growth by interfering with the cell cycle, and inducing apoptosis and necrosis in cancer cells (Daniluk et al., 2022; Huang et al., 2016). Melittin's ability to selectively target cancer cells while sparing normal cells makes it a promising candidate for enhancing the efficacy of traditional cancer therapies (Pandey et al., 2023).

Melittin, a water-soluble peptide from bee venom, has garnered attention for its potential in cancer therapy. It exhibits direct cytotoxic effects on cancer cells and modulates immune responses. Studies have shown that melittin can induce cell cycle arrest and apoptosis, regulate pathways involved in metastasis, angiogenesis, and inflammation, and interact with signaling molecules like Bax, Bcl-2, caspases, and NF- $\kappa$ B. Its ability to inhibit growth and induce apoptosis has been enhanced when delivered in niosomes, making it a promising candidate for cancer treatment. Recent research focuses on improving melittin's selectivity and reducing its toxicity through nanotechnology and combination with conventional drugs (Haque et al., 2023; Pandey et al., 2023).

CD147, or EMMPRIN or basigin, is a transmembrane glycoprotein extensively implicated in cancer progression. Found in various human tumors, CD147 plays a multifaceted role by stimulating the secretion



of matrix metalloproteinases (MMPs) and cytokines. It modulates cellular processes such as proliferation, apoptosis, migration, metastasis, and differentiation, particularly in hypoxic environments. CD147 expression is closely associated with tumor invasion, metastasis, and angiogenesis, rendering it a significant biomarker for cancer prognosis and diagnosis. Additionally, CD147 is a promising therapeutic target, with monoclonal antibodies against it showing potential efficacy, notably in treating hepatocellular carcinoma (Huang et al., 2023; Xiong et al., 2014).

This glycoprotein is notably overexpressed in a broad spectrum of aggressive human cancers, spanning from central nervous system tumors to those of the head and neck, breast, lung, gastrointestinal tract, genitourinary system, skin, and hematological and musculoskeletal systems. Its pivotal role extends to cancer cell proliferation, survival, angiogenesis, metabolic reprogramming, immune evasion, invasion, and metastasis (Nyalali et al., 2023).

The interaction between Cyclophilin A (CypA) and CD147 plays a pivotal role in cancer development. CypA, with its peptidyl-prolyl cis-trans isomerase activity, binds to CD147, influencing multiple cellular functions and activating downstream signaling pathways crucial in cancer pathology. This interaction is associated with increased tumor growth, metastasis, therapeutic resistance, and poor patient prognosis. Studies using The Cancer Genome Atlas (TCGA) database have shown a significant correlation between the overexpression of CypA and CD147 and advanced cancer stages and lower survival rates. Consequently, the CypA/CD147 axis is considered a promising target for anticancer therapy, with the potential to improve treatment outcomes (Bakhtiyari et al., 2023; Han & Jung, 2022; Nyalali et al., 2023; Xiong et al., 2014).

This study aims to investigate the anticancer potential inherent in the interactions between melittin peptides and the CD147 receptor, providing a comprehensive structural and functional analysis of ligand-target interactions. The study aims to elucidate the structural determinants influencing their biological activity through detailed examination and comparison of the structural properties of various melittin peptides and their binding affinities with the CD147 receptor. Additionally, the study explores the role of hydrophobic interactions in forming and stabilizing receptor-ligand complexes, shedding light on the thermodynamic stability of these interactions under different temperature conditions. Computational tools are utilized in molecular docking studies to reveal the *in silico* structures of receptor-ligand complexes, where receptors consist of proteins and ligands may consist of another protein or small molecule (Oner et al., 2024), and to assess the

potential anticancer properties of the examined ligands, providing valuable insights into their therapeutic potential. Overall, this study aims to contribute to the existing knowledge by providing insights into the interactions between melittin peptides and the CD147 receptor, shedding light on their structural and functional aspects.

## MATERIAL and METHODS

The Protein Data Bank (PDB), a database storing the structures and related information of biological macromolecules, was utilized to select materials for the research. The molecule structures corresponding to the three-dimensional structure of the Ig1 domain of CD147 with PDB ID 5XF0 (Jin et al., 2018), as well as the structures with PDB IDs 2MLT, 6O4M, 3QRX, 8AHT, and 8AHS, represent melittin, one of the most essential toxins of *Apis mellifera*. The ligand structures, excluding the receptor structure 5XF0, were validated by depositors using X-ray diffraction and submitted to the Worldwide Protein Data Bank (wwPDB). It is evident from the publicly released X-ray Structure Validation Reports that they are valid PDB entries. As of the date of the study (2024), 13 entries related to melittin were identified on the official website of the PDB: <https://www.rcsb.org/>. NMR-based structures for ligands were excluded from this study, focusing solely on the five entries obtained through X-ray diffraction methods. This decision was made due to the limited number of NMR-resolved structures available in the PDB (three entries) and the lack of comparability with X-ray crystallographic structures, as normalized real-space R-value (RSRZ) outliers do not apply to NMR. Consequently, a comparative analysis of CD147 interactions between NMR and X-ray crystallographic structures was deemed outside the scope of this study. The interaction between the receptor and ligand molecules was visualized using the molecular surface method in PyMOL.

The Uniprot PDBsum tool was used to analyze the receptor-ligand complexes' PDB files. This tool enabled the visualization of interactions among polypeptide chains, residues, and atoms, encompassing hydrogen bonds, non-bonded contacts, and salt bridges.

The amino acid sequences of the ligands were obtained from the RCSB Protein Data Bank. Subsequently, sequence-specific parameters, including hydrophobicity, hydrophobicity, amphipathicity, hydrophilicity, charge, isoelectric point, and molecular weight were predicted using the AntiCP 2.0 server with default settings (Model 1, SVM threshold: 0.45), as described by Agrawal et al., (Agrawal et al., 2021). The analysis of hydrophobicity was further validated using the ProtScale program from The SIB Swiss Institute of Bioinformatics,

employing the Kyte–Doolittle method with a scoring window size of 3 and assigning a relative weight of 100% to the window edges compared to the window center. To assess the anti-cancer activity of the ligands, the ENNACT web server was employed, and anti-cancer activity scores were predicted as normalized sigmoid scores ranging from 0 to 1, following the method described by Timmons et al. (Timmons & Hewage, 2021).

The ClusPro 2.0 server (Desta et al., 2020; Kozakov et al., 2013, 2017) was employed for docking purposes, utilizing default settings. Following the completion of the docking process, the resulting receptor-ligand complexes were saved in PDB format as optimized docking models, considering the center scores and the number of members. The receptor 5XF0's A chain was selected for the docking process. In this molecule, 20 conformers were loaded into the PDB, of which the first conformer was used for docking. The ligands 2MLT (in homotetramer form, using the A chain for docking) and 6O4M (in homotetramer form, using the B chain for docking) consist of 26 amino acid residues each, with X-ray diffraction resolutions of 2.00 Å (Eisenberg et al., 1990) and 1.27 Å (Kurgan et al., 2019), respectively. 3QRX (in heterodimer form, using the B chain for docking) consists of 20 amino acid residues with an X-ray diffraction resolution of 2.20 Å (Sosa et al., 2011). 8AHT (in heterohexamer form, using the F chain for docking) consists of 25 amino acid residues with an X-ray diffraction resolution of 2.20 Å (Dürvanger et al., 2023). 8AHS (in heterodimer form, using the C chain for docking) consists of 23 amino acid residues with an X-ray diffraction resolution of 2.48 Å (Dürvanger et al., 2023).

Subsequently, the PDB file containing the receptor-ligand complexes was submitted to the Protein

Binding Energy Prediction (PRODIGY) server to assess the strength of protein-protein interactions and their thermodynamic stabilities under specific conditions, including temperatures of 25 °C and 40 °C. This analysis predicted the free energy change ( $\Delta G$ , kcal.mol<sup>-1</sup>) and the dissociation constant Kd (M).

### Statistical analyses

Each receptor-ligand complex is represented by only a single observation, which limits the options for statistical analysis. Descriptive statistics, including minimum, maximum, and arithmetic mean, have been calculated to summarize the findings. However, the results are purely descriptive, and no statistical tests can be applied without additional data.

## RESULTS and DISCUSSION

The limited number of structural models of melittin peptides used in this study constrains the generalizability of the findings across all anticancer mechanisms. Additionally, observed limitations related to the models' structural quality may affect the binding results' reliability. Therefore, considering the limited sample size and structural constraints, these findings should be interpreted with caution.

Upon sorting the ligands' scores obtained from the RCSB PDB, the ranking revealed that 2MLT, 6O4M, 3QRX, 8AHT, and 8AHS were arranged in descending order based on their score magnitude. Accordingly, graphs illustrating the overall measures, including free R-value (Rfree), clash score, Ramachandran outliers percent, sidechain outliers percent, and RSRZ outliers percent for the ligands, are presented in Table 1.

Table 1. Percentile ranks for ligands

*Çizelge 1. Ligandlar için yüzdelerik sıralamalar*

Metric	2MLT	6O4M	3QRX	8AHT	8AHS
<b>Rfree</b>	NA	0,235	0,334	0,265	0,303
<b>Clashscore</b>	2	3	8	3	4
<b>Ramachandran outliers (%)</b>	0	0	1,2	0	0
<b>Sidechain outliers (%)</b>	7,1	0	2,9	0,6	0
<b>RSRZ outliers (%)</b>	0	1,7	4,2	4,8	6,7

The table presents percentile ranks for the ligands, with better values highlighted in shades of green and worse in shades of red. The intensity of the green color indicates a higher degree of improvement, while the intensity of the red color signifies a greater degree of deterioration. The quantitative representation of these values is depicted through blue data bars. This adapted form of percentile rank from RCSB PDB visually represents metrics within the table.

A common characteristic of all selected models was their classification as Hydrophobic-favored models within the ClusPro 2.0 server. The scores and coefficients of these models and those of the other models, including Balanced, Electrostatic-favored, and VdW+Elec, are delineated in Table 2. Additionally, it illustrates the outcomes from the

PRODIGY server regarding receptor-ligand docking, presenting the Gibbs free energy ( $\Delta G$ ) and dissociation constant (Kd) metrics to assess the binding affinity and thermodynamic stability of protein-protein interactions under variable temperature conditions. It was discerned that among the receptor-ligand complexes, the 5XF0-8AHT

complex exhibited the most substantial  $\Delta G$  and binding affinity, indicating a robust interaction. Conversely, the weakest  $\Delta G$  and binding affinity were identified in the 5XF0-3QRX complex. Moreover, an

increase in temperature was correlated with a consistent diminution in binding affinity, congruent with the observed trends in  $\Delta G$  and binding affinity.

Table 2. Summary of binding properties and interaction characteristics of receptor-ligand complexes from molecular docking simulations in ClusPro 2.0

Çizelge 2. ClusPro 2.0 kullanılarak yapılan moleküler bağlanma simülasyonlarından elde edilen reseptör-ligand komplekslerinin bağlanma özellikleri ve etkileşim karakteristiklerinin özeti

Receptor-Ligand Complex	Cluster/Center	Balanced	Electrostatic-favored	Hydrophobic-favored	VdW+Elec	$\Delta G$ [kcal.mol <sup>-1</sup> ]	Kd [M] at 25.0 °C/40.0 °C
5XF0-2MLT	Cluster	8	6	13	0	-9.6	8.4e-08/1.8e-07
	Center	-587.0	-623.9	-807.7	-185.7		
5XF0-6O4M	Cluster	1	8	1	2	-9.4	1.3e-07/2.9e-07
	Center	-584.1	-684.3	-932.6	-153.5		
5XF0-3QRX	Cluster	1	2	6	1	-8.8	3.5e-07/7.2e-07
	Center	-591.3	-597.1	-915.7	-127.8		
5XF0-8AHT	Cluster	3	5	0	5	-10.6	1.7e-08/4.1e-08
	Center	-608.3	-676.6	-854.4	-162.3		
5XF0-8AHS	Cluster	7	5	5	9	-9.8	6.7e-08/1.5e-07
	Center	-588.6	-617.4	-908.2	-141.0		
Total	Mean (Cluster/Center)	4.0/-591.86	5.2/-639.86	5.0/-883.72	3.4/-154.06	-9.64	1.3e-07/2.76e-07
	Minimum (Cluster/Center)	1/-608.3	2/-684.3	0/-932.6	0/-185.7	-10.6	1.7e-08/4.1e-08
	Maximum (Cluster/Center)	8/-584.1	8/-597.1	13/-807.7	9/-127.8	-8.8	3.5e-07/7.2e-07
	SD (Cluster/Center)	3.32/9.55	2.17/38.45	5.15/51.59	3.65/21.96	0.65	1.29e-07/2.63e-07

This table summarizes the receptor-ligand complex analyses derived from molecular docking simulations. The parameters include interaction counts categorized as Balanced, Electrostatic-favored, Hydrophobic-favored, and VdW+Elec, as well as binding energy ( $\Delta G$ ) in kcal/mol and dissociation constants (Kd) at 25.0 °C and 40.0 °C. The Total, Mean, Minimum, Maximum, and Standard Deviation (SD) values are provided for both cluster and center metrics, highlighting the analyzed complexes' binding properties and structural quality.

Table 3 encapsulates the predictive analytics of ligands as procured from the AntiCP 2.0 server, detailing the SVM score of each ligand, descriptors of its aqueous interactions, and its chemical attributes. Table 3 also showcases the forecasted anticancer efficacy of therapeutic peptides, as determined by the ENNAACT server. This includes a comprehensive listing of the ligand's amino acid composition and a normalized sigmoidal probability score (PROB score) from 0 to 1. Trained explicitly on a dataset consisting of 861 anticancer peptides and an equal number of non-anticancer peptides through machine learning techniques, the AntiCP 2.0 server (Agrawal, 2021) conducted anticancer scoring for the ligands. The SVM score for 2MLT reached its maximum level, followed sequentially by 8AHT, 8AHS, and 3QRX. Similarly, the ENNAACT server, trained using neural network algorithms, indicates the notably high potential anticancer activity of 2MLT due to its elevated PROB score, followed in descending order by

8AHT, 8AHS, and 3QRX, a finding corroborated by the AntiCP 2.0 server.

The visualizations involve examining the structural details of the interactions between the 5XF0 receptor and the ligands 2MLT, 6O4M, 3QRX, 8AHT, and 8AHS, revealing their chain structures and specific interactions (Figure 1). This structural examination provided insights into the chain structures and interactions of the receptor-ligand complexes, shedding light on their molecular characteristics and potential binding modes.

Structural analyses of 2MLT revealed four hydrogen bonds formed between Ser204, Glu118, and Glu17 residues of 5XF0 and Lys23 and Arg24 residues of 2MLT, accompanied by three salt bridges linking Glu118 and Glu177 residues of 5XF0 with Arg24 and Lys21 residues of 2MLT. Moreover, 79 non-bonded contacts were observed among different amino acids.

Table 3. Predictive analytics of ligands and forecasted anticancer efficacy from AntiCP 2.0 and ENNAACT servers.

*Çizelge 3. Ligandların tahmini analizi ve AntiCP 2.0 ve ENNAACT sunucularından öngörülen antikanser etkinliklerinin tahmini.*

ID	Seq.	SVM	Hpho.	Hpat.	Amph.	Hphl.	Ch.	pI	Mw	PROB
2MLT, 6O4M	GIGAVLKVLTTGLPALISWIKRKRQ	1.0	-0.08	0.27	0.71	-0.20	5.0	12.03	2847.91	0.997
3QRX	IGAVLKVLTTGLPALISWIK	0.64	0.18	1.37	0.37	-0.72	2.0	10.02	2093.95	0.905
8AHT	GIGAVLKVLTTGLPALISWIKRKRQ	0.93	-0.06	0.42	0.69	-0.21	5.0	12.03	2719.76	0.995
8AHS	IGAVLKVLTTGLPALISWIKRKR	0.84	-0.04	0.63	0.69	-0.24	5.0	12.03	2534.54	0.990
Total	Mean	0.85	0.00	0.67	0.62	-0.34	4.25	11.53	2549.04	0.97
	Minimum	0.64	-0.08	0.27	0.37	-0.72	2.00	10.02	2093.95	0.91
	Maximum	1	0.18	1.37	0.71	-0.20	5.00	12.03	2847.91	1.00
	SD	0.135	0.105	0.423	0.142	0.218	1.299	0.870	285.39	0.039

Abbreviations: Seq. (Sequence of ligand); SVM (Support Vector Machine score); Hpho. (Hydrophobicity); Hpat. (Hydropathicity); Amph. (Amphipathicity); Hphl. (Hydrophilicity); Ch (Charge); pI (Isoelectric Point); Mw (Molecular Weight); PROB (Normalized Sigmoidal Probability Score). The Total, Mean, Minimum, Maximum, and Standard Deviation (SD) values are provided for analyzed complexes.

Similarly, for 6O4M, five hydrogen bonds involving Glu114, Glu120, Met123, and Asn152 residues of 5XF0 and Arg24, Lys23, Lys21, and Thr10 residues of 6O4M were identified, along with three salt bridges connecting Glu114, Glu120, and Glu168 residues of 5XF0 with Arg24, Lys23, and Lys21 residues of 6O4M. Additionally, 70 non-bonded contacts were noted among various amino acids. Furthermore, structural analysis of 3QRX revealed three hydrogen bonds between Asp144, Glu177, and Arg201 residues of 5XF0 and Trp19, Lys7, and Val8 residues of 3QRX, along with one salt bridge between Glu177 residue of 5XF0 and Lys7 residue of 6O4M. In addition, 60 non-bonded contacts were identified among different amino acids. Moreover, for 8AHT, six hydrogen bonds formed between Glu114, His115, Asn152, Val160, and Glu172 residues of 5XF0 and Lys7, Lys23, and Lys21 residues of 8AHT, accompanied by two salt bridges linking Glu114 and Glu172 residues of 5XF0 with Lys7 and Lys21 residues of 8AHT. Besides, 67 non-bonded contacts were observed among various amino acids. Lastly, structural analysis of 8AHS unveiled five hydrogen bonds involving Gln100, Met123, Arg166, and Glu168 residues of 5XF0 and Leu9, Lys23, and Leu16 residues of 8AHS, along with one salt bridge between Glu168 residue of 5XF0 and Lys23 residue of 8AHS. Additionally, 69 non-bonded contacts were noted among different amino acids.

According to the results presented by the PDBsum server regarding the interactions between the receptor and ligands, various bonding characteristics among residues have been identified. For the 5XF0-2MLT interaction, hydrogen bonds were found to have lengths ranging from a minimum of 2.73 Å to a maximum of 2.85 Å. In comparison, salt bridges ranged from a minimum of 2.59 Å to a maximum of 2.76 Å, and non-bonded contacts ranged from a minimum of 2.56 Å to a maximum of 3.89 Å. In the

case of the 5XF0-6O4M interaction, hydrogen bonds ranged from a minimum of 2.59 Å to a maximum of 3.30 Å, salt bridges ranged from a minimum of 2.48 Å to a maximum of 2.75 Å, and non-bonded contacts ranged from a minimum of 2.48 Å to a maximum of 3.90 Å. Regarding the 5XF0-3QRX interaction, hydrogen bonds had lengths ranging from a minimum of 2.48 Å to a maximum of 2.91 Å, with the salt bridge length being 2.48 Å, and non-bonded contacts ranging from a minimum of 2.48 Å to a maximum of 3.88 Å. For the 5XF0-8AHT interaction, hydrogen bonds ranged from a minimum of 2.47 Å to a maximum of 2.77 Å, salt bridges ranged from a minimum of 2.47 Å to a maximum of 2.58 Å, and non-bonded contacts ranged from a minimum of 2.47 Å to a maximum of 3.88 Å. Lastly, in the 5XF0-8AHS interaction, hydrogen bonds had lengths ranging from a minimum of 2.48 Å to a maximum of 3.28 Å, with the salt bridge length being 2.48 Å, and non-bonded contacts ranged from a minimum of 2.48 Å to a maximum of 3.88 Å.

The hydrophobicity indices of the ligands were ascertained using the Kyte–Doolittle scale via the ProtScale tool, as depicted in Figure 2. Based on this scale, the hydrophobicity values observed for 2MLT and 6O4M are significantly elevated. Due to the identical sequences of these peptides, hydrophobicity values are presented in a single graph. Similarly, it is observed that the other peptides exhibit high hydrophobicity, attributed to their similar sequences.

The 2MLT melittin peptide was resolved using X-ray diffraction, with a resolution of 2.00 Å. Although the Rfree value was not specified, a clash score value of 2 indicates a low clash score. The peptide, with a Ramachandran outlier percent of 0%, generally falls within acceptable torsion angle limits; however, the sidechain outlier value of 7.1% suggests some sidechains may have abnormal conformations.



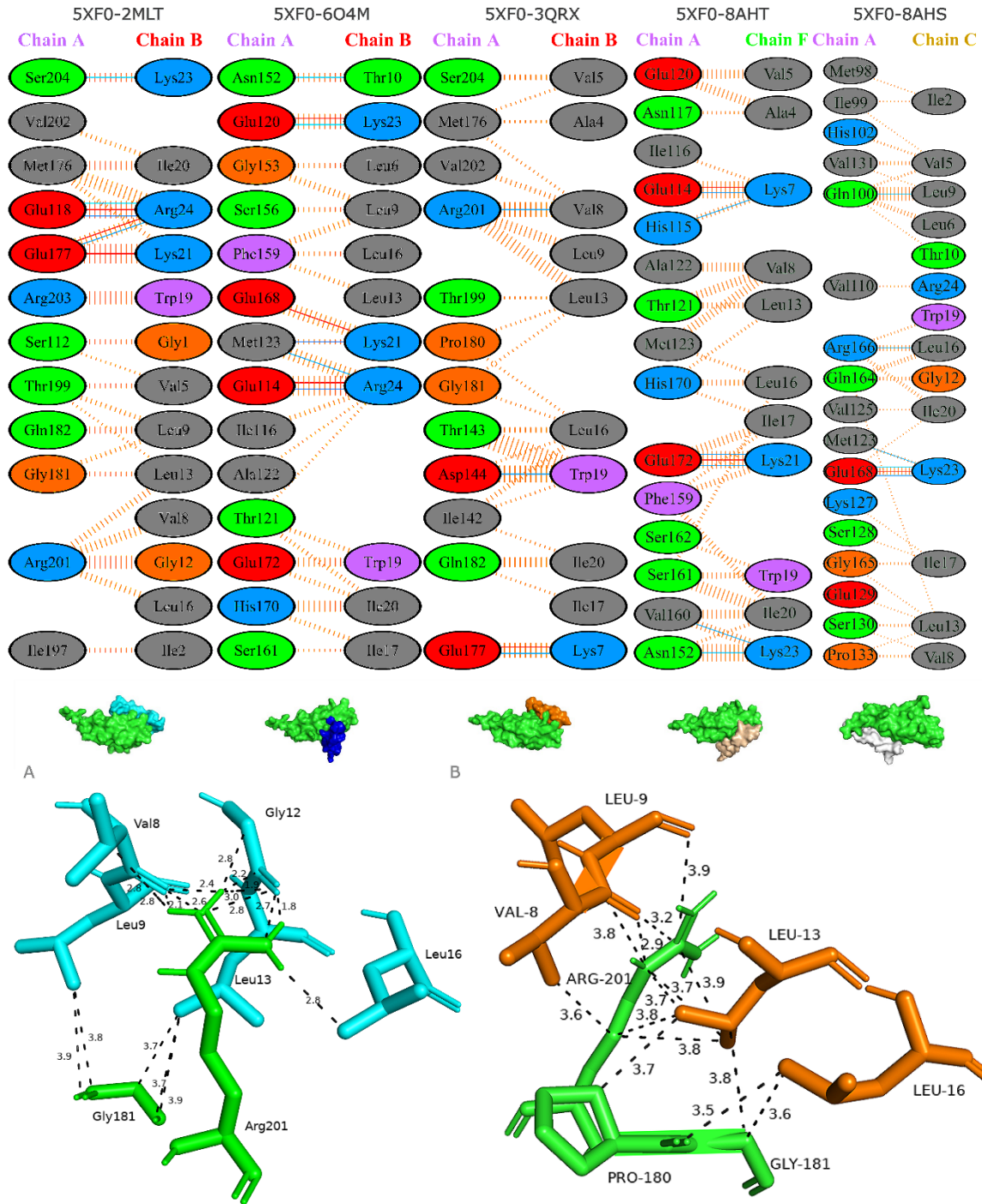


Figure 1. Visualization of interactions between the 5XF0 receptor A chain and ligands via PDBsum and PyMOL

*Note.* The interactions between the A chain of the 5XF0 receptor and various chains of the ligands are visualized in PDBsum. Discrete vertical red lines represent non-bonded contacts, horizontal red lines denote salt bridges, and horizontal blue lines indicate hydrogen bonds. Using PyMOL software, the interactions are further illustrated, with the receptor depicted in green and the ligands in cyan, blue, orange, wheat, and white. Panel A focuses on the interactions between 5XF0 (green) and 2MLT (cyan), indicating distances in angstroms (Å) with dashed lines. Panel B highlights interactions between 5XF0 (green) and 3QRX (orange), also showing distances in Å with dashed lines.

*Şekil 1. 5XF0 reseptörü A zinciri ile ligandlar arasındaki etkileşimlerin PDBsum ve PyMOL ile görselleştirilmesi*

*Not.* 5XF0 reseptörünün A zinciri ile ligandların çeşitli zincirleri arasındaki etkileşimler PDBsum'da görselleştirilmiştir. Ayrık dikey kırmızı çizgiler bağımsız etkileşimleri, yatay kırmızı çizgiler tuz köprülerini ve yatay mavi çizgiler hidrojen bağlarını gösterir. PyMOL yazılımı kullanılarak etkileşimler, yeşil renkle gösterilen reseptör ve camgöbeği, mavi, turuncu, buğday ve beyaz ligandlar ile daha ayrıntılı olarak gösterilmektedir. Panel A, 5XF0 (yeşil) ve 2MLT (camgöbeği) arasındaki etkileşimlere odaklanır ve mesafeleri kesikli çizgilerle angstrom (Å) cinsinden göstermektedir. Panel B, 5XF0 (yeşil) ve 3QRX (turuncu) arasındaki etkileşimleri vurgulamakta ve ayrıca mesafeleri kesikli çizgilerle Å cinsinden göstermektedir.

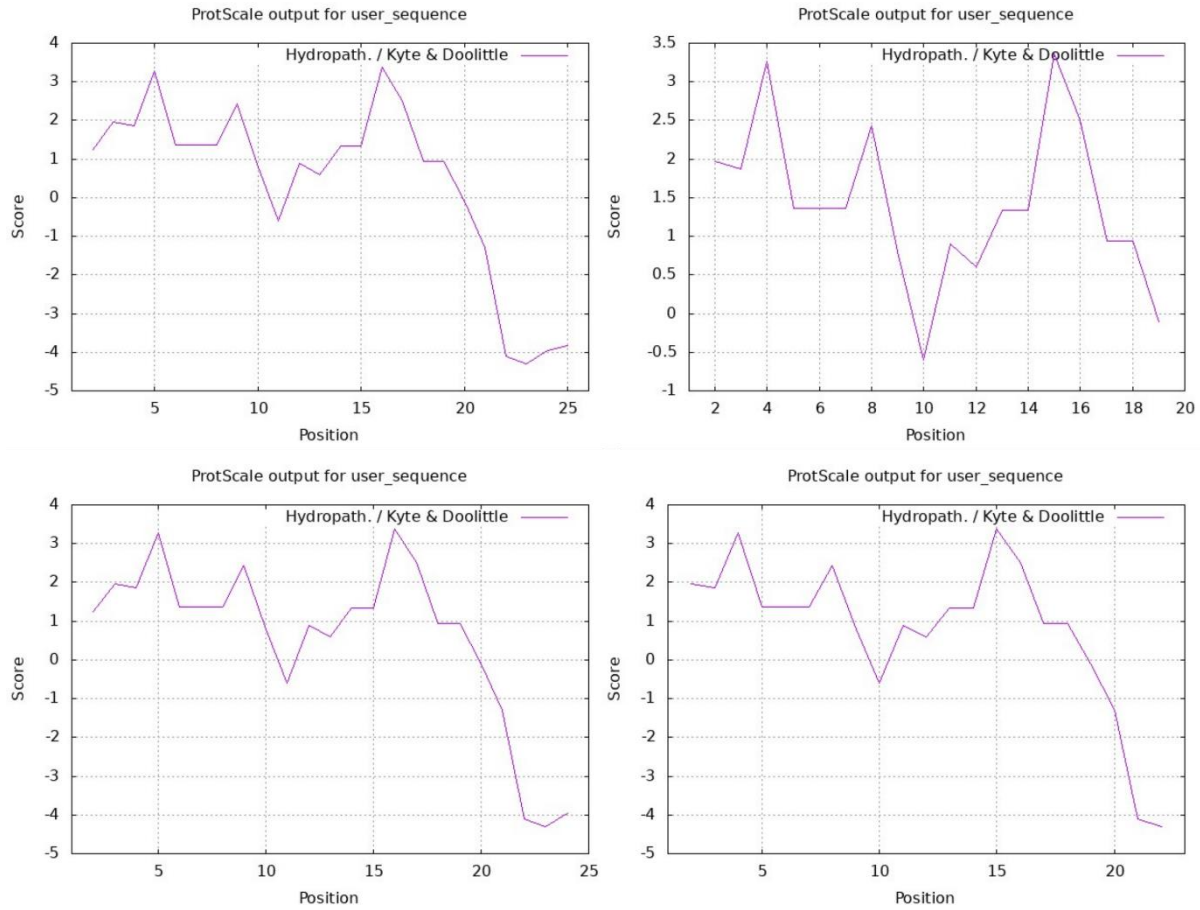


Figure 2. Assessment of ligand hydrophobicity using the Kyte-Doolittle scale

*Note.* The sequences in the images on the upper left correspond to 2MLT and 6O4M, while the sequence on the right pertains to 3QRX. The sequences in the pictures below correspond to 8AHT on the left and 8AHS on the right. The horizontal axis indicates amino acid positions, while the vertical axis illustrates hydrophobicity levels. Positive values denote the presence of hydrophobic amino acids in the peptide sequence.

*Şekil 2. Kyte-Doolittle ölçeği kullanarak ligandların hidrofobiklik değerlendirilmesi*

*Not.* Üst panelde soldaki resimde gösterilen diziler 2MLT ve 6O4M'ye karşılık gelirken, sağdaki dizi 3QRX'e aittir. Alt paneldeki resimlerde gösterilen diziler solda 8AHT'ye ve sağda 8AHS'ye karşılık gelmektedir. Yatay eksen amino asit konumlarını gösterirken dikey eksen hidrofobiklik seviyelerini gösterir. Pozitif değerler, peptid dizisinde hidrofobik amino asitlerin varlığını belirtmektedir.

The RSRZ outlier value of 0% generally falls within acceptable limits. The 6O4M melittin peptide has a structural model with a resolution of 1.27 Å and a Rfree value of 0.235, indicating good agreement with experimental data. With a clash score value of 3, the peptide is considered acceptable regarding clashes. The Ramachandran outlier percent of 0% and sidechain outlier value of 0% indicate generally acceptable structural quality; however, the RSRZ outlier value of 1.7% suggests some atoms may have abnormal conformations. The 3QRX melittin peptide has a structural model with a resolution of 2.20 Å but a weak performance fitting experimental data, with an Rfree value of 0.334. Additionally, the peptide has a clash score value of 8, indicating significant problems with clashes. The peptide demonstrates poor structural quality with a Ramachandran outlier

percent of 1.2% and a sidechain outlier value of 2.9%. The RSRZ outlier value of 4.2% indicates that some atoms may have abnormal conformations. Finally, the 8AHT and 8AHS melittin peptides have resolutions of 2.20 Å and 2.48 Å, respectively, with Rfree values of 0.265 and 0.303, indicating moderate agreement. However, with a clash score value of 4 and an RSRZ outlier value of 6.7%, the structural quality of 8AHS appears slightly lower compared to 8AHT. Based on the results presented, it can be observed that the structural quality and characteristics of the melittin peptides varied among the complexes analyzed. The 2MLT complex exhibited a relatively favorable structural profile, with low clash scores and acceptable torsion angle limits. In contrast, the 3QRX complex showed weaker performance in fitting experimental data, indicating potential issues with its

structural integrity. However, it should be noted that in terms of molecular binding mode, 3QRX exhibits more remarkable similarity to the CD147/CypA interaction than 2MLT. These findings suggest that the structural properties of the melittin peptides may influence their interactions with the receptor and ultimately impact their biological activity. Further investigations into the structural determinants of these peptides are warranted to elucidate their therapeutic potential and inform drug design strategies.

The selected models were consistently classified as Hydrophobic-favored within the ClusPro 2.0 server, indicating the significant involvement of hydrophobic interactions in both the formation and stabilization of receptor-ligand complexes. Notably, the 5XF0-8AHT complex exhibited the highest  $\Delta G$  and binding affinity ( $-10.6 \text{ kcal.mol}^{-1}$ ;  $1.7\text{e-}08 \text{ M}$ ), suggesting a robust and stable interaction. Conversely, the 5XF0-3QRX complex displayed the weakest  $\Delta G$  and binding affinity ( $-8.8 \text{ kcal.mol}^{-1}$ ;  $3.5\text{e-}07 \text{ M}$ ), indicative of a comparatively weaker interaction. Furthermore, an increase in temperature correlated with a consistent decrease in binding affinity across the receptor-ligand complexes, aligning with the observed changes in  $\Delta G$  and binding affinity, thus indicating a temperature-dependent impact on the stability of protein-protein interactions. In summary, these findings underscore the crucial role of hydrophobic interactions in receptor-ligand complex formation. They offer valuable insights into their binding affinity and thermodynamic stability under varying temperature conditions. Such insights contribute significantly to our understanding of the molecular mechanisms governing protein-protein interactions and hold implications for drug discovery and design endeavors.

The AntiCP 2.0 server, trained on a dataset of anticancer and non-anticancer peptides, conducted anticancer scoring for the ligands. The SVM score for 2MLT attained the maximum level (1.0), followed by 8AHT (0.93), 8AHS (0.84), and 3QRX (0.64). Similarly, the ENNACT server, employing neural network algorithms, indicates the high potential anticancer activity of 2MLT based on its elevated PROB score (0.997), followed by 8AHT (0.995), 8AHS (0.990), and 3QRX (0.905). These findings collectively support the potential anticancer properties of the examined ligands, as highlighted by both computational tools.

The structural examination of molecular complexes has provided insights into receptor-ligand complexes' chain structures and interactions, elucidating their molecular characteristics and potential binding modes. Four and five hydrogen bonds in the 2MLT and 6O4M complexes, respectively, indicate strong interactions with the 5XF0 receptor. Additionally, identifying three salt bridges in these complexes

suggests further binding reinforcement. Conversely, three hydrogen bonds and a single salt bridge in the 3QRX complex indicate fewer interactions. The presence of six and five hydrogen bonds in the 8AHT and 8AHS complexes, respectively, demonstrates robust binding between the receptor and ligand. However, the number of salt bridges and non-bonded motifs in these complexes plays a significant role in binding stability and the diversity of specific interactions. These findings contribute to a detailed understanding of the molecular interactions between ligands and receptors, providing a valuable foundation for comprehending the respective ligands' biological activities and therapeutic potentials.

The upregulation of CypA and CD147 in the signaling cascade of malignant cells has been implicated in the initiation and progression of cancer (Han & Jung, 2022; Yurchenko et al., 2010). CypA facilitates signal transduction by forming complexes with Pro180 and Pro211 residues of CD147, with Glu218 playing a pivotal role in this process (Han & Jung, 2022). Recent research has highlighted the regulatory role of Pro180-Gly181 in the CypA/CD147 binding mechanism, identifying Arg201 as a critical residue for this interaction (Yang et al., 2022). Our investigation similarly revealed non-covalent contacts between Gly181 and Leu9, Leu13, and Arg201 with Leu13, Val8, Gly12, and Leu16 in the interaction between 5XF0 and 2MLT, and notably, non-bonded interactions involving Pro180 and Gly181 with Leu13, Leu16, and Arg201 with Val8, Leu9, Leu13 in the case of 3QRX, suggesting specific amino acid interactions independent of chemical bonding. Furthermore, a hydrogen bond between Arg201 and Val8 was identified. While the binding of CypA to CD147 induces conformational changes akin to a molecular chaperone, further exploration of the non-bonded interactions observed with 2MLT and 3QRX warrants investigation in subsequent studies.

The analysis of interactions between the receptor (5XF0) and ligands revealed diverse bonding characteristics across different complexes. Hydrogen bonds, salt bridges, and non-bonded contacts were identified as crucial interaction types. These findings from PDBsum highlight the eclectic nature of interactions between the receptor and ligands, providing valuable insights into their binding mechanisms and potential functional implications.

The hydrophobicity indices obtained using the Kyte-Doolittle scale via the ProtScale tool reveal significant attributes of the ligands under scrutiny. The ligands exhibit elevated hydrophobicity, presumably stemming from their similar sequences. In particular, the sequence 3QRX, which corresponds to IGAVLKVLTTGLPALISWIK, shows a significantly hydrophobic nature, whereas the sequences 2MLT and 6O4M, represented by



GIGAVLKVLTGTPALISWIKRKRQQ, possess relatively lower hydrophobicity. Additionally, variations in amphipathicity and hydrophilicity properties are observed across different sequences. These observations underscore the hydrophobic nature of the ligands under examination and emphasize the potential ramifications of their hydrophobic properties in diverse biological processes and interactions, warranting further investigation in subsequent analyses.

In conclusion, the analysis of the 3QRX ligand and its interaction with the 5XF0 receptor reveals crucial insights into receptor-ligand interactions. Despite structural deficiencies such as clashes and abnormal conformations, 3QRX exhibits a molecular binding mode that closely resembles the CD147/CypA interaction, suggesting potential similarities in their biological mechanisms. Furthermore, the notably hydrophobic nature of the 3QRX sequence may influence its interaction with the receptor. However, the 5XF0-3QRX complex demonstrates the weakest binding affinity among the studied complexes, indicating a weaker interaction. These findings emphasize the significance of considering both structural and sequence-based characteristics when assessing the efficacy of receptor-ligand interactions, offering valuable insights into the molecular basis of protein-protein interactions.

## CONCLUSION

To summarize, the comprehensive analysis presented in this study sheds light on various aspects of receptor-ligand interactions and their implications. Structural examinations elucidated the complexes' molecular characteristics and binding modes, highlighting the significance of hydrogen bonds, salt bridges, and non-bonded contacts. Computational tools provided insights into the potential anticancer properties of the ligands, with notable variations in hydrophobicity observed across different sequences. The classification of models as Hydrophobic-favored underscores the role of hydrophobic interactions in complex stability. Moreover, temperature-dependent effects on binding affinity emphasize the dynamic nature of protein-protein interactions. These findings deepen our understanding of molecular interactions and provide valuable insights for drug discovery and design efforts in various biomedical applications. Further research into these complexes' structural determinants and therapeutic potentials is warranted to advance our knowledge and facilitate the development of novel therapeutics.

## Contribution of Authors

BD: Design, Perform, Analyze, Write and editing, Project Administration.

## Conflict of Interests and Ethical Statement

None.

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## Evaluation of Raftlin and 8-iso-prostaglandin Levels on the Clinical Severity of Cases with Covid-19 in Türkiye: A Cross-Sectional Study

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### ABSTRACT

COVID-19 is an infectious respiratory disease caused by the severe acute respiratory syndrome Coronavirus 2 (SARS-CoV-2). Raftlin is a large lipid raft protein found in cell membranes and plays a crucial role in inducing autoimmune and vascular inflammatory responses. Although there have been few studies on 8-iso-prostaglandin F<sub>2α</sub> (8-iso-PGF<sub>2α</sub>) as a marker of oxidative stress in COVID-19, the levels of raftlin in the disease have not been previously investigated. The objective of in this study was to evaluate the levels of Raftlin and 8-iso-PGF<sub>2α</sub> in COVID-19 patients. We analyzed clinical findings from three groups: healthy controls, patients with a mild course of COVID-19, and patients with a severe course of COVID-19. We measured the levels of Raftlin and 8-iso-PGF<sub>2α</sub> in serum samples using ELISA. This results showed that the levels of Raftlin and 8-iso-PGF<sub>2α</sub> were higher in patients with severe COVID-19 compared to the control and mild course groups. Furthermore, a significant positive correlation was found between Raftlin and 8-iso-PGF<sub>2α</sub> in the severe COVID-19 group. Raftlin was identified as a potentially important biomarker in COVID-19 patients. This study is valuable as it is the first to investigate changes in Raftlin and 8-iso-PGF<sub>2α</sub> levels in COVID-19 disease.

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## Türkiye'de COVID-19 Olgularının Klinik Şiddeti Üzerine Raftlin ve 8-izoprostaglandin Düzeylerinin Değerlendirilmesi

### ÖZET

COVID-19, şiddetli akut solunum sendromu SARS-CoV-2'nin neden olduğu bulaşıcı bir solunum yolu hastalığıdır. Raftlin, hücre zarlarında bulunan büyük bir lipid sal proteindir ve otoimmün ve vasküler inflamatuvar yanıtların indüklenmesinde önemli rol oynar. COVID-19'da oksidatif stresin bir belirteci olarak 8-iso-prostaglandin F<sub>2α</sub> (8-iso-PGF<sub>2α</sub>) ile ilgili az sayıda çalışma olmasına rağmen, hastalığıdaki raftlin seviyeleri daha önce araştırılmamıştır. Çalışmamızın amacı, COVID-19 hastalarında Raftlin ve 8-iso-PGF<sub>2α</sub> düzeylerini değerlendirmektir. Üç gruptan elde edilen klinik bulguları analiz edildi: sağlıklı kontroller, hafif COVID-19 seyri olan hastalar ve şiddetli COVID-19 seyri olan hastalar. ELISA kit kullanarak serum örneklerinde Raftlin ve 8-iso-PGF<sub>2α</sub> seviyelerini ölçtük. Sonuçlarımız, Raftlin ve 8-iso-PGF<sub>2α</sub> seviyelerinin şiddetli COVID-19 hastalarında kontrol ve hafif seyirli gruplara kıyasla daha yüksek olduğunu göstermiştir. Ayrıca, şiddetli COVID-19 grubunda Raftlin ve 8-iso-PGF<sub>2α</sub> arasında anlamlı bir pozitif korelasyon bulunmuştur. Raftlin, COVID-19 hastalarında potansiyel olarak önemli bir biyobelirteç olarak tanımlanmıştır. Bu çalışma, COVID-19 hastalığında Raftlin ve 8-iso-PGF<sub>2α</sub> seviyelerindeki değişiklikleri araştıran ilk çalışma olması açısından değerlidir.

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## INTRODUCTION

COVID-19 is one of the most serious public health problems of recent years. Investigating the pathogenetic mechanisms of the disease and new therapeutic targets is of great importance due to high mortality as well as serious economic and social consequences (Hui ve ark., 2019). One aspect of the pathogenesis of COVID-19 that is still unclear is the great heterogeneity of response among patients, from asymptomatic course to severe symptoms (The Novel Coronavirus Pneumonia Emergency Response Epidemiology Team, 2020). Symptoms tend to be more aggressive and fatal in more vulnerable groups, including the elderly, patients with chronic diseases, patients receiving immunosuppressive therapy, and pregnant women (Hui et al., 2020; The Novel Coronavirus Pneumonia Emergency Response Epidemiology Team, 2020). COVID-19 is believed to rarely affect infants, so body reactivity studies mainly focus on adult patients (Guan et al., 2020).

Reactive oxygen species (ROS) are formed during metabolic activity under physiological conditions in living organisms (Kurutas, 2016). Activation of antimicrobial immune cells, neutrophils, and macrophages and the production of proinflammatory cytokines depend on ROS (Miraloglu et al., 2016; Juan et al., 2021; Muhammed et al., 2021). ROS have an important role in the development of an antiviral immune response through the formation of type I interferon (Nour et al., 2020). ROS also play an important role as mediators of cell signalling pathways, but this does not require a negative effect on cell structures due to compensation by antioxidant mechanisms (Angel et al., 2018). Overproduction of ROS, including COVID-19, can stimulate inflammatory signalling cascades through the increased genomic expression of protein kinases, transcription factors, and proinflammatory regulators, leading to hyperactivation of the immune system. This may lead to the development of oxidative damage (Saeki et al., 2003).

Lipid peroxidation caused by ROS is an important pathological process involving the oxidation of polyunsaturated fatty acids in biological membranes (Bilgen et al., 2019). Oxidative stress through lipid peroxidation is thought to play an important role in the development of many diseases, including COVID-19. Isoprostanes, a family of prostaglandin-like compounds, are by-products of free radical catalysed oxidation of arachidonic acid. 8-iso-prostaglandin F<sub>2</sub>α (8-iso-PGF<sub>2</sub>α), an important isoprostane, is very stable

in body fluids and tissues and is therefore considered the most ideal index for the detection of excessive chemical lipid peroxidation and the amount of free circulation (Bilal et al., 2021). 8-iso-PGF<sub>2</sub>α have been measured in various bodyfluids, such as urine, blood, bile, pericardial fluid, and cerebrospinal fluid, as indicators of lipid peroxidation (Milne et al., 2008; Kaviarasan et al., 2009; Pratico et al., 2004).

Lipid rafts are membrane microdomains enriched in saturated phospholipids, sphingolipids, and cholesterol (Muhammed et al., 2020). They are involved in various cellular processes, including signal transduction, endocytosis and exocytosis, entry of obligate intracellular pathogens, and production of pathological protein forms associated with various diseases (Zarkovic et al., 2022). Lipid rafts have a diverse and different protein composition. Raftlin (RFTN), the main lipid raft protein, is found in B cells and is responsible for the regulation of B cell antigen receptor (BCR) signalling. RFTN also plays an important role in the induction of autoimmune response and vascular inflammatory response. RFTN is a parameter used in the pathophysiology of a vascular inflammatory response to diagnose inflammatory diseases and to characterize the immune response (Lahaie et al., 1998).

Lipid rafts are glycoprotein (sphingolipid) and cholesterol-rich regions containing receptor proteins. Lipid rafts are membrane microdomains enriched in saturated phospholipids, sphingolipids and cholesterol. They have a diverse but distinct protein composition and are involved in a variety of cellular processes, including polarised traffic, signal transduction, endo- and exocytosis, entry of obligate intracellular pathogens and production of pathological protein forms associated with Alzheimer's and prion diseases (Lee et al., 2014).

RFTN levels have not been previously investigated in COVID-19 disease. However, there are limited studies on the use of 8-iso-PGF<sub>2</sub>α as an oxidative stress marker in COVID-19. In this study, we aimed to evaluate the changes in RFTN and 8-iso-PGF<sub>2</sub>α levels in COVID-19 patients.

## MATERIALS AND METHOD

The research was designed as a cross-sectional study. Patients who applied to Tokat Gaziosmanpaşa University Faculty of Medicine Hospital between 01.05.2021 - 01.12.2021, received a definitive diagnosis of COVID-19 and received outpatient treatment and stayed in home isolation for 14 days; infectious

diseases, chest diseases, general internal medicine, ear, nose and throat, etc. Intubated patients in need of intensive care and mechanical ventilation who received inpatient supportive treatment in hospital wards were brought to the Anesthesiology and Reanimation Intensive Care Unit, and patients transferred from in-hospital internal medicine and infection wards and external centres were included. This study consisted of a total of 75 patients randomly selected and included patients who were in isolation at home (n=25), hospitalized in the ward (n=25), and receiving supportive treatment in the intensive care unit (n=25). In addition, 25 healthy individuals were selected as the control group. Sociodemographic data of the patients and control subjects included in the study are presented in Table 1. This study was approved by Tokat Gaziosmanpasa University Faculty of Medicine Local Ethics Committee (21-KAEK-099).

### Biochemical Analysis

Blood samples obtained from patients and control subjects were taken between 08.00 and 11.00 in the morning and after 8 to 12 hours of fasting because they were fasting venous blood samples. The samples were sent to Tokat Gaziosmanpasa University Faculty of Medicine, Department of Biochemistry. Fasting venous blood samples (10 ml) of the study participants were placed in anticoagulant-free tubes and centrifuged at 5000 g for 10 minutes at 4°C in a device (Hettich MIKRO 220 R; Andreas Hettich GmbH & Co. KG Tuttleen, Germany). After centrifugation, sera were separated and stored in acid-washed tubes at -20°C until RFTN and 8-iso-PGF2α analyses. The levels

of these parameters were analyzed in the Department of Medical Biochemistry, Tokat Gaziosmanpasa University. RFTN and 8-iso-PGF2α levels were measured by quantitative sandwich enzyme immunoassay technique (ELISA) using a commercial kit (Mybiosource, USA) according to the manufacturer's instructions.

### Statistical analysis

The data obtained at the end of the data collection phase were transferred to the computer environment and analyzed. Data were analyzed using the Statistical Package for Social Sciences (SPSS 27.0) software. Mann-Whitney U test was used to determine the degree of differences in serum levels (ng/mL) between the severe, mild, and control groups of COVID-19. Data were expressed as mean and standard deviation (SD). An Independent sample t-test was used to compare normally distributed variables. The chi-square test was used to compare the groups. Spearman correlation test was used for the relationship between variables. A receiver operating characteristic (ROC) curve was plotted to test the diagnostic performance of RFTN and 8-iso-PGF2α in patients with COVID-19. The association of RFTN and 8-iso-PGF2α with the severity of COVID-19 was visually shown by scatter plots. The significance level was accepted as **p<0.05**.

### RESULTS and DISCUSSION

When the general gender distribution of the patients was analyzed, 40 (50.6%) were male and 35 (49.4%) were female. Sociodemographic data of the patient and control groups are given in Table 1.

Table 1 Socio-demographic characteristics of the individuals in the patient and control groups.

*Çizelge 1. Hasta ve kontrol gruplarındaki bireylerin sosyo-demografik özellikleri.*

Parameters	Patient (n=75)	Control (n= 25)	P değeri
Age (years)	43.8 ± 13.8	40.2 ± 6.8	0,28
Gender			0,20
Male	40 (70%)	14 (52.4%)	
Female	35 (30%)	11 (47.6%)	
BMI(kg/m <sup>2</sup> )	21.9 ± 2.2	21.1 ± 1.1	0,15
Symptoms			
Mild	20 (%80)	0 (%)	
Moderate	20 (%80)	0 (%)	
Severe	20 (%80)	0 (%)	
Comorbidities			
No	40 (%80)	0 (%)	
Hypertension	6(%80)	0 (%)	
Diabetes	7(%80)	0 (%)	

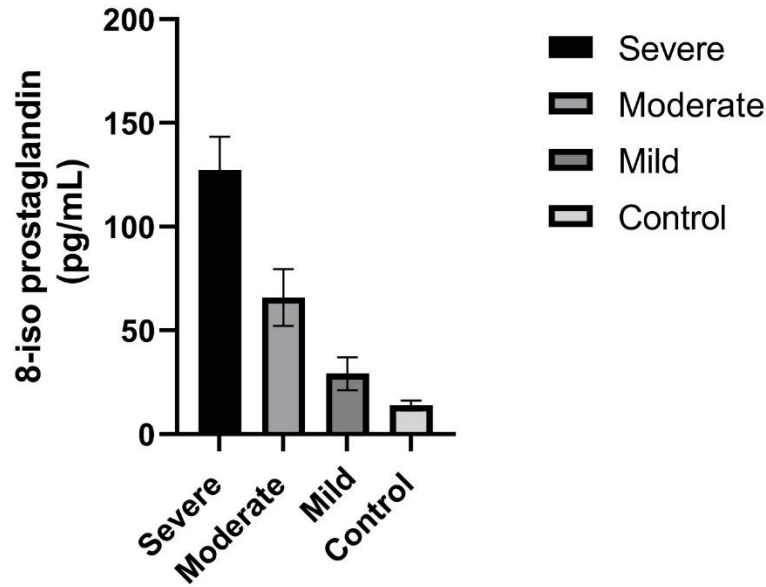
As shown in Table 2, Figures 1 and 2, serum RFTN and 8-iso-PGF2α levels were significantly higher in severe COVID-19 patients compared to the control group (p<0.001). Moreover, RFTN and 8-iso-PGF2α levels were significantly higher in severe COVID-19 patients compared to mild and moderate COVID-19 patients (p<0.001).

As shown in Table 3, a significant correlation was found according to the correlation analysis between the groups for RFTN levels (p<0.001, r=0.691). In Table 4, a significant positive correlation was found between the groups for 8-isoPG levels (p<0.001, r=0.711).

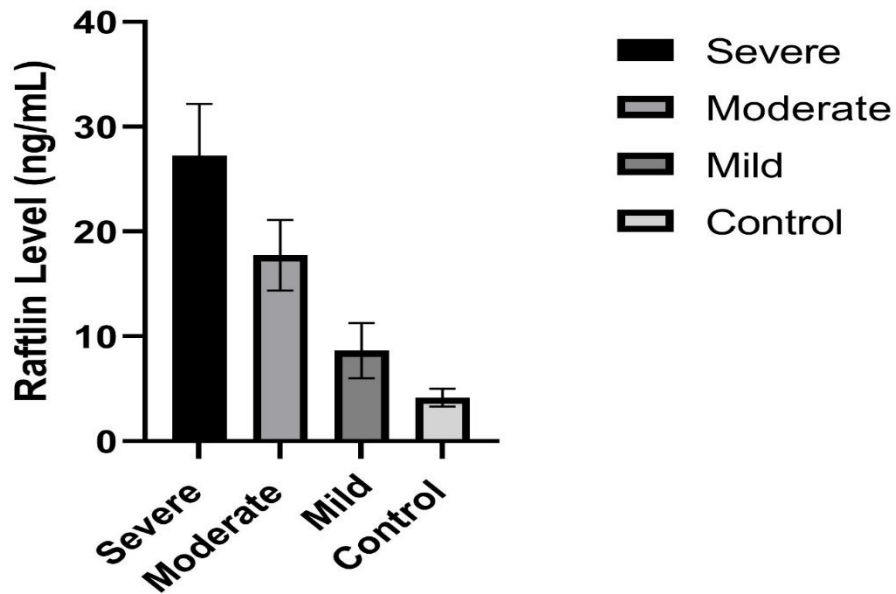


Table 2 The results of Raftlin and 8-iso prostaglandin levels between patient and healthy controls  
Çizelge 2. Raftlin ve 8-iso prostaglandin düzeylerinin hasta ve sağlıklı kontroller arasındaki sonuçları.

Variable		Covid Severe (n=25)	Covid Moderate (n=25)	Covid Mild (n=25)	Control (n=25)	p-value
Raftlin-1 (ng/mL)	Median (Q1-Q3)	27.25 (22.37-17.96)	17.75 (14.39-21.45)	8.65 (6.02-11.27)	4.16 (3.32-5.0)	<0.001
8-iso prostaglandin (pg/mL)	Median (Q1-Q3)	127.25 (111.15-143.35)	65.9 (52.27-79.53)	29.2 (21.26-37.14)	13.90 (11.48-16.3)	<0.001



Şekil 1. Gruplar arasında 8-iso prostaglandin düzeyleri  
Figure 1. 8-iso prostaglandin levels between groups



Şekil 2. Gruplar arasında Raftlin düzeyleri  
Figure 2. Raftlin levels between groups

Table 3 Correlation results between groups for RFTN levels

Çizelge 3. RFTN düzeyleri için gruplar arasındaki korelasyon sonuçları.

		Raftlin	Groups
Raftlin	r value	1,000	,691**
	p-value	.	,0001
	N	85	85
Groups	r value	,691**	1,000
	p-value	,0001	.
	N	85	85

\*\* . Correlation is significant at the 0.01 level (2-tailed).

Table 4 Correlation results between groups for 8-isoPG levels

Çizelge 4. 8-isoPG levels düzeyleri için gruplar arasındaki korelasyon sonuçları.

		8-isoPG	Groups
8-isoPG	r value	1,000	,711**
	p-value	.	,0002
	N	85	85
Groups	r value	,711**	1,000
	p-value	,0002	.
	N	85	85

\*\* . Correlation is significant at the 0.01 level (2-tailed).

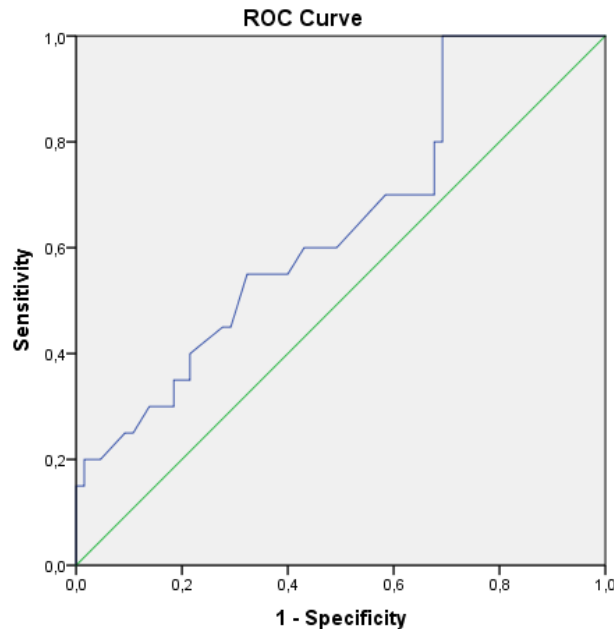
The ROC curve AUC, cut-off value, specificity, and sensitivity values of RFTN are shown in Figure 3 and Table 5. Accordingly, the cut-off value of RFTN was found to be 9.5 ng/mL, sensitivity 85, and specificity 79. In addition, ROC curve AUC, cut-off value,

specificity, and sensitivity values for 8-iso-PGF2α are shown in Figure 4 and Table 6. The cut-off value of 8-iso-PGF2α was found to be 97.8 pg/mL, sensitivity 88, and specificity 85. (P<0.05).

Table 5 ROC curve AUC cut-off, specificity, and sensitivity values for Raftlin

Çizelge 5. Raftlin için ROC eğrisi AUC kesme, özgülük ve duyarlılık değerleri.

Risk Factor	AUC 95%	P	Cut off	Sensitivity (%)	Specificity
0.846 (0.702-0.889)	<0.001	9.5	85	79	



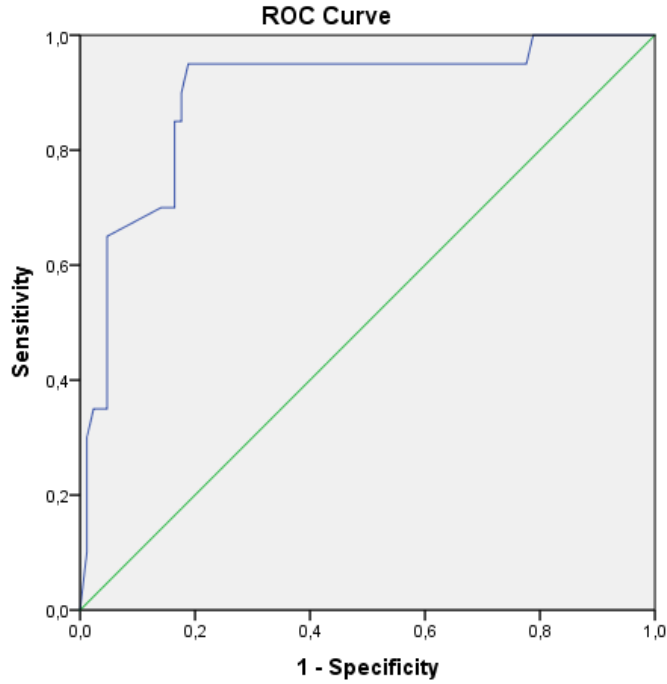
Diagonal segments are produced by ties.

Şekil 3. Bu eğri, doğru-pozitif oranı ve doğru-negatif oranı bilgilerini birleştirir ve AUC, Raftlin'in genel ayırt edici gücünün bir ölçüsüdür.

Figure 3. This curve combines the information of the true-positive rate and the true-negative rate, and the AUC is a measure of the overall discriminative power of Raftlin.

Table 6 ROC curve AUC cut-off, specificity, and sensitivity values for 8-iso-PGF2α  
Çizelge 6. 8-iso-PGF2α için ROC eğrisi AUC kesme, özgülük ve duyarlılık değerleri.

Risk Factor	AUC 95%	P	Cut off	Sensitivity (%)	Specificity
0.896	(0.813-0.978)	<0.001	97.8	88	85



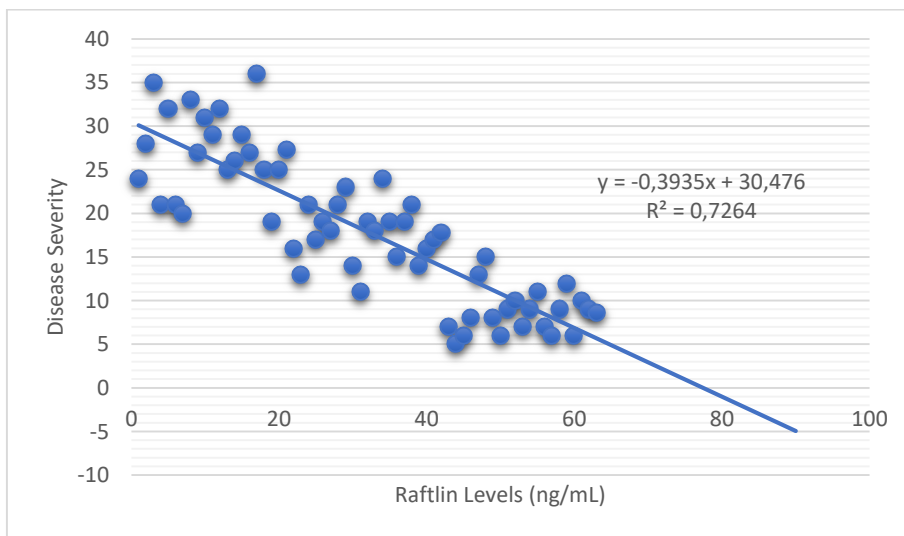
Diagonal segments are produced by ties.

Şekil 4. Bu eğri, doğru-pozitif oranı ve doğru-negatif oranı bilgilerini birleştirir ve AUC, 8-iso prostaglandin 'in genel ayırt edici gücünün bir ölçüsüdür.

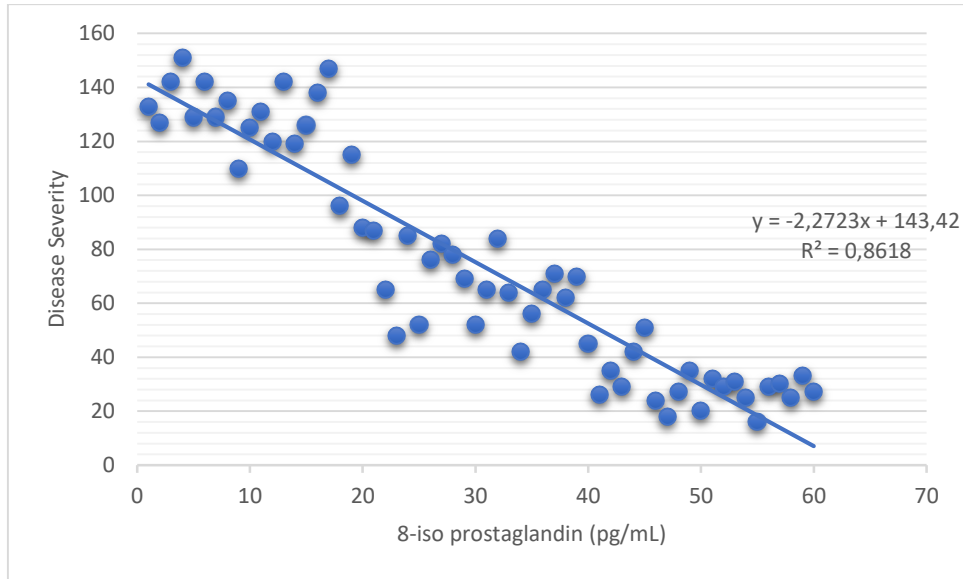
Figure 4. This curve combines the information of the true-positive rate and the true-negative rate, and the AUC is a measure of the overall discriminative power of 8-iso prostaglandin.

A scatter plot was drawn to further reveal the relationship between disease severity and RFTN (Figure 5). According to the scatter plot, as the severity of the disease decreases, RFTN levels also decrease. The R<sup>2</sup> value of 0.72 reveals a significant relationship between this results.

A scatter plot was drawn to further reveal the relationship between disease severity and 8-iso-PGF2α (Figure 6). According to the scatter plot, as the severity of the disease decreases, the level of raftlin also decreases. The R<sup>2</sup> value of 0.86 reveals that there is a significant relationship between this results.



Şekil 5. Hastalık şiddeti ve raftlin arasındaki dağılım grafiği ilişkisi  
Figure 5. Scatter plot relationship between disease severity and raftlin.



Şekil 6. Hastalık şiddeti ve 8-izoprostaglandin arasındaki dağılım grafiği ilişkisi  
*Figure 6. Scatter plot relationship between disease severity and 8-iso prostaglandin.*

To this knowledge, this is the first study to evaluate serum RFTN and 8-iso-PGF $2\alpha$  levels in patients with COVID-19. We believe that the groups we compared were homogeneous enough to make a valid assessment of these biomarkers for COVID-19 patients. Four main findings were observed in this study: First, elevated serum levels of RFTN and 8-iso-PGF $2\alpha$  were detected in severe COVID-19 patients compared to mild COVID-19 patients and control subjects. Second, RFTN and 8-iso-PGF $2\alpha$  levels showed good diagnostic performance in severe COVID-19 patients. Third, we found a positive and significant correlation between RFTN and 8-iso-PGF $2\alpha$  levels in the COVID-19 patient group.

In areas where free radicals are formed, membrane lipids may be sensitive to peroxidation and this may lead to the formation of isoprostanes. Isoprostanes are released by the action of phospholipases and are stable products in circulation. High isoprostane levels in plasma and other body fluids indicate the presence of oxidative stress and are frequently used as biomarkers of damage in various cells and tissues (Muhammed et al., 2020; Zarkovic et al., 2022). However, circulating isoprostanes may also function as intracellular signalling molecules. They generally cause vasoconstriction in most vascular systems and constrict smooth muscles in the lymphatic, uterine, gastrointestinal, and tracheobronchial systems (Lahaie et al., 1998; Derouiche et al., 2020). In this study, COVID-19 infection alone caused a 2-3-fold increase in the 8-iso-PGF $2\alpha$  level mediated by the Fenton reaction. This finding showed that 8-iso-PGF $2\alpha$  may be an important predictive marker of cumulative oxidative stress and worsening of the condition of the patient infected with COVID-19. The increase in free radicals followed by an increase in 8-iso-PGF $2\alpha$  is the first evidence that 8-iso-PGF $2\alpha$ , a marker of oxidative

stress, may play a role in the pathogenesis of COVID-19 infection. There are also studies showing that decreased antioxidant levels in severe SARS-CoV-2 patients are accompanied by increased oxidative stress, as evidenced by higher levels of reactive oxygen and nitrogen species as well as lipid peroxidation (Muhammed et al., 2021; Muhammed et al., 2020; Noonog et al., 2023). This may indicate a poor prognosis in patients with COVID.

Lipid rafts play a very important role in cell signalling mechanisms. In the literature, the role of lipid rafts using a G-protein-coupled receptor system or tyrosine kinase receptor system in signalling mechanisms is discussed (Saeki et al., 2003). RFTN is an important lipid raft protein identified from Raji B cells. It is required for the regulation of lipid rafts and signal transduction of B cell antigen receptors (Saeki et al., 2003). In addition, RFTN plays a role in the stimulation of the nucleocapsid complex during TLR3 activation and autoimmune responses (Schmidt et al., 2009; Saeki et al., 2009; Watanabe et al., 2011). RFTN also plays a role in the pathophysiology of sepsis and vascular inflammatory response (Bae et al., 2008; Bae et al., 2004; Pike, 2003). In this study, it was shown that RFTN levels were significantly higher in the COVID-19 group compared to the control group. No study on RFTN in COVID-19 was found in the literature. Therefore, we could not compare this RFTN results obtained from COVID-19 patients. Increased RFTN levels in COVID-19 may result from increased inflammation due to COVID-19. The factors that trigger the disease in SARS-CoV-2-infected individuals are not fully understood, and the severe development of the disease does not appear to be related only to viral load and may also include an inadequate interferon response (Saeki et al., 2003).



Correlation results constitute an important finding of this study. The correlation coefficient is indicated by the symbol 'r'. While the 'r' value  $\leq 0.35$  represents a weak correlation, values between 0.36 and 0.67 indicate a moderate correlation. Values between 0.68 and 0.90 indicate a high correlation and values between 0.90 and 1.0 indicate a very high correlation (Taylor, 1990). In this study, a strong positive correlation was found between the severity of COVID-19 disease and RFTN and 8-iso-PGF2 $\alpha$  levels. No study investigating the relationship between RFTN and 8-iso-PGF2 $\alpha$  levels in COVID-19 was found in the literature. Therefore, we could not compare this results. A high correlation coefficient may form the basis for the detection of biomarkers. In conclusion, increased levels of 8-iso-PGF2 $\alpha$  and RFTN in COVID-19 may play a role in the pathogenesis and progression of the disease.

Currently, new diagnostic biomarkers need to be identified to aid in the diagnosis of COVID. In ROC analysis, the discrimination of a value is assessed according to the area under the curve (AUC). The ROC curve is categorized as: 0.9-1 = very good, 0.8-0.9 = good, 0.7-0.8 = moderate, 0.6-0.7 = poor, and  $<0.6$  = failure (Demirhan et al., 2023). The diagnostic values of biomarkers have been studied in some recent clinical studies and bacterial and viral infectious diseases. Tekin et al. showed that increased serum CRP levels may be a diagnostic biomarker in various bacterial and viral infectious diseases (Tekin et al., 2017). In their study, they calculated the sensitivity and specificity of CRP parameters in detecting bacterial infection as 60.26% and 45.61%, respectively, and AUC: 0.534 in ROC analysis (Karahan et al., 2021). Determined the cut-off value of plasma cytomegalovirus (CMV) virus load detected by PCR to predict the diagnosis of cytomegalovirus gastrointestinal disease (CMV-GIHD) by ROC analysis. Accordingly, they obtained AUC: 0.88, 78% sensitivity, 100% specificity, 100% positive predictive value, and 87% negative predictive value in ROC analysis in CMV-GIH positive and CMV-GIH negative solid organ transplant recipients carrying CMV virus (Karahan et al., 2021). To this knowledge, this is the first study to test the diagnostic value of the ROC curve RFTN and 8-iso-PGF2 $\alpha$  levels in patients with COVID-19. In this study, the AUC for RFTN was 0.846 and the AUC for 8-iso-PGF2 $\alpha$  was 0.896. This finding indicates that serum levels of RFTN and 8-iso-PGF2 $\alpha$  have very good diagnostic value. We do not claim that the levels of RFTN and 8-iso-PGF2 $\alpha$  are new methods that should be used as diagnostic biomarkers in patients with COVID-19. We believe, that further studies should be performed in larger and more homogeneous groups to test the diagnostic value of RFTN and 8-iso-PGF2 $\alpha$  in patients with COVID-19.

## CONCLUSION and RECOMMENDATIONS

However, this study has several limitations, such as the sample size, because it may not be easy to find associations between variables, and the small size may not provide a representative distribution of the population, and this was due to the presence of only 75 patients. Second, free radicals could have been directly determined but this was not possible due to their high reactivity, short half-life, and labile nature; instead, their most reliable and stable metabolite, 8-iso-PGF2 $\alpha$ , was measured as a marker of oxidative stress. The results of RFTN and 8-iso-PGF2 $\alpha$ , which we present for the first time in COVID-19 patients, suggest that RFTN and 8-iso-PGF2 $\alpha$  may be a determinant biomarker in the prognosis of this disease. We think that the results of this study will lead to other studies and will be more supportive of new data.

**Ethics Committee Approval Number: 21-KAEK-099**

## Summary of Researchers' Contribution Rate Declaration

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

## Conflict of Interest Statement

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

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## LC-MS/MS Analysis and Biological Activities of Different Parts of *Ziziphora capitata* L.

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### ABSTRACT

The *Ziziphora* species, classified under the Lamiaceae family, have a strong aromatic property. *Ziziphora* species have been used in folk medicine as sedative, gastric, aphrodisiac, bloating, and degassing. In the current study, the phenolic and flavanoid content of ethanol extracts of *Ziziphora capitata* L. species of flower, leaf, branch, mixed, and root parts was determined by the LC-MS/MS device. In addition, the antioxidant and cytotoxic activities of the extracts, as well as their inhibitory effects on enzymes (antihypertensive, AchE (acetylcholinesterase), BchE (butyrylcholinesterase), elastase, tyrosinase, collagenase and urease), were determined. The LC-MS/MS results showed that quinic acid (25578, 5842, 25171, 14055, 10597  $\mu\text{g g}^{-1}$ , respectively) was found in higher amounts in flower, leaf, branch, mixed, and root extracts of *Z. capitata* species compared to other components. Additionally, rosmarinic acid (17097  $\mu\text{g g}^{-1}$ ), cynaroside (8432), and hesperidin (8067) were found to be major components. It was observed that the flower extract of the species exhibited strong antioxidant activity ( $\text{IC}_{50}$ : 37.18 $\pm$ 1.36  $\mu\text{g mL}^{-1}$ , 9.89 $\pm$ 0.45,  $\text{A}_{0.5}$ : 16.27 $\pm$ 0.02, respectively) in DPPH, ABTS and CUPRAC methods. It was concluded that the leaf extract of *Z. capitata* species had a strong cytotoxic effect on HT-29 (colon cancer cell line) (viability %: 9.26 $\pm$ 0.69). It was observed that the root part of the species exhibited higher activity in butyrylcholinesterase (BChE) enzyme inhibition activity (inhibition %: 40.56 $\pm$ 0.88) than other parts. It was determined that *Z. capitata* extracts did not show acetylcholinesterase, urease, tyrosinase, elastase, collagenase, and antihypertensive enzyme activity or showed low activity. As a result, it is thought that the flower extract of the *Z. capitata* species has better results in terms of the examined parameters, whereas the leaf extract needs to be subjected to more detailed *in vitro* and *in vivo* research conducted to be used in the pharmaceutical industry as a result of its cytotoxic effect against colon cancer cell lines.

### Biochemistry

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*Ziziphora capitata*  
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## *Ziziphora capitata* L. Türünün Farklı Kısımlarının LC-MS/MS Analizi ve Biyolojik Aktiviteleri

### ÖZET

Lamiaceae familyasının altında sınıflandırılan *Ziziphora* türleri güçlü aromatik özelliğe sahiptir. Halk hekimliğinde *Ziziphora* türleri yatıştırıcı, midevi, afrodisyak, şişkinlik ve gaz giderici olarak kullanılmıştır. Mevcut çalışmada *Ziziphora capitata* L. türünün çiçek, yaprak, dal, karışık ve kök kısımlarının etanol ekstratlarının fenolik ve flavanoid içeriği LC-MS/MS cihazı ile belirlenmiştir. Ayrıca ekstratların antioksidan, sitotoksik etkileri ile enzim inhibisyon aktiviteleri (antihipertansif, AchE (asetilkolinesteraz), BchE (bütilkolinesteraz), elastaz, tirozinaz, kollajenaz ve üreaz) belirlenmiştir. LC-MS/MS sonuçlarına göre *Z. capitata* türünün çiçek, yaprak, dal, karışık ve kök ekstratlarında kinik asidin (sırasıyla, 25578, 5842, 25171, 14055, 10597  $\mu\text{g g}^{-1}$ ) diğer bileşenlerden daha yüksek içeriğe sahip olduğu tespit

### Biyokimya

### Araştırma Makalesi

### Makale Tarihçesi

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### Anahtar Kelimeler

*Ziziphora capitata*  
LC-MS/MS  
Antioksidan  
Sitotoksik  
Kinik asit



edilmiştir. Ayrıca rosmarinik asit ( $17097 \mu\text{g g}^{-1}$ ), sinarozit (8432) ve hesperidin (8067) bileşiklerinin majör bileşenler olduğu bulunmuştur. DPPH, ABTS ve CUPRAC yöntemlerinde türün çiçek ekstresinin güçlü antioksidan aktivitesi (sırasıyla:  $\text{IC}_{50}$ :  $37.18 \pm 1.36 \mu\text{g mL}^{-1}$ ;  $9.89 \pm 0.45$ ;  $A_{0.5}$ :  $16.27 \pm 0.02$ ) sergilediği görülmüştür. *Z. capitata* türünün yaprak ekstresinin HT-29 (kolon kanseri hücre hattı) üzerine (% canlılık:  $9.26 \pm 0.69$ ) güçlü sitotoksik etki gösterdiği belirlenmiştir. Bütirilkinesteraz (BChE) enzim inhibisyon aktivitesinde türün kök kısmının (%inhibison:  $40.56 \pm 0.88$ ) diğer kısımlarından daha yüksek aktivite sergilediği görülmüştür. *Z. capitata* türünün etanol ekstrelerinin asetilkolinesteraz, üreaz, tirozinaz, elastaz, kollajenaz ve antihipertansif enzim aktivitesi göstermediği veya düşük aktivite gösterdiği belirlenmiştir. Sonuç olarak, *Z. capitata* türünün çiçek ekstresinin incelenen parametreler açısından daha iyi sonuçlara sahip olduğu, yaprak ekstresinin ise kolon kanseri hücre hattı üzerindeki sitotoksik etkisinden dolayı türün ilaç endüstrisinde kullanılabilmesi için *in vitro* ve *in vivo* olarak detaylı araştırmalara tabi tutulması gerektiği düşünülmektedir.

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## INTRODUCTION

Plants are the starting point of the ancient medical system, which has been practiced for thousands of years and continues to provide novel treatments to the world. (Gurib-Fakim, 2006). Medicinal and aromatic plants have been used for centuries as spices and for the prevention of diseases. (Li, 2006; Christaki et al., 2012; Youssif et al., 2024). Medicinal plants are becoming increasingly important because of their potential to prevent and treat diseases. (Yu et al., 2021). Almost all cultures have used medicinal plants to treat diseases (Bozyel et al., 2019). Ensuring the safety, quality and efficacy of herbal products has become an important theme in today's world. (Singh, 2015).

The genus *Ziziphora*, belonging to the family Lamiaceae, is represented by five species and six taxa. In Turkey, it is grown in Western Anatolia, the Mediterranean Region and the Central and Eastern Anatolia regions. (Kaya & Dirmenci 2012; Satıl & Selvi, 2020). *Ziziphora* species are either annual or perennial herbs (Kaya & Dirmenci 2012). In the traditional medicinal practices of Turkey and Iran, *Ziziphora* species have been utilized for their sedative, stomach-soothing, aphrodisiac, anti-flatulence, and carminative properties (Sezik & Tümen, 1984; Kaya & Dirmenci, 2012; Selvi et al., 2015). In Turkey, species from this genus are referred to as 'Dağ Reyhani', 'Filiskin otu', or 'Nane ruhu'. It is also known that the dried plant parts of this genus are used as herbal tea and spices. (Baytop, 1999; Kaya & Dirmenci, 2012; Selvi et al., 2015). *Z. capitata* species contains terpenoids, flavonoids, essential oils, and phenolic

acids (Ghazanfari et al., 2013; Youssif et al., 2024). It is also stated that this species has various pharmacological and cytotoxic effects on humans (Mohammadhosseini et al., 2016; Youssif et al., 2024). The essential oil composition of certain *Ziziphora* species from Iran has been studied before and was found to be abundant in oxygenated monoterpenes, including pulegone. The main constituents of the essential oil of the Iranian species *Z. clinopodioides* subsp. *rigida* were identified as piperitenone, thymol, pulegone and *p*-menth-3-en-8-ol (Ebrahimi et al., 2009).

In the literature review of *Z. capitata* species, it was found that germacrene D, (*Z*)- $\beta$ -osimene, (*E*)- $\beta$ -osimene, limonene,  $\beta$ -caryophyllene, hexadecanoic acid and bicyclogermacrene were the main components in its essential oil. Additionally, the antibacterial activity of *Z. capitata* oil was measured (Aghajani et al., 2008). Cytotoxic activity analysis of *Z. capitata* species against MCF-7 cell line and antioxidant, phenolic, and flavonoid analysis were performed (Mohammadhosseini et al., 2016; Youssif et al., 2024). There is no study on the inhibition of urease, AChE, BChE and tyrosinase enzymes by ethanol extract of *Z. capitata* species. In this sense, this study is the first of its kind.

In this study, ethanol extracts of flower, branch, mixed, root, and leaf parts of *Z. capitata* species were prepared. Phytochemical content analysis of the prepared extracts was performed by LC-MS/MS. The toxic effects of the extracts on PDF (healthy cell line) and cytotoxic effects on HT-29 and MCF-7 were determined and revealed. In addition, *in vitro*



antioxidant capacity (DPPH, ABTS, CUPRAC), acetylcholinesterase (AChE), butyrylcholinesterase (BChE), antihypertensive, elastase, collagenase, tyrosinase and urease enzyme inhibitory activities of the extracts were determined.

Table 1. Names of studied species, extract yield, and herbarium number

*Çizelge 1. Çalışılan türlerin isimleri, ekstre verimi ve herbarium numarası*

Species Name	Code	Extract Yield %	Herbarium No
<i>Ziziphora capitata</i> flower	ZCC	5.42	
<i>Ziziphora capitata</i> leaf	ZCY	3.61	
<i>Ziziphora capitata</i> branch	ZCD	1.26	M.Fırat 32648 (VANF)
<i>Ziziphora capitata</i> mixed	ZCKA	6.32	
<i>Ziziphora capitata</i> root	ZCKO	1.18	

### Extraction and LC-MS Analysis

*Z. capitata* species were separated into leaf, branch, flower, root and mixed parts. The separated parts were ground into powder with a grinder and 10 g of each type were weighed. Then, ethanol (50 mL, 3x24 hours) was added to the plant samples. After solvent evaporation processes, crude extracts were obtained (Akdeniz et al., 2021). The extracts were adjusted to final concentrations of 1000 µg mL<sup>-1</sup> prior to LC-MS/MS injection. The phytochemical composition of the examined plant species was evaluated using a previously established and validated LC-MS/MS method. In this method, 53 compounds, particularly found in natural products, with phenolic acid and flavonoid form, and 3 internal standards were analyzed (Yilmaz, 2020).

### Total Flavonoid-Phenolic Content, Antioxidant and Cytotoxic Activity Analyses

Total phenolic (as pyrocatechol equivalent) and flavonoid contents of *Z. capitata* ethanol extracts (ZCC, ZCY, ZCD, ZCKA, and ZCKO) were calculated (as quercetin equivalent) (Slinkard & Singleton, 1977; Moreno et al., 2000). To evaluate the antioxidant activity of *Z. capitata* species ZCC, ZCY, ZCD, ZCKA, and ZCKO extracts, DPPH (free radical scavenging), ABTS (cation radical scavenging) methods were used and the values were calculated as IC<sub>50</sub>. Additionally, the extracts were evaluated using the CUPRAC (Copper (II) reduction capacity) method and the results were calculated as A<sub>0.5</sub>. (Blois, 1958; Re et al., 1999; Apak et al., 2004; Tural et al., 2024). Butylated hydroxytoluene (BHT) and α-tocopherol were applied as references. Additionally, the method established by Mojarraba et al. (2013) was employed, with slight modifications, to evaluate the toxic and cytotoxic activities of the species ZCC, ZCY, ZCD, ZCKA, and ZCKO extracts.

## MATERIAL and METHOD

### Plant Material

The species name, code, extract yield, and herbarium number of the used part of the *Ziziphora capitata* L. plant collected in Van province in 2016 are given in Table 1.

### Enzyme Activities

#### Cholinesterase enzyme activity

To inhibit the enzymes acetylcholinesterase and butyrylcholinesterase, the method developed by Ellman et al. (1961) was used. In this method, galantamine was used as a reference when measuring enzyme inhibition.

#### Urease enzyme activity

Urease enzyme inhibition activity of ZCC, ZCY, ZCD, ZCKA, and ZCKO extracts was determined using the method developed by Zahit et al. (2015). Thiourea was used as reference.

#### Tyrosinase enzyme activity

Tyrosinase enzyme inhibition activity of of ZCC, ZCY, ZCD, ZCKA, and ZCKO extracts was determined using the method developed by Hearing & Jiménez (1987). Kojic acid was used as a reference in this method.

#### Elastase and Collagenase enzyme activities

To determine the antiaging effects of the samples, elastase (Kraunsoe et al., 1999) and collagenase (Thiring et al., 2009) inhibitory activity determinations were performed. N-succinyl-(Ala)<sub>3</sub>-nitroanilide and N-(3-[2-Furyl]acryloyl)-Leu-Gly-Pro-Ala were used as substrates in elastase and collagenase enzyme inhibitory activity determinations, respectively. Oleanolic acid and epicatechin gallate were used as standard references.

#### Hypertensive enzyme activity

The method described by Kwon et al. (2006) was used with minor variations. Lisinopril was used as standard.

## RESULTS and DISCUSSION

### LC-MS/MS analysis

According to the LC-MS/MS results of ethanol extracts

of *Z. capitata* species (ZCC, ZCY, ZCD, ZCKA, and ZCKO), the main components observed in all extracts were quinic acid (25578, 5842, 25171, 14055, 10597,  $\mu\text{g g}^{-1}$ , respectively), cynaroside (4349, 6371, 1417, 8432, 106), hesperidin (3246, 8067, 1554, 3016, ND) and rosmarinic acid (17097, 3663, 6950, 4179, 12013). It was determined that the compound with the highest

amount in all studied parts of the *Z. capitata* species was quinic acid. It is seen that all extracts studied contain caffeic acid and the highest amount belongs to ZCKO extract (4088). It was concluded that the leaf extract of *Z. capitata* (ZCY) contains higher hesperidin (8067) than other extracts (Table 2 and Figure 1 and 2).

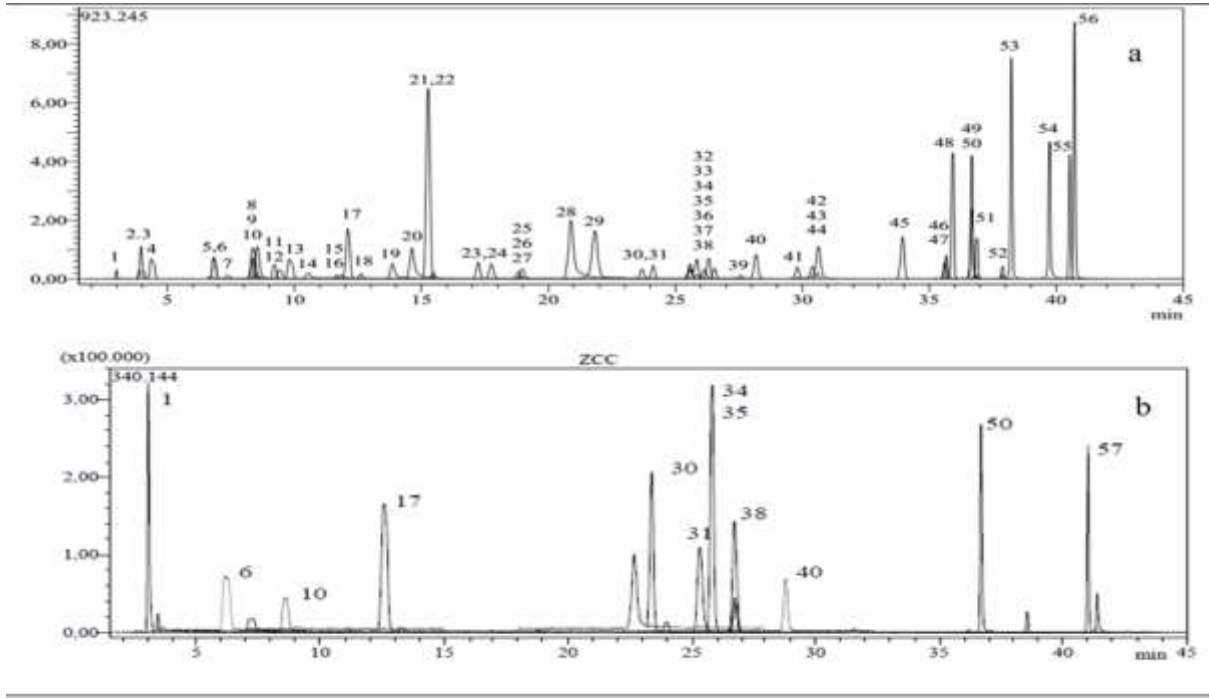


Figure 1. a) TIC chromatogram of the standards mixture analyzed by the LC-MS/MS ( $1 \mu\text{g mL}^{-1}$ ). b) LC-MS/MS chromatogram of ethanol extract of flower part of *Z. capitata* species

Şekil 1. a) LC-MS/MS ile analiz edilen standart karışımının TİK kromatogramı ( $1 \mu\text{g mL}^{-1}$ ). b) *Z. capitata* türünün çiçek kısmının etanol özütünün LC-MS/MS kromatogramı

There is no research on the phytochemical content of the *Z. capitata* species in the literature. However, some components found in the species were reported to be determined qualitatively by UPLC-QTOF-MS/MS (Youssif et al., 2024). Ahmedi et al. (2021) identified 44 compounds following LC-MS examination of the ethanol extract from the *Z. clinopodioides* Lam. species. Taheri et al. (2023) identified the main constituents of *Z. clinopodioides* as quercetin, rutin and apigenin (16738.85, 15004.45,  $106.25 \mu\text{g g}^{-1}$ , respectively). Ozkan et al. (2020) also conducted a

study in which it was determined that the ethanol extracts of the root and aerial parts of the *Z. clinopodioides* species contained high amounts of quinic acid ( $14721.04 \pm 120.71$ ,  $9020.51 \pm 73.97 \mu\text{g g}^{-1}$ , respectively), malic acid ( $2179.04 \pm 24.62$ ,  $1972.95 \pm 22.29 \mu\text{g g}^{-1}$ ) and rhoifolin ( $3593.31 \pm 338.13$ ,  $1044.74 \pm 98.31 \mu\text{g g}^{-1}$ ), which is parallel to this study. Compared with previous studies, the major compounds of *Z. capitata* appear to be partially different from *Ziziphora clinopodioides*.

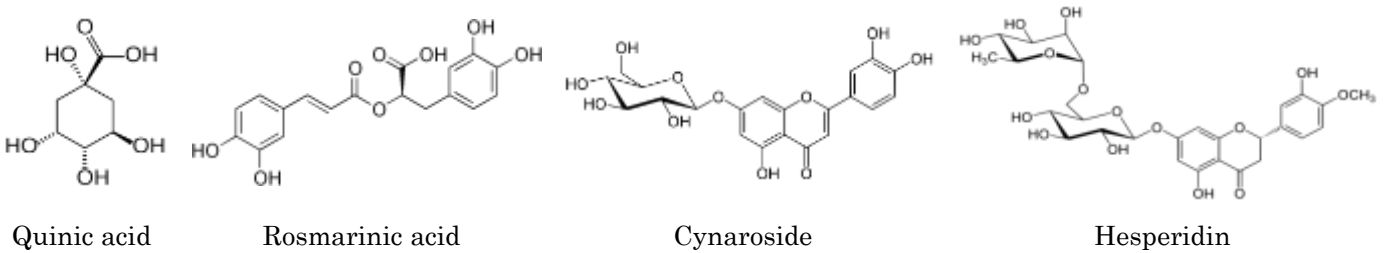


Figure 2. Major components of the species

Şekil 2. Türün major bileşikleri

Table 2. Phenolic and flavonoid contents of the studied species by LC-MS ( $\mu\text{g}$  analyte  $\text{g}^{-1}$  extract)  
*Çizelge 2. Çalışılan türlerinin LC-MS ile fenolik ve flavonoid içerikleri ( $\mu\text{g}$  analit  $\text{g}^{-1}$  ekstre)*

No	Analytes	Retention time (min)	Parent ion ( $m/z$ ) <sup>a</sup>	MS <sup>2</sup> (Collision energy) <sup>b</sup>	Quantification ( $\mu\text{g}$ analyte $\text{g}^{-1}$ extract) <sup>c</sup>				
					ZCC	ZCY	ZCD	ZCKA	ZCKO
1	Quinic acid	3.0	190.8	93.0	25578	5842	25171	14055	10597
6	Protocatechuic acid	6.8	152.8	108.0	1132	894	1395	1436	1879
9	Chlorogenic acid	8.4	353.0	85.0	ND	ND	ND	ND	157
10	Protocatechuic aldehyde	8.5	137.2	92.0	576	655	514	502	1201
17	Caffeic acid	12.1	179.0	134.0	1580	1362	2352	1692	4088
19	Vanillin	13.9	153.1	125.0	ND	ND	79	ND	60
24	<i>p</i> -Coumaric acid	17.8	163.0	93.0	130	167	76	407	65
28	Coumarin	20.9	146.9	103.1	10	ND	12	ND	ND
29	Salicylic acid	21.8	137.2	65.0	525	113	526	387	279
30	Cynaroside	23.7	447.0	284.0	4349	6371	1417	8432	106
33	Rutin	25.6	608.9	301.0	2605	5774	1144	2293	ND
34	isoquercitrin	25.6	463.0	271.0	1733	1842	1107	1443	ND
35	Hesperidin	25.8	611.2	449.0	3246	8067	1554	3016	ND
38	Rosmarinic acid	26.6	359.0	197.0	17097	3663	6950	4179	12013
40	Cosmosiin	28.2	431.0	269.0	714	847	147	1642	86
42	Astragalın	30.4	447.0	255.0	229	249	140	303	ND
47	Quercetin	35.7	301.0	272.9	121	79	122	85	ND
50	Luteolin	36.7	284.8	151.0/175.0	651	827	162	1705	61
52	Kaempferol	37.9	285.0	239.0	ND	ND	ND	ND	19
53	Apigenin	38.2	268.8	151.0/149.0	49	13	12	108	50
55	Chrysin	40.5	252.8	145.0/119.0	2	ND	27	10	156
56	Acacetin	40.7	283.0	239.0	242	238	207	694	4320

<sup>a</sup>Parent ion ( $m/z$ ): Molecular ions of standards (mass-to-charge ratio). <sup>b</sup>MS<sup>2</sup> (CE): MRM fragments for the related molecular ions (CE refers to related collision energies of the fragment ions). <sup>c</sup>Values in  $\mu\text{g g}^{-1}$  ( $w w^{-1}$ ) of plant ethanol extract. <sup>d</sup>IS: Internal standard. ND: not detected. Numbers on the far left raw indicate the standard phytochemical compounds. (Components that are working but not detected: Fumaric acid, Aconitic acid, Gallic acid, Epigallocatechin, Catechin, Gentisic acid, Tannic acid, Epigallocatechin gallate, 1,5-Dicaffeoylquinic acid, 4-OH Benzoic acid, Epicatechin, Vanillic acid, Syringic acid, Syringic aldehyde, Daidzin, Epicatechingallate, Piceid, Ferulic acid-D3-ISd, Ferulic acid, Sinapic acid, Miquelianin, Rutin-D3-ISd,  $\sigma$ -Coumaric acid, Genistin, Ellagic acid, Quercitrin, Nicotiflorin, Fisetin, Daidzein, Quercetin-D3-ISd, Naringenin, Hesperedin, Genistein, Amentoflavone)

### Total Flavonoid-Phenolic Content Results

When the total phenolic contents of ZCC, ZCY, ZCD, ZCKA, and ZCKO extracts of *Z. capitata* species were examined, it was determined quantitatively as ZCKO>ZCC>ZCY>ZCD>ZCKA (63.49±3.00, 51.98±1.82, 46.43±0.52, 40.08±1.82 and 33.93±0.42  $\mu\text{g}$  Pes  $\text{mg}^{-1}$  extract, respectively). It is possible to rank the total flavonoid content as ZCKA>ZCY>ZCC>ZCD>ZCKO (45.14±0.32, 33.14±0.89, 16.86±0.36, 10.57±0.00, and 8.14±0.06,  $\mu\text{g}$  QEs  $\text{mg}^{-1}$  extract, respectively). It was found that the extract from the root part of the species had the highest total phenolic content (ZCKO), while the mixed part of the species (ZCKA) had the highest total flavonoid content (Table 3). In the study by Youssif et al. (2024), the quantitative analysis results of various active components, including phenolics and flavonoids, of the *Z. capitata* species were determined as 180.10±0.6  $\text{mg g}^{-1}$  (gallic acid equivalent) and 40±0.8  $\text{mg g}^{-1}$  (quercetin equivalent), respectively. It is evident that this study is parallel to the present study in terms of total flavonoid amount.

### Antioxidant Activity Results

The results indicated that all extracts showed substantial antioxidant activity in each of the three methods. In the DPPH free radical scavenging method, all extracts showed significant antioxidant activity relative to BHT as a standard, with the ZCC extract ( $\text{IC}_{50}$ : 37.18±1.36  $\mu\text{g mL}^{-1}$ ) displaying the best activity. In the ABTS cation radical method, it was found that all extracts showed strong antioxidant activity, and the ZCKO ( $\text{IC}_{50}$ : 8.00±0.07  $\mu\text{g mL}^{-1}$ ) extract showed stronger activity. According to the CUPRAC copper reduction method, all extracts were found to exhibit strong antioxidant effects, and the ZCC ( $\text{A}_{0.5}$ : 16.27±0.02  $\mu\text{g mL}^{-1}$ ) extract had stronger activity (Table 3).

The antioxidant capacity of *Z. capitata* ( $\text{IC}_{50}$ : 206.6±1.3  $\mu\text{g mL}^{-1}$ ) was measured by the DPPH method in the study conducted by Mohammadhosseini et al. (2016). In the study conducted by Abad and Nadaf (2023), the essential oil of the *Ziziphora persica* Bunge was determined to have an  $\text{IC}_{50}$  of 34.20±1.32  $\mu\text{g mL}^{-1}$

according to the DPPH method. When compared with previous studies, it was determined that *Z. capitata* species showed similarly high antioxidant activity.

### Cytotoxic Activity

The toxic effects of ZCC, ZCY, ZCD, ZCKA, and ZCKO ethanol extracts of the species were studied against, as well as their cytotoxic effects against HT-29 and MCF-7. The percentage viability values of *Z. capitata* species extracts were determined at a 200 µg mL<sup>-1</sup> concentration. It was determined that all extracts did not have toxic effects on PDF cell lines. ZCY extract showed a very high cytotoxic effect on the HT-29 cell

line (viability %: 9.26±0.69). It was also found that ZCC extract had a good cytotoxic effect against the MCF-7 cell line (viability %: 47.72±0.81) (Table 3).

In the literature, in the study conducted by Youssif et al. (2024), hexane (46.7±1.68 µg mL<sup>-1</sup>), chloroform (50.3±1.79 µg mL<sup>-1</sup>), ethyl acetate (108±3.18 µg mL<sup>-1</sup>), 95% ethanol (377±9.72 µg mL<sup>-1</sup>), and water (407±11.28 µg mL<sup>-1</sup>) extracts of *Z. capitata* species showed moderate cytotoxic activity against MCF-7 cell line. When the current study was compared with the previous study, moderate cytotoxic activity against MCF-7 was detected in the samples in both studies.

Table 3. Total phenolic, flavonoid, antioxidant and cytotoxic activity results of the studied extracts

Çizelge 3. Çalışılan ekstrelerinin toplam fenolik, flavonoid, antioksidan ve sitotoksik aktivite sonuçları

Sample s	Phenolic Content (µg Pes mg <sup>-1</sup> extract) <sup>b</sup>	Flavonoid Content (µg QEs mg <sup>-1</sup> extract) <sup>c</sup>	IC <sub>50</sub> (µg mL <sup>-1</sup> ) <sup>d</sup>		A <sub>0.5</sub>		Vitality (%) <sup>e</sup>	
			DPPH Free Radical	ABTS Cation Radical	CUPRAC	HT29	MCF7	PDF
ZCC	51.98±1.82	16.86±0.36	37.18±1.36	9.89±0.45	16.27±0.02	232.61±2.77	47.72±0.81	126.65±0.95
ZCY	46.43±0.52	33.14±0.89	48.43±0.94	13.83±0.16	27.17±0.03	9.26±0.69	116.19±2.85	95.00±2.75
ZCD	40.08±1.82	10.57±0.00	60.91±1.25	23.34±0.26	26.16±0.02	207.60±0.94	90.71±1.37	89.98±3.81
ZCKA	33.93±0.42	45.14±0.32	54.52±1.24	15.55±0.25	22.84±0.01	200.25±6.48	73.93±0.72	89.33±3.59
ZCKO	63.49±3.00	8.14±0.06	37.40±0.98	8.00±0.07	23.14±0.03	245.42±1.00	73.72±3.38	105.09±0.65
α-TOC	-	-	13.10±0.52	10.48±0.63	14.49±0.11	-	-	-
BHT	-	-	62.15±0.35	13.62±0.28	7.69±0.01	-	-	-

<sup>a</sup>Values are given as the average and standard deviation of 3 parallel measurements. <sup>b</sup>Pyrocatechol equivalent phenolic content. (y = 0.0408(µg) + 0.0383 (r<sup>2</sup>: 0.9951) ). <sup>c</sup>Quercetin equivalent flavonoid content. (y = 0.0355 (µg) + 0.0673 (r<sup>2</sup>: 0.9975) ). <sup>d</sup>Results are given as IC<sub>50</sub> values. <sup>e</sup>vitality (%) values at 200 ppm concentration. ZCC: *Z. capitata* flower, ZCY: *Z. capitata* leaf, ZCD: *Z. capitata* branch, ZCKA: *Z. capitata* mixed, ZCKO: *Z. capitata* root.

### Enzyme Inhibition Activity

AChE, BChE, hypertensive, urease, collagenase, tyrosinase, and elastase enzyme inhibition activities of ZCC, ZCY, ZCD, ZCKA, and ZCKO ethanol extracts of the species were tested (Table 4). When the results were examined, it was found that the extracts were

inactive in the AChE enzyme inhibition test, all extracts were active in the BChE enzyme inhibition test, and ZCKA, ZCKO, and ZCY extracts (40.25±1.62, 40.56±0.88, and 40.25±1.62, respectively) showed moderate activity. The extracts were found to exhibit low or no inhibitory activity against urease, tyrosinase, elastase, collagenase, and hypertensive enzymes.

Table 4. Anticholinesterase, urease, tyrosinase, elastase, collagenase, and antihypertensive enzyme activity results of the studied species

Çizelge 4. Çalışılan türlerinin antikolinesteraz, üreaz, tirozinaz, elastaz, kolajenaz ve antihipertansif enzim aktivite sonuçları

Samples	Inhibition (%) <sup>a</sup>						
	AChE	BChE	Urease	Tyrosinase	Elastase	Collagenase	ACE
ZCC	5.07±0.97	24.87±0.73	13.55±0.69	17.25±0.16	AD	8.79±0.12	AD
ZCY	AD	40.25±1.62	AD	4.71±0.31	AD	9.63±0.08	AD
ZCD	AD	20.09±0.73	AD	AD	AD	7.45±0.05	AD
ZCKA	AD	40.25±1.62	AD	14.25±0.14	AD	7.28±0.06	AD
ZCKO	AD	40.56±0.88	AD	AD	AD	10.47±0.12	AD
Galantamine <sup>b</sup>	89.12±0.64	76.10±0.23	-	-	-	-	-
Thiourea <sup>b</sup>	-	-	94.64±0.16	-	-	-	-
Kojic acid <sup>b</sup>	-	-	-	91.64±0.23	-	-	-
Oleanolic acid <sup>b</sup>	-	-	-	-	44.32±0.20	-	-
Epicatechin <sup>b</sup> gallate <sup>b</sup>	-	-	-	-	-	43.80±0.12	-
Lisinopril <sup>b</sup>	-	-	-	-	-	-	97.68±0.42

<sup>a</sup>Values are given as the mean and standard deviation of 3 parallel measurements (200 µg mL<sup>-1</sup>). <sup>b</sup>Standard item. AD: Inactive. ZCC: *Z. capitata* flower, ZCY: *Z. capitata* leaf, ZCD: *Z. capitata* branch, ZCKA: *Z. capitata* mixed, ZCKO: *Z. capitata* root.



No studies were found in the literature regarding elastase, AChE, BChE, hypertensive, collagenase, tyrosinase, and urease enzyme inhibition activities of *Z. capitata*. This study is the first of its kind in this regard. However, when we look at the studies on other species belonging to the *Ziziphora* genus, in the study conducted by Ozkan et al., (2020), AChE, BChE, tyrosinase and urease enzyme inhibition activities of ethanol extracts of the aerial and root parts of the *Z. clinopodioides* species were determined and it was emphasized that only the extract of the aerial part of the species exhibited low activity in the tyrosinase enzyme inhibition test (inhibition %:  $8.60 \pm 0.87$ ) and the extracts were not active in other enzymes. Sarıkurkcu et al. (2019) measured the tyrosinase enzyme inhibition activities of ethyl acetate ( $IC_{50}$ :  $1.40 \pm 0.06$  mg mL<sup>-1</sup>), methanol ( $1.25 \pm 0.01$ ), and water ( $2.71 \pm 0.42$ ) extracts of *Z. taurica* subsp. *cleonioides*. In another study conducted by Tomczyk et al. (2019), tyrosinase inhibition activities of ethyl acetate ( $IC_{50}$ :  $1.37 \pm 0.07$  mg mL<sup>-1</sup>), methanol ( $1.46 \pm 0.06$ ), and water ( $2.29 \pm 0.13$ ) extracts of *Z. taurica* subsp. *taurica* species were determined. When the results were examined, it was determined that the butyrylcholinesterase and tyrosinase inhibition activity of the *Z. capitata* species differed compared to other species.

## CONCLUSION

This study involved the preparation of ethanol extracts from the flower, leaf, branch, mixed, and root components of the *Z. capitata* species, with their contents analyzed using LC-MS/MS and compared across 53 phytochemicals. The total phenolic and flavonoid contents of all extracts, cytotoxic activity, antioxidant capacities measured by DPPH, ABTS, and CUPRAC techniques, as well as the inhibition rates of hypertensive, urease, AChE, BChE, tyrosinase, elastase, and collagenase enzymes were assessed and compared. The LC-MS/MS analysis of *Z. capitata* revealed quinic acid, cynaroside, hesperidin, and rosmarinic acid as the predominant constituents in all extracts. The flower extract of *Z. capitata* species exhibited greater quantities of these components compared to other extracts. The total phenolic content of the root extract of the examined species was found to be superior to that of other extracts. The overall flavonoid concentration was greater in the mixed extract. All extracts from the *Z. capitata* species exhibited significant antioxidant activity, with the flower extract demonstrating the highest level of activity among them. *Z. capitata* leaf extract exhibited significant cytotoxicity against HT-29, although other extracts shown no cytotoxic impact on this cell line. The flower extract exhibited moderate cytotoxic activity against MCF-7, while the mixed and root extracts also shown moderate cytotoxic activity. Upon evaluation of enzyme inhibitory activity, it was

ascertained that only the flower extract shown activity against AChE and urease enzymes, exhibiting moderate activity. The BChE enzyme inhibition assay revealed that combined root and leaf extracts had moderate inhibitory efficacy. The flower extract exhibits superior activity compared to other extracts in the tyrosinase enzyme inhibition assay.

Consequently, it turned out that the flower extract of the *Z. capitata* species exhibited superior chemical composition and biological activity. Furthermore, given that the *Z. capitata* leaf extract exhibited a very high cytotoxic effect against the HT-29 cell line, the authors suggest that the *Z. capitata* species warrants further comprehensive *in vitro* and *in vivo* investigations for potential incorporation into the pharmaceutical sector.

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## Contribution Rate Statement Summary of Researchers

Plant material: A.E, M.F.; Experimental studies: S.Y., M.V.C, I.Y., M.A.Y., E.C.K., M.C.; Data compilation and article writing: S.Y., M.C., A.E.

## Conflict of Interest

The authors of the articles declare that they have no conflict of interest.

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## *Ziziphora clinopodioides* Lam. Türünün Kültür İle Doğal Ortamlarda Yetişen Örneklerinin Kimyasal ve Biyolojik Yönden Detaylı İncelenmesi

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### ÖZET

Lamiaceae familyası ilaç, gıda, kozmetik ve parfümeri sektörleri için önemli bir kaynaktır. Lamiaceae familyasına ait olan *Ziziphora clinopodioides* Lam. türü çok eski zamanlardan beri halk hekimliğinde kullanılmaktadır. Mevcut çalışmada *Z. clinopodioides* türünün doğal ve kültür örneklerinin toprak üstü kısımlarının etanol ekstraktlarının toplam fenolik ve flavonoid içeriği, antioksidan, sitotoksik ve enzim (AChE, BChE, tirozinaz, üreaz, elastaz, kolajenaz ve ACE) inhibisyon aktivitelerinin belirlenmesi amaçlanmaktadır. Ayrıca türün aroma içerikleri GC-MS/FID ile belirlenip, etanol ekstraktlarının fitokimyasal bileşimi LC-MS/MS ile tespit edilmiştir. Aroma analizi sonuçlarına göre *Z. clinopodioides* türünün kültür örneğinin majör bileşenleri pulegon (%39.83), *cis*-menton (%21.36), *trans*-menton (%16.64), doğal örneğin majör bileşenleri ise pulegon (%62.42), neoizomentol (%5.93) ve *cis*-pulegon oksit (%5.47) olarak tespit edilmiştir. LC-MS/MS sonuçlarına göre türün kültür ve doğal örneklerinde kinik asit (sırasıyla, 25.841, 15.694 mg analit g<sup>-1</sup> ekstre), rosmarinik asit (6.804, 25.523) ve asasetin (6.115, 10.764) majör bileşenler olarak tespit edilmiştir. Ayrıca kültür örneğinde hesperidin (5.725) daha yüksek olduğu tespit edilmiştir. Enzim aktivitesi sonuçlarına göre türün kültür ve doğal örneklerinin orta düzeyde bütirilkolinesteraz enzim inhibisyon aktivitesi (sırasıyla, % inhibisyon: 45.14±1.40; 43.57±0.73) gösterdiği belirlenmiştir. Üreaz enzim inhibisyon aktivitesinde ise kültür örneğinin orta düzeyde aktivite gösterdiği (% inhibisyon: 43.64±0.39) fakat doğal örneğin aktivite göstermediği, ayrıca kültür örneğinin yüksek antihipertansif (% inhibisyon: 81.6±1.19) aktivite gösterdiği belirlenmiştir. Sonuçlar genel olarak değerlendirildiğinde türün kültür örneğinin doğal örneğe kıyasla yüksek antioksidan, bütirilkolinesteraz, üreaz ve antihipertansif aktivite gösterdiği belirlenmiştir.

### Biyokimya

### Araştırma Makalesi

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### Anahtar Kelimeler

Lamiaceae

GC-MS

Pulegon

Tarımsal kültür

Antihipertansif

## The Detailed Chemical and Biological Analysis of *Ziziphora clinopodioides* Lam. Species Growing in Cultural and Natural Environments

### ABSTRACT

The Lamiaceae family is an important resource for the pharmaceutical, food, cosmetic, and perfumery sectors. The species *Ziziphora clinopodioides* Lam., belonging to the Lamiaceae family, has been used in folk medicine since ancient times. The aim of the present study was to determine the total phenolic and flavonoid contents, antioxidant, cytotoxic and enzyme (AChE, BChE, tyrosinase, urease, elastase, collagenase and ACE) inhibition activities of ethanol extracts of the aerial parts of natural and cultivated samples of the species *Z.*

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*clinopodioides*. In addition, the aroma contents of the species were determined by GC-MS/FID, and the phytochemical composition of the ethanol extracts was determined by LC-MS/MS. According to the aroma analysis results, the major components of the culture sample of the *Z. clinopodioides* species were determined as pulegone (39.83%), *cis*-menthone (21.36%), *trans*-menthone (16.64%), while the major components of the natural sample were determined as pulegone (62.42%), neoisomenthol (5.93%) and *cis*-pulegone oxide (5.47%). According to LC-MS/MS results, quinic acid (25.841, 15.694 mg analyte g<sup>-1</sup> extract, respectively), rosmarinic acid (6.804, 25.523), and acacetin (6.115, 10.764) were detected as major compounds in cultured and natural samples of the species. Also, hesperidin (5.725) was higher in culture sample. According to the enzyme activity results, it was determined that cultured and natural samples of the species showed moderate butyrylcholinesterase enzyme inhibition activity (inhibition %: 45.14±1.40; 43.57±0.73, respectively). In urease enzyme inhibition activity, it was determined that culture sample showed moderate activity (inhibition %: 43.64±0.39) but natural sample did not show activity, and culture sample showed high antihypertensive activity (inhibition %: 81.6±1.19). When the results were evaluated in general, it was determined that the cultured sample of the species showed higher antioxidant, butyrylcholinesterase, urease, and antihypertensive activity compared to natural sample.

#### Keywords

Lamiaceae  
GC-MS  
Pulegone  
Agricultural culture  
Antihypertensive

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## GİRİŞ

İnsanoğlu geçmişten günümüze kadar beslenmek, yaralarını iyileştirmek ve hastalıklarını tedavi etmek gibi çok çeşitli amaçlar için bitkilerden yararlanmışlardır. M.Ö. 5000'li yıllarda insanların tedavi amaçlı kullandıkları 250 adet bitkinin varlığı tespit edilmiştir (Göktaş & Gıdık, 2019). Hititler, Mısırlılar ve Sümerler gibi çok çeşitli medeniyetlerin yıllar boyunca hastalıkları tedavi etmek için bitkilerden yararlandıkları bildirilmiştir (Göktaş & Gıdık, 2019). Dünya Sağlık Örgütüne (WHO) göre günümüzde kullanılan farmasötik ilaçların %25'i tıbbi bitkilerden üretilmektedir. Yine FAO (Gıda ve Tarım Örgütü)'ya göre dünya genelinde satılan ilaçların %30'unun bitkilerden elde edildiği ifade edilmiştir (Acıbuca & Budak, 2018). Son yıllarda endüstriyel alanlarda kullanımı artış gösteren tıbbi ve aromatik bitkilere verilen önem giderek artmaktadır (Oğan & Cömert, 2022). Dünya Sağlık Örgütü verilerine göre dünyada tedavi amacıyla kullanılan bitki ile gıdalara aroma verici olarak kullanılan bitkilerin sayısının yaklaşık olarak 20.000 olduğu rapor edilmiştir.

Lamiaceae familyası ilaç, gıda, kozmetik ve parfümeri gibi farklı ekonomik endüstriyel alanlar için önemli bir kaynaktır (Karayel, 2019; Yılmaz ve ark., 2023).

Lamiaceae familyasının bir üyesi olan *Z. clinopodioides* Lam., çok yıllık aromatik bir bitki türü olup besin kaynağı olarak kullanılabilir. *Z. clinopodioides* eski çağlardan beri halk hekimliğinde kullanılmaktadır (Sahakyan & Petrosyan, 2022). *Z. clinopodioides*, Anadolu'da yaygın olarak bulunan yenilebilir bir tıbbi bitkidir. Bitkinin yaprakları, çiçekleri ve sapı Türkiye'de sıklıkla yabani sebze olarak veya aroma ve yiyeceklere lezzet katmak için katkı maddesi olarak kullanılmaktadır. *Z. clinopodioides* bitkisinin kurutulmuş toprak üstü kısımları, yaprakları, çiçekleri ve gövdesi, özellikle Türkiye ve İran'da, aroma ve lezzet verici olarak yoğurt ve peynir gibi süt ürünlerinde kullanılmaktadır. Yerel olarak 'Kırnanesi' olarak bilinen bitki, halk arasında iştah açıcı, gaz giderici, antiseptik ve yara iyileştirici olarak kullanılmaktadır (Meral ve ark., 2002; Ozturk & Ercisli, 2007; Senejoux ve ark., 2012; Kazeminia ve ark., 2024). *Z. clinopodioides* bitkisinin uçucu yağ, askorbik asit ve flavonoidler açısından zengin olduğu bilinmektedir. Uçucu yağının farmakolojik etkisinden sorumlu olduğu ve uçucu yağının ana bileşenlerinin  $\alpha$ -pinen,  $\beta$ -pinen, limonen, menton, izomenton, pulegon ve timol olduğu rapor edilmiştir (Hayta & Bağcı, 2016).

Bu çalışmada *Z. clinopodioides* türünün hem kültür hem de doğal örneklerinin GC-MS/FID ile aroma analizi, LC-MS/MS ile fitokimyasal içerik analizi yapılarak kimyasal bileşimi ortaya konmuştur. Türün hem kültür hem de doğal örneklerinin ABTS, DPPH ve CUPRAC yöntemleri ile antioksidan kapasitesi tespit edilmiştir. Ayrıca kolinesteraz (AChE ve BChE), üreaz, tirozinaz, elastaz, kolajenaz ve anjiyotensin dönüştürücü enzim (ACE) inhibisyon aktivite tayinleri yapılmıştır. Türünün kültür ve doğal örneklerinin kimyasal ve biyolojik açıdan karşılaştırılmasına ilişkin literatürde çalışma bulunmaması ve bu durumun ilk defa bu çalışmada ortaya konması çalışmayı özgün kılmaktadır.

## MATERYAL ve METOD

### Bitki Materyali ve Kültür Koşulları

*Ziziphora clinopodioides* Lam. bitkisinin ekstre verimi, toplanma yeri, zamanı ve herbaryum numarası Çizelge 1'de verilmiştir. Kültür için kullanılan tohumlar, GAP Uluslararası Tarımsal Araştırma ve Eğitim Merkezi (GAPUTAEM) kampüs alanında doğal olarak yetişen bitkilerden elde edilmiştir. Akdeniz ve ark. (2021) çalışmasındaki şartlar uygulanarak kültür bitkisi yetiştirilmiştir (Çizelge 1).

Türün kültür çalışmaları GAPUTAEM deneme alanında gerçekleştirilmiştir. Tarla denemesinde materyal olarak *Z. clinopodioides* tohumları kullanılmıştır. Tohumlar GAPUTAEM kampüs alanında doğal olarak yetişen bitkilerden elde edilmiştir. Ağustos 2013'te tohumlar doğal ortamlarından toplanmıştır. Bu tohumlar su dolu bir

kaba aktarılmış ve bir gece bekletilmiştir. Suyun üstüne çıkan tohumlar kullanılmamış, su altında kalanlar kullanılmıştır. Tohum yatağı için, ince elekten geçirilmiş yakılmış koyun gübresi, orman toprağı ve nehir kumunun eşit oranda karışımından oluşan bir harç hazırlanmıştır. Harç, delikli plastik tüplere (10x25 cm) doldurulmuştur. Tohumlar, Kasım ayında her tüpte 3-4 tohum olacak şekilde, 3-4 cm derinliğe ekilmiştir. Tohum yatağı, tohumlar çimlenene kadar süzgeçle sulanmıştır. Fideler 5-10 cm boya ulaştıktan sonra erken ilkbaharda (Şubat-Mart ayları sonrası) tarlaya dikilmiştir. Bunun için tarlaya 30x30x30 cm boyutlarında çukurlar açılmıştır. Her çukura 2 L su konulduktan sonra, fideler çukura yerleştirilmiş, üzeri toprakla doldurulmuş ve sıkıştırılmıştır. Dikim yılı ilk kuruluş yılı olarak kabul edildiğinden, hasat ikinci yılda yapılmıştır. Deneme alanı Dicle Nehri kıyısındaki bodrum alanda kurulmuştur. Bu alan deniz seviyesinden yaklaşık 609 m yükseklikte olup 37°56'29.36"K (kuzey enlemi) ve 40°15'16.07"D (doğu boylamı) koordinatlarında bulunmaktadır. Bölge iklimi Akdeniz iklimi özelliklerine sahiptir. Yazları genellikle sıcak ve kurak, kışları ise soğuk ve yağışlıdır. Uzun yıllara ait iklim bulgularına göre her yıl toplam yağış miktarı 454 mm, ortalama sıcaklık ise 15.8°C'dir (Meteoroloji Müdürlüğü'nün Diyarbakır uzun yıllar ortalaması). Genel toprak özellikleri Dicle nehri sularının taşıdığı materyal üzerinde oluşmuş büyük toprak grubuna ait alüvyonlu topraklardır (Tekin ve ark., 2017). Doğal olarak yetişen türlerin toplandığı alan ile türün kültüre alındığı alanın iklim koşulları, bölge aynı olduğu için hemen hemen aynıdır.

Çizelge 1. Çalışılan türlerin isimleri, ekstre verimi, toplanma yeri, toplanma zamanı ve herbaryum numaraları  
Table 1. Names of studied species, extract yield, collection place, collection time and herbarium numbers

Tür İsmi	Etanol Ekstre Verimi %	Kod	Toplanma Yeri	Toplanma Zamanı	Herbaryum No
<i>Z. clinopodioides</i> (Kültür)	2.94	ZC1	Project Number: TAGEM /17/A07/P09/013	2015	M.Fırat 32745(VANF)
<i>Z. clinopodioides</i> (Doğal)	5.0	ZC2	Diyarbakır	2015	M.Fırat 32745(VANF)

### Ekstraksiyon İşlemi

Bitkinin kültür ve doğal örneklerinin toprak üstü kısımları toplanıp nemsiz ve gölge bir ortamda kurutulmuştur. Kurutulan örnekler toz haline getirilip ekstrelerini hazırlamak için 10g tartılmıştır. Daha sonra tartılan örnek üzerine 50 mL etanol ilave edilerek 8 saat bekletilmiştir. 8 saat sonra ultrasonik su banyosunda oda sıcaklığında 30 dk. bekletilmiş ve süzme işlemi yapılmıştır. Bu işlem 3 defa tekrarlandı daha sonra toplam süzüntüden evaporatör yardımıyla çözücüsünden kurtarılıp ham ekstre elde edilmiştir (Akdeniz ve ark., 2021).

Aroma analizi numune ekstraksiyonu SPME (Katı Faz Mikroekstraksiyon) yöntemi ile yapılmıştır. Doğrudan kuru bitkinin kendisinden alınan 1-2 g örnek 20 mL'lik Headspace vialine alınarak 40 °C'de 15 dk boyunca inkübasyona bırakılmıştır. İnkübasyon süresince açığa çıkan aroma bileşikleri kullanılan SPME fiberi tarafından adsorplanmış ve örnek Headspace örnekleme bloğu ile GC-MS/FID cihazına numune gönderilmiştir. NIST ve Wiley GC-MS kütüphaneleri bileşenlerin belirlenmesinde kullanılmıştır (Yigitkan ve ark., 2022).

### LC-MS/MS Analizi

### Headspace-GC-MS/FID Cihazı ile Aroma Analizi

*Z. clinopodioides* kültür (ZC1) ve *Z. clinopodioides* doğal (ZC2) örneklerinin etanol ekstraları LC-MS/MS enjeksiyonu öncesinde son konsantrasyonları 1000 µg mL<sup>-1</sup>'ye ayarlanmıştır. İncelenen örneklerin fitokimyasal bileşimi, daha önce belirlenmiş ve doğrulanmış LC-MS/MS yöntemi kullanılarak gerçekleştirilmiştir. Bu yöntemde, özellikle doğal ürünlerde bulunan, fenolik asit ve flavonoid yapılarında olan 53 bileşik ve 3 iç standart bakımından analiz edilmiştir (Yılmaz, 2020).

### Toplam Flavonoid-Fenolik İçerik, Antioksidan ve Sitotoksik Aktivite Analizleri

*Z. clinopodioides* türünün toprak üstü etanol ekstralarının toplam fenolik içeriği pirokatekole eşdeğer ve toplam flavonoid içerikleri kuersetine eşdeğer olarak hesaplanmıştır (Slinkard & Singleton, 1977; Moreno ve ark., 2000). Türün antioksidan aktivitesini tespit etmek için DPPH (serbest radikal giderim yöntemi), ABTS (katyon radikali giderim aktivitesi yöntemi) kullanılmış ve değerler IC<sub>50</sub> olarak hesaplanmıştır. Ayrıca ekstralar CUPRAC (Bakır (II) indirgeyici kapasite) yöntemi ile antioksidan aktivite tayini yapılmış ve sonuçlar A<sub>0.5</sub> olarak hesaplanmıştır (Blois, 1958; Re ve ark., 1999; Apak ve ark., 2004). Bütillenmiş hidroksitoluen (BHT) ve α-tokoferol referans olarak kullanılmıştır. Ayrıca ekstralarının toksik ve sitotoksik aktivitelerini tespit etmek için Mojarraba ve ark. (2013) tarafından geliştirilen yöntemde ufak modifikasyonlar yapılarak gerçekleştirildi.

### Enzim Aktiviteleri

#### Kolinesteraz enzim aktivitesi

Çalışılan ekstraların antikolinesteraz aktivitesinin tespiti için Ellman ve ark. (1961) tarafından geliştirilen asetilkolinesteraz (AChE) ve bütirilkolinesteraz (BChE) enzim inhibisyonuna dayalı yöntem kullanılmıştır. Referans olarak galantamin kullanılmıştır.

#### Üreaz enzim aktivitesi

Örneklerin etanol ekstralarının üreaz enzim inhibisyon aktivitesini tespit etmek için Zahid ve ark. (2015) tarafından geliştirilen yöntem kullanılmıştır. Üreaz enzim inhibisyon aktivitesi için tiyüöre referans olarak kullanılmıştır.

#### Tirozinaz enzim aktivitesi

Türün etanol ekstralarının tirozinaz enzim inhibisyon aktivitesi Hearing & Jiménez (1987)'in geliştirdiği yöntemle yapılmıştır. Tirozinaz enzim inhibisyon aktivitesi için referans olarak kojik asit kullanılmıştır.

### Elastaz ve Kolajenaz enzim aktiviteleri

Örneklerin antiaging (yaşlanma karşıtı) etkilerini belirlemek için elastaz (Kraunsoe ve ark., 1996) ve kolajenaz (Thiring ve ark., 2009) inhibisyon aktivite tayinleri yapılmıştır. Elastaz ve kolajenaz enzim inhibitör aktivite tayinlerinde sırasıyla substrat olarak N-süksinil-(Ala)<sub>3</sub>-nitroanilide ve N-(3-[2-Furyl]acryloyl)-Leu-Gly-Pro-Ala, standart olarak ise oleanolik asit ve epikateşin gallat kullanılmıştır. (Akdeniz ve ark., 2021; Yigitkan ve ark., 2022).

### Hipertansif enzim aktivitesi

Kwon ve ark. (2006) tarafından geliştirilen yöntemde küçük modifikasyonlar yapılarak kullanılmıştır. Standart olarak lisinopril kullanılmıştır.

## BULGULAR ve TARTIŞMA

### Headspace-GC-MS/FID Cihazı ile Aroma Analizi

*Z. clinopodioides* türünün doğal ve kültür örneklerinin aroma içerikleri Çizelge 2'de verilmiştir. GC-MS/FID sonuçlarına göre ZC1 örneğinin aroma içeriğinin %95.22'si aydınlatılmış ve 19 bileşen belirlenmiştir. ZC2 örneğinin aroma içeriğinin ise %90.92'si aydınlatılmış ve 19 bileşen tespit edilmiştir. ZC1'in majör bileşenlerinin pulegon (%39.83), *cis*-menton (%21.36) ve *trans*-menton (%16.64) olduğu belirlenmiştir. ZC2 örneğinin majör bileşenleri ise pulegon (%62.42), neoizomentol (%5.93) ve *cis*-pulegon oksit (%5.47) olduğu tespit edilmiştir (Çizelge 2 ve Şekil 1).

Sahakyan ve Petrosyan (2022) tarafından yapılan çalışmada, Ermenistan'da toplanan *Z. clinopodioides* türünden elde edilen uçucu yağın ana bileşenleri, sırasıyla %42.1, %9.7, %8.22, %7.35 ve %5.9 konsantrasyonlarıyla pulegon, izomenton, 1,8-sineol, piperiton ve neomentol olarak rapor edilmiştir. İran'dan toplanan *Z. clinopodioides* türünün uçucu yağ bileşenleri hidrodistilasyon yoluyla elde edilmiş ve GC-MS ile analiz edilmiştir. Analiz sonucunda bitkinin uçucu yağında 27 bileşen tanımlanmıştır. Ana bileşikler pulegon (%44.5), terpineol (%14.5), metil asetat (%10.9), izo-neomentol (%7.1) ve 1,8-sineol (%4.1) olarak tespit edilmiştir (Behravan ve ark., 2007). Başka bir çalışmada *Z. clinopodioides* türünün 9 farklı büyüme evresinin uçucu yağ bileşimi GC-MS ile analiz edilmiş ve major bileşenler, pulegon (%77.48-87.3), *p*-mentanon (%2.79-12.39), *trans*-izopulegon (%1.04-2.06), *d*-limonen (%0.51-3.03) ve karvon (%1.5-4.48) olarak tespit edilmiştir (Ding ve ark., 2014). Alp ve ark. (2016) tarafından, *Z. clinopodioides* türünün Türkiye'deki Çoruh vadisinin farklı noktalarında yerel olarak toplam 8 takson toplanmış ve bunların toprak üstü kısımlarından elde edilen uçucu yağlar GC-MS ile analiz edilmiştir. Yağın %92.91'ini temsil eden 17 bileşik tanımlanmıştır. Tüm örneklerin başlıca bileşenleri

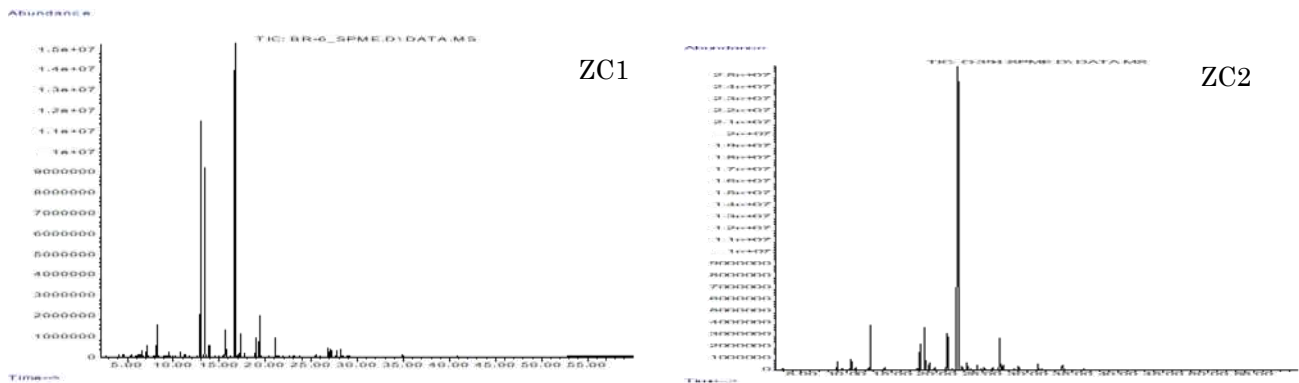
pulegon (%40.13-51.13), 1,8-sineol (%6.18-9.34), limonen (%6.98-9.78), mentol (%6.57-8.83),  $\beta$ -pinen (%2.15-6.88), menton (%4.45-5.47), piperitenon (%4.44-7.03) ve piperiton (%2.98-4.56) olarak tespit edilmiştir. Ozturk ve Ercisli (2007) tarafından yapılan başka bir çalışmada Türkiye'nin Doğu kesiminde toplanan *Z. clinopodioides* türünün toprak üstü kısımlarından elde edilen uçucu yağların ana

bileşenleri pulegon (%31.86), 1,8-sineol (%12.21), limonen (%10.48), mentol (%9.13),  $\beta$ -pinen (%6.88), menton (%6.73), piperitenon (%5.30) ve piperiton (%4.18) olarak tespit edilmiştir. Sonuçlar değerlendirildiğinde mevcut çalışma majör bileşikler açısından literatürdeki çalışmalarla paralellik göstermektedir.

Çizelge 2. ZC1 ve ZC2 örneklerinin aroma sonuçları  
Table 2. Aroma results of ZC1 and ZC2 samples

No	RI <sup>a</sup>	Bileşenler	ZC1	ZC2	No	RI <sup>a</sup>	Bileşenler	ZC1	ZC2
1	935	$\alpha$ -Pinene	0.20 <sup>b</sup>	0,77	17	1148	<i>cis</i> -Menthone	21.36	-
2	952	Camphene	-	0.14	18	1157	Menthone	-	3.14
3	975	1-Octene-3-ol	-	0.16	19	1164	<i>tr</i> -Menthone	16.64	-
4	975	Sabinene	0.12	-	20	1167	Neoisomenthol	-	5.93
5	980	$\beta$ -Pinene	0.34	1.10	21	1181	Isopulegone	1.05	-
6	984	3-Octanone	-	0.10	22	1213	Verbenone	1.81	-
7	991	$\beta$ -Myrcene	-	0.07	23	1221	<i>cis</i> -Pulegone oxide	-	5.47
8	997	Mesitylene	0.40	-	24	1243	Pulegone	39.83	62.42
9	1000	Decane	0.80	-	25	1246	Carvenone	0.89	-
10	1026	$\sigma$ -Simen	-	0.18	26	1258	Piperitone	2.02	-
11	1031	D-Limonene	0.81	0.26	27	1290	Thymol	1.64	-
12	1032	Eucalyptol	2.29	4.65	28	1301	Carvacrol	3.61	0.98
13	1060	$\gamma$ -Terpinen	-	0.20	29	1346	Piperitenone	-	4.58
14	1091	Terpinolene	-	0.05	30	1381	Copaene	-	0.24
15	1100	4-Thujanol	0.37	-	31	1392	$\beta$ -Bourbonen	-	0.48
16	1100	Undecane	0.32	-	32	1489	Germaecrene D	0.72	-
<b>Toplam Tanımlanan (%)</b>								<b>95.22</b>	<b>90.92</b>

<sup>a</sup>Ahkonma indeksi, <sup>b</sup>% içerik, ZC1: *Z. clinopodioides* kültür örneği, ZC2: *Z. clinopodioides* doğal örneği



Şekil 1. Çalışılan türlerin GC-MS kromatogramları; ZC1: *Z. clinopodioides* kültür örneğinin aroma bileşenlerinin Headspace-GC-MS toplam iyon kromatogramı, ZC2: *Z. clinopodioides* doğal örneğinin aroma bileşenlerinin Headspace-GC-MS toplam iyon kromatogramı

Figure 1. GC-MS chromatograms of the studied species; ZC1: Headspace-GC-MS total ion chromatogram of aroma components of *Z. clinopodioides* culture sample, ZC2: Headspace-GC-MS total ion chromatogram of aroma components of *Z. clinopodioides* natural sample

### LC-MS/MS Analizi

Her iki ekstrede de kinik asit (sırasıyla, 25.841, 15.694 mg analit g<sup>-1</sup> ekstre), rosmarinik asit (6.804, 25.523) ve asasetin (6.115, 10.764)'nin majör bileşenler olduğu görülmektedir. Kültür örneğinde

farklı olarak hesperidin (5.725) bileşenin yüksek miktarda olduğu tespit edilmiştir. ZC1 ekstresinde kinik asit ve hesperidin bileşenlerin miktarlarının ZC2 ekstresine kıyasla daha yüksek olduğu görülmektedir (Çizelge 3 ve Şekil 2-3).

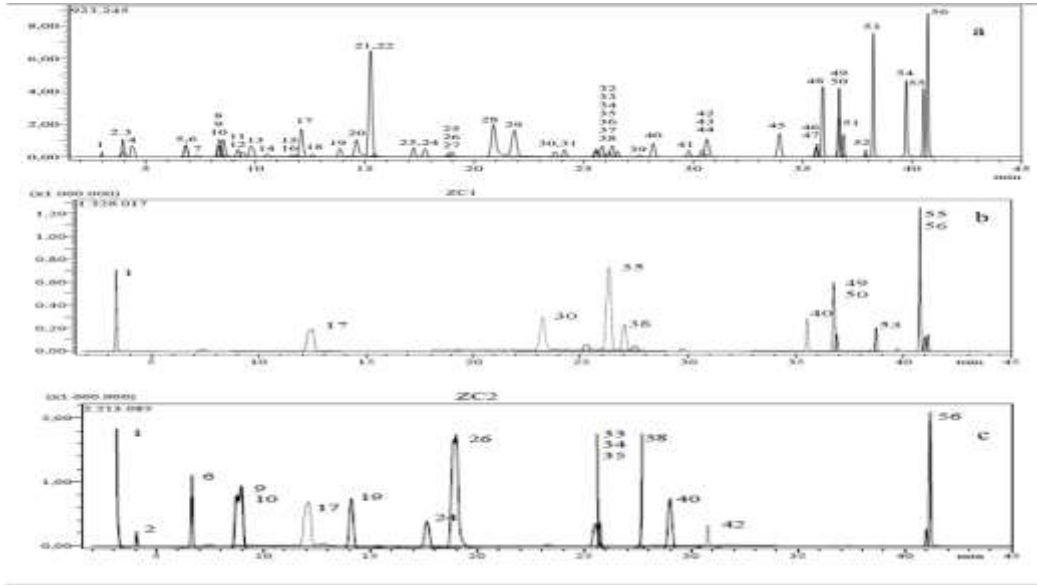


Çizelge 3. *Z. clinopodioides* türünün etanol ekstrelerinin LC-MS/MS ile fitokimyasal bileşenlerin kantitatif tayini (mg analit g<sup>-1</sup> ekstre)

Table 3. Quantitative determination of phytochemical components of ethanol extracts of *Z. clinopodioides* species by LC-MS/MS (mg analyte g<sup>-1</sup> extract)

No	Analitler	RT <sup>a</sup>	Ana iyon (m/z) <sup>b</sup>	MS <sup>2</sup> (Çarpışma enerjisi) <sup>c</sup>	Miktar (mg analit g <sup>-1</sup> ekstre) <sup>d</sup>	
					ZC1	ZC2
1	Quinic acid	3.0	190.8	93.0	25.841	15.694
2	Fumaric acid	3.9	115.2	40.9	0.728	0.111
3	Aconitic acid	4.0	172.8	129.0	nd <sup>e</sup>	nd
4	Gallic acid	4.4	168.8	79.0	0.013	nd
5	Epigallocatechin	6.7	304.8	219.0	nd	nd
6	Protocatechuic acid	6.8	152.8	108.0	0.746	1.204
7	Catechin	7.4	288.8	203.1	nd	nd
8	Gentisic acid	8.3	152.8	109.0	0.271	nd
9	Chlorogenic acid	8.4	353.0	85.0	0.156	0.534
10	Protocatechuic aldehyde	8.5	137.2	92.0	0.055	0.557
11	Tannic acid	9.2	182.8	78.0	nd	nd
12	Epigallocatechin gallate	9.4	457.0	305.1	nd	nd
13	1,5-Dicaffeoylquinic acid	9.8	515.0	191.0	nd	nd
14	4-OH Benzoic acid	10.5	137.2	65.0	0.223	nd
15	Epicatechin	11.6	289.0	203.0	nd	nd
16	Vanilic acid	11.8	166.8	108.0	nd	nd
17	Caffeic acid	12.1	179.0	134.0	1.141	2.426
18	Syringic acid	12.6	196.8	166.9	nd	nd
19	Vanillin	13.9	153.1	125.0	nd	0.276
20	Syringic aldehyde	14.6	181.0	151.1	nd	0.061
21	Daidzin	15.2	417.1	199.0	nd	nd
22	Epicatechin gallate	15.5	441.0	289.0	nd	nd
23	Piceid	17.2	391.0	135/106.9	nd	nd
24	p-Coumaric acid	17.8	163.0	93.0	0.532	0.174
25	Ferulic acid-D3-IS <sup>h</sup>	18.8	196.2	152.1	is	is
26	Ferulic acid	18.8	192.8	149.0	1.155	0.138
27	Sinapic acid	18.9	222.8	193.0	nd	nd
28	Coumarin	20.9	146.9	103.1	nd	nd
29	Salicylic acid	21.8	137.2	65.0	0.460	0.171
30	Cynaroside	23.7	447.0	284.0	2.902	0.449
31	Miquelianin	24.1	477.0	150.9	nd	nd
32	Rutin-D3-IS <sup>h</sup>	25.5	612.2	304.1	is	is
33	Rutin	25.6	608.9	301.0	0.182	1.061
34	isoquercitrin	25.6	463.0	271.0	0.065	0.987
35	Hesperidin	25.8	611.2	449.0	5.725	1.082
36	σ-Coumaric acid	26.1	162.8	93.0	nd	nd
37	Genistin	26.3	431.0	239.0	nd	nd
38	Rosmarinic acid	26.6	359.0	197.0	6.804	25.523
39	Ellagic acid	27.6	301.0	284.0	nd	nd
40	Cosmosiin	28.2	431.0	269.0	0.737	0.226
41	Quercitrin	29.8	447.0	301.0	nd	nd
42	Astragalın	30.4	447.0	255.0	0.025	0.174
43	Nicotiflorin	30.6	592.9	255.0/284.0	nd	nd
44	Fisetin	30.6	285.0	163.0	nd	nd
45	Daidzein	34.0	253.0	223.0	nd	nd
46	Quercetin-D3-IS <sup>h</sup>	35.6	304.0	275.9	is	is
47	Quercetin	35.7	301.0	272.9	0.008	nd
48	Naringenin	35.9	270.9	119.0	0.469	0.079
49	Hesperetin	36.7	301.0	136.0/286.0	1.014	0.004
50	Luteolin	36.7	284.8	151.0/175.0	0.864	0.140
51	Genistein	36.9	269.0	135.0	nd	nd
52	Kaempferol	37.9	285.0	239.0	nd	nd
53	Apigenin	38.2	268.8	151.0/149.0	0.377	0.152
54	Amentoflavone	39.7	537.0	417.0	nd	nd
55	Chrysin	40.5	252.8	145.0/119.0	0.339	0.752
56	Acacetin	40.7	283.0	239.0	6.115	10.764

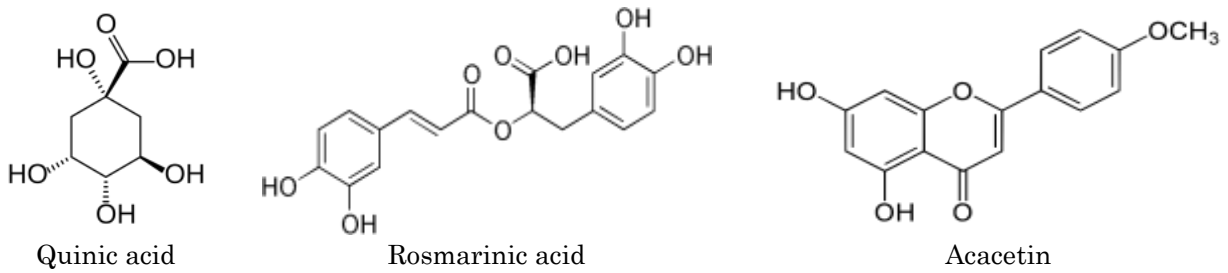
<sup>a</sup>RT: Tutulma süresi, <sup>b</sup>Ana iyon (m/z): Standart bileşiklerin moleküler iyonları (kütle/şarj oranı), <sup>c</sup>MS<sup>2</sup> (ÇE): İlgili moleküler iyonlar için MRM fragmentleri, <sup>d</sup>Etanol ekstresi için mg g<sup>-1</sup> cinsinden değerler, <sup>e</sup>nd: Tespit edilmedi, <sup>h</sup>is: İç standart, ZC1 *Z. clinopodioides* kültür örneğinin etanol ekstresi, ZC2: *Z. clinopodioides* doğal örneğinin etanol ekstresi



Şekil 2. a) LC-MS/MS ile analiz edilen standartın ( $1 \mu\text{g mL}^{-1}$ ) TIC kromatogramı, b) ZC1 etanol ekstresinin LC-MS/MS kromatogramı, c) ZC2 etanol ekstresinin LC-MS/MS kromatogramı  
Figure 2. a) TIC chromatogram of the standard ( $1 \mu\text{g mL}^{-1}$ ) analyzed by LC-MS/MS, b) LC-MS/MS chromatogram of ZC1 ethanol extract, c) LC-MS/MS chromatogram of ZC2 ethanol extract

Özkan ve ark. (2020) tarafından yapılan çalışmada *Z. clinopodioides* türünün toprak üstü ve kök kısımlarının etanol ekstrelerinin LC-MS sonucunun başlıca bileşikleri, kinik asit, malik asit ve rhoifolin olarak tespit edilmiştir. Toprak üstü kısım ekstraktındaki bileşik miktarları sırasıyla  $9020.51 \pm 73.97 \mu\text{g g}^{-1}$ ,  $1972.95 \pm 22.29 \mu\text{g g}^{-1}$  ve  $1972.95 \pm 22.29 \mu\text{g g}^{-1}$  olarak hesaplanmıştır. Kök

ekstraktında ise bileşik miktarları sırasıyla  $14721.04 \pm 120.71 \mu\text{g g}^{-1}$ ,  $2179.04 \pm 24.62 \mu\text{g g}^{-1}$  ve  $3593.31 \pm 338.1 \mu\text{g g}^{-1}$  olarak belirlenmiştir. Ayrıca özütlerin hesperidin, naringenin, rutin, apigenin ve izokersetin gibi bazı flavonoidler ve bazı fenolik asitler içerdiğini ifade etmişlerdir. Mevcut çalışma önceki çalışmayla karşılaştırıldığında majör bileşen olarak her iki çalışmada kinik asit tespit edilmiştir.



Şekil 3. Türün majör bileşikleri  
Figure 3. Major components of the species

### Toplam Flavonoid-Fenolik İçerik Analizleri

Kültür örneğinin toplam fenolik ve flavonoid miktarlarının doğal örneğine kıyasla yaklaşık olarak iki katına eşit olduğu tespit edilmiştir (Çizelge 4).

Tian ve ark. (2011) tarafından yapılan çalışmada, *Z. clinopodioides* türünün etil asetat, kloroform, *n*-butanol ve etanol ekstrelerinin toplam fenolik ve toplam flavonoid miktarları incelenmiş ve sonuçlar yüzde (%) olarak hesaplanmıştır. Sonuçlar incelendiğinde, etil asetat ekstresinin (%19.27) yüksek miktarda polifenolik içerdiği, kloroform (%4.99) ve *n*-butanol (%3.94) ekstrelerinin ise az

miktarda polifenolik içerdiği belirtilmiştir. Petrol eteri (%0.23) ve etanol (%1.64) ekstrelerinin ise neredeyse hiç polifenolik içermediği bildirilmiştir. Ayrıca, *Z. clinopodioides*'in toplam flavonoid içeriği etil asetat (%65.61), kloroform (%14.36) ve *n*-butanol (%10.76) ekstrelerinde bulunduğu rapor edilmiştir. Alp ve ark. (2016) tarafından yapılan çalışmada, *Z. clinopodioides* türünün toprak üstü kısmının Türkiye'nin Çoruh Vadisi'ndeki 8 ekotipinin toplam fenolik içeriğinin, 43.41 ile 55.71 mg gallik asit eşdeğeri  $100 \text{ g}^{-1}$  taze ağırlık arasında değiştiği bildirilmiştir. Aliakbarlu ve Shamelı (2013)

tarafından yapılan çalışmada farklı gelişim evrelerinde olan *Z. clinopodioides* türünün toplam fenolik içeriğinin 9.91 ile 12.80 mg g<sup>-1</sup> arasında değiştiğini rapor etmişlerdir. Ding ve ark. (2014) tarafından yapılan çalışmada ise *Ziziphora clinopodioides* Lam. türünün 9 farklı büyüme evresi boyunca toplam fenolik ve toplam flavonoid analizi yapılmıştır. Sonuçlar toplam fenolik içerik için 9.91±0.18 ile 12.80±0.04 mg g<sup>-1</sup> arasında, toplam flavonoid miktarları ise 29.84±0.18 ile 50.63±0.59 mg g<sup>-1</sup> arasında olduğu tespit edilmiştir.

### Antioksidan Aktivite Analizi

*Z. clinopodioides* türünün DPPH (serbest radikal giderim yöntemi), ABTS (katyon radikali giderim aktivitesi yöntemi) ve CUPRAC (bakır(II) indirgeyici kapasite) yöntemlerine göre antioksidan aktivite tayinleri yapılmıştır. Standart olarak BHT ve  $\alpha$ -TOC kullanılmıştır. Sonuçlar değerlendirildiğinde ZC1 ekstresinin DPPH serbest radikal giderim yönteminde (IC<sub>50</sub>: 62.95±1.15 µg mL<sup>-1</sup>) standart olarak kullanılan BHT'ye (IC<sub>50</sub>: 62.15±0.35) benzer antioksidan aktiviteye sahip olduğu görülmektedir. ZC2 ekstresinin ise orta düzeyde aktiviteye (145.61±1.94) sahip olduğu görülmektedir. ABTS katyon radikali yönteminde de ZC1 örneğinin

(40.81±0.98), ZC2 (49.29±0.24) örneğinden daha yüksek antioksidan aktivite gösterdiği belirlenmiştir. CUPRAC yöntemine göre her iki ekstrenin hemen hemen birbirine eşit oranda antioksidan aktivite sergiledikleri belirlenmiştir (Çizelge 4).

Salehi ve ark. (2005) tarafından yapılan çalışmada, İran'dan toplanan *Z. clinopodioides* türünün antioksidan aktivitesi DPPH yöntemiyle incelenmiştir. Türün metanol ekstresinin serbest radikal temizleme aktivitesinin diğer tüm ekstrlerden daha iyi olduğu (IC<sub>50</sub>: 30.7 µg mL<sup>-1</sup>) belirlenmiştir. Alp ve ark. (2016) tarafından yapılan çalışmada *Z. clinopodioides* türünün 8 taksonun uçucu yağlarının DPPH yöntemine göre IC<sub>50</sub> değerlerinin 3.60 ile 4.20 mg mL<sup>-1</sup> arasında olduğu belirlenmiştir. Taheri ve ark. (2023) tarafından yapılan çalışmada, *Z. clinopodioides* türünün 14 popülasyonunun antioksidan aktiviteleri değerlendirmek için DPPH ve FRAP yöntemleri kullanılmıştır. Sonuçlara göre DPPH için en yüksek değerler sırasıyla 1. ve 13. popülasyonlarda 4.61±0.4 ve 7.59±0.26 µg mL<sup>-1</sup> iken, FRAP için sırasıyla 6. ve 1. popülasyonlarda 328.61±5.54 ve 292.84±2.85 mg g<sup>-1</sup> olarak bulunmuştur. Önceki çalışmalar ve mevcut çalışma, türün yüksek antioksidan aktivite gösterdiğini ortaya koymaktadır.

Çizelge 4. Ekstrelerin toplam fenolik, flavonoid içerikleri, toksik ve sitotoksik aktiviteleri ile antioksidan aktivite sonuçları

Table 4. Total phenolic, flavonoid contents, toxic and cytotoxic activities, and antioxidant activity results of the extracts

Örnekle r	Fenolik İçeriği (µg Pes mg <sup>-1</sup> ekstrakt) <sup>b</sup>	Flavonoid İçeriği (µg QEs mg <sup>-1</sup> ekstrakt) <sup>c</sup>	IC <sub>50</sub> (µg mL <sup>-1</sup> ) <sup>d</sup>		A 0.5		% Canlılık <sup>e</sup>	
			ABTS Katyon Radikali	DPPH Serbest Radikali	CUPRAC	HT29	MCF7	PDF
ZC1	42.39±1.14	13.44±0.25	40.81±0.98	62.95±1.15	60.52±1.45	176.25±1.80	81.63±0.82	157.78±5.60
ZC2	23.81±0.00	6.57±0.99	49.29±0.24	145.61±1.94	62.53±0.03	60.28±0.60	101.90±0.33	263.5±3.43
BHT	-	-	13.62±0.28	62.15±0.35	7.69±0.01	-	-	-
$\alpha$ -TOC	-	-	10.48±0.63	13.10±0.52	14.49±0.11	-	-	-

<sup>a</sup>Değerler 3 paralel ölçümün ortalaması ve standart sapması olarak verilmiştir. <sup>b</sup>Pirokatekole eşdeğer fenolik içerik. ( $y = 0.0498(\mu\text{g}) + 0.0434 (r^2: 0.9918)$ ). <sup>c</sup>Kersetine eşdeğer flavonoid içerik. ( $y = 0.0535 (\mu\text{g}) + 0.0748 (r^2: 0.9960)$ ). <sup>d</sup>Sonuçlar IC<sub>50</sub> değerleri olarak verilmiştir. <sup>e</sup>200 ppm konsantrasyondaki % canlılık değerleri. ZC1: *Z. clinopodioides* kültür örneğinin etanol ekstresi, ZC2: *Z. clinopodioides* doğal örneğinin etanol ekstresi

### Sitotoksik Aktivite

*Z. clinopodioides* türünün ZC1 ve ZC2 örneklerinin etanol ekstrlerinin toksik etkileri PDF (sağlıklı birincil dermal fibroblast hücre hattı) hücre hattına ve sitotoksik etkileri HT-29 (kolon kanseri hücre hattı) ve MCF-7 (meme kanseri hücre hattı) hücre hattına karşı incelenmiştir. Sonuçlar incelendiğinde ekstraktların yüzde canlılık değerleri 200 µg mL<sup>-1</sup> konsantrasyonunda, ZC2 örneği kolon kanseri hücre hattı (HT-29) üzerinde iyi düzeyde (% canlılık: 60.28±0.60) sitotoksik etki göstermiştir. Ayrıca ZC1 örneğinin meme kanseri hücre hattına (MCF-7) karşı orta düzeyde (81.63±0.82) sitotoksik etki gösterdiği bulunmuştur. Her iki ekstrenin PDF (sağlıklı hücre

hatları) üzerine toksik etki göstermediği bulunmuştur (Çizelge 4).

### Enzim İnhibisyon Aktivitesi

Çalışılan her iki ekstrenin AChE, BChE, üreaz, tirozinaz, elastaz, kolajenaz ve ACE enzim inhibisyon aktivite ölçümleri yapılmıştır. Enzim aktivitesi sonuçlarına göre kültür ve doğal örneklerin orta düzeyde (sırasıyla, % inhibisyon: 45.14±1.40; 43.57±0.73) bütirilkinesteraz enzim inhibisyon aktivitesi gösterdiği belirlenmiştir. Üreaz enzim inhibisyon aktivitesi açısından değerlendirdiğimizde ise kültür örneğinin orta düzeyde aktivite gösterdiği (% inhibisyon: 43.64±0.39) fakat doğal örneğin aktivite göstermediği tespit edilmiştir. Ayrıca kültür

örneğin yüksek antihipertansif (% inhibisyon: 81.6±1.19) aktivite gösterdiği belirlenmiştir (Çizelge 5).

Ozdemir ve ark. (2013) tarafından yapılan çalışmada, *Z. clinopodioides* türünün özütü sodyum fosfat tampon içinde hazırlanmış ve AChE enzim inhibisyonu aktivitesi ölçülmüştür. 200 µg mL<sup>-1</sup> konsantrasyonda yaklaşık olarak % 40'lık bir inhibisyon aktivitesi sergilemiştir. Özkan ve ark. (2020) tarafından yapılan çalışmada *Z. clinopodioides*

türünün toprak üstü ve kök kısımlarının etanol ekstrelerinin antikolinesteraz (AChE ve BChE), tirozinaz ve üreaz enzim inhibisyon aktivite testi gerçekleştirilmiştir. Ekstrelerin AChE, BChE ve üreaz enzim inhibisyon testinde aktif olmadıkları, sadece toprak üstü ekstresinin tirozinaz enzim inhibisyon testinde zayıf aktivite (% inhibisyon: 8.60±0.87; standart olarak kullanılan kojik asit: 95.26±0.23) gösterdiği tespit edilmiştir.

Çizelge 5. Ekstrelerin antikolinesteraz, üreaz, tirozinaz, elastaz, kolajenaz ve antihipertansif enzim aktivite sonuçları

Table 5. Anticholinesterase, urease, tyrosinase, elastase, collagenase, and antihypertensive enzyme activity results of the extracts

Örnekler	AChE (% inh.) <sup>a</sup>	BChE (% inh.) <sup>a</sup>	Üreaz (% inh.) <sup>a</sup>	Tirozinaz (% inh.) <sup>a</sup>	Elastaz (% inh.) <sup>a</sup>	Kolajenaz (% inh.) <sup>a</sup>	ACE (% inh.) <sup>a</sup>
ZC1	A.D	45.14±1.40	43.64±0.39	A.D	A.D	10.04±0.12	81.6±1.19
ZC2	A.D	43.57±0.73	A.D	7.49±0.37	A.D	A.D	74.7±0.26
Galantamin <sup>b</sup>	80.54±1.43	76.73±0.46	-	-	-	-	-
Tiyüreb	-	-	95.74±0.67	-	-	-	-
Kojik asit <sup>b</sup>	-	-	-	93.64±1.23	-	-	-
Oleanolik asit <sup>b</sup>	-	-	-	-	44.32±0.20	-	-
Epikateşin gallat <sup>b</sup>	-	-	-	-	-	43.80±0.12	-
Lisinopril <sup>b</sup>	-	-	-	-	-	-	97.68±0.42

<sup>a</sup>Değerler 3 paralel ölçümün ortalaması ve standart sapması olarak verilmiştir (200 µg mL<sup>-1</sup>). <sup>b</sup>Standart madde. A.D: Aktif değil. ZC1: *Z. clinopodioides* kültür örneğinin etanol ekstresi. ZC2: *Z. clinopodioides* doğal örneğinin etanol ekstresi.

## SONUÇ ve ÖNERİLER

Bu çalışmada, *Z. clinopodioides* türünün kültür ile doğal örneklerinin toprak üstü kısımlarının etanol ekstresi hazırlanarak, GC-MS ile aroma analizi, LC-MS/MS ile fitokimyasal içeriği tespit edilip karşılaştırılmıştır. Her iki ekstrenin toplam fenolik ve flavonoid içeriği, sitotoksik aktivitesi, DPPH, ABTS ve CUPRAC yöntemleriyle antioksidan kapasiteleri, AChE, BChE, üreaz, tirozinaz, elastaz, kolajenaz ve ACE enzim inhibisyon aktiviteleri tespit edilip karşılaştırılmıştır. *Z. clinopodioides* kültür örneğinin aroma analiz sonucuna göre majör bileşenleri pulegon, *cis*-menton ve *trans*-menton olarak belirlenmiştir. Doğal örneğin ise majör bileşenleri pulegon, neoizomentol ve *cis*-pulegon oksit olduğu tespit edilmiştir. LC-MS/MS sonuçlarına göre kinik asit, rosmarinik asit ve asasetin majör bileşenler olarak tespit edilmiştir. Kültür örneğinin fenolik ve flavonoid içeriğinin doğal örneğe göre yaklaşık olarak iki kat daha fazla olduğu tespit edilmiştir. Kültür örneğinin genel olarak daha yüksek antioksidan aktivite gösterdiği belirlenmiştir. Enzim aktivitesi sonuçlarına göre kültür ve doğal örneklerin orta düzeyde bütirikolinesteraz enzim inhibisyon aktivitesi gösterdiği belirlenmiştir. Üreaz enzim inhibisyon aktivitesi açısından değerlendirdiğimizde ise kültür örneğinin orta düzeyde aktivite gösterdiği fakat doğal örneğin aktivite göstermediği

bulunmuştur. Ayrıca kültür örneğinin yüksek antihipertansif aktivite gösterdiği belirlenmiştir. Sonuç olarak *Z. clinopodioides* türünün kültüre alınmasının kimyasal içeriği ve biyolojik aktiviteleri üzerinde olumlu sonuçlar doğurduğu söylenebilir.

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## Çıkar Çatışması Beyanı

Makale yazarları aralarında herhangi bir çıkar çatışması olmadığını beyan ederler.

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## Phyllanthaceae Familyası Yeni Cins (*Phyllanthus* L.) Kaydı: Türkiye Damarlı Bitkilerine İstilacı Yabancı bir İlave

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### ÖZET

*Phyllanthus* L. cinsi ve *P. tenellus* Roxb. (Phyllanthaceae) türü, Türkiye florası için yeni kayıt olarak verilmektedir. Bitkinin Türkçe adı sera nohutu olarak önerilmektedir. Türkiye’de seralarda istilacı yabancı bir ot olan bitkinin tanınmasını kolaylaştırmak üzere teknik çizimi ve fotoğrafları sunulmaktadır. Cinsin ve türün detaylı betimleri de verilmektedir. Türün örnekleri, KTÜ Orman Fakültesi araştırma ve uygulama serası ile Orman Genel Müdürlüğü, Of Orman Fidanlığı seralarından toplanmıştır. KD Anadolu, nemli iklimi nedeniyle pek çok yabancı bitki taksonuna ev sahipliği yapmaktadır. Son yıllarda yapılan çalışmalar, bu bölgeden çok sayıda yabancı bitki taksonunun Türkiye florasına kaydedildiğini vurgulamaktadır.

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## A New Generic Record of Phyllanthaceae (*Phyllanthus* L.): Addition to the Alien Invasive Vascular Flora of Türkiye

### ABSTRACT

In this study, the genus *Phyllanthus* L. and its species *P. tenellus* Roxb. (Phyllanthaceae) are reported as a new record for the flora of Türkiye. The Turkish name of the species is suggested as sera nohutu (greenhouse chickpea). Technical drawings and photographs are given to facilitate the identification of the plant, which is an invasive weed in greenhouses in Türkiye. A description of the genus and species are also provided in detail. Specimens of the species were collected from research and application the greenhouse of the Faculty of Forestry in Karadeniz Technical University (KTÜ) and Of Forest Nursery Directorate greenhouses. NE Anatolia hosts many alien plant taxa, primarily due to its humid climate. Recent studies highlighted that many alien plant taxa have been recorded in the flora of Türkiye from this region.

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### INTRODUCTION

Despite different types of greenhouses, in general, they are special structures which are designed in order to regulate especially the temperature and humidity of the environment inside and they are usually associated with horticulture and the exhibition of both native and exotic plants. Thus, they are centers of the introduction of alien plant species and potentially may become sources of dispersion of some taxa (Galera & Ratyńska, 1999). In Türkiye, the studies on the weed of greenhouses are very limited in that they are generally on determining yield losses in crops (Arslan et

al., 2012; Torun, 2022). In addition to causing yield and labor loss, early detection and rapid response of invasive alien species is important in terms of protecting biodiversity. As in the present study, early detection of non-native (alien, introduced, adventive) plant species' intentional or unintentional introduction is the first step of such studies.

The genus *Phyllanthus* L. is not represented in the native vascular flora of Türkiye and is a member of the family Euphorbiaceae, one of the largest families of flowering plants. This family is lately divided into three families such as Euphorbiaceae, Phyllanthaceae, and

Picrodendraceae (Chase et al., 2002). Hereafter, *Phyllanthus* is a member of Phyllanthaceae which is pantropical and the second-most species-rich family segregated from Euphorbiaceae (Bouman et al., 2021) with approx. 2000 species (Kullayiswamy et al., 2021) and some 59 genera (Hoffmann et al., 2006; Serdar et al., 2008) worldwide. Phyllanthaceae has already been divided into seven subgenera (*Kirganelis*, *Isocladius*, *Embllica*, *Phyllanthodendron*, *Eriococcus*, *Xylophylla* and *Phyllanthus*) and the subgenus *Phyllanthus* comprise the two sections; *Phyllanthus* and *Urinaria* (Chakrabarty & Balakrishnan, 2018; Kullayiswamy et al., 2021).

Phyllanthaceae is known with 2 native genera in Turkish vascular flora such as *Andrachne* L. and *Flueggea* Willd. (Güner et al., 2012). The present paper underlined that by adding the new genus, *Phyllanthus* L., the number of genera of Phyllanthaceae of Flora of Türkiye reached three. *Phyllanthus* is represented throughout the tropical and subtropical regions of the world (Webster, 1957; Wu et al., 2008; Bouman et al., 2018). The genus, with almost 900 species, is the largest and highly diverse genus in the family Phyllanthaceae (Goaverts et al., 2000). The word

*Phyllanthus* comes from two Ancient Greek words “phyllon” and “anthos” (mean leaf and flower respectively) they refer to the appearance of flowers from the edge of the leaves (Pasha et al., 2013). Many of the *Phyllanthus* species share a distinctive vegetative specialization known as “phyllanthoid branching” with leaves on the main stem reduced to scale-like leaves (cataphylls), lack laminate leaves on the main stem (Webster, 1956; Kathriarachchi, 2006; Ralimanana & Cable, 2020).

*Phyllanthus tenellus* Roxb. is native to Angola, Comoros, Madagascar, Mauritius, Mozambique, Réunion, SW. Arabian Peninsula, Tanzania, Yemen, and Morocco, and regarded as a naturalized plant in more than 30 countries (Figure 1). Due to its rapid flowering and explosively dehiscent fruits, it is able to become invasive out of its natural distribution range in at least 30 countries (POWO, 2024; Khamar et al., 2022). Other than its native lands, this taxon occupies different habitats such as forest margins, forest clearings, and cultivated lands (Coode et al., 1982), 0-2050 m above sea level in Madagascar (Ralimanana & Hoffmann, 2011).

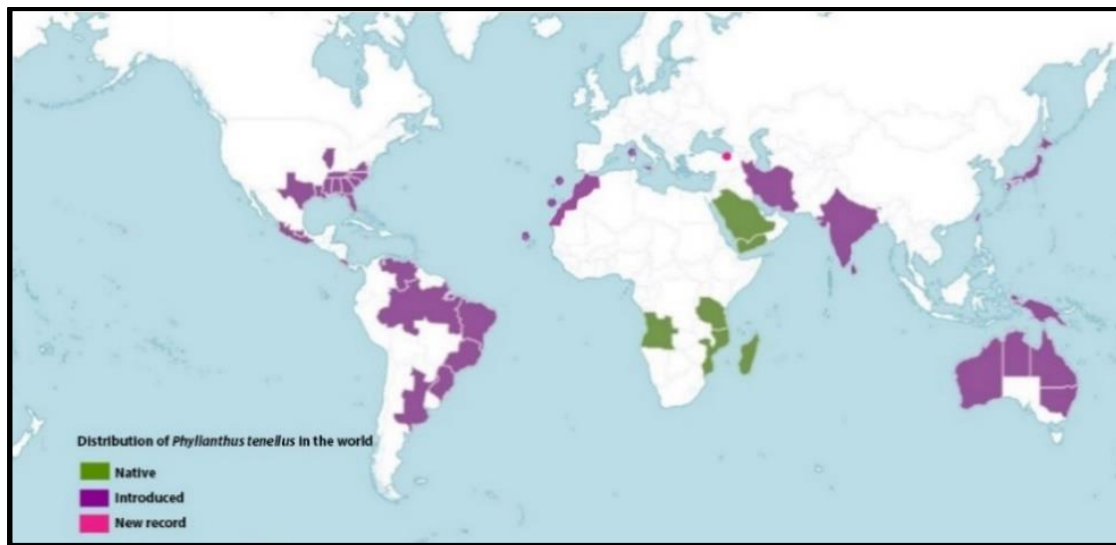


Figure 1. Distribution of *P. tenellus* in the World (Modified from Khamar et al., 2022 and POWO, 2024).  
Şekil 1. *P. tenellus*'ün Dünyadaki yayılışı (Khamar et al., 2022 ve POWO, 2024'den uyarlanmıştır).

The new record genus and its species, an invasive greenhouse weed, record reported here which has not been reported yet from Türkiye prior to the present paper.

## MATERIAL and METHOD

The studied materials of *P. tenellus* were collected from different propagation greenhouses in Trabzon province located in NE Anatolia. All specimens were stored at KATO Herbarium (in Karadeniz Technical University, Faculty of Forestry) in Trabzon. After careful examination of the collected materials of *Phyllanthus* (KATO: 24451!, 24452! and 24453!),

which were collected from A7 (Karadeniz Technical University, Kanuni Campus in Ortahisar District) and A8 (Forest Nursery in Of District) Trabzon province, and checking with proper literature (Webster, 1957, 1970; Ralimanana & Hoffmann 2011; Crisafulli et al., 2011; Zare et al., 2015; Khamar et al., 2022), *P. tenellus* was determined as a new alien greenhouse weed record for the flora of Türkiye.

## RESULTS and DISCUSSION

Webster (1956) underline that the main characters used to distinguish the present genus from other 59 genera of the family are the apetalous flowers, absence



of pistillodes and staminodes and the presence of a disc or disc glands in the flowers and branching (phyllanthoid) system. This genus, which is morphologically diverse, shows many differences in terms of flowers, seeds and habit.

*Phyllanthus* L., Sp. Pl. 2: 981. 1753.

**Type:** *P. maderaspatensis* L.

The description of the genus below is based on the treatments of Wu et al. (2008), Ralimanana & Hoffmann (2011), and Pasha et al. (2013).

Trees, shrubs, or herbs phyllanthoid branching present or absent; mostly monoecious; glabrous or with simple hairs. Simple leaves petiolate or sessile; alternate, opposite, subopposite or spirally arranged, entire, sometimes revolute, usually symmetric, often reduced and scale-like on main stems; stipules small, deciduous or persistent; venation pinnate. Stipules varied, entire to fimbriate. Inflorescences are usually axillary, on leafy branches; pedicels delicate, bracteoles 2 per flower. Flowers pedicellate, glabrous in both sexes; pedicels terete, tepals 5–6. Male flowers: sepals imbricate; filaments entirely free, apetalous; disk glands 3–6, various shapes; stamens 2–6; filaments free or connate; pistillodes absent. Female flowers: sepals as in male or more; disk glands free or connate into an annulus, surrounding ovary; ovary usually 3-loculed; styles 3, short, free or shortly connate, bifid. Fruit is usually a dehiscent, globose capsule, occasionally baccate or drupaceous. Seeds 2 per locule, trigonous; surface smooth, sculptured, or striate.

### Recommended Dichotomous Key to Genera of Phyllanthaceae in Türkiye

Adding the genus *Phyllanthus*, the number of genera of *Phyllanthaceae* in Turkish flora reached three and a new key to genera was recommended for the Flora of Türkiye as seen below:

1. Monoecious or dioecious, shrubs or herbs (in Türkiye); flowers apetalous

2. Leafy branches resembling pinnate leaves (phyllanthoid); male flowers without pistillode; stamens 2-6 and shorter than sepal ..... **Phyllanthus**

2. Shoots never resembling pinnate leaves; male flowers with pistillode; stamens 4-7 and clearly longer than sepal ..... **Flueggea**

1. Monoecious, subshrubs or perennial herbs (in Türkiye); flowers with developed petals ..... **Andrachne**

*Phyllanthus tenellus* Roxb. Flora Indica 2(3):668 (Roxburgh, 1832). Type: India. Calcutta, Wallich 7892 A, Figure 2.

**Syn.:** *Phyllanthus brisbanicus* F. M. Bailey, *Diasperus tenellus* (Roxb.) Kuntze, *D. corcovadensis* (Müll. Arg.) Kuntze (Crisafulli et al., 2011)

**Common names:** Mascarene Island leaf flower, Long-

stalked *Phyllanthus*, Long stalked leaf flower.

**Description:** Monoecious, annual herb, up to 50 cm in height. Somewhat woody at base (in Türkiye), glabrous plant. Stem smooth with spirally arranged cataphylls; phyllanthoid branching, branches terete and striate. Cataphylls and cataphyllary stipules triangular, entire, glabrous. Stipules entire, persistent, membranous, triangular, glabrous. Leaves 7 – 15 per branch, elliptic to ovate, acute or obtuse at the tip, 6 – 10 × 3 – 9 mm, petioles terete, glabrous. Cymules axillary on deciduous branchlets; inflorescence with 1-2 male flowers and 1 female flower on proximal parts or 1 female flower on distal parts of plagiotropic branches. Pedicels of staminate flowers up to 1.5 mm; calyx lobes 5, entire, 1-veined, whitish, stamens 5 and entirely free, filaments ca. 0.2-0.3 mm long; disc glands 5. Pedicels of pistillate flower (2.5-) 3-8 mm; calyx lobes 5, ovate, acute or obtuse; disk entire; ovary smooth; styles free, spreading. Capsules depressed globose or globose, greenish, glabrous, 1.7-1.9 mm broad, seed pale brown, densely papillose, 0.8-0.9 mm.

**Type:** India. Botanic Gardens, Calcutta, Wallich 7892 A ex p. (holotype K).

**Turkish Name:** Sera nohutu (The new scientific Turkish name suggested according to the guide of Menemen et al., (2016)).

**Collected localities:** A7 Trabzon – Ortahisar District, Kanuni Campus, Research and application greenhouse of Faculty of Forestry in KTÜ, 60 m, 05.06.2024, KATO 24451!; Drawn specimen (figure 2), ibid!, KATO 24452!; A8 Trabzon – Of District, General Directorate of Forestry, Greenhouses of Of Forest Nursery, 6 m. 15.06.2024, KATO 24453!, (Figure 3, 4).

Because of its distribution in greenhouses in Türkiye, pot soil is an important vector for easily dispersing diaspores of the species, and especially the lower altitudes of the Eastern Black Sea region of Türkiye are at risk of easy invasion. Furthermore, this region has extremely appropriate climate condition for the species which easily grows in forest clearings or margins, and cultivated fields (Coode et al., 1982) and the species is eager to moderate temperature.

While the time and vector of introduction into Trabzon province are uncertain, it could have probably been disseminated by pot soil of both many ornamental and fruit (blueberry) plants commonly produced and used in the region. Due to reports of its naturalization in Italy (Crisafulli, 2011), Iran (Zare et al., 2015), Sumatera (Hariri et al., 2020), Morocco (Khamar et al., 2022), with more than 30 other countries (POWO,

2024), the distribution of *P. tenellus* in NE Anatolia is not surprising. While the species is indicated as an invasive taxon both in agricultural areas and nurseries, some records showed that it is able to be introduced in urban areas (Crisafulli, 2011; Hariri et al., 2020).

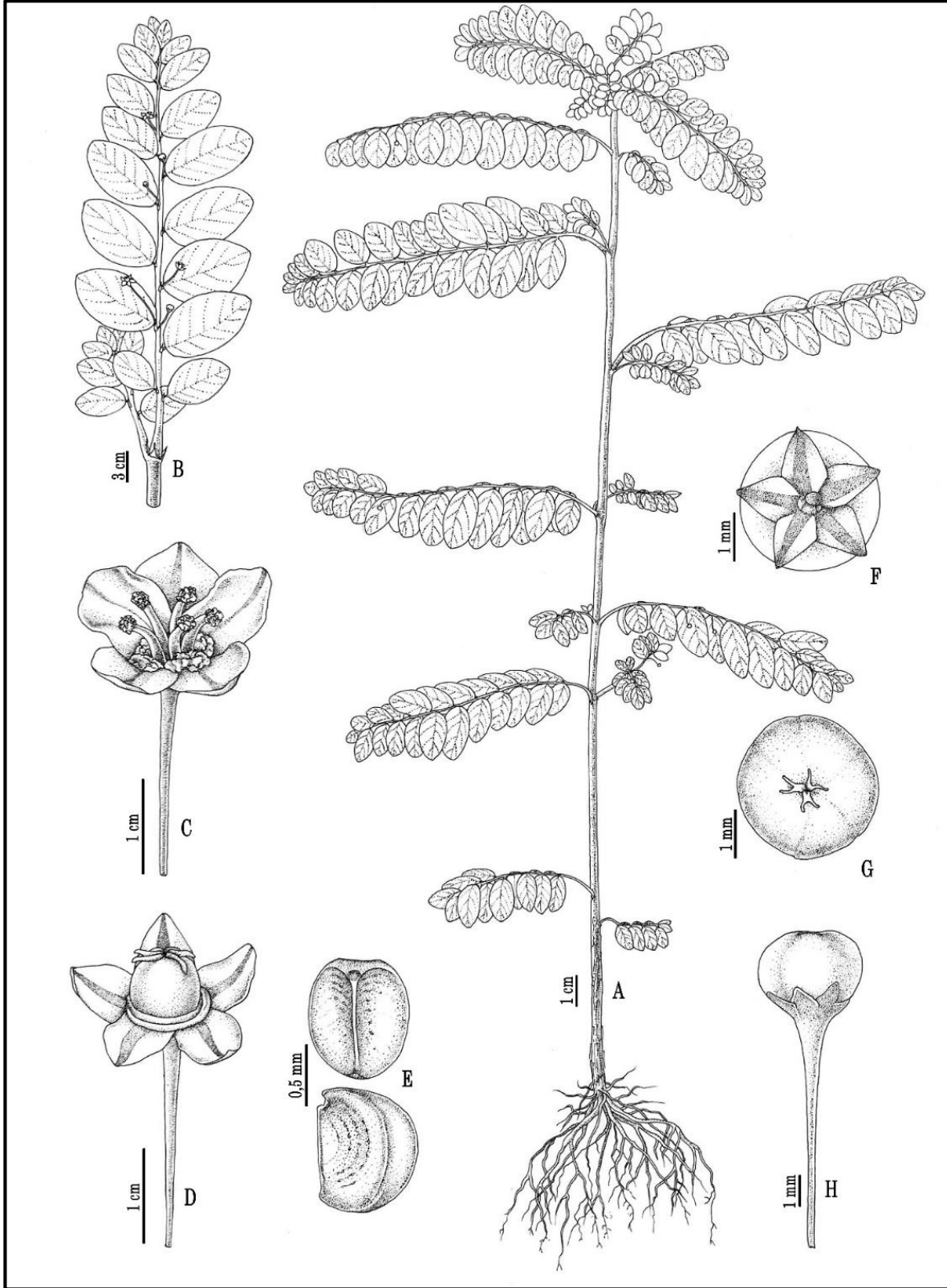


Figure 2. *Phyllanthus tenellus*: A–Habit; B–Branches and leaves with stipule and phyllodium; C– Staminate flower; D– Pistillate flower; E–Seeds (ventral (up) and lateral (down) views); F, G, H–Fruit (back, top and side view, respectively) (Drawing by Melike Çiğdem).

Şekil 2. *Phyllanthus tenellus*: A–Bitkinin habitusu; B–Kulakçık ve stipullu dallar; C– Erkek çiçek; D– Dişi çiçek; E– Tohumlar (üstte karın ve altta yan taraf görünümü); F, G, H– Meyve görünümleri (sırasıyla; sırt, üst ve yan) (Melike Çiğdem tarafından çizilmiştir).



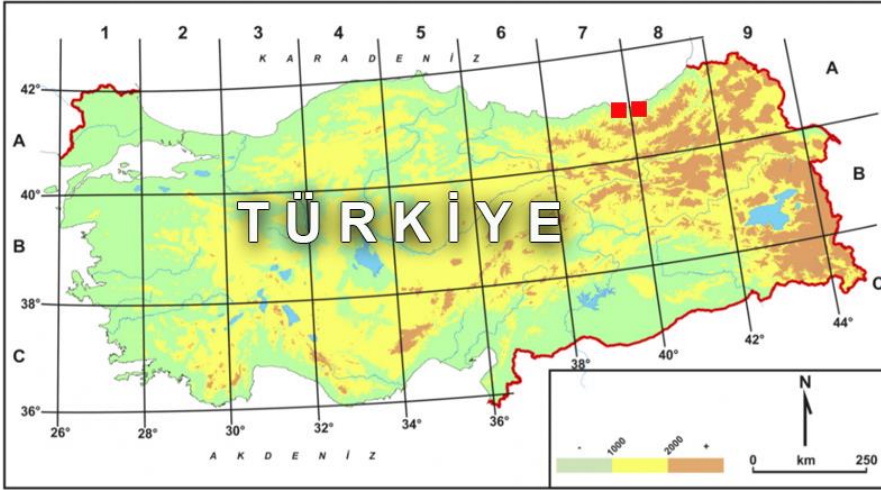


Figure 3. Distribution of *P. tenellus* (■) in Türkiye (Adapted from Güner & Ekim, 2014).

Şekil 3. *P. tenellus* (■)'un Türkiye'deki yayılışı (Güner ve Ekim, 2014'den uyarlanmıştır).

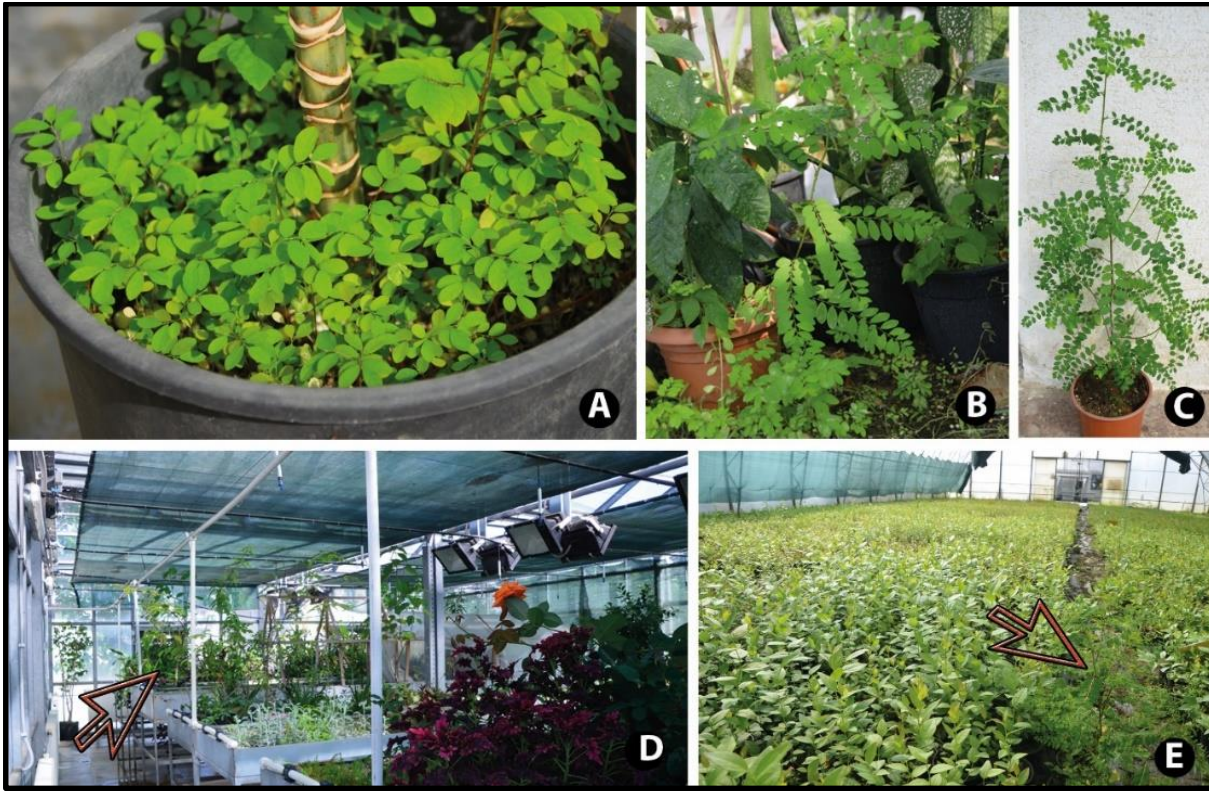


Figure 4. **A, B** – Individuals in different pots with different ornamental exotic plants in the greenhouse; **C** – An individual in the pot of a dead blueberry seedling; **D** – In the greenhouse bench in KTÜ; **E** – Together with blueberry seedlings in Of Forest Nursery.

Şekil 4. **A, B** – Serada farklı egzotik süs bitkileri ile farklı saksılardaki bireyler; **C** – Ölü bir maviyemiş fidesi saksısındaki bir birey; **D** – KTÜ'de sera tezgahlarında; **E** – Of Orman Fidanlığında maviyemiş fidanlarıyla birlikte.

Unfortunately, in the Eastern Black Sea Region, the weather is rainy throughout the year, which is suitable for this species to be carried out of greenhouses unintentionally and introduced into the natural places. Furthermore, diaspores may be dispersed quickly by the blueberry pots distributed/sold by Of Orman

Nursery. For this reason, cleaning *P. tenellus*, already a weed of greenhouses in Türkiye, seedlings pots, and eradicating individuals of the species in greenhouses before seed/fruit maturation will be an important weed control activity before its distribution.

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## Conflict of Interest

The author declares that he has no conflict of interest.

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## The Therapeutic Potential of Berberine and Resveratrol in Type 2 Diabetes Treatment: Pharmacokinetic and Bioactivity Properties, and Molecular Docking Analysis

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### ABSTRACT

Type 2 diabetes (T2D), typically characterized by insulin resistance, is a metabolic disorder that occurs when the body cannot use insulin effectively or does not produce enough insulin. In the treatment of T2D, insulin, metformin, and sulfonylureas are commonly used. Given the limitations of current treatment options, there is a strong need for intensive efforts in the discovery of new drugs. Berberine exhibits antidiabetic effects and possesses anti-inflammatory and antioxidant properties. Resveratrol is another natural compound that has been extensively researched due to its antioxidant and anti-inflammatory characteristics. This study aimed to investigate the interactions between berberine and resveratrol with proteins related to or causing T2D, including ADIPOR1 (PDB-ID: 6ks1), ADIPOR2 (PDB-ID: 5lxg), TNF- $\alpha$  (PDB-ID: 7kpb), PTP1B (PDB-ID: 4i8n), GLUT1 (PDB-ID: 4pyp), IGF-IR (PDB-ID: 8eyr), IGF1 (PDB-ID: 6pyh), ADAMTS9 (PDB-ID: 3ppv), and SPHK2 (PDB ID: 4v24). SwissADME was used to assess the pharmacokinetic properties of berberine and resveratrol. Molecular docking was performed to analyze the interactions between these ligands and the specified proteins. Additionally, the potential bioactivity features of compounds were determined. Protein-protein interactions were obtained from the STRING database. The study data indicated that both compounds have high blood-brain barrier (BBB) penetration and gastrointestinal absorption ability (HIA). Besides, berberine exhibited the highest binding affinity with GLUT4 (-10.1 Kcal/mol), GLUT1 (-9.3 Kcal/mol), and SPHK2 (-9.3 Kcal/mol), while resveratrol showed strong binding with SPHK2 (-9.0 Kcal/mol) and TNF- $\alpha$  (-8.7 Kcal/mol) and. All proteins displayed binding energies of more than -7 Kcal/mol, suggesting that both berberine and resveratrol hold promise as potential drug candidates for T2D.

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## Berberin ve Resveratrolün Tip 2 Diyabet Tedavisindeki Terapötik Potansiyeli: Farmakokinetik ve Biyoyararlılık Özellikleri ile Moleküler Docking Analizi

### ÖZET

Tip 2 diyabet (T2D), tipik olarak insülin direnci ile karakterize edilen ve vücudun insülini etkili bir şekilde kullanamadığı veya yeterli insülin üretmediği bir metabolik bozukluktur. T2D tedavisinde insülin, metformin ve sülfonilüreler yaygın olarak kullanılmaktadır. Mevcut tedavi seçeneklerinin sınırlamaları göz önüne alındığında, yeni ilaçların keşfinde yoğun çabalara ihtiyaç vardır. Berberin, antidiyabetik, anti-inflamatuvar ve antioksidan özelliklere sahiptir. Resveratrol, antioksidan ve anti-enflamatuvar özellikleri nedeniyle kapsamlı bir şekilde araştırılan bir başka doğal bileşiktir. Bu çalışmanın amacı, berberin ve resveratrolün ADIPOR1 (PDB-ID: 6ks1), ADIPOR2 (PDB-ID: 5lxg), TNF- $\alpha$  (PDB-ID: 7kpb), PTP1B (PDB-ID: 4i8n), GLUT1 (PDB-ID: 4pyp), IGF-IR (PDB-ID: 8eyr), IGF1 (PDB-ID: 6pyh), ADAMTS9 (PDB-ID: 3ppv) ve SPHK2 (PDB ID: 4v24) dahil olmak üzere T2D ile ilişkili veya T2D'ye neden olan proteinlerle olan etkileşimlerini araştırmaktır. Berberin ve resveratrolün farmakokinetik özelliklerini değerlendirmek

### Moleküler Biyoloji

### Araştırma Makalesi

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### Anahtar Kelimeler

Berberine  
Moleküler yerleştirme  
Resveratrol  
Tip 2 diyabet

için SwissADME kullanılmıştır. Bu ligandlar ile belirtilen proteinler arasındaki etkileşimleri analiz etmek için moleküler yerleştirme yapılmıştır. Ayrıca, bileşiklerin potansiyel biyoyararlılık özellikleri belirlenmiştir. Protein-protein etkileşimleri STRING veri tabanından elde edilmiştir. Çalışma verileri, her iki bileşiğin de yüksek kan-beyin bariyeri (BBB) penetrasyonu ve gastrointestinal emilim yeteneğine (HIA) sahip olduğunu göstermiştir. Bunun yanında, berberin GLUT4 (-10.1 Kcal/mol), GLUT1 (-9.3 Kcal/mol) ve SPHK2 (-9.3 Kcal/mol) ile en yüksek bağlanma afinitesini gösterirken, resveratrol SPHK2 (-9.0 Kcal/mol) ve TNFR1 (-8.7 Kcal/mol) ile güçlü bağlanma göstermiştir. Tüm proteinler, -7 Kcal/mol'den daha yüksek bağlanma enerjileri sergilemiş, bu da berberin ve resveratrolün T2D için potansiyel ilaç adayları olarak umut verici olduğunu göstermektedir.

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## INTRODUCTION

Type 2 diabetes (T2D) is a metabolic disorder primarily distinguished by insulin resistance, signifying a condition where the body's ability to utilize insulin effectively is compromised, often accompanied by insufficient insulin production (American Diabetes Association, 2020). It is estimated that by the year 2045, approximately 783 million adults worldwide will have T2D (Magliano et al., 2021). Genetic, epigenetic, and environmental factors (such as obesity, unhealthy eating habits, lack of physical activity, etc.) are associated with the etiology of the disease (Prasad and Groop, 2015; Ghosh et al., 2022). In the treatment of T2D, insulin, metformin, and sulfonylureas are commonly used, and in some cases, surgical options may be considered (Davies et al., 2018; Gebrie et al., 2021). Additionally, maintaining healthy eating habits and regular physical activity are important for keeping blood sugar levels under control. It is known that multiple signaling pathways are involved in the development of T2D. Some of these include glucose transporter 4 (GLUT4), c-Jun N-terminal kinase (JNK), AMP-activated protein kinase (AMPK), and peroxisome proliferator-activated receptor gamma (PPAR-gamma) (Wen et al., 2022; Sanches et al., 2023).

Berberine is a natural compound derived from plants and has been used in traditional medicine for centuries. It has anti-diabetic, anti-inflammatory, antioxidant, and regulatory effects on lipid metabolism (Utami et al., 2023). It can exhibit antidiabetic activity by increasing insulin sensitivity and reducing glucose production. Molecular studies related to berberine's antidiabetic activity have suggested that berberine may activate an enzyme called AMPK, which can enhance insulin sensitivity and improve glucose metabolism (Xu et al., 2014).

Resveratrol is a natural polyphenol compound found

primarily in grapes and red wine. Numerous studies have shown that resveratrol is a potent antioxidant capable of combating free radicals in the body, preventing cellular damage, and reducing the aging process (Meng et al., 2021; Rudrapal et al., 2022). Additionally, it has been reported to have beneficial effects on cardiovascular health, including vasodilation, blood pressure regulation, and cholesterol level management (Rui et al., 2021). Furthermore, research has explored its potential in slowing down the development of neurological diseases (Andrade et al., 2018). Finally, there is a hypothesis that resveratrol may help regulate blood sugar levels, increase insulin sensitivity, and thereby reduce the risk of T2D (Zhu et al., 2017).

Molecular docking studies are a computer-based computational and modeling approach aimed at predicting how a drug candidate or a chemical compound can interact with a target protein or molecule, specifically identifying the binding site or interaction region (Meng et al., 2011). These studies are crucial for exploring drug-target interaction mechanisms in the design and optimization of new drugs. Also, it can be used to assess the potential repurposing of existing drugs by examining their interactions with different target proteins.

In this context, the aim of this study is not only to analyze the potential antidiabetic activity of berberine using a molecular docking approach but also to evaluate the antidiabetic, pharmacokinetic, and bioactivity effectiveness of resveratrol in comparison to berberine. Understanding the interactions between berberine and resveratrol with proteins associated with T2D (adiponectin receptor 1 (ADIPOR1) and adiponectin receptor 2 (ADIPOR2), tumor necrosis factor-alpha (TNF- $\alpha$ ), protein tyrosine phosphatase 1B (PTP1B), glucose transporter 1 (GLUT1), glucose

transporter 4 (GLUT4), insulin-like growth factor 1 receptor (IGF-IR), insulin-like growth factor 1 (IGF1), a disintegrin and metalloproteinase with thrombospondin motifs 9 (ADAMTS9), sphingosine kinase 2 (SPHK2) is crucial for uncovering the drug potentials of these compounds for antidiabetic activity. Molecular docking studies play a significant role in elucidating how these compounds interact with specific proteins, providing valuable insights into their potential therapeutic applications in the context of T2D.

## MATERIAL and METHOD

### Prediction of Druglikeness

To assess the drug likeness of berberine and resveratrol, their SMILES format was retrieved from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>), and the SwissADME website (<https://www.swissadme.ch/>) was utilized to analyze their absorption, bioavailability, distribution, metabolism, and excretion properties. Furthermore, the compounds' passage through the gastrointestinal wall and blood-brain barrier (BBB) was evaluated based on the BOILED-Egg data (Daina et al., 2017).

### Selection of Targets Related to Berberine and Resveratrol

Berberine and resveratrol underwent target prediction through the SwissTargetPrediction database, which can be accessed at <https://www.swisstargetprediction.ch/>. This tool utilizes computational methods to anticipate probable protein targets with which these substances could potentially interact. To ensure accuracy and uniformity, the standardized gene names linked to these anticipated targets were then obtained through the UniProt platform, yielding an exhaustive compilation of proteins associated with berberine and resveratrol.

### Molecular Docking

In this research, molecular docking served as a validation technique, employing computer simulations to forecast the binding affinity between receptors and ligands. Docking analyses were performed involving berberine and resveratrol, as well as target proteins whose 3D structures were retrieved from the PubChem database. The 3D structures of the selected target proteins associated with T2D were sourced from the Protein Data Bank (RCSB PDB) database (<https://www.rcsb.org/>). These chosen targets encompassed ADIPOR1 (PDB-ID: 6ks1), ADIPOR2 (PDB-ID: 5lxb), TNF- $\alpha$  (PDB-ID: 7kpb), PTP1B (PDB-ID: 4i8n), GLUT1 (PDB-ID: 4pyp), GLUT4 (PDB ID: 7wsn), IGF-IR (PDB-ID: 8eyr), IGF1 (PDB-ID: 6pyh),

ADAMTS9 (PDB-ID: 3ppv), and SPHK2 (PDB ID: 4v24). Optimal ligand binding conformations within the target proteins were determined using the AutoDock tool. This tool assessed the binding conformations based on their free binding energy, employing a scoring function. Subsequently, additional optimization for molecular docking of the target protein structures was conducted using AutoDock 4.2 software (<https://cadd.labshare.cn/cb-dock2/php/blinddock.php>). Data regarding berberine and resveratrol were acquired through the online platform DeepDataSource ([molinstincts.com](http://molinstincts.com)).

### Protein-Protein Interaction (PPI) Network

Target interactions were determined through the String platform (<https://www.string-db.org/>). In order to pinpoint central nodes and crucial proteins within the PPI network, calculations for centrality degrees, encompassing betweenness, closeness, and subgraph centrality, were executed utilizing Cytoscape without any external intervention.

### Suggested Bioactivity

A thorough evaluation of the possible bioactive characteristics of berberine and resveratrol is crucial for understanding their unique impacts and mechanisms of operation. To predict their potential bioactivity, we utilized the PASS platform (<http://www.way2drug.com/passonline/index.php>). This platform is renowned for its exceptional predictive capabilities, encompassing 3678 different activity types, and maintaining an average accuracy rate of around 95%. These predictions are derived solely from the structural formulas of the compounds.

## RESULTS

### SWISS Adme Property

The SMILES format of berberine (<https://pubchem.ncbi.nlm.nih.gov/compound/2353>) and resveratrol (<https://pubchem.ncbi.nlm.nih.gov/compound/445154>) was obtained by transferring it to the SwissADME platform (<http://www.swissadme.ch/>). The canonical SMILES of berberine were COC1=C(C2=C[N+]3=C(C=C2C=C1)C4=CC5=C(C=C4CC3)OCO5)OC and resveratrol C1=CC(=CC=C1C=CC2=CC(=CC(=C2)O)O)O. The three-dimensional configuration of compounds can be observed in Figure 1A and 1B. Berberine's and resveratrol's drug-likeness was comprehensively assessed using filters based on Lipinski (Pfizer), Ghose (Amgen), Veber (GSK), Egan (Pharmacia), and Muegge (Bayer). In terms of drug-like properties, it was found that both compounds exhibit favorable drug-like properties according to all filters (Figure 1A and 1B).

The Swiss ADME bioavailability radar assesses six



different physicochemical properties. These properties are LIPO (lipophilicity), SIZE, POLAR (polarity), INSOLU (insolubility), INSATU (insaturation), and FLEX (flexibility). Berberine is situated within a favorable range for bioavailability, complying with all

of these properties. However, resveratrol complies with all of these properties except for INSATU. This analysis could assist in evaluating berberine and resveratrol as a potential drug candidate.

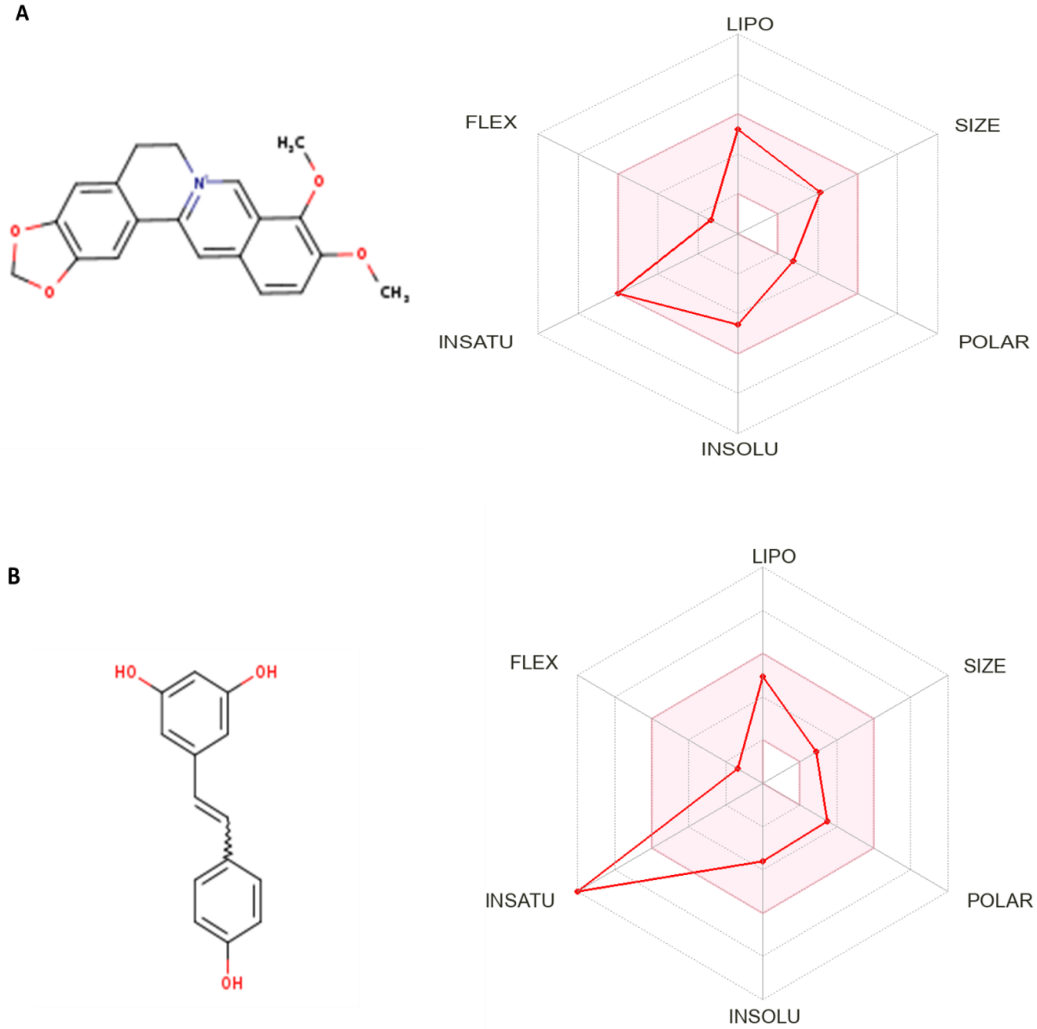


Figure 1. (A) Berberine 3D conformer and Swiss ADME properties (B) Resveratrol 3D conformer and SwissADME properties (right side of the figure) (<http://www.swissadme.ch/index.php#>). The oral bioavailability radar takes into account six physicochemical properties. Within this radar, the optimal range for each property is highlighted in pink, indicating the most favorable conditions. These properties include lipophilicity (XLOGP3) falling between -0.7 and +5.0, molecular weight (MW) ranging from 150 to 500 g/mol, polarity measured by the topological polar surface area (TPSA) between 20 and 130 Å<sup>2</sup>, solubility with a log S not exceeding 6, saturation levels with a sp<sup>3</sup> hybridization fraction of at least 0.25, and flexibility restricted to no more than 9 rotatable bonds.

Şekil 1. (A) Berberin ve resveratrol 3D konformerleri ve (B) SwissADME özellikleri (şeklin sağ tarafı) (<http://www.swissadme.ch/index.php#>). Oral biyoyararlanım radarı, altı fizikokimyasal özelliği dikkate alır. Bu radarda, her bir özellik için optimal aralık pembe renkte vurgulanmış olup en uygun koşulları belirtir. Bu özellikler arasında -0.7 ile +5.0 arasındaki lipofilite (XLOGP3), 150 ile 500 g/mol arasındaki molekül ağırlık (MW), 20 ile 130 Å<sup>2</sup> arasında ölçülen tepe polar yüzey alanı (TPSA) ile polarite, çözünürlük log S'nin 6'yı geçmemesi, en az 0.25 sp<sup>3</sup> hibridizasyon fraksiyonu uygunluk seviyeleri ve 9'dan fazla dönebilen bağla sınırlı olmayan esneklik yer alır.

According to the BOILED-Egg diagram, both compounds have the high BBB penetration and gastrointestinal (GI) tract. However, berberine

possesses the substructure of P-glycoprotein (PGP), while resveratrol does not have this substructure. The details are explained in Figure 2.

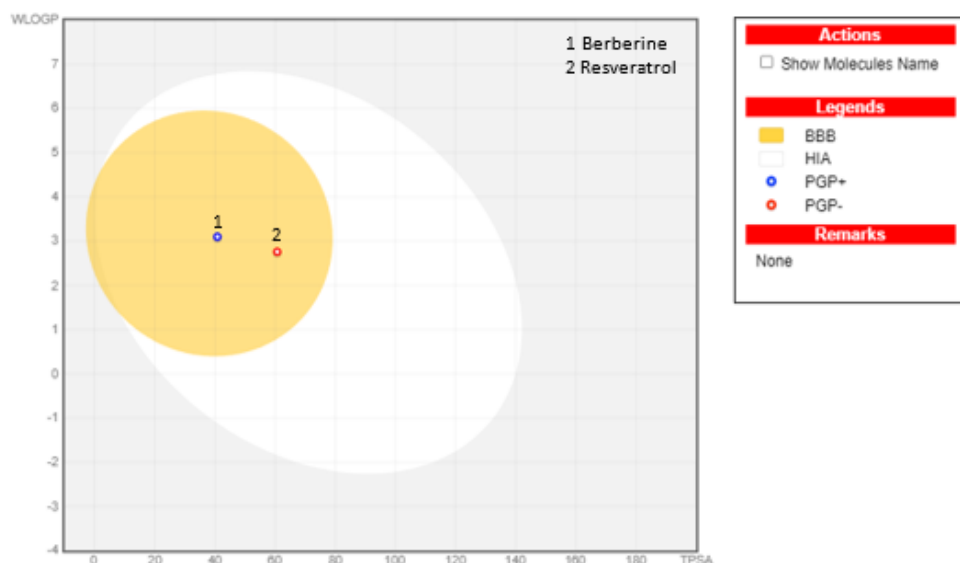


Figure 2. The BOILED-Egg diagram. The compounds within the white ellipse are likely to be efficiently absorbed by the GI tract. Those within the yellow ellipse, resembling the yolk, are expected to have a high likelihood of crossing the BBB and reaching the central nervous system. The blue points indicate compounds that are anticipated to be substrates of PGP+ and may be actively transported out of the brain or GI (1). On the other hand, the red points represent compounds predicted not to be substrates of PGP- (2).

*Şekil 2. BOILED-Egg diyagramı. Beyaz elips içindeki bileşiklerin sindirim sistemi tarafından etkili bir şekilde emilmesi muhtemeldir. Sarı elips içindeki, yumurtanın sarısına benzeyen bileşiklerin, kan-beyin bariyerini geçme ve merkezi sinir sistemine ulaşma olasılığı yüksektir. Mavi noktalar, PGP+ substratı olması beklenen ve beyin veya sindirim sistemi dışına aktif olarak taşınabilecek bileşikleri gösterir (sol taraf). Diğer yandan, kırmızı noktalar, PGP- substratı olmayan bileşikleri temsil eder (sağ taraf).*

It was observed that berberine differs from resveratrol in terms of molecular weight, H-bond donor and acceptor count, lipophilicity (LogP), topological polar surface area (TPSA), rotatable bond count, solubility (Log S), van der Waals volume, and P-gp substrate properties. Although both compounds show high

gastrointestinal absorption, berberine cannot cross the blood-brain barrier (BBB). Additionally, it was observed that berberine also differs from resveratrol in terms of P-gp substrate properties. While berberine can be expelled by P-glycoprotein, resveratrol does not exhibit this characteristic (Table 1).

Table 1. Physicochemical Properties of Berberine and Resveratrol Based on Bioavailability and Drug Similarity Analyses

*Çizelge 1. Biyoyararlanım ve İlaç Benzerliği Analizlerine Dayalı Berberin ve Resveratrol'ün Fizikokimyasal Özellikleri*

Physicochemical Property	Berberine	Resveratrol
Molecular Weight (g/mol)	336.38	228.25
H-Bond Donors	4	3
H-Bond Acceptors	7	3
LogP (lipophilicity)	1.91	2.78
TPSA (Topological Polar Surface Area) Å <sup>2</sup>	127.59	60.69
Rotatable Bonds (Flexibility)	5	1
Log S (Solubility)	-4.95	-3.51
VdW Volume Å <sup>3</sup>	299.70	207.24
BBB Permeability	No	Yes
Gastrointestinal Absorption	High	High
P-gp Substrate	Yes	No
CYP1A2 Inhibitor	No	No
CYP2C19 Inhibitor	Yes	No
CYP2C9 Inhibitor	Yes	No
CYP2D6 Inhibitor	No	No
CYP3A4 Inhibitor	No	No
Synthetic Accessibility Score	3.29	2.84

### Molecular docking

Nine common targets were selected as potential targets of berberine and resveratrol in the treatment of T2D. Their binding affinity with these ligands is provided in Table 1. Among the selected targets, it was determined that the GLUT1, GLUT4, and SPHK2 proteins had the best binding energy values of -10.1, -9.3, and -9.3 Kcal/mol for berberine, respectively. Additionally, ADIPOR1 (-9.0 Kcal/mol), ADIPOR2 (-8.1 Kcal/mol), TNF- $\alpha$  (-9.0 Kcal/mol), PTP1B (-8.5

Kcal/mol), IGF-IR (-8.6 Kcal/mol), IGF1 (-8.3 Kcal/mol) and ADAMTS9 (-7.4 Kcal/mol) also exhibited significantly high binding energies. However, it was determined that TNF- $\alpha$  and SPHK2 protein had the best binding energy, with a value of -8.7 and -9.0 Kcal/mol for resveratrol. In addition, ADIPOR1 (-7.1 Kcal/mol), ADIPOR2 (-7.7 Kcal/mol), GLUT4 (-8.4 Kcal/mol), PTP1B (-7.2 Kcal/mol), GLUT1 (-7.3 Kcal/mol), IGF-IR (-7.5 Kcal/mol), IGF1 (-7.3 Kcal/mol) and ADAMTS9 (-7.1 Kcal/mol) also exhibited significantly high binding energies (Table 2).

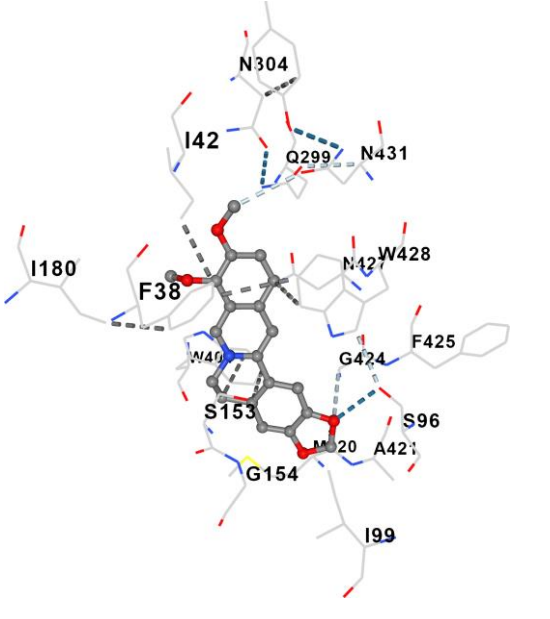
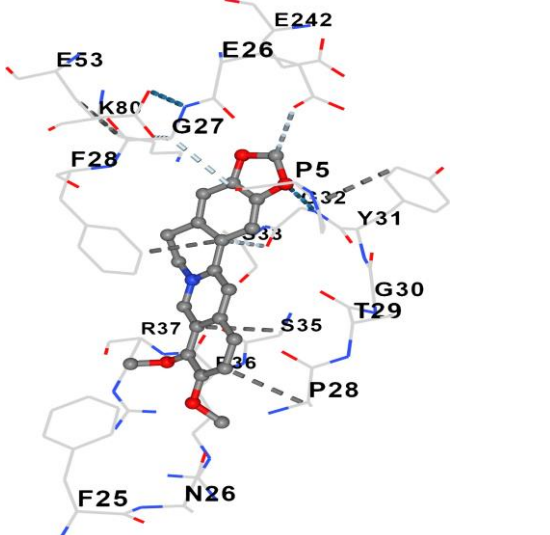
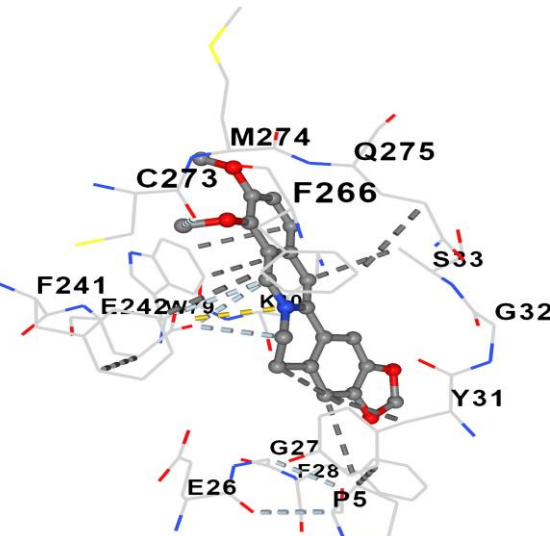
Table 2. Interaction of berberine and resveratrol with target proteins

*Çizelge 2. Hedef proteinler ile berberine ve resveratrolün interaksyonu*

Target Protein	Binding Affinity (Kcal/mol)	Cavity volume (Å <sup>3</sup> )	Contact residues	Ligand-Molecule Interaction
<b>Berberine</b>				
ADIPOR1	-9.0	4297	Chain A: LEU215 ILE216 SER219 PHE220 LEU274 GLY275 GLY278 VAL279 THR282 MET300 PHE303 PHE304 MET306 ALA307 TYR310 HIS351	
ADIPOR2	-8.1	440	Chain H: GLN39 SER40 PRO41 GLY42 LYS43 SER44 VAL93 TYR95 Chain L: ALA9 GLN38 LYS39 GLN40 GLY41 SER85 TYR87 GLY100 GLY101 THR102 LYS103	

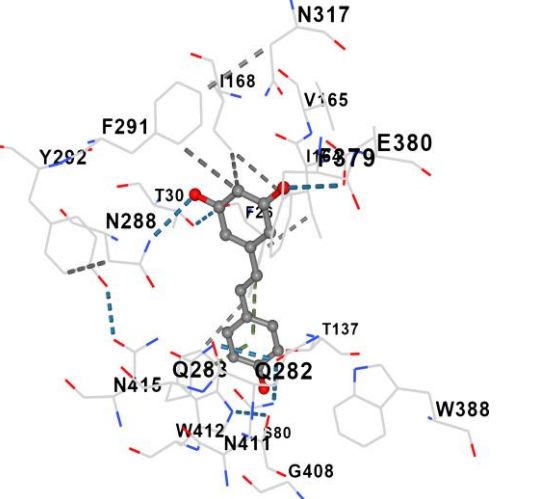
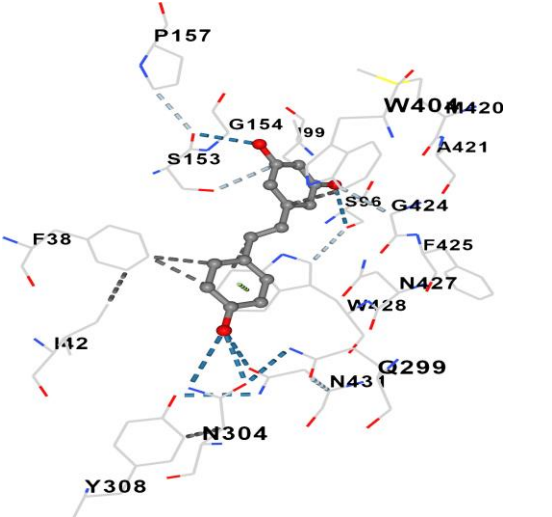
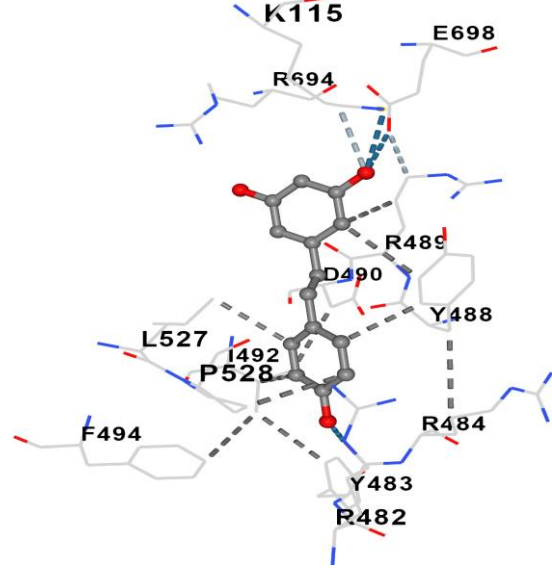
TNF- $\alpha$	-9.0	25422	Chain A: LEU75 ILE97 LYS98 SER99 PRO100 GLU116 Chain C: PRO113 TRP114 TYR115 GLU116 Chain L: TYR49 Chain H: GLY102 TYR103 TRP107	
PTP1B	-8.5	234	Chain A: TYR46 ASP48 VAL49 ASP181 PHE182 CYS215 SER216 ALA217 GLY218 ILE219 GLY220 ARG221 GLN262	
GLUT1	-9.3	1429	Chain A: PHE26 THR30 GLN161 ILE164 VAL165 ILE168 GLN282 GLN283 ILE287 ASN288 PHE291 ASN317 THR321 SER324 LEU325 VAL328 PHE379 GLU380 GLY384 PRO385 TRP388 PHE389 ASN411	



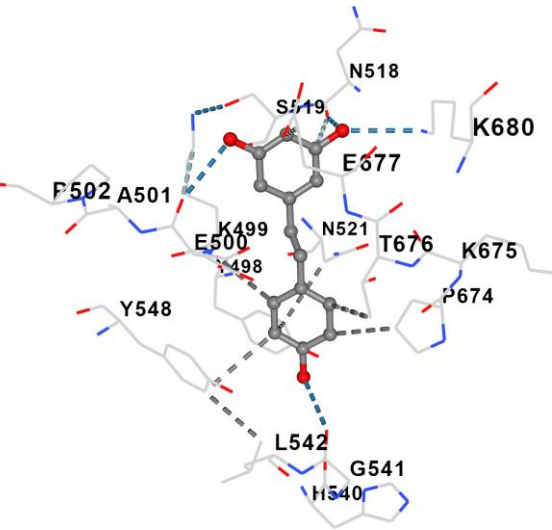
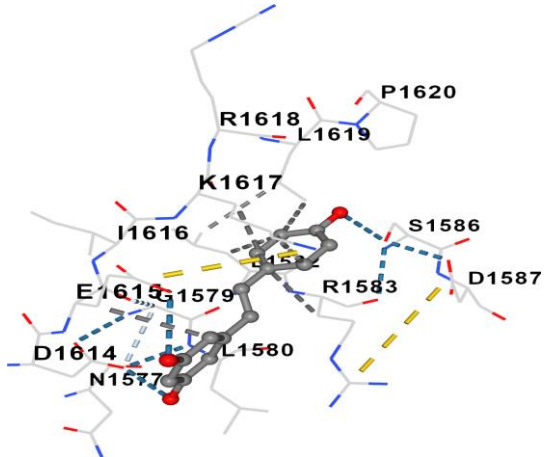
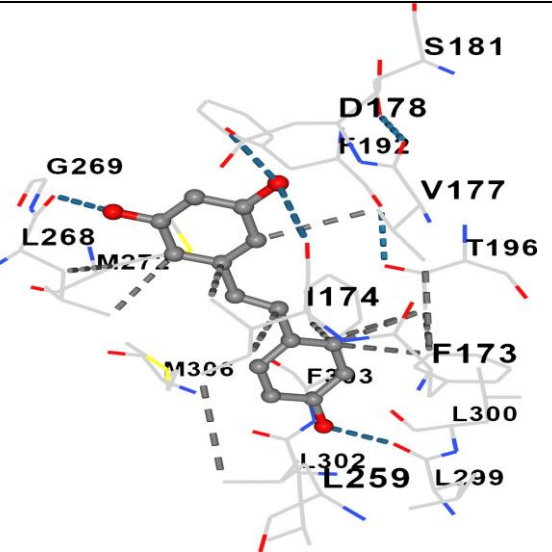
GLUT4	-10.1	3458	Chain A: PHE38 ILE42 SER96 ILE99 SER153 GLY154 ILE180 GLN299 ASN304 TYR308 TRP404 MET420 ALA421 GLY424 PHE425 ASN427 TRP428 ASN431	 <p>3D molecular model of GLUT4 binding site. The ligand is shown in stick representation with red oxygen and blue nitrogen atoms. Dashed lines indicate hydrogen bonds between the ligand and residues: N304, I42, Q299, N431, I180, F38, W40, S153, G154, M20, A421, S96, F425, G424, N42, W428, and I99.</p>
IGF-IR	-8.6	9791	Chain B: PHE700 SER704 Chain A: PRO5 GLU26 PHE28 HIS30 GLU53 TYR54 LYS80 LEU81 PHE82 THR316 Chain D: THR29 GLY30 TYR31 GLY32 SER35 ARG37 ALA38	 <p>3D molecular model of IGF-IR binding site. The ligand is shown in stick representation with red oxygen and blue nitrogen atoms. Dashed lines indicate hydrogen bonds between the ligand and residues: E53, K80, F28, G27, E242, E26, P5, Y31, G30, T29, R37, F36, S35, P28, F25, and N26.</p>
IGF1	-8.3	3062	Chain B: TYR31 GLY32 SER33 Chain D: PRO5 GLU26 GLY27 PHE28 TRP79 LYS80 PHE241 GLU242 PHE266 CYS273 MET274 GLN275	 <p>3D molecular model of IGF1 binding site. The ligand is shown in stick representation with red oxygen and blue nitrogen atoms. Dashed lines indicate hydrogen bonds between the ligand and residues: M274, Q275, C273, F266, S33, G32, F241, E242, W79, K10, Y31, G27, F28, E26, and P5.</p>
ADAMTS9	-7.4	127	Chain A: GLY1579 LEU1582 ARG1583 SER1586 ASP1587	

			ASP1614 GLU1615 ILE1616 LYS1617 ARG1618 LEU1619 PRO1620	
SPHK2	-9.3	10611	Chain A: LEU194 PHE197 LEU198 LEU200 ALA201 ALA202 ARG296 LEU300 Chain B: MET190 LEU194 PHE197 LEU198 LEU200 ALA201 ARG296 ALA297 LEU300 Chain C: LEU194 PHE197 LEU198 LEU200 ALA201 ARG296 ALA297 LEU300	
<b>Resveratrol</b>				
ADIPOR1	-7.1	4297	Chain A: TYR209 SER210 ILE212 ALA213 ILE216 ALA249 ALA253 ARG267 PHE271 LEU274 GLY275 TYR310 ILE311 GLY313 ALA314 PHE340 VAL344	

ADIPOR2	-7.7	4654	<p>Chain A: TRP114 ASP117                      PHE201 HIS202 TYR205                      GLU209 SER212 ARG213                      SER216 ASP219 TYR220                      ALA270 ARG275 ARG278                      TYR328 HIS348</p>	
TNF- $\alpha$	-8.7	25422	<p>Chain A: LEU75 LEU76                      THR77 ILE97 ASN137                      ARG138                      Chain L: LEU46 TYR49                      LEU54 ALA55 ASP56                      Chain H: ALA31 TYR32                      TYR33 TYR53 GLU99                      ALA100 TYR101 TRP107</p>	
PTP1B	-7.2	234	<p>Chain A: TYR46 ARG47                      ASP48 VAL49 ASP181                      PHE182 CYS215 SER216                      ALA217 ILE219 GLY220                      ARG221 GLN262 GLN266</p>	

GLUT1	-7.3	1429	Chain A: PHE26 THR30 SER80 THR137 VAL165 ILE168 GLN282 GLN283 ASN288 PHE291 ASN317 PHE379 GLU380 TRP388 GLY408 ASN411 TRP412 ASN415	
GLUT4	-8.4	3458	Chain A: PHE38 ILE42 SER95 SER96 ILE99 GLY150 SER153 GLY154 PRO157 GLN299 ASN304 TYR308 TRP404 MET420 ALA421 GLY424 PHE425 ASN427 TRP428 ASN431	
IGF-IR	-7.5	1571	Chain B: LYS115 Chain A: ARG482 TYR483 ARG484 TYR488 ARG489 ASP490 ILE492 PHE494 LEU527 PRO528 ALA691 ARG694 GLU698 Chain C: ALA8	



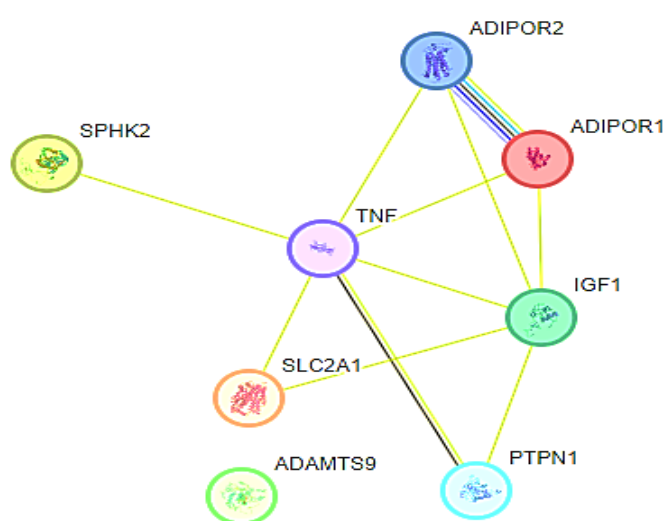
IGF1	-7.3	2118	<p>Chain A: TYR498 LYS499                      GLU500 ASN518 SER519                      ASN521 HIS540 GLY541                      LEU542 TYR548                      Chain D: PRO674 LYS675                      THR676 GLU677 LYS680</p>	
ADAMTS9	-7.1	127	<p>Chain A: ASN1577                      GLY1579 LEU1580                      LEU1582 ARG1583                      SER1586 ASP1587                      ASP1614 GLU1615                      ILE1616 LYS1617                      ARG1618 LEU1619                      PRO1620</p>	
SPHK2	-8.7	26771	<p>Chain A: ARG24 GLY26                      LYS27 GLY111 SER112                      ASN131 PHE173 ILE174                      VAL177 ASP178 GLU180                      SER181 LYS183 TYR184                      ARG185 ARG186 GLY188                      GLU189 PHE192 THR196                      LEU198 LEU200 LEU259                      LEU268 GLY269 MET272                      VAL290 LEU299 LEU300                      LEU302 PHE303 MET306                      LEU319                      Chain B: MET190                      PHE192 THR193 THR196                      PHE197 GLU271 MET272                      PHE303 LEU304 ALA305                      MET306 GLU307                      Chain C: ARG186</p>	

The interactions between berberine and target proteins were assessed using the AutoDock tool, which utilizes a scoring function based on binding free energies to evaluate binding conformations. Subsequently, the structures of target proteins were subjected to molecular docking optimization, and the analysis was conducted using AutoDock 4.2 software (<https://cadd.labshare.cn/cb-dock2/php/blinddock.php>). The cavity volume ( $\text{\AA}^3$ ) value, which represents the size of the binding pockets in the protein, along with protein-ligand interactions from the molecular docking analysis, is provided in Table 2. The contact points between residues and bonds show the amino acids and binding configurations between ligands and target

proteins. The blue dashed lines represent hydrogen bond interactions, the yellow dashed lines represent hydrophobic interactions, and the red dashed lines represent electrostatic interactions (Table 2).

### Constructing a Protein-Protein Interaction Network (PPI)

To explore protein-protein interactions and identify 9 shared targets, we utilized the String database (<https://www.string-db.org/>). These interactions were instrumental in uncovering the functional connections and associations among the proteins. Next, the data was imported into Cytoscape v3.7.2 to construct a PPI network composed of 7 nodes and 10 edges (Figure 3).



**Figure 3.** Protein interaction network analysis  
*Şekil 3. Protein etkileşim ağı analizi*

### Succession of bioactivities

Bioactivity prediction was performed using the platform accessible at "<http://www.way2drug.com/passonline/predict.php>." The predictive model for the bioactivity spectrum is founded on a Bayesian approach. Within this tool, predictions are generated based on the Pa:Pi (active to inactive ratio) at three distinct threshold levels: Pa > 30%, Pa > 50%, and Pa > 70%. The results of these forecasts are presented in terms of Pa (probability of activity) and Pi (probability of inactivity). Compounds exceeding a Pa value of 0.7 are considered promising candidates for the specified biological activity (Table 3).

### DISCUSSION

In the present study, the therapeutic effect of berberine and resveratrol was evaluated in situ for type 2 diabetes (T2D) using a molecular docking approach. Molecular docking studies are employed to

understand how a molecule affects a target protein. In this study, molecular docking was performed to assess the binding status of berberine and resveratrol with the 3D structures of proteins associated with the T2D, including ADIPOR1 and ADIPOR2, TNF- $\alpha$ , PTP1B, GLUT1, GLUT4, IGF-IR, IGF1, ADAMTS9, SPHK2. These proteins can influence various processes in T2D, including insulin signaling, cell death, inflammation, and cell growth. Therefore, understanding the roles of these proteins in the pathogenesis and treatment of T2D is crucial. In the realm of molecular docking studies, the term "low-energy binding" typically signifies a robust and stable interaction between a ligand and a protein, indicating a strong affinity.

Understanding and regulating the function of GLUT1 and especially GLUT4 proteins can play a significant role in the treatment and prevention of T2D. GLUT1 is responsible for the uptake of glucose into cells and can also transport some lipids besides glucose (Pragallapati and Manyam, 2019). Considering that

lipid metabolism disorders are common in individuals with T2D and can increase the risk of cardiovascular diseases, controlling them is essential (Martín-Timón et al., 2014; Rubino et al., 2016; Petersen and Shulman, 2018). In addition to GLUT1, GLUT4 is also a carrier protein located on the cell membrane that regulates the entry of glucose into the cell. In the case of T2D, glucose cannot be taken up by GLUT4 proteins, and the body develops insulin resistance. Overcoming insulin resistance and ensuring the effective functioning of GLUT4 proteins are important for effective treatment. Molecular binding analysis showed that berberine exhibited the best binding to the GLUT4 protein (-10.1 Kcal/mol), followed by the GLUT1 protein (-9.3 Kcal/mol). However, resveratrol,

although less tightly bound compared to berberine, has binding energies above -7 kcal/mol for both GLUT1 and GLUT4. Additionally, another significant protein in the pathogenesis of T2D is SPHK2. SPHK2 regulates cellular responses by modulating lipid signal pathways in cells, which can affect inflammation and oxidative stress levels, potentially contributing to the development of T2D (Qi et al., 2021). The data from this study showed that berberine and resveratrol exhibit a high binding affinity with the SPHK2 enzyme (-9.3 and -9.0 Kcal/mol, respectively). The high binding affinities of these compounds with this enzyme demonstrated that they should be considered target enzymes in advanced studies related to T2D.

**Table 3.** The prospective biological activity spectrum associated with berberine and resveratrol  
*Çizelge 3. Berberin ve resveratrol ile ilişkilendirilen olası biyolojik aktivite spektrumu*

Activity	Pa	Pi
<b>Berberine</b>		
P-glycoprotein substrate	0.408	0.040
GABA aminotransferase inhibitor	0.424	0.039
<b>Resveratrol</b>		
APOA1 expression enhancer	0.923	0.002
JAK2 expression inhibitor	0.912	0.003
Sugar-phosphatase inhibitor	0.835	0.011
Aldehyde oxidase inhibitor	0.831	0.007
Fatty-acyl-CoA synthase inhibitor	0.823	0.004
Glucan endo-1,6-beta-glucosidase inhibitor	0.816	0.006
Glucose oxidase inhibitor	0.799	0.012
Beta glucuronidase inhibitor	0.752	0.003
GABA aminotransferase inhibitor	0.719	0.004
Insulysin inhibitor	0.707	0.008
Gluconate 2-dehydrogenase (acceptor) inhibitor	0.737	0.038

Adiponectin is a hormone secreted from adipose tissue known for its ability to increase insulin sensitivity in the body (Khoramipour et al., 2021). In other words, sufficient production of adiponectin and the effective functioning of adiponectin receptors can help cells use insulin more effectively. Adiponectin can reverse this condition by reducing insulin resistance. Adiponectin binds to cells through two different receptors, namely ADIPOR1 and ADIPOR2 (Thundyil et al., 2012). These receptors facilitate the uptake of adiponectin hormone into cells and initiate signaling pathways that convey its effects. The function of ADIPOR1 and ADIPOR2 includes regulating intracellular signal transduction and contributing to metabolic processes, including increasing insulin sensitivity (Li et al., 2022). They have been investigated as potential drug targets for the treatment and prevention of T2D (Deng et al., 2023). Medications or therapeutic approaches aimed at enhancing the activity of these receptors can help increase insulin sensitivity, thereby assisting in the management of T2D. The present study showed that berberine exhibits binding energies of -9.0 Kcal/mol and -8.1 Kcal/mol with ADIPOR1 and ADIPOR2,

respectively. On the other hand, resveratrol has binding energies of -7.1 Kcal/mol with ADIPOR1 and -7.7 Kcal/mol with ADIPOR2. Berberine binds to these receptors with lower energy values compared to resveratrol.

TNF- $\alpha$  can potentially harm insulin-producing beta cells in the pancreas, leading to a reduction in insulin production and the progression of T2D (Rehman and Akash, 2016). Furthermore, TNF- $\alpha$  can negatively affect insulin receptors on the cell surface, causing cells to perceive insulin less effectively. Additionally, TNF- $\alpha$  can disrupt glucose metabolism, contributing to elevated blood sugar levels (Wondmkun, 2020). In the treatment of T2D, drugs that aim to reduce the effects of TNF- $\alpha$  work towards enhancing insulin sensitivity by controlling inflammation (Li et al., 2023). In this study, both drug candidates showed nearly identical binding energies with TNF- $\alpha$ , with berberine at -9.0 Kcal/mol and resveratrol at -8.7, indicating that both drug candidates have the potential to modulate inflammation associated with T2D.

Drug-likeness is a crucial concept in drug research and development, determining a molecule's potential as a drug and predicting its pharmacokinetic profile (Bickerton et al., 2012). Pharmaceutical companies use various filters, including Lipinski's Rule of Five, the Ghose filter, the Veber filter, and the Mudgee filter, to establish drug-likeness criteria for compounds like berberine and resveratrol. According to Lipinski's Rule of Five, compounds must meet specific criteria, including a molecular weight below 500 daltons, limited hydrogen bond donors and acceptors, and a suitable log P (octanol-water partition coefficient). The Ghose filter assesses criteria such as molecular weight, LogP, atom count, and molar refractivity. The Veber filter considers rotatable bonds and polar surface area, while the Mudgee filter examines ring and bond counts (Kralj et al., 2023). According to SwissADME properties, both berberine and resveratrol exhibited drug-like characteristics according to Lipinski's five rules, Ghose, Veber, Egan, and Muegge filters. Also, both have high GI absorption and BBB permeability. Additionally, significant bioactivity analysis indicated that resveratrol exhibited characteristics such as enhancing APOA1 expression at 0.923 Pa. Apolipoprotein A1 (APOA1) is an apolipoprotein associated with High-Density Lipoprotein (HDL), a lipoprotein. APOA1 plays a crucial role as a structural component of HDL, contributing to HDL's functions in cholesterol transport and metabolism (Mangaraj et al., 2016). Due to its ability to raise HDL levels and potentially improve metabolic health and reduce cardiovascular risk, it is believed that APOA1 may have the potential to reduce the risk of T2D (Zvintzou et al., 2023). Higher HDL levels are generally associated with a lower risk of T2D. Furthermore, bioactivities with Pa values greater than 0.7, which may be associated with T2D, are listed in Table 3. When examining the predicted bioactivity properties of resveratrol, it is evident that it has a significantly greater number of activities related to T2D compared to berberine. Additionally, berberine's bioactivities had Pa values below 0.7. While the binding energy of resveratrol to proteins associated with T2D was like that of berberine in molecular docking analysis, their predicted bioactivities are quite distinct. Furthermore, resveratrol has been observed to possess characteristics such as a sugar-phosphate inhibitor (Pa 0.8305), glucose oxidase inhibitor (Pa 0.799), beta-glucuronidase inhibitor (Pa 0.752), and insulysin inhibitor (Pa 0.707).

The results of this study indicate that both berberine and resveratrol should be considered as potential drug candidates for T2D treatment. Molecular docking studies revealed that berberine and resveratrol have high binding affinities to critical proteins associated with T2D. Particularly, it is strong binding to GLUT1, GLUT4, TNF- $\alpha$ , and SPHK2 protein, suggesting that

berberine and resveratrol modulate inflammation, glucose intake, and insulin resistance. These findings emphasize the importance of considering berberine and resveratrol as drug candidates with multiple targets for T2D treatment.

## CONCLUSION

Swiss ADME data showed that berberine aligns with favorable bioavailability characteristics, while resveratrol lacks INSATU compliance. According to the BOILED-Egg diagram, both compounds demonstrate high BBB permeability and effective GI absorption. Molecular docking studies have demonstrated the high binding affinities of berberine and resveratrol to key proteins associated with T2D. These results underscore the significance of considering these compounds as promising drug candidates with multiple targets for the treatment of T2D. Future research should further investigate the efficacy of berberine and resveratrol in T2D treatment through more comprehensive experiments supported by clinical studies. Additionally, the pharmacokinetics and safety of berberine and resveratrol should be explored in greater detail, especially regarding their potential roles in early T2D diagnosis, treatment response monitoring, and risk reduction factors.

## Contribution of Authors

SC: Designed, performed, analyzed, wrote, reviewed and edited.

## Conflict of Interest

The author declares no conflict of interest.

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## Preliminary Insights into the Phylogeny of *Colchicum* Species

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### ABSTRACT

*Colchicum* has played a vital role in the field of medicine due to its inherent properties, with its importance extending from the past to the present. DNA barcoding is an effective tool for the identification and conservation of plant species. This study tested the application of the *18S rRNA* gene for the phylogenetic construction of *Colchicum* species. The phylogenetic UPGMA (unweighted pair group method with arithmetic mean) tree and STRUCTURE analysis were conducted to assess the consistency of the results for 33 *Colchicum* specimens and one outgroup species, utilizing the *18S rRNA* partial gene. The results of the study showed that DNA barcoding using *18S rRNA* was an effective method for identifying *Colchicum* species, with 97.76% of the tested sequences being successfully determined. This study contributes to the field of phylogenetic analysis of *Colchicum* species by illustrating the utility of the *18S rRNA* gene for phylogenetic construction, underscoring its significance in advancing our understanding of plant genetics. The nucleotide sequences obtained for species identification in this study were uploaded to the barcode of life data system (BOLD), contributing to internationally accessible genetic data.

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## *Colchicum* Türlerinin Filogenisine İlişkin Ön Bilgiler

### ÖZET

*Colchicum*, sahip olduğu doğal özellikleri nedeniyle tıp alanında önemli bir rol oynamış olup, önemi geçmişten günümüze kadar uzanmaktadır. DNA barkodlama, bitki türlerinin tanımlanması ve korunması için etkili bir araçtır. Bu çalışma, *Colchicum* türlerinin filogenetik yapısının oluşturulmasında *18S rRNA* geninin uygulamasını test etmiştir. 33 *Colchicum* örneği ve bir dış grup türü kullanılarak, *18S rRNA* kısmi geni ile filogenetik UPGMA (aritmetik ortalama ile ağırlıksız çift grup yöntemi) ağacı ve STRUCTURE analizi yapılmıştır. Çalışmanın sonuçları, *18S rRNA* kullanılarak yapılan DNA barkodlamanın *Colchicum* türlerinin tanımlanmasında etkili bir yöntem olduğunu ve test edilen dizilerin %97.76'sının başarılı bir şekilde belirlendiğini göstermiştir. Bu çalışma, *18S rRNA* geninin filogenetik yapı oluşturmadaki faydasını göstererek *Colchicum* türlerinin filogenetik analizine katkıda bulunmakta ve bitki genetiğini anlama konusunda ilerlememize olanak sağlamaktadır. Tür tanımlaması için elde edilen nükleotid dizileri, uluslararası erişilebilir genetik verilere katkıda bulunmak amacıyla yaşam barkodu veri sistemine (BOLD) yüklenmiştir.

### Bitki Biyoteknolojisi

### Araştırma Makalesi

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### Anahtar Kelimeler

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Filogenetik analiz  
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## INTRODUCTION

*Colchicum*, a genus of herbaceous plants belonging to the Liliaceae family, is commonly utilized both as an ornamental species and for its medicinal properties,

primarily due to the presence of alkaloids. In today's medical field, it assumes a vital role in addressing many global diseases, whether used in isolation or conjunction with different pharmaceuticals (Toplan et

al., 2016). In addition to alkaloids, *Colchicum* species also include flavonoids, phenolic acids, tannins, and fatty acids (Hailu et al., 2021). *Colchicum* species face several challenges in terms of conservation. The loss of their habitats due to urbanization and land development, as well as agricultural activities, poses a significant threat to their survival. Additionally, over-harvesting for medicinal purposes has led to a decline in wild populations, and illegal trade further jeopardizes rare and endangered *Colchicum* species. Climate change also poses a significant threat to their habitats, with disruptions in precipitation patterns and extreme weather events affecting their survival (Chandra, 2016; Rather et al., 2022).

Beyond the preservation of biodiversity, accurate taxonomic identification carries considerable significance in the domain of herbal or traditional medicine (Techen et al., 2014). In response to the challenges posed by traditional taxonomy, various genomic methods have been suggested. Among these, DNA barcoding, initially introduced by Hebert et al. (2003) has proven highly effective in both the determination of existing species and the discovery of unknown ones. DNA barcoding employs short, uniform, and variable DNA regions (<1000 bp) to assess and classify species (Savolainen et al., 2005). Due to its simplicity and greater accuracy compared to the difficulties and subjectivity involved in morphology-based taxonomy classification, DNA barcoding is growing in popularity (Kress, 2017). While the *cytochrome c oxidase 1 (COI)* gene in the mitochondrial genome is widely accepted as a universal barcode marker for animals, an equivalent universal barcode for plants remains to be identified (Kress & Erickson, 2008). In the realm of plant life, the employment of DNA barcoding is not ideally suited due to its slow progression and limited variation. As a result, the search for acceptable plant barcode regions has shifted to the nuclear and chloroplast genomes, which have higher substitution rates (Hollingsworth et al., 2011). Due to their ability to distinguish between different plant species, various potential regions, including *matK*, *rbcL*, *trnH-psbA*, *trnL-F*, *ITS*, *18S rRNA*, and *5S rRNA*, have been extensively studied (Mishra et al., 2016).

Barcoding efforts focus on the region comprising the V4 hypervariable domain of the *18S rRNA* gene, which is roughly 400 base pairs (bp) in length and is highly variable, making it useful for species-level identification. They are widely used markers for plants concerning their discrimination capacity, and due to their conserved nature, and their widespread availability across plant taxa (Srivastava et al., 2016). The first *18S rRNA* sequences of angiosperms were of maize (Messing et al., 1984), rice (Takaiwa et al., 1984), soybean (Eckenrode et al., 1985), and Poaceae (Hamby & Zimmer, 1988). Also, the study that

provides a comprehensive comparison of the *18S RNA* and *rbcL* sequences of various plant species (Nickrent & Soltis, 1995) and concludes that *18S rDNA* sequences provide an adequate dataset for conducting phylogenetic analyses at more comprehensive taxonomic levels within angiosperms (Soltis et al., 1997) provided a framework for future studies on selecting the *18S rRNA* gene for phylogenetic analysis and DNA barcoding in plants.

UPGMA (unweighted pair group method with arithmetic mean) is a widely used method in phylogenetic analysis for constructing dendrograms based on genetic distance. The utility of the UPGMA method in phylogenetic and population structure analyses has been demonstrated in various studies, underscoring its relevance for diverse plant taxa (Demirel et al., 2022; Karakan et al., 2024). In this preliminary study, the UPGMA method was employed to construct the phylogenetic relationships among 33 *Colchicum* specimens using nucleotide sequences from the *18S rRNA* gene region, and STRUCTURE analysis was used to validate the phylogenetic tree data with population structure. The *18S rRNA* gene is chosen for phylogenetic studies due to its highly conserved nature, which allows for the comparison of evolutionary relationships across diverse taxa. Its relatively slow rate of evolution provides stable and reliable markers for inferring deeper evolutionary lineages and relationships among species.

## MATERIAL and METHOD

### Plant material

In this preliminary study, three individuals from each of the 11 *Colchicum* species were collected to provide an initial insight into the genetic diversity within the genus. Detailed information on the specimens, including the season of collection, is provided in Table 1. All specimens were supplied by the Republic of Türkiye Ministry of Agriculture and Forestry, Atatürk Horticultural Central Research Institute in Yalova. As an outgroup, *Androcymbium dregei* (obtained from GenBank with the accession number: JQ405011.1) from the Colchicaceae family was used. The choice of *Androcymbium dregei* as an outgroup was based on its phylogenetic distance within the family, providing a robust reference for comparative analysis.

### DNA extraction, PCR amplification, and sequencing

Genomic DNA from leaf tissue was extracted using the CTAB method developed by Doyle & Doyle (1987). Partial *18S rRNA* gene amplifications by the polymerase chain reaction (PCR) were performed using 2 ng total genomic DNA as a template in a 25 µL reaction mixture, consisting of PCR buffer (1 X), 4 mmol/L MgCl<sub>2</sub>, 0.8 mmol/L each primer, 0.2 mmol/L each dNTP, and 0.5 U Taq DNA polymerase. Primers designed using Primer3, flanking the *18S rRNA* gene



regions, are as follows: *18S rRNA* Forward: 5'- ATT GTG ACG GAG CCG GGC GAG G -3', *18S rRNA* Reverse: 5'- CGG AGT TTG GTG GGG ACG TAC C -3'; The PCR program employed in this study followed these steps: an initial denaturation step at 94°C for 3 minutes, followed by 39 cycles consisting of denaturation at 94°C for 1 minute, annealing at 53°C

for 1 minute, and extension at 72°C for 1 minute. The process concluded with a final extension at 72 °C for 10 minutes. The PCR amplifications were carried out on a Veriti™ 96-Well Fast Thermal Cycler. The PCR products were visualized using electrophoresis on agarose gel (1.5%).

Table 1. Genotype codes and seasonal information of 11 *Colchicum* species were used in this study.  
 Çizelge 1. Çalışmada kullanılan 11 *Colchicum* türünün genotip kodları ve mevsimsel bilgileri.

No	Species	Genotype Code	Season
1		C4803A	
2	<i>C. balansae</i> Planchon	C4810	Autumn
3		C4821	
4		C0730	
5	<i>C. baytopiorum</i> C.D. Brickell	C0740	Late-autumn
6		C0748	
7		C4819	
8	<i>C. macrophyllum</i> B.L. Burt	C4822	Autumn
9		C4823	
10		C0605	
11	<i>C. bivonae</i> Guss.	C1412	Autumn
12		C1703	
13		C0749	
14	<i>C. boissieri</i> Orph.	C0751	Autumn
15		C3503	
16		C3304	
17	<i>C. cilicicum</i> (Boiss.) Dammer	C5103	Autumn
18		C5104	
19		C2710	
20	<i>C. polyphyllum</i> Boiss. & Heldr.	C3108	Autumn
21		C3308	
22		C0101	
23	<i>C. serpentinum</i> Woron. ex Misch.	C4227	Winter
24		C4601	
25		C2802	
26	<i>C. speciosum</i> Steven	C3701	Autumn
27		C5301	
28		C0733	
29	<i>C. stevenii</i> Kunth	C0735	Late-autumn
30		C0765	
31		C2003	
32	<i>C. burttii</i> Meikle	C4801	Winter
33		C4814	

Purification of PCR products was carried out by the 'ChargeSwitch-Pro PCR Clean-up Purification' protocol. Cycle sequencing reactions were performed using the "Big Dye-Kit Standard" protocol. DNA sequencing was performed with the "ABI 310 Genetic Analyzer" sequencing system.

### Sequence analysis

After the sequencing step, the chromatograms acquired were subjected to further examination through Geneious Prime version 2023.2.1. To ensure

the accuracy of each sequence, a comprehensive examination of the peaks corresponding to individual nucleotides was conducted, followed by the generation of a consensus sequence after eliminating segments of lower quality. The molecular taxonomic identity of the resulting consensus sequence was authenticated using the nucleotide blast tool (BLASTN - basic local alignment search tool-nucleotide) available at the National Center for Biotechnology Information (NCBI). This tool assesses nucleotide/protein sequences by comparing them to sequence databases,

determining identities based on percentage similarity, and considering the E-value.

The phylogenetic analysis was performed in the “CLC Genomics Workbench 23.0.2” program by the “UPGMA” procedure, using the “Kimura 80” model of evolution with confidence measured through 1000 replicates of bootstrap.

For population structure analysis based on *18S rRNA* sequence information, the determination of SNPs was first performed in the “Mega 6” program. STRUCTURE analysis was conducted using the software application “STRUCTURE (version 2.3.4)” to categorize the genotypes based on the approach proposed by Pritchard et al. (2000). During this analysis, various models (K=1-10) were tested after 100,000 iterations of the markov chain monte carlo (MCMC) algorithm, along with customized statistical methods, to identify the most suitable population count. In addition, each model was performed 10 times with 10.000 length burning period. To calculate the  $\Delta K$

value of the populations, “STRUCTURE HARVESTER” (Earl & Vonholdt, 2012) software was used, and the number of subpopulations was identified. For the K and  $\Delta K$  values obtained for this purpose, the data determines the optimum K value based on the probability value reached (Evanno et al., 2005). The genotypes that have a probability  $\geq 0.80$  are classified as 'pure lineage', whereas those with a probability  $\leq 0.80$  are classified as 'mixed ancestry'.

## RESULTS

### PCR amplification and sequencing

The PCR products obtained from the amplification of the *18S rRNA* gene region were analyzed by agarose gel electrophoresis (Figure 1). Clear bands of approximately 200 bp were observed for all samples. These results indicate that the target gene region was successfully amplified and that the PCR conditions used in the study were reliable.

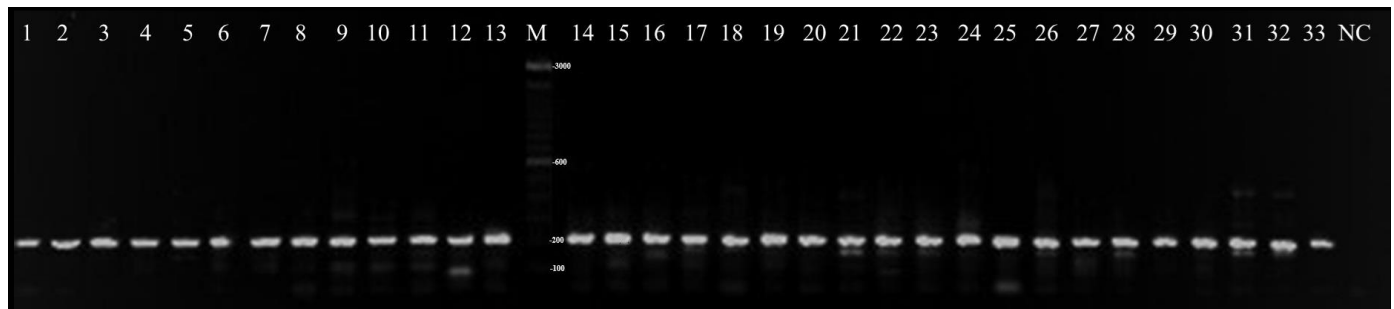


Figure 1. Agarose gel electrophoresis of PCR products for the *18S rRNA* gene region of *Colchicum* specimens (1-33). M: molecular weight marker (100-3000 bp); NC: negative control.

Şekil 1. *Colchicum* örneklerinin (1-33) *18S rRNA* gen bölgesine ait PCR ürünlerinin agaroz jel elektroforezi. M: moleküler ağırlık markeri (100-3000 bp); NC: negatif kontrol.

The sequence information for 33 *Colchicum* specimens is given in Appendix 1. The lengths of the *18S rRNA* sequences used for the analyses ranged from 131 to 145 bp. GC content is 52.5%. The identification of species was carried out through nucleotide blast, where the highest similarity score reached 97.76%, and the E value was minimal at  $8e-56$ . The sequencing data has been registered in the BOLD data system under the CSDB project code ([www.boldsystems.org](http://www.boldsystems.org)).

Before performing the phylogenetic analyses, a multiple sequence alignment was conducted to assess the genetic variation among the *Colchicum* species, as shown in Figure 2. This figure presents the multiple sequence alignment of various *Colchicum* species, highlighting the nucleotide variations within the *18S rRNA* region. This alignment provides insights into the genetic diversity among the species and supports the efficacy of *18S rRNA* as a suitable barcode for species identification.

### Phylogenetic analysis

UPGMA tree based on *18S rRNA* sequence data

identified 33 *Colchicum* specimens, and each was well supported with high bootstrap values (50%-100%). The phylogenetic tree (Figure 3) was grouped by seasonal state, with each group represented by a specific color. Additionally, to distinguish individual species, the nodes were color-coded, with each species assigned a unique color.

The phylogenetic tree provided data for the classification of *Colchicum* species into two primary groups, labeled as Group I and Group II, with two subgroups within each major group. IA-IB and IIA-IIB, respectively (Figure 3). The IA subgroup contains *C. boissieri* Orph., *C. cilicicum*; the IB subgroup contains *C. bivonae* Guss.; the IIA subgroup contains *C. polyphyllum* Boiss. & Heldr.; the IIB group is divided into two branches: IIC and IID. The IIC subgroup contains *C. baytopiorum* C.D. Brickell, *C. stevenii*; the IID group is also divided into two branches: The IIE and IIF. The IIE subgroup contains *C. burttii*, *C. serpentinum* Woron. ex Misch.; the IIF subgroup contains *C. speciosum* Steven, *C. macrophyllum* B.L. Burt, *C. balansae* Planchon.





### Structure analysis

The model displaying the greatest  $\Delta K$  value was chosen as the optimal model for characterizing the population. A genetic identity threshold of  $\geq 0.80$  was adopted to distinguish genotypes as subpopulation individuals within the selected best model (K). Genotypes falling below this threshold were not included in any specific subpopulation and were regarded as genetically mixed individuals (Fukunaga et al., 2005).

In this study, the population structure of the 33 *Colchicum* specimens with the peak of delta K was observed at  $K = 3$ , indicating the presence of three main populations (clusters, Q1–Q3) (Figure 4a). 33 *Colchicum* specimens have membership coefficients of 0.80 and higher and are therefore likely to be pure. The classification of accessions into populations based on this model-based structure is shown in Figure 4b. The

STRUCTURE analysis corroborated the findings of the dendrogram analysis, revealing the presence of three distinct clusters within the population. Out of the 33 specimens, there were 3 genotypes in the first subpopulation (19, 20, 21), 12 genotypes in the second subpopulation (28, 6, 9, 1, 5, 29, 4, 3, 30, 8, 2, 7), and 6 genotypes in the third subpopulation (13, 14, 15, 16, 17, 18). The first subpopulation consists of 10 genotypes (11, 31, 33, 22, 32, 12, 10, 27, 26, 25) and is a combination of genotypes from both the first and second populations. The two remaining genotypes in the second subpopulation, 23 and 24, are a combination of the genotypes from the first and second populations. The combination of genotypes from both the first and second populations includes 10 genotypes (11, 31, 33, 22, 32, 12, 10, 27, 26, 25), while the remaining two genotypes, 23 and 24, are also a blend of genotypes from the first and second populations.

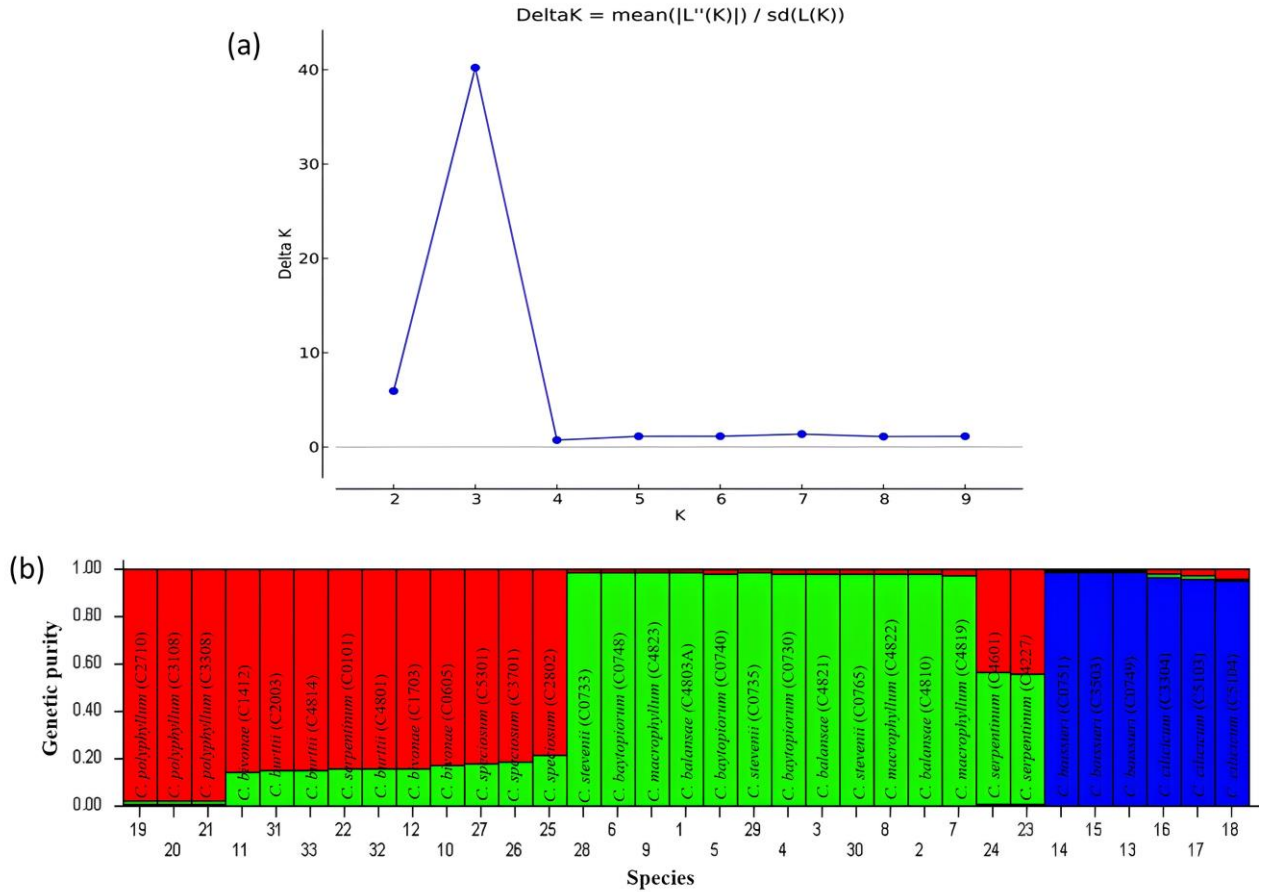


Figure 4. Results of the clustering analysis performed using STRUCTURE Version 2.3.4 (a) Delta K values for various assumed population numbers (K) in the STRUCTURE analysis, and (b) The Q-plot illustrating the clustering of 33 *Colchicum* specimens at the  $K = 3$  clustering level using the set of discriminative single-nucleotide polymorphisms (SNPs). Different subpopulations are represented by distinct colors (first subpopulation: red, second subpopulation: green, third subpopulation: blue).

Şekil 4. STRUCTURE Versiyon 2.3.4 kullanılarak gerçekleştirilen kümeleme analizi sonuçları (a) STRUCTURE analizinde varsayılan farklı popülasyon sayıları (K) için Delta K değerleri ve (b) Ayırt edici tek nükleotid polimorfizmleri (SNP'ler) kullanılarak  $K = 3$  kümeleme düzeyinde 33 *Colchicum* örneğinin kümelemesini gösteren Q-plot. Farklı alt popülasyonlar farklı renklerle temsil edilmiştir (birinci alt popülasyon: kırmızı, ikinci alt popülasyon: yeşil, üçüncü alt popülasyon: mavi).



## DISCUSSION

As in this study, many studies have demonstrated the effectiveness of the *18S rRNA* gene in plant barcoding. Dong et al. (2003) used DNA sequences from the *5S rRNA* spacer, the *ITS* region, and the *18S rRNA* coding region to identify the phylogenetic relationships of ten *Astragalus* species generally found in China. Their research indicated the highest sequence similarity between *A. membranaceus* and *A. membranaceus* var. *mongholicus*, whereas other *Astragalus* species exhibited less significant genetic relationships. Safhi et al. (2022) demonstrated the efficiency of DNA barcoding using nuclear regions (*ITS* and *18S rRNA*) for the identification of *C. gileadensis* accessions. Aykut (2020) contributed to the field of DNA barcoding by highlighting the importance of using *rDNA* regions for identifying and evaluating relationships of taxonomically problematic species, specifically *Quercus* species in Türkiye. The study found that both regions include intergenic spacer (*IGS*), *5S rRNA* genes, and *ITS1*, *ITS2*, *18S rRNA*, *5.8S rRNA*, and *25S rRNA* genes, which are useful tools for DNA barcoding of *Quercus* species. Based on the study by Bae (2009), the region containing three genes, including the *18S rRNA* gene, of *Atractylodes japonica* Koidz was successfully amplified and sequenced, revealing a close phylogenetic relationship with other members of the Compositae family. Yang et al. (1999) estimated the phylogenetic relationships among nine genera in four tribes of the Brassicaceae family using nuclear ribosomal DNA sequences, including the *18S rRNA* gene, revealing distinct groupings and suggesting closer relationships between certain genera. Soltis et al. (2000) conducted a phylogenetic analysis of 560 angiosperms and seven outgroups using a combined data set that included the *18S rDNA* gene. Their study provided the most highly resolved and strongly supported topology for angiosperms to date.

So far, there has been no study to identify *Colchicum* species with *18S rRNA* gene region. However, there are identifications of *Colchicum* species in the literature by both morphological and molecular methods. In the differentiation of *Colchicum* species, it is seen that markers such as AFLP and RAPD and gene regions such as *ITS*, *trnL-trnH* are frequently used on a molecular basis. Several studies have investigated the identification of *Colchicum* species using molecular markers. Karakas et al. (2014) conducted molecular analyses of *Colchicum* species using AFLP molecular marker from Türkiye, contributing to a better understanding of genetic variations within the genus. Persson et al. (2011) analyzed the phylogenetic relationships among *Colchicum* species using *trnL-trnF IGS*, *trnL intron*, *trnH-psbA IGS*, *trnY-trnD IGS*, *atpB-rbcL IGS*, and *rps16 intron* plastid regions, morphological and

chromosomal characteristics, and non-coding chloroplast DNA sequence data. Sahin et al. (2020) used the *trnL-trnF* chloroplast gene region and the *ITS* nuclear gene region to determine the phylogenetic relationships of 52 *Colchicum* species. Consistent with the research findings, the *ITS* and *trnL-trnF* analyses revealed a close relationship between *C. baytopiorum* C.D. Brickell, and *C. stevenii*. Also, the *trnL-trnF* phylogenetic tree supported the findings of Persson et al. (2011) that *C. balansae* Planchon and *C. macrophyllum* B.L. Burt were close species in the same clade. Similarly, this analysis revealed the close relationship of these two species. Tuyel et al. (2020) assessed genetic variation patterns in 16 *Colchicum* species, utilizing RAPD markers and analyzing the chloroplast DNA sequence *trnL-trnF*. This study confirmed conclusions of Tuyel et al. (2020) about the relationship between *C. speciosum* Steven and *C. balansae* Planchon based on RAPD data. Gandhi et al. (2023) utilized DNA barcoding to accurately identify two varieties of *Colchicum*, specifically *Colchicum autumnale* L. and *Colchicum luteum* Baker. The DNA barcodes used in this study included the *rbcL* and *psbA-trnH* regions, which were effective in distinguishing between the two species. This method proved essential in differentiating the species, especially given the lack of prior literature on the DNA barcoding of *Colchicum luteum* Baker.

Sahin et al. (2021) examined the floristic diversity of the *Colchicum* genus in Türkiye by analyzing 52 species based on 36 morphological traits using principal component analysis (PCA). Their study highlighted significant morphological differences, particularly in flowering time, perigon tube structure, and leaf characteristics, aiding in species characterization. They recommended the need for further investigation to gain a complete understanding of the biogeography and evolutionary aspects of these species. Sahin et al. (2021) found the same seasonal patterns in species grouping in their morphological characterization analysis utilizing the same samples as in this study. When comparing this study with Sahin et al. (2021) in terms of the relationship among species, it was determined that in both studies, *C. baytopiorum* C.D. Brickell and *C. stevenii*; *C. burttii* Meikle and *C. serpentinum* Woron. ex Miscz.; *C. speciosum* Steven and *C. balansae* Planchon were found to be closely related to each other. Furthermore, in both studies, *C. polyphyllum* Boiss. & Heldr. From autumn species were found to be closer to *C. baytopiorum* C.D. Brickell and *C. stevenii* Kunth species from late-autumn species. The findings demonstrated concurrence between the morphological and genetic identification approaches.

When comparing the morphological study by Düşen and Sümbül (2007) on *Colchicum* species with the

similarities observed in this study, *C. speciosum* Steven and *C. cilicicum* (Boiss.) Dammer as well as *C. balansae* Planchon and *C. cilicicum* (Boiss.) Dammer was identified as similar species in their research. However, in this study, *C. cilicicum* (Boiss.) Dammer is grouped distantly from *C. balansae* Planchon and *C. speciosum* Steven, while *C. balansae* Planchon and *C. speciosum* Steven are clustered on the same branch (IIF). Additionally, while Düşen and Sümbül (2007) classified *C. serpentinum* Woron. ex Misch and *C. stevenii* as closely related species, this study finds them on the same main branch but in different sub-branches, with *C. serpentinum* Woron. ex Misch in IIE and *C. stevenii* in IIC.

In this preliminary study, analysis of the complementary phylogenetic tree and STRUCTURE results revealed that each sample clustered within its respective species. Furthermore, species occurring in late autumn and winter formed distinct clusters corresponding to their seasonal groups. Notably, autumn species also displayed close clustering, except *C. polyphyllum* Boiss. & Heldr., which deviated from this pattern. In the STRUCTURE analysis, *C. polyphyllum* Boiss. & Heldr. (first subpopulation) was observed to form a distinct group within its species, independent of the other species. Within the phylogenetic tree, *C. cilicicum* (Boiss.) Dammer and *C. boissieri* Orph. are closely grouped in group 1A. This clustering is corroborated by the STRUCTURE analysis, which identifies these species within the third subpopulation. *C. stevenii* Kunth and *C. baytopiorum* C.D. Brickell are classified together in the IIC group on the phylogenetic tree and in the second subpopulation in the STRUCTURE analysis. Additionally, the STRUCTURE analysis, which assigns *C. balansae* Planchon and *C. macrophyllum* B.L. Burt to the second subpopulation, supports the close grouping of these species within group IIF in the phylogenetic tree. This research also highlighted the close relationships among *C. stevenii* Kunth and *C. baytopiorum* C.D. Brickell; *C. balansae* Planchon, *C. speciosum* Steven, and *C. macrophyllum* B.L. Burt; and *C. burttii* Meikle and *C. serpentinum* Woron. ex Misch. These findings are consistent with previous studies on the identification of *Colchicum* species.

## CONCLUSION

This study represents a preliminary effort to elucidate the phylogenetic relationships among *Colchicum* specimens using the *18S rRNA* gene region. While the findings provide valuable initial insights, they also highlight the need for further research incorporating additional molecular markers to enhance the robustness and accuracy of the phylogenetic analyses. Future studies should consider using multiple barcode genes, such as *matK*, and *rbcL*, to obtain a more comprehensive understanding of the genetic diversity

and evolutionary relationships within the *Colchicum* genus. This approach will enable more detailed and reliable reconstructions of species relationships, ultimately contributing to a more nuanced understanding of the taxonomy and evolution of this important plant group.

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## Contribution Rate Statement Summary of Researchers

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

## Conflict of Interest

The authors have declared no conflict of interest.

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## Effects of Some Bacterial Isolates on Vase Life of Cut Rose Flowers

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### ABSTRACT

To reduce the impact of rapid temperature changes during transportation on cut flowers, continuous cooling should be provided throughout the process. The frigid transportation conditions required to transport cut flowers from their production facilities to the point where they will be marketed are quite expensive. Preliminary applications that can significantly reduce this cost should be made. One of these applications is the use of symbiotic psychrotolerant bacteria, which thrive at low temperatures, to improve the transportation and shelf life of cut flowers. Cut roses are commercially transported and stored at +2-4°C. In this study, the application of psychrotolerant bacteria isolated from plants aims to investigate their effects on the transportation and vase life of cut roses under unfavorable transport temperature conditions (+10 °C), aiming to minimize potential quality losses. For this objective, 18 bacterial strains were isolated from the leaf apoplasts of cold-resistant wild plants. These strains, which exhibit cold tolerance and the ability to block ethylene synthesis in plants, were used both separately and in combination. Bacterial solutions were applied to cut rose leaves (*Rosa hybrida* L. cv. Samourai), and all treatment groups were kept in controlled conditions at +10°C for 9 days. Control 1 and Control 2 were treatments in which only pure water was sprayed on the green leaves of cut roses at temperatures of +2 °C and +10 °C, respectively. At the end of the experiment, the cut roses were evaluated according to vase life, fresh weight, visual quality, and cold damage (CD). The longest vase life was determined in D3 (DT-10 isolate-*Bacillus cereus*), D4 (DT-11 isolate-*Bacillus cereus*), M1 (DT-17 isolate-*P. proteolytica*) and K1+B8 (*Bacillus cereus* + *S. kitahiroshimense*) applications. These applications, which have obtained the highest vase lifetimes, and B5, B8, D1, D2, K3, Ç1, Ç2, M2, and D1 + Ç1 applications were in the same statistical group. The D3, D4, M1, and K1+B8 treatments increased the vase life of cut roses by 55.55% compared to Control 2. Control 1 and D1+Ç1 treatments showed the highest relative fresh weight increase in 66.06% and 64.28%, respectively. Among the groups, the D2 (DT-6 isolate-*Bacillus cereus*) had the greatest visual quality score. The lowest CD% was observed in the application involving the D2 isolate, correlating with the highest visual quality determined under the same D2 application. The study revealed that postharvest application of *Bacillus cereus* isolates (especially DT-6 and DT-10) to cut roses increased their vase life.

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## Bazı Bakteriyel İzolatların Kesme Gül Çiçeklerinin Vazo Ömrüne Etkileri

### ÖZET

Kesme çiçeklerin, taşıma sırasındaki ani sıcaklık değişimlerinden daha az etkilenmeleri için taşıma sürecinde kesintisiz soğutma yapılması gerekmektedir. Kesme çiçeklerin üretim yerlerinden pazarlanacakları noktaya taşınmasında gerekli olan soğuk taşıma koşulları çok maliyetli olmaktadır. Bu maliyetin azaltılmasında etkili olabilecek ön uygulamalar yapılmalıdır. Bu uygulamalardan biri düşük sıcaklıklarda yaşayabilen psikrotolerant bakterilerin kesme çiçeklerin taşınması ve

### Bahçe Bitkileri

### Araştırma Makalesi

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sonrasında raf ömrünün uzatılmasında uygulanabilmesidir. Kesme güller ticari olarak +2-4°C'de taşınmakta veya depolanmaktadır. Bu çalışmada, bitkilerden izole edilen psikrotolerant bakterilerin uygulanmasının, kesme güllerin uygun olmayan taşıma sıcaklığı koşullarında (+10 °C) taşınması ve vazo ömrü üzerindeki etkilerini araştırmak ve olası kalite kayıplarını minimuma indirmek amaçlanmıştır. Bunun için soğuğa dayanıklı yabancı bitkilerin yaprak apoplastından izole edilen, soğuğa tolerans gösteren ve etilen sentezini inhibe etme yeteneği gösteren 18 bakteri suşu hem ayrı ayrı hem de kombinasyon halinde hazırlanmıştır. Daha sonra kesilmiş kesme güllerin (*Rosa hybrida* L. cv. Samourai) yapraklarına bakteriyel solüsyonlar uygulandı ve tüm uygulama grupları 9 gün boyunca +10°C'de kontrollü koşullar altında tutulmuştur. Kesme güllerin yeşil yapraklarına sadece saf su püskürtülen kontrol grubu uygulamalar olan Kontrol 1 Kontrol 2 uygulaması, sırası ile +2 °C ve +10 °C sıcaklık koşullarında bekletilmişlerdir. Deneme sonunda, kesme güller vazo ömrü, taze ağırlık, görsel kalite ve soğuk hasarına (CD) göre değerlendirildi. En uzun vazo ömrü D3 (DT-10 izolatı- *Bacillus cereus*), D4 (DT-11 izolatı- *Bacillus cereus*), M1 (DT-17 izolatı- *P. proteolytica*) ve K1+B8 (*Bacillus cereus* + *S. kitahiroshimense*) uygulamalarında belirlendi. En yüksek vazo ömrü elde edilen bu uygulamalar ile B5, B8, D1, D2, K3, Ç1, Ç2, M2 ve D1+Ç1 (karışık) uygulamaları aynı istatistiksel grupta yer almıştır. D3, D4, M1 ve K1+B8 uygulamaları Kontrol 2 ile karşılaştırıldığında kesme güllerin vazo ömrünü %55,55 oranında arttırmıştır. En yüksek bağıl taze ağırlık artışı Kontrol 1 (%66,06) ve D1+Ç1 (%64,28) uygulamalarında tespit edildi. Gruplar arasında en yüksek görsel kalite puanını bakteriyel uygulama D2 (DT-6 isolate- *Bacillus cereus*) almıştır. En düşük CD% değeri D2 bakteri izolatının kullanıldığı uygulamada belirlenmiştir. En iyi görsel kalite puanı da minimum CD%'nin gözlemlendiği D2 uygulamasında saptanmıştır. Çalışmada, kesme güllere hasat sonrası *Bacillus cereus* izolatları (özellikle DT-6 ve DT-10) uygulanmasının, vazo ömrünü artırdığı ortaya koyulmuştur.

#### Anahtar Kelimeler

Apoplast  
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Kesme çiçek  
Taşıma

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## INTRODUCTION

Cut flowers, a key commodity in the ornamental plant industry, rank as the most traded group globally. The most crucial factor in facilitating the trade of these products is their ability to be mass-produced and easily transported (Barlas et al., 2019). The prolonged vase and/or shelf life significantly influences the pricing of both cut flowers and potted plants. Disruptions during transportation, a final stage from sowing to consumer reach, can lead to a loss of value or render the flowers less valuable than their production cost. This disturbance also impacts consumer demand and satisfaction with cut flowers (Onozaki et al., 2001). It has been reported that 25% of cut flowers produced in the world are lost during the transportation and storage process (Ünsal, 2022). In cut flower production for domestic consumption in our country, it is estimated that the quality loss rate of cut flowers that

occurs during the transportation and storage process is around 30-50%, especially due to the inability to provide a cold chain. However, according to research conducted with exporters, it was reported that the highest cost during cut flower production belongs to transportation costs at 35% (Sönmez, 2012). It has been reported that the average vase life of different types of cut flowers decreases by 32% after 4 days of transportation (Kazaz, 2015).

Studies conducted on various cut flowers have shown that post-harvest lifespan depends on the respiration rate, and flower lifespan decreases with increased respiration. Respiratory speed increases in direct proportion to temperature. For these reasons, the temperature after production or harvest must be reduced to the lowest temperature at which the flower or plant will not be damaged, and it must be ensured that the cold chain is not broken during storage and

transportation throughout all marketing channels. The temperature just above the freezing point, 0°C, is the appropriate storage temperature for many products. Low temperatures can control metabolism, reduce the consumption of stored compounds and the amount of water lost through respiration, and limit the development of pathogens (Da Silva, 2003; Jahnke et al., 2020). Additionally, low temperatures between 0 °C and 2 °C are recommended for storing rose-cut flowers (Jahnke et al., 2022). Despite all these justifications and requirements, cut flowers and potted plants are generally stored or transported at high temperatures in various marketing channels. This situation reduces quality by increasing water loss and accelerating metabolism and ultimately causes damage to everyone, from the flower producer to the consumer. Uninterrupted cooling is required for cut flowers during the transportation process so that they are less affected by sudden temperature changes during transportation (Çelikel, 2020). The cold transport conditions required to move cut flowers from production areas to market points are very costly and often overlooked. To effectively reduce these costs, preventive measures that are often deemed necessary are implemented. One of these measures is the use of psychrophilic (cold-loving) bacteria in the transport of cut flowers. These bacteria thrive at low temperatures (-5 to 15 °C) and exhibit strong adaptations to tolerate such conditions (Arda, 2000). Some psychrophilic microorganisms were isolated from various environments such as seas, oceans, soil, fish, and vegetables (Graumann, 1996). For example, psychrophilic bacteria isolated from subalpine soil in the Northwestern Indian Himalayas have been shown to increase cold tolerance in wheat (Selvakumar et al., 2008).

Additionally, such bacteria have also been shown to exist in apoplastic areas of certain plants. The apoplast is a continuous space in plants formed by inter and extracellular spaces, cell walls, and dead cells (e.g. xylem) that lie outside the cell membranes. For a plant, the apoplast is a dynamic region where most processes such as growth, nutrition, signal perception, and stress response regulation occur (Atici & Nalbantoglu, 1999). Certain psychrophilic bacteria living symbiotically in the apoplast of some plants can significantly contribute to the plant's abiotic stress tolerance. For example, Tiryaki et al. (2019) demonstrated the success of psychrophilic bacteria isolated from the leaf apoplasts of 14 cold-tolerant wild plants and 2 different crops in increasing cold stress tolerance in beans (*Phaseolus vulgaris* L.). On the other hand, studies have reported that bacteria extend vase life, depending on the type of bacteria present in the vase solution (van Doorn et al. 1991; Jacob & Kim, 2010; Carlson et al., 2015). For instance, two strains of *Pseudomonas fulva* and *Escherichia coli*, also known as biocontrol bacteria,

increased the vase life of cut *Zinnia elegans* (Carlson et al., (2015). Naing et al. (2017) also noted that *Enterobacter cloacae* was able to extend the vase life of cloves by 3 days and played an important role in the biological control of microorganisms causing petal senescence. According to our knowledge, there is no existing research attempting to determine the impact of psychrophilic Plant Growth-Promoting Bacteria (PGPB) isolated from cold-resistant wild plants on the vase life of cut flowers in cut flower transportation.

The cold transportation conditions required to transport cut flowers from their production sites to the point where they will be marketed are very costly. Preliminary applications that can be effective in reducing this cost should be made. One of these applications is that psychrophile (cold-loving) bacteria, which can live at low temperatures and have high adaptations to tolerate this, can be applied to the transportation of cut flowers. Cut roses are commercially transported and stored at +2-4 °C. The main purpose of this research is to examine the effects of treating cut roses with psychrophile bacteria and then transporting them under inappropriate transportation temperature conditions (+10 °C) on the vase life of cut roses and reducing possible quality losses. For this purpose, in our study, the effects of 18 different bacterial isolates with psychrophilic properties isolated from the leaf apoplast (intercellular) of some cold-resistant wild plants were investigated in extending the vase life and reducing quality losses that may occur during the transportation of cut roses. The results of this study are believed to contribute to reducing quality and plant losses during transportation, a crucial issue in the cut flower sector, while also aiding in lowering transportation and storage costs and extending the vase life of cut flowers.

## MATERIAL and METHOD

### Plant Material

The research was carried out, between April and December 2022, in the climate chamber and application laboratory of the Atatürk University Faculty of Science Department of Biology. The red standard type 'Samourai' cut rose cultivar belonging to the *Rosa hybrida* L. species was used as plant material. According to the catalog data of the breeder company, the petal color of the Samourai variety is red, the flower stem length is 80-90 cm, the vase life is 10-12 days, the bud length is 5.0-6.0 cm, the number of petals is 35-40 (Anonymous, 2022). In the world cut rose trade, the market share of red varieties is 30%. In Turkey, this rate is 80% (Ünsal, 2022). In addition, since the Samourai cultivar is among the most-grown rose varieties in Turkey, it was chosen as the plant material in the research.



## Harvesting Cut Flowers

Cut rose flowers were harvested early in the morning from the greenhouse of a company that produces commercial cut roses in Antalya (Turkey) on 11 November 2024, at commercial harvest maturity (as the petals begin to curl back) (Ueyama & Ichimura, 1998). After harvesting, it was soaked in water for 3-4 hours. The cut roses were given to an agreed bus

company within the same hours, and they reached us within 22 hours by road. No cooling conditions are provided for bus transportation. It was ensured that the cut roses have as many leaves as possible, their leaves are not cleaned, and they are packaged in a way that will minimize damage during transportation (Figure 1). The experiment was set up by applying the cut flowers as soon as they arrived.



Figure 1. The cut roses were packaged in a way (placed in perforated cardboard boxes) that will minimize damage during transportation

Şekil 1. Kesilen güller taşıma esnasında zarar görmeyecek şekilde (delikli karton kutulara konularak) paketlenmiştir

## Preparation of Bacterial Isolates

In order to isolate psychrophilic bacteria, 14 wild and 2 cultivated plants (Table 1) species resistant to cold were utilized, collected from Mount Palandöken in Erzurum Province, Atatürk University campus (day/night temperatures of  $-1^{\circ}\text{C}/-20^{\circ}\text{C}$ ), and the city center of Erzincan (day/night temperatures of  $5^{\circ}\text{C}/-2^{\circ}\text{C}$ ) (Tiryaki et al., 2019). Bacteria were isolated from the leaf apoplasts of these plants and after purification, species identification was made according to 16S rRNA sequence analysis and Vitek technique. Twenty plant growth-regulating bacteria (PGPB) isolates belonging to 10 bacterial species were identified from the leaf apoplast of plants (Table 1). These are *Sphingobacterium faecium* (isolates: DT-1, DT-2, DT-3, DT-9, DT-15), *S. kitahiroshimense* (DT-4), *Staphylococcus intermedius* (DT-5), *Bacillus cereus* (DT-6, DT-10, DT-11, DT-12), *Pseudomonas fragi* (DT-7, DT-8), *P. chloropaphis* (DT-14), *P. fluorescens* (DT-16, DT-18), *P. proteolytica* (DT-17, DT-19), *Raoultella ornithinolytica* (DT-13), *Brevibacterium frigoritolerans* (DT-20) (Tiryaki et al., 2019). These bacterial isolates were freshly cultured one week before the cut roses arrived and were prepared separately in liquid media. The bacterial concentration of each solution was adjusted to  $10^8$  CFU/ml.

## Post-harvest Carrying out Pre-Treatment

The flowers, which were harvested at a length of 70 cm in the producer's greenhouse and brought to the processing house, were packaged and placed in perforated cardboard boxes and brought by road to the

laboratory (Erzurum) where the study would be carried out within approximately 22 hours. The bottom parts of the flowers brought to the laboratory were cut at an angle to a length of 2.5 cm.

## Application of Bacterial Solutions

Application groups consisted of 18 bacterial isolates and 2 control groups (pure water), each with three repetitions. The solutions from the application groups were sprayed to fully moisten the green leaves of cut roses. Applications were made once to the cut roses. The control-2 received no bacterial application; only pure water was sprayed on the green leaves, and these were placed at  $+10^{\circ}\text{C}$  in the dark (Figure 2a-f). Following the completion of the spraying process, the cut roses were enveloped in paper packages and positioned in perforated boxes. These boxes were then situated in a climate chamber adjusted to environmental conditions ( $+10^{\circ}\text{C}$  in the dark) within the transport vehicle. Concurrently, another application group was prepared, where roses were kept in the dark, enclosed in paper packages, and placed in perforated boxes at  $0^{\circ}\text{C}$  (Çelikel, 2020), just above the freezing point, with pure water sprayed solely on the leaves (Figure 2a-f). The applications and their corresponding codes are detailed in Table 2. The roses earmarked for application were stored in water. For 5 days, cut roses were kept at both temperatures ( $+10^{\circ}\text{C}$  and  $0^{\circ}\text{C}$ ). On the fifth day, to initiate the vase life experiment (Figure 2 g-h), the lower portions of the flowers were cut at an oblique angle to a length of 2.5 cm, and any remaining leaves in the vase water were manually removed.



Table 1. Bacteria and the plants from which they were isolated (Tiryaki et al. 2019).

Çizelge 2. Bakteriler ve izole edildikleri bitkiler (Tiryaki ve ark. 2019).

Plant	Bacteria	Isolate	Plant	Bacteria	Isolate
<i>Onosma isauricum</i>	<i>Sphingobacterium faecium</i>	DT-1	<i>Fragaria vesca</i>	<i>Bacillus cereus</i>	DT-11
<i>Verbascum cheiranthifolium</i>	<i>Sphingobacterium faecium</i>	DT-2	<i>Taraxacum sieheanum</i>	<i>Bacillus cereus</i>	DT-12
<i>Chenopodium botrys</i>	<i>Sphingobacterium faecium</i>	DT-3	<i>Galanthus gracilis</i>	<i>Raoultella ornithinolytica</i>	DT-13
<i>Chenopodium foliosum</i>	<i>Sphingobacterium kitahiroshimense</i>	DT-4	<i>Galanthus gracilis</i>	<i>Pseudomonas chloropaphis</i>	DT-14
<i>Myosotis alpestris</i> sp.	<i>Staphylococcus intermedius</i>	DT-5	<i>Galanthus gracilis</i>	<i>Sphingobacterium faecium</i>	DT-15
<i>Capsella bursa-pastoris</i> sp.	<i>Bacillus cereus</i>	DT-6	<i>Colchicum speciosum</i>	<i>Pseudomonas fluorescens</i>	DT-16
<i>Artemisia austriaca</i>	<i>Pseudomonas fragi</i>	DT-7	<i>Colchicum speciosum</i>	<i>Pseudomonas proteolytica</i>	DT-17
<i>Draba nemorosa</i>	<i>Pseudomonas fragi</i>	DT-8	<i>Scilla siberica</i>	<i>Pseudomonas fluorescens</i>	DT-18
<i>Raphanus raphanistrum</i>	<i>Sphingobacterium faecium</i>	DT-9	<i>Scilla siberica</i>	<i>Pseudomonas proteolytica</i>	DT-19
<i>Trifolium repens</i>	<i>Bacillus cereus</i>	DT-10	<i>Erodium cicutarium</i>	<i>Brevibacterium frigoritolerans</i>	DT-20

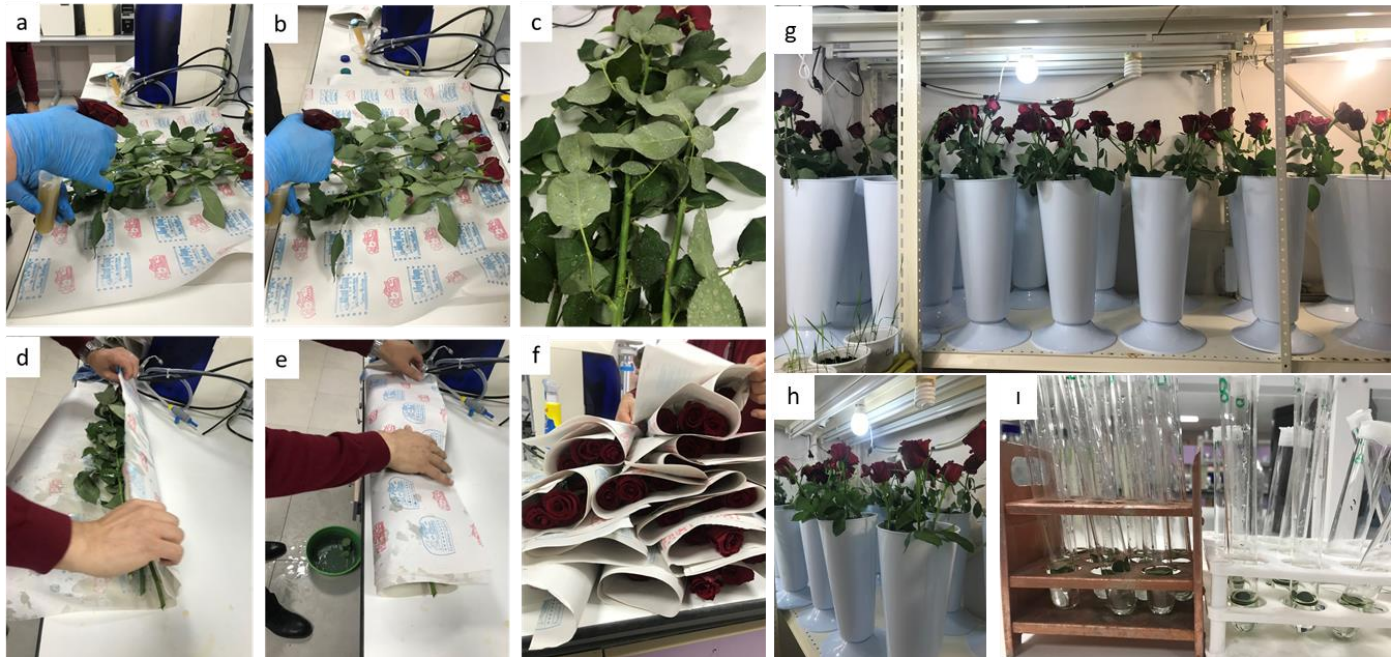


Figure 2. Spraying the leaves of cut roses with application solutions until they are completely wet, then wrapping them in paper again and placing them in a dark environment at +10°C (a-f); roses removed on the 5th day of bacterial treatment and placed in vases in climate chamber conditions (g-h); stage of construction process determining the % cold damage on leaves (i)

Şekil 2. Kesme güllerin yapraklarına tamamen ıslanacak şekilde uygulama solüsyonları püskürtülerek tekrar kağıda sarılarak +10 °C'de (a-f) karanlık ortama hazırlanması; güller bakteri uygulamalarının 5. gününde çıkarılıp iklim odası koşullarında vazolara yerleştirildi (g-h); yapraklarda soğuktan kaynaklanan zararın %'sini belirleme aşaması (i)

### Conditions for Determining Vase Life

The vase life of flowers was assessed under controlled conditions in a growth chamber with a temperature of 21±2.0°C, relative humidity of 65±5%, light intensity of 1000 lux, and a day length of 12 hours (Ueyama & Ichimura, 1998; Lü et al., 2010). The vases utilized for the vase life studies had a volume of 5 liters and were filled with 3 liters of pure water. Each vase

accommodated three cut roses (Figure 2 g-h).

### Parameters Examined in Cut Flowers

**Vase life (days):** Vase life for cut rose flowers are accepted as the number of days from the day the flowers are placed in the vase (start) to the day when the petals begin to fade, and the flower necks begin to bend (Ichimura et al. 1999; Lü et al. 2010).

Table 2. The applications and codes used in the study.

Çizelge 3. Çalışmada yapılan uygulamalar ve kodları.

Applications	Bacteria	Temperature / Light
1 Control-1		+2 °C Darkness
2 Control-2		10 °C Darkness
3 B1 (DT-1 isolate)		
4 B3 (DT-2 isolate)		
5 B4(DT-3 isolate)	<i>Sphingobacterium faecium</i>	10 °C Darkness
6 B5 (DT-9 isolate)		
7 B6 (DT-15 isolate)		
8 B8 (DT-4 isolate)	<i>S. kitahiroshimense</i>	10 °C
9 D1 (DT-5 isolate)	<i>Staphylococcus intermedius</i>	Darkness
10 D2 (DT-6 isolate)		
11 D3 (DT-10 isolate)		
12 D4 (DT-11 isolate)	<i>Bacillus cereus</i>	10 °C Darkness
13 K1 (DT-12 isolate)		
14 K2 (DT-7 isolate)		
15 K3 (DT-8 isolate)	<i>Pseudomonas fragi</i>	10 °C Darkness
16 G1 (DT-14 isolate)	<i>P. chloropaphis</i>	10 °C Darkness
17 Ç1 (DT-16 isolate)		
18 Ç2 (DT-18 isolate)	<i>P. fluorescens</i>	10 °C Darkness
19 M1 (DT-17 isolate)		
20 M2 (DT-19 isolate)	<i>P. proteolytica</i>	10 °C Darkness
21 K1+B8 [(DT-12 isolate) + (DT-4 isolate)]	<i>Bacillus cereus</i> + <i>S. kitahiroshimense</i>	10 °C
22 D1+Ç1 [(DT-5 isolate) + (DT-16 isolate)]	<i>Staphylococcus intermedius</i> + <i>P. fluorescens</i>	Darkness

**Visual quality:** Fading, darkening, bluing, curling backwards, drying and falling off of the petals of all flowers, blooming and bending of the flowers, and yellowing, drying and falling off of the leaves were taken into consideration and evaluated on a 1-5 scale (Score 1: very bad, Score 2: bad, Score 3: medium, Score 4: good, Score 5: very good). Visual quality assessment was made on the 8th day of the flowers' vase life.

**Viability Test (Membrane permeability):** By measuring cold damage (%) in control and bacteria-applied plant leaves, the role of the applied bacteria in preventing cold damage in the leaves was determined (Figure 2i). The determination of this parameter was carried out as follows: 0.1 g of fresh leaf sample was placed in each of the 20 test tubes. The tubes were placed collectively in a water bath that could be

adjusted up to -16°C (alcohol mixed with water was added to prevent the interior of the bath from freezing) and all tubes were kept for 10 minutes at each degree from -1 to -16°C. 4 ml of pure water was put into each tube taken from the bath and these tubes were kept at 4°C for 24 hours. Later, the amount of ions transferred to pure water by thawing the frozen leaf in these tubes was measured with an electrical conductometer and the values were converted to cold damage according to the method of Griffith et al. (1992) and Taşgın et al. (2003).

**Fresh weight (FW):** Branch weights of the flowers used in the experiment were calculated daily by taking the difference between the weights of vases with and without flowers on a digital scale sensitive to 0.01 g. During the vase life of the flowers, proportional fresh

weight measurements were made at 2-day intervals (Day 1, Day 3, Day 5, Day 7, and Day 9). The following formula was used to calculate the proportional fresh weight (He et al., 2006).

$$FW (\%) = (At/At=0) \times 100$$

At: Branch weight on day t (e.g. 1st, 2nd, 3rd, etc.)

At=0: Initial (day 0) weight of the branch

### Statistical Analysis

The study was set up with 3 replications according to the Randomized Plot Trial Design and a total of 189 flowers were used, 3 flowers in each replication. The numerical data obtained were subjected to analysis of variance (ANOVA) using the IBM SPSS version 25.0 package program, and the Duncan multiple comparison method ( $p < 0.05$ ) was used to determine the differences between the averages.

## RESULTS and DISCUSSION

**Vase life:** In the ornamental plants sector, the vase life of cut flowers is one of the fundamental criteria for

evaluating quality. The vase life of cut rose flowers is generally short due to both wilting of flowers and neck bending (Ünsal, 2022). In this research, the necessary cold transportation conditions were set up (+10 °C) in the transportation of cut rose flowers from their production areas to the point where they will be marketed, and then the bacteria isolated from the leaf apoplast of the cold-tolerant plants (14 wild and 2 cultivated) were applied to the leaves of cut flowers of the rose plant (*Rosa hybrida* L. 'Samourai'). Among the bacterial applications, the lowest vase life was obtained from Control 1, B3, and K1 isolates. The applications with the longest vase life were determined numerically as D3, D4, M1, and K1 + B8. Both petals and green leaves maintained their (in D3, D4, M1, and K1 + B8 applications) vitality until the 9th day when the vase life period was evaluated. These applications, which have obtained the highest vase lifetimes, and B5, B8, D1, D2, K3, Ç1, Ç2, M2, and D1 + Ç1 applications were in the same statistical group (Table 3). When compared to Control 2, D3, D4, M1, and K1 + B8 applications extended the vase life of cut roses by 55.55%.

Table 3. Effects of the bacterial isolates on vase lifetime (days), visual quality scores, and % cold damage on leaves of cut roses

Çizelge 4. Bakteri izolatlarının kesme gül yapraklarında vazo ömrü (gün), görsel kalite puanları ve soğuk zarar yüzdesine etkileri.

Applications	Vase life (days)	Visual quality (1-5 scale)	Damage% ( $\mu$ S)
Control 1 (+2 °C)	1.00±0.000 g ***	1.33	75.55±3.410 <sup>ns</sup>
Control 2 (+10 °C)	5.00±1.000 ef	3.33	50.42±0.530
B1	6.00±1.000 cde	4.33	33.44±2.340
B3	1.00±0.000 g	4.33	29.83±1.880
B4	5.67±0.577 de	3.00	28.33±1.873
B5	8.00±1.732 ab	3.33	20.13±0.650
B6	4.00±1.000 f	3.33	49.25±0.820
B8	8.67±0.577 ab	2.67	23.26±0.280
D1	8.33±1.154 ab	4.67	40.47±0.480
D2	8.67±0.577 ab	5.00	12.63±1.150
D3	9.00±0.000 a	3.33	30.05±1.040
D4	9.00±0.000 a	4.67	32.20±0.934
K1	2.00±1.000 g	1.67	34.37±0.760
K2	7.00±1.000 bcd	3.00	33.62±0.730
K3	8.00±1.732 ab	4.00	24.69±0.730
G1	4.00±1.000 f	1.00	46.39±0.610
Ç1	8.00±1.000 ab	4.67	35.22±0.660
Ç2	7.33±0.577 abc	1.67	37.60±1.470
M1	9.00±0.000 a	3.67	25.07±0.270
M2	8.33±0.577 ab	2.67	99.50±0.866
K1 + B8	9.00±0.000 a	4.67	72.02±1.390
D1 + Ç1	7.67±1.527b	3.33	55.08±0.210
<i>F value</i>	F(25.112)= 0.000		F(741.975)= 0.000

ns: insignificant at  $p > 0.05$ , statistically significant at probability level of \*\*\* $P < 0.001$ . Note: There is no difference between the means indicated with the same letter at the 5% significance level.

Some studies have reported that bacteria extend vase life, depending on the type of bacteria present in the vase solution (van Doorn et al., 1991; Jacob & Kim, 2010; Carlson et al., 2015). Carlson et al. (2015), reported that two bacterial strains (*Pseudomonas*

*fulva* and *Escherichia coli*), also known as biocontrol bacteria, increased the vase life of cut *Zinnia elegans*. Additionally, Naing et al. (2017) reported that *Enterobacter cloacae*, a biocontrol bacterium, can extend the vase life of cloves by 3 days and plays an



important role in the biological control of microorganisms that cause petal senescence. Previous studies were generally carried out by adding beneficial bacteria to the vase solution. In this context, it was not possible to directly compare the present research results because we did not find the relevant literature.

**Visual quality:** Visual quality, the common day when deterioration started in all applications, was the 8th day of vase's life. Therefore, the visual quality parameter was evaluated on the 8th day of vase's life. In the present study, data regarding the visual quality scores of flowers are given in Table 3. It was determined that the flowers received 1 to 5 quality points on the scale created to evaluate the visual quality of the flowers. Among the applications, bacteria application D2 received the highest visual quality score (5 points). D1, D4, Ç1, K1, and K1 + B8 applications received 4.67 from this rating. In these applications, deformations began in both flowers and leaves. In general, cut roses maintained their visual quality criteria in the vase for 9 days with D2, D1, D4, Ç1, K1 and K1 + B8 applications. Control-2 and Ç2 applications dried out due to the climate chamber problem before they were taken into vase life trials, and the flowers belonging to the groups to which B5, B6, K1, and G1 bacterial isolates were applied started to bend before they fully opened, and neck bending continued in the flowers in the following days of vase life.

Some endophytic bacteria that live symbiotically with plants contain ACC deaminase (1-aminocyclopropane-1-carboxylic acid deaminase) enzyme, which prevents the synthesis of ethylene produced in the plant. Thanks to this enzyme, these bacteria suppress ethylene synthesis in the plant, causing positive effects on delaying senescence and longevity. ACC deaminase activity of bacterial isolates isolated from cold-tolerant wild plants used in the research was determined as a result of the research conducted by Tiryaki (2015). It has been reported that there are differences in ACC deaminase activity among isolates and that the bacterial isolate with the highest ACC deaminase activity is K2 [*Brevibacterium frigoritolerans* (DT-20)] (Tiryaki, 2015). As a result of the present research, the applications that received the highest visual quality scores, D2, D1, D4, Ç1, K1 and K1+B8, contain *Staphylococcus intermedius* (D1), *Bacillus cereus* (D2, D4), *P. fluorescens* (Ç1, K1) and *Bacillus cereus* + *S. kitahiroshimense* (K1 + B8) isolates.

**Viability Test:** Electrolyte leakage in tissues increases due to the damage to cell membranes after chilling or freezing as a result of low temperatures (Jha et al., 2019). Due to the increase in membrane permeability, the electrolyte exchange between cells and the external environment increases. Increased electrolyte leakage is directly related to chilling or cold damage (CD%) in

cells and tissues (Campos et al., 2003). In this research, 18 different endophytic bacteria isolated from the leaf apoplast of cold-resistant wild plants and containing enzymes that inhibit ethylene synthesis in the plant were applied. When the CD% values on green leaves given in Table 3 are examined, the highest cold damage was determined in the application of Control 1 (+2 °C). Leaf application of all bacterial isolates was made at +10 °C. In this case, the cold damage determined to be 50% and above was determined in Control-2, M2, K1 + B8, and D1 + Ç1. The lowest CD% was determined in the D2 bacterial isolate (Table 3).

The cold damage parameter on leaves is generally used as a stress parameter. In the study, cold damage levels in leaves have been analyzed to determine stress tolerance or degree of protection from stress damage. In the literature, we have not come across a study that determines the DH% for a plant that is kept at a low temperature such as + 10 °C for a certain period after PGPB is applied to a plant and then kept in a vase at room temperature conditions. Therefore, it is not possible to directly compare the data with a similar study. Studies are determining the effects of PGPBs applied under low-temperature conditions on plant %DH (Ait Barka et al., 2006; Turan et al., 2013; Tiryaki, 2015). However, the present study's subject and purpose are different from these studies and comparisons cannot be made. Psychrophile (cold-loving) bacteria, which can live at low temperatures and have high adaptations to tolerate this, have reproductive temperatures between 0-4°C, and their enzymes can show activity between -5°C and +20°C (Arda, 2000). Many researchers have isolated psychrophile microorganisms from various environments such as seas, oceans, soils, fish, milk, meat, and vegetables (Graumann, 1996). It was concluded that the psychrophile (cold-loving) bacteria used in the present study, especially D2, has been reached and can be applied in the transportation of cut flowers.

It has been shown that psychrophile PGPBs isolated from cold-tolerant wild plants can increase low-temperature tolerance in some plants (Selvakumar et al., 2008). In addition to surviving at extremely low temperatures, "psychrophiles" also secrete active biomolecules that can promote plant growth in low-temperature ranges (Rondón et al., 2019; Yadav et al., 2016; Balcázar et al., 2015). In the D2 application, where the minimum CD% was determined, the best visual quality score was also determined. It is thought that this practice may have played a role in increasing tolerance to water loss in cut roses.

**Fresh weight (FW):** The effect of different bacterial isolate applications on relative fresh weight and the weight changes that occurred during the 9-day vase period are given in Figure 3.



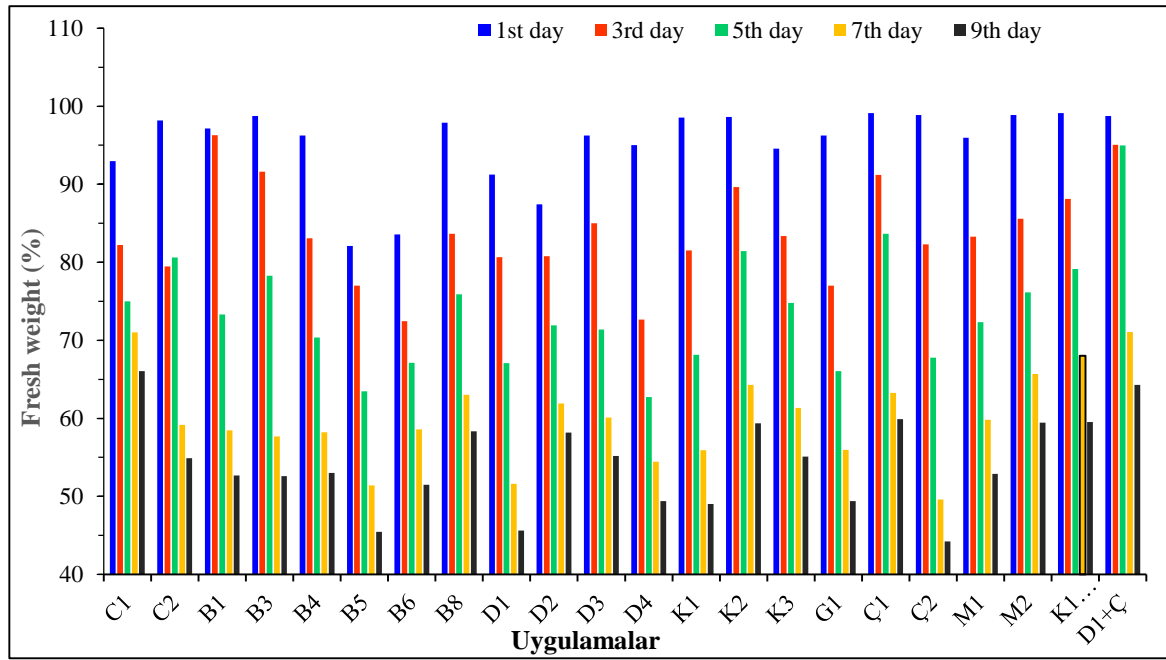


Figure 3. Effects of the bacterial isolates on the relative fresh weight (%) of cut roses. C1: Control (+2 °C); C2: Control (+10 °C). (*F* values: 1<sup>st</sup> day  $F(2.023)=0,24$ ; 3<sup>rd</sup> day  $F(1.290)=0,233$ ; 5<sup>th</sup> day  $F(2.159)=0,016$ ; 7<sup>th</sup> day  $F(1.101)=0,382$ ; 9<sup>th</sup> day  $F(1.225)=0,278$ ). Statistically significant at probability level of  $P<0.05$ .

Şekil 3. Bakteri izolatlarının kesme güllerin bağıl taze ağırlığı (%) üzerine etkileri.

It was determined that the applications were significantly effective ( $p\leq 0.001$ ) on the relative FW change of cut rose. At the end of the first day of vase life, the highest relative FW increase among the applications was determined in all applications except the application groups in which B5 and B6 bacterial isolates were applied. The lowest relative FW increase was seen in the B5 application. At the end of the 9th day of the study, the highest FW weight increase was detected in Control 1 (66.06%) and D1+Ç1 applications (64.28%) (Figure 3).

Researchers reported that the proportional fresh weight increases in cut roses were first between days 3-9 st and started to decrease after the 9th day (Ichimura et al., 1999; Alaey et al., 2011; Tuna, 2012). Ichimura et al. (2002) also reported that the relative FW of cut roses increased until the 3rd day in control and until the 6th day in different flower preservatives. Although the results obtained in the present study are generally compatible with the above literature, it is observed that the proportional FW change varies according to bacterial isolates. It is thought that this may be due to the variety used and trial conditions. In the current study, only tap water was placed in the vases of all application groups as a vase solution. The flower stems of the cut roses at the beginning of the experiment were cut to a length of 1-1.5 cm and included in the experiment. The decrease in fresh weight ratios has nothing to do with nutritional or vase solution content. We think that the proportional changes in FW may be due to +10 °C conditions not being optimum. Especially Control-2 and Ç2

applications suffered from drying damage to petals and leaves before they were placed in the vase. Additionally, we think that the shedding of green leaves, which could not be foreseen before the experiment, may be misleading in the measurement of these weights. The present research, in which a method that has not been done or applied before, is the first of these important studies. We believe that it can serve as a guide for future studies.

## CONCLUSION

In the current research, bacteria isolated from the leaf apoplast of 16 different cold-resistant plants were separately applied to the plant leaves to partially create the necessary cold transportation conditions for the transportation of cut rose flowers from the greenhouse to the point where they will be marketed. At the same time, two different bacterial formulations prepared with two binary bacterial strains were applied. The effects of the applications on the parameters affecting the vase life of cut roses were examined and evaluations were made. It has been determined that applications in which D3, D4, M1, and K1 + B8 bacterial isolates are used in the transportation of cut flowers are promising applications in terms of ensuring less cooling, minimal loss in cut flower quality, extending vase life, and reducing cooling costs. In general, with D2, D1, D4, Ç1, K1, and K1+B8 applications, cut roses maintained their visual quality criteria in the vase for 9 days. The present study is the first to aim to provide less cooling and extend vase life by using psychrophile PGPBs in

the transportation of cut flowers. Research should be continued by enriching the number and content of studies for this purpose.

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## Author contribution statement

DT is the primary author of this article. Investigation, Methodology DT, FPK; Formal analysis, and Writing-Original Draft – FPK, ÖA, DT; References, Data Collection and/or Processing – DT; Analysis and/or Interpretation, Critical Review and Edit- AD. All authors read and approved the final manuscript.

## Declaration of Interests

The authors declare that there are no potential conflicts of interest regarding the authorship, research, and publication of this manuscript.

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## Farklı Dalga Boylu Led Aydınlatma Uygulamalarının Domateste Fide Kalitesi Üzerine Etkileri

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### ÖZET

Bu çalışma, farklı dalga boylarına sahip led lambalar kullanılarak oluşturulan aydınlatma ortamlarının domates fidelerinin kaliteleri üzerine olan etkilerinin araştırılması ve fide üreticilerinin teslimatlarında herhangi bir sebeple meydana gelebilecek gecikmeler olması durumunda fidelerde boy ve kalite kontrolünün sağlanarak üreticilerin bu sıkıntılarında bir çözüm bulmak amacıyla yürütülmüştür. Bu amaçla öncelikle iklim odasında aynı ışık yoğunluğuna sahip farklı dalga boylarında (kırmızı, mavi, yeşil, beyaz-günışığı karışımı-kontrol) led lambalar altında dikim aşamasından itibaren “Ferguson F1” domates çeşidine ait fideler 10 gün süre ile yetiştirilmiştir. Daha sonra domates fideleri, boy uzamasını baskılamada etkili olduğu belirlenen mavi ışığın farklı oranlarda (%100, %50, %25 ve %12.5) kontrol olarak kullanılan beyaz-günışığı karışımı lambalara eklenmesiyle elde edilen ışık koşullarına maruz bırakılmıştır. Son olarak da farklı oranlarda mavi ışığın uzun dönemdeki etkilerinin kalıcılığını ortaya koyabilmek amacıyla fideler tekrar beyaz-günışığı koşullarına maruz bırakılmıştır. Fide aşamasında kullanılan farklı dalga boyları arasından domates fidelerinin gelişimini baskılayan dalga boyunun mavi ışık olduğu belirlenmiştir. Ayrıca, bitki gelişimini teşvik eden ve fide boyunda artışlara neden olan ışığın ise kırmızı ışık olduğu ortaya konmuştur. Farklı oranlarda mavi ışık koşulları altında yetiştirilen domates fidelerinde mavi ışık oranı azaldıkça bitki yaş ağırlığında ve bitki boyunda önemli seviyede artışlar elde edilmiştir. Farklı oranlardaki mavi ışığın fide kalitesi üzerine uzun süreli etkisine bakıldığında uygulama sonrası kontrol aydınlatma koşullarına alınan domates fidelerinin kalitesi üzerinde mavi ışığın etkisinin geçici olduğu ve fidelerde büyümenin devam ettiği görülmüştür. Bu sonuçlar fide boyunun baskılanmasında %100 mavi ışığın kullanılabileceğini ve mavi ışığın etkisinin hazır fide yetiştiriciliğinde kullanılan kimyasal maddeler gibi kalıcı olmadığını göstermiştir.

### Bahçe Bitkileri

### Araştırma Makalesi

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### Anahtar Kelimeler

*Lycopersicon lycopersicum*

Dalga Boyu

Led Lamba

Fide Boy Kontrolü

## Effects of Different Wavelength-Led Lighting Applications on Seedling Quality in Tomato

### ABSTRACT

This study was conducted to investigate the effects of lighting environments created using LED lamps with different wavelengths on the quality of tomato seedlings and to find a solution to the problems of transplant producers in case of any delays in deliveries, by ensuring control over the height and quality of the seedlings. For this purpose, seedlings of the “Ferguson F1” tomato variety were first grown for 10 days under LED lamps with different wavelengths (red, blue, green, white-daylight mix-control) with the same light intensity in a climate chamber from the transplanting stage. Subsequently, the tomato seedlings were exposed to light conditions obtained by adding blue light, which was determined to be effective in suppressing stem elongation, in different proportions (100%, 50%, 25%, and 12.5%) to the white-daylight mix lamps used as control. Finally, to demonstrate the long-term effects and persistence of different proportions of blue light, the seedlings were once

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again exposed to white-daylight conditions. Among the different wavelengths used during the seedling stage, blue light was determined to suppress the growth of tomato seedlings. Additionally, it was found that red light promoted plant growth and caused a significant increase in seedling height. Significant increases in plant fresh weight and plant height were also obtained in tomato seedlings grown under different blue light conditions as the proportion of blue light decreased. When the long-term effect of different proportions of blue light on seedling quality was considered, it was observed that the effect of blue light on the quality of tomato seedlings transferred to control lighting conditions after application was temporary and the growth of the seedlings continued. These results have shown that 100% blue light can be used to suppress seedling height and that the effect of blue light is not as permanent as the chemicals used in the transplant cultivation industry.

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## GİRİŞ

İnsan hayatına 1980'li yılların sonlarında girmeye başlamış olan ve aydınlatma amacıyla bitki yetiştiriciliğinde büyük avantajlar sağlayan led lambalar; 1990'lı yılların başında bitki yetiştiriciliği ile ilgili çalışmalar yapan bilim insanların merakını uyandırmış ve yetiştiricilikte özellikle aydınlatmanın yetersiz kaldığı durumlarda kullanılabilirliği araştırılmaya başlanmıştır (Bula ve ark., 1991, Bayhan & Avcı, 2019). Led lambaların bitki yetiştiriciliğinde kullanılması ile birlikte özel spektrum aralıkları geliştirilmiş; bu da bitkinin büyüme hızı, fotosentez verimliliği, biyokütle üretimi ve çiçeklenme gibi belirli özelliklerinin geliştirebilmesi ya da kontrol edilmesine olanak sağlamıştır. Böylece bitkilerde istenen özellikleri elde etmek ve bitkilerin kalitesi ile verimliliğini artırmaya yönelik ışığın kullanılabilirliği ispatlanmıştır (Ouzounis ve ark., 2015, Çağlayan & Ertekin, 2018).

Bitki yetiştiriciliğinin hemen hemen tüm aşamalarında ışığın dalga boylarının üretim amacına uygun olarak kullanılması, yetiştiriciliğin kalitesini artırmakla birlikte kontrollü bir fide üretim sistemine olanak sağlamakta ve fide kalitesini artırarak hem fide üreticisinin hem de fide alıcısının iyi sonuçlar almasını sağlamaktadır. Günümüz koşullarında ise sebze üreticileri artık üretim materyali olarak kullanılan fideleri genellikle kendisi üretmemekte bunun yerine hazır üretilmiş şekilde temin etmektedir. Bu sebeplerden dolayı hazır fide üretim sektörü son yıllarda ülkemizde büyük bir gelişme göstermiş ve sayıları 210'un üzerinde olan üretici firmalarda yılda yaklaşık 6.5 milyar sebze fidesi üretilmektedir (Yetiştir & Ellialtıoğlu, 2022). Hazır fide üreten firmalar, tohum ekim zamanını üreticiden gelen talepler doğrultusunda ayarlayarak istenilen zamanda teslimat yapmakta ve üreticilerin isteklerine

cevap vermektedir. Fakat üreticinin bazı nedenlerden dolayı yetiştiricilik yapılacak olan ortamı ya da araziye zamanında hazırlayamaması gibi durumlarda firmaların teslimat yapamamasından dolayı fideler, seralarda büyüme ve gelişmeye devam etmektedir. Büyüme ve gelişmenin devam etmesi, fide boyunun aşırı uzamasına (istenilen hazır fide formunu aşması) dolayısıyla fide kalitesinin düşmesine yol açmaktadır (Başak & Demir, 2022, Çakırer Seyrek, 2024). Bu problemi ortadan kaldırmak amacıyla fide üreticileri büyümeyi engelleyici bazı kimyasal maddeler kullanılmakta fakat bu maddelerin etkisi istenilenden daha uzun sürelerde devam edebilmekte ve fideler yetiştirme yerlerine dikildiklerinde bile büyüme ve gelişme olumsuz etkilenebilmektedir (Uslu, 2002). Şu ana kadar fidelerde boy kontrolü amacıyla daminozide, butralin, chlormequat, chlorpropham, 3 maleichydrazide ve mepiquat chloride gibi engelleyici etki gösteren giberellin sentez inhibitörleri ile gece sıcaklığını gündüz sıcaklığından yüksek tutmak gibi yöntemler kullanılmaktadır (Mohamed, 2022). Giberellin sentez inhibitörleri hem tek yıllık hem de çok yıllık bitkilerde bitki boy ve sürgün uzamasının kontrolünde etkili olmaktadır (Ergun ve ark., 2007). Ancak, bu kimyasalların etkileri uygulamadan sonra uzun süre devam edebilmektedir. Örneğin, patlıcan fideleri ile yapılan bir çalışmada paclobutrazol uygulamasının fide gelişimini gereğinden fazla baskıladığı bu yüzden tarlaya dikilen fidelerde gelişme ve büyümenin durmasının bir süre daha devam ettiği ve hasatta gecikmeye neden olduğu bildirilmiştir (Geboloğlu ve ark., 2015).

Bitkilerin yaşamsal faaliyetlerini sürdürdüğü ışık dalga boyları insan gözüne benzer bir spektrum aralığına sahiptir (Deram, 2013). Araştırmalar, fotosentez etkinliği için en önemli dalga boylarının mavi ve kırmızı dalga boylarında olduğunu göstermektedir ve fotosentetik verimliliğin optimum

olduğu zirve noktası 440 (mavi) ve 670 (kırmızı) nm'de (+/- 10 nm) bulunmaktadır (McCree, 1971). Bununla birlikte, ışığın kalitesinin bitki büyüme ve gelişmesinde etkilerinin oldukça değişken olduğu bilinmektedir. Örneğin, domates fidelerinde yapılan bir çalışmada, yetiştiricilikte kullanılan 230 ve 300  $\mu\text{mol m}^{-2} \text{s}^{-1}$  PPF'de mavi ışığın bitki büyümesini baskıladığı ve fide boylarının uzamasını engellendiği bulunmuştur (Kaya, 2022). Yine domates yetiştiriciliğinde sadece kırmızı ışığın kullanımının boy uzamasını ve yaprak büyümesini teşvik ettiği fakat fotosentezde rol alan enzimlerin aktivitesini düşürdüğü bildirilmiştir (Izzo ve ark., 2020). Aynı çalışmada yetiştiricilikte kullanılan mavi ışığın ise daha kısa boylu kompakt yapılı fidelerin oluşumuna neden olduğu ve fotosentetik kapasiteyi ise teşvik ettiği belirlenmiştir. Buna karşılık patlıcan fideleri üzerine yapılan bir çalışmada mavi ışığın fide boy uzamasını, yaprak gelişimini ve fotozsentetik aktiviteyi teşvik ettiğini ve kırmızı ışığın ise bu parametreleri baskıladığı belirlenmiştir (Di ve ark., 2021).

Bu çalışmada, sebze yetiştiriciliğinde üreticilerin farklı dalga boyları kullanarak bitki boy kontrolünü kimyasal madde kullanmadan sağlayabilme olanakları araştırılmıştır. Bu çalışmanın ülkemizdeki sebze fidesi üretimi ve farklı dalga boyları üzerine etkileri bakımından ileride yapılacak çalışmalar için de yardımcı olması amaçlanmıştır. Çalışma, farklı dalga boylarına sahip led lambalar kullanılarak oluşturulan ortamların domates fidelerinin kaliteleri üzerine olan etkilerinin araştırılması ve fide üretiminde teslimatının herhangi bir sebeple gecikmesi durumunda fidelerde boy ve kalite kontrolünün sağlanarak fide üreticilerinin bu sıkıntısına bir çözüm bulmak amacıyla kurgulanmıştır. Bu amaca yönelik olarak öncelikle kullanılan farklı dalga boyları arasında büyümeyi baskılayan dalga boyunun ortaya konması amaçlanmıştır. Daha sonra, büyümeyi baskılayan dalga boyundaki lambaların değişik oranlarda güneş ışığını taklit eden beyaz-gün ışığı lambalarla belirli oranda karıştırılarak elde edilen ışık koşullarında yetiştirilmesinin fidelerin büyüme ve gelişmeleri üzerine etkilerinin belirlenmesi ve böylece hazır fide yetiştiriciliğinde alternatif fide boy kontrolü yöntemi olarak kullanılabilme olanağının araştırılması hedeflenmiştir.

## MATERYAL ve YÖNTEM

### Materyal

Çalışma Kahramanmaraş Sütçü İmam Üniversitesi Ziraat Fakültesi Bahçe Bitkileri Bölümüne ait tohum ve sebze fizyolojisi laboratuvarına ait iklim odasında yürütülmüştür. Araştırmada materyal olarak Ferguson F<sub>1</sub> domates çeşidi (Antalya Tarım, Antalya) kullanılmıştır. Ferguson F<sub>1</sub> sırik domates çeşidi, kaliksi uzun ve kalın olup boğum araları kısadır.

Bahar dikimlerine uygun olan bu domates çeşidinin meyvesi hafif dilimli ve yuvarlak olup 220-240 g arası meyve ağırlığı vardır.

### Yöntem

Bitkilerin yetiştirildiği iklim odasında farklı dalga boylarına sahip 100  $\mu\text{mol m}^{-2} \text{s}^{-1}$  ışık yoğunluğunu homojen bir şekilde sağlayabilen farklı sayı ve renklerdeki led lambalar (mavi, kırmızı ve yeşil) kullanılarak homojen bir ışık sistemi kurulmuştur. Kontrol olarak da aynı ışık yoğunluğuna sahip beyaz ve gün ışığı karışımı (%50-50 oranında) led lambalar kullanılmıştır. Mavi led lambaların üretici beyanı esasına göre ışığın sahip olduğu dalga boyu 456 nm, kırmızı lambaların 665 nm, yeşil lambaların ise 510 nm'dir. Kontrol grubu olarak kullanılan beyaz led lambaların dalga boyu ise görünür spektrum (400-700 nm) aralığıdır (Çağlayan & Ertekin., 2016) ve gün ışığının spektrum aralığı ise 360-780 nm dalga boyunu kapsayan tam spektrumlu ışık olduğu bildirilmiştir (Bohar ve ark., 2017). Bitkilerin yetiştirildiği iklim odasında raflar, farklı dalga boyuna ait led lambalarla ışıklandırılmış ve ışığın yandaki ya da karşıdaki bitkilere etki etmemesi için rafların aralarına ve önlerine ışık geçirmez levhalar ve örtüler eklenmiştir.

Domates tohumları içerisinde 3:1 oranında torf ve perlit bulunduran viyollere ekilmiş ve viyoller sıcaklığı 25 °C'ye ayarlanmış iklim odasına konmuştur. Araştırmada, farklı dalga boyuna sahip led lambaların fide kalitesi üzerindeki etkilerini ortaya koymak amacıyla aşağıda belirtilen aydınlatma yöntemleri uygulanmıştır.

### Fide Aşamasında Farklı Dalga Boylarındaki Işığın Fide Kalitesi Üzerine Etkisi

Fideler 3-4 yapraklı hazır fide formuna gelene (dikim aşaması) kadar kontrol uygulaması (beyaz ve gün ışığı karışımı) altında yetiştirilmiştir. Yetiştiricilik süresi boyunca 2 kez ½ oranında Hoagland çözeltisi ve 2 kez demir sülfat gübrelemesi yapılmıştır. Fideler dikim aşamasına ulaştıklarında 10 gün süreyle 100  $\mu\text{mol m}^{-2} \text{s}^{-1}$  ışık yoğunluğuna sahip farklı dalga boylarındaki led lambalarla (%100 kırmızı, %100 mavi, %100 yeşil ve %100 kontrol) aydınlatılmış raflara taşınmışlardır. Her bir uygulama 4 kez tekrar edilmiş ve her tekerrürde 8 adet bitki yer almıştır. Belirtilen sürenin sonunda bitkiler üzerinde aşağıda belirtilen fenolojik ölçümler ve analizler yapılmıştır.

### Farklı Oranlarındaki Mavi Işığın Fide Kalitesi Üzerine Etkisi

Yukarıda belirtilen araştırma sonrasında bitki boy uzamasını baskılayan dalga boyunun mavi olduğu belirlenmiştir. Kontrol uygulamasında kullanılan led lambalar (beyaz ve gün ışığı karışımı) altında yetiştirilen fideler 3-4 yapraklı aşamaya ulaştıklarında 10 gün süreyle %0 (kontrol), %12.5, %25, %50 ve %100 oranında mavi ışık eklenmiş kontrol

ışığı aydınlatma koşullarına alınmışlardır. Bütün uygulamalarda  $100 \mu\text{mol m}^{-2} \text{s}^{-1}$  ışık yoğunluğu elde etmek için farklı sayıda led lamba kullanılmıştır. Her bir uygulama 4 kez tekrarlanmış ve her tekerrürde 8 fide yetiştirilmiştir. Sürenin sonunda bitkiler üzerinde aşağıda belirtilen fenolojik ölçümler ve analizler yapılmıştır

**Farklı Oranlardaki Mavi Işığın Fide Kalitesi Üzerine Kalıcı Etkilerinin Araştırılması**

Yukarıda belirtilen ışık koşullarında 10 gün süresince bırakılan fidelerin bir kısmı üzerinde aşağıda belirtilen fenolojik ölçümler alınmış ve analizler yapılmıştır. Fide kalitesindeki meydana gelen değişikliklerin kalıcı olup olmadığını ortaya koymak amacıyla daha sonra beyaz ışık-gün ışığı karışımı (kontrol) koşullarına alınarak 10 gün daha ortamda bırakılan fidelerde sürenin sonunda aşağıda belirtilen fenolojik ölçümler ve analizler yapılmıştır. Her bir uygulama 4 kez tekrarlanmış ve her tekerrürde 8 fide yetiştirilmiştir.

Uygulamalar arasındaki farkı ortaya koymak amacıyla aşağıda belirtilen ölçüm ve analizler yapılmıştır.

**Fiziksel ölçümler**

**Fide boyu (cm):** Toprak seviyesinden bitkinin büyüme ucuna kadar olan mesafe dijital kumpas aracılığıyla tekerrürdeki tüm fidelerde belirlenmiştir.

**Fide gövde kalınlığı (çap, mm):** Fidelerin gövde çapı toprak seviyesinde dijital kumpas aracılığıyla tekerrürdeki tüm fidelerde belirlenmiştir.

**İlk gerçek yaprağa kadar olan gövde uzunluğu boyu (cm):** Toprak seviyesinden ilk gerçek yaprağa kadar olan mesafe dijital kumpas aracılığıyla tekerrürdeki tüm fidelerde belirlenmiştir.

**Fide yaş ağırlığı (g):** Her bir fidenin (toprak üstü kısım) taze ağırlığı hassas terazide tartılarak belirlenmiştir.

**Fide kuru ağırlığı (g):** Fideler etüvde  $72 \text{ }^\circ\text{C}$ 'de 48 saat süre ile kurutulduktan sonra ağırlıkları hassas terazi kullanılarak belirlenmiştir.

**Kök yaş ağırlığı (g):** Fideler toprak seviyesinden kesilmiş ve kökler akan su altına yıkandıktan sonra kurutma kâğıdı ile yüzey kurutma yapılmış ve ağırlıkları belirlenmiştir.

**Kök kuru ağırlığı (g):** Kökler etüvde  $72 \text{ }^\circ\text{C}$ 'de 48 saat süre ile kurutulduktan sonra ağırlıkları hassas terazi kullanılarak belirlenmiştir.

**Yaprak alanı:** Bitkilerin yaprak alanları (petiyoller hariç) LICOR LI-3000 serisi yaprak alanı ölçer yardımı ile her bir tekerrürden rastgele seçilen 3 bitki üzerinde belirlenmiştir.

**Laboratuvar analizleri**

**Klorofil miktarı:** Bitkiler arasından (her uygulamanın her bir tekerrüründen) tesadüfen alınan  $0.5 \text{ g}$  yaprak örneği %80'lik aseton ile ekstrakt edildikten sonra

klorofil a ve klorofil b pigmentlerinin konsantrasyonları, filtre edilmiş ekstrakt solüsyonunun abosorbanslarının Optima SP-3000 Plus model spektrofotometrede  $645 \text{ nm}$  ve  $663 \text{ nm}$ 'de okunmasından sonra Gunes ve ark. (2007)'de belirtilen aşağıdaki formüle göre hesaplanmıştır.

$\text{Klorofil a (mg g}^{-1} \text{ taze ağırlık-TA)} = 11.75 \times A_{663} - 2.35 \times A_{645}$

$\text{Klorofil b (mg g}^{-1} \text{ taze ağırlık)} = 18.61 \times A_{645} - 3.96 \times A_{663}$

**Malondialdehid (MDA) içeriği:** Bitki dokularında serbest radikallerin oluşturduğu hasardan dolayı oluşan bir bozulma ürünü olan MDA içeriği Zhang ve ark. (2005)'de belirtilen yöntemle göre belirlenmiştir. Bunun için  $0.25 \text{ g}$  yaprak örneği (her uygulamada bulunan her bir tekerrürden) %0.1 trikloroasetik asit (TCA) ile ezilerek  $6000 \text{ g}$ 'de  $5 \text{ dk}$  süre ile santrifüj edilmiştir. Elde edile süpernatanttan  $1 \text{ mL}$  alınarak içinde %20 TCA bulunan %0.5'lik tiobarbütrik asit (TBA)'den  $4 \text{ mL}$  ilave edilmiş ve sonrasında  $100 \text{ }^\circ\text{C}$ 'de  $30 \text{ dk}$  kaynatıldıktan sonra buz banyosuna  $5 \text{ dk}$  süre ile konulmuştur. Sonrasında spektrofotometrede  $450$ ,  $532$  ve  $600 \text{ nm}$ 'de absorban okumaları yapılmış ve MDA içeriği aşağıda belirtilen formüle göre hesaplanmıştır.

$\text{MDA (}\mu\text{mol g}^{-1} \text{ TA)} = 6.45 \times (A_{532} - A_{600}) - 0.56 \times A_{450}$

**$\text{H}_2\text{O}_2$  (Hidrojen peroksit) içeriği:** Yapraklarda stres sonucu oluşan serbest bir radikal olan  $\text{H}_2\text{O}_2$  miktarının tayini Alexieva ve ark. (2001)'de belirtilen yöntem kullanılarak gerçekleştirilmiştir. Bunun için  $0.25 \text{ g}$  yaprak örneği (her uygulamada her tekerrüründen alınmış) buz üzerinde porselen havan içerisinde  $3 \text{ mL}$  %0.1'lik TCA ilave edilerek ezilmiştir. Örnekler pipet ile çekilerek,  $2 \text{ mL}$ 'lik ependorf tüplere konmuş soğutuculu santrifüjde  $4 \text{ }^\circ\text{C}$ 'de  $10.000 \text{ 'de}$   $10 \text{ dk}$  santrifüj edilmiştir. Sonrasında  $15 \text{ mL}$  hacime sahip tüplere sırası ile  $75 \mu\text{L}$  supernatant,  $75 \mu\text{L}$  K-P tampon çözeltisi ve  $1.5 \text{ mL}$  KI (potasyum iyodür) konulmuş ve vorteks ile  $30 \text{ sn}$  karıştırıldıktan sonra spektrofotometrede  $390 \text{ nm}$ 'de okunmuştur. Kör numune oluştururken supernatant yerine saf su kullanılmış ve diğer işlemlerde aynı şekilde tekrarlanmıştır. Farklı yoğunlukta  $1-1000 \text{ nmol}$  yoğunluk aralığında  $\text{H}_2\text{O}_2$  içeren çözeltiler kullanılarak standart eğri elde edilmiş ve örneklerin  $\text{H}_2\text{O}_2$  içeriği standart eğriden elde edilen denkleme göre  $\mu\text{mol g}^{-1} \text{ TA}$  olarak hesaplanmıştır.

**İstatistik Analizler**

Araştırma sonucu elde edilen veriler SAS 8.1 istatistik paket programı kullanarak 4 tekerrürlü olarak tesadüf parselleri deneme desenine göre tek yönlü varyans analizine tabi tutulmuş ve uygulamalar arasındaki farklılıkların belirlenmesinde LSD (asgari önemli fark) testi kullanılmıştır.

## BULGULAR ve TARTIŞMA

### Farklı dalga boylarındaki ışığın domates fideleri üzerine etkileri

Çizelge 1 incelendiğinde 10 gün süreyle mavi ışığa maruz bırakılan fidelerin boylarının diğer ışık uygulamalarına kıyasla önemli seviyede daha kısa olduğu ve mavi ışığın bitki boy uzaması üzerinde engelleyici bir etkiye sahip olduğu belirlenmiştir (Şekil

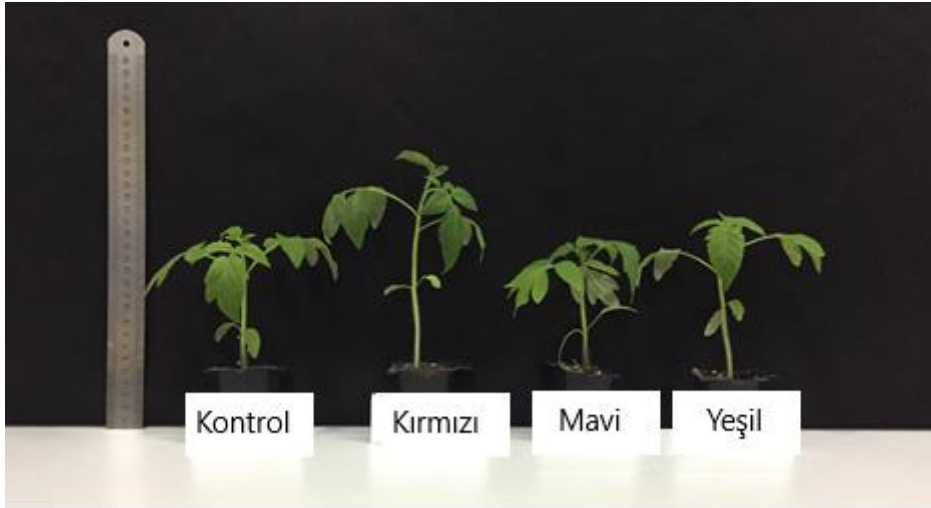
1). Farklı dalga boylarındaki led lambalar altında yetiştiricilik yapmanın gövde kalınlığı üzerine olan etkisine bakıldığında, en kalın fidelerin kırmızı ışık altında yetiştirilen fideler olduğu ve diğer tüm ışık uygulamalarındaki fidelerin önemli seviyede daha ince gövde kalınlığına sahip olduğu görülmüştür. Işık kalitesinin domates fidelerinin ilk gerçek yaprağa kadar olan uzunluğu ve yaprak sayısı üzerine önemli bir etkisinin olmadığı belirlenmiştir (Çizelge 1).

Çizelge 1. Işık kalitesinin domates fidelerinin ilk gerçek yaprak boyu, fide boyu, gövde çapı ve yaprak sayısı üzerine etkisi

Table 1. The effects of light quality on the distance to first true leaves, seedling height, stem diameter and leaf number of tomato seedlings.

Uygulamalar	İlk gerçek yaprak boyu (cm)	Fide Boyu (cm)	Gövde çapı (mm)	Yaprak sayısı (adet)
Kontrol	5.79 ± 0.17	15.33 <sup>a</sup> ± 0.65	2.63 <sup>b</sup> ± 0.13	4.46 ± 0.14
Kırmızı	6.04 ± 0.23	16.14 <sup>a</sup> ± 0.30	3.12 <sup>a</sup> ± 0.05	4.62 ± 0.09
Mavi	5.35 ± 0.41	12.00 <sup>b</sup> ± 0.80	2.77 <sup>b</sup> ± 0.08	4.17 ± 0.10
Yeşil	5.62 ± 0.13	15.33 <sup>a</sup> ± 0.66	2.63 <sup>b</sup> ± 0.13	4.43 ± 0.14
p değeri	0.3341	0.0027	0.0193	0.1205
LSD <sub>0.05</sub>	-	1.95	0.32	-

\*: P<0.05 Farklı harflerle gösterilen değerler istatistiksel olarak birbirinden farklıdır.



Şekil 1. Fide aşamasında farklı dalga boyunda ışığa maruz kalan domates fideleri  
Figure 1. Tomato seedlings are exposed to light of different wavelengths at the seedling stage.

Veriler incelendiğinde ışık kalitesinin bitki yaş ve kuru ağırlığını önemli seviyede etkilediği görülmüş ve en yüksek bitki yaş ve kuru ağırlığı (sırasıyla 17.14 ± 0.51 g ve 1.47 ± 0.05 g) kırmızı ışık altında yetişen fidelerde ölçülmüştür. Kırmızı ışık altında yetişen fideleri kontrol ve yeşil ışığa maruz kalan fideler takip etmiş ve mavi ışık altında yetişen fidelerin ise en düşük bitki yaş ve kuru ağırlığına (sırasıyla 10.90 ± 0.24 g ve 0.77 ± 0.04 g) sahip olduğu belirlenmiştir. Işık kalitesinin kök yaş ve kuru ağırlığı üzerine önemli bir etkisinin ise olmadığı gözlenmiştir (Çizelge 2).

Yukarıda bahsedilen sonuçlar farklı renkte led lambalar altında yetiştirilen hazır fide aşamasına ulaşmış domates fidelerinde kırmızı ışığın fide boyunun uzamasını teşvik ettiğini, buna karşılık mavi

ışığın ise fidelerin gelişimlerini ve dolayısıyla boylarını önemli seviyede baskıladığını ortaya koymuştur. Kırmızı ışığın bitki boy uzamasını ve fotosentez hızını teşvik ettiği bilinmekte ayrıca bu dalga boyunun fizyolojik aktiviteler bakımından en etkin dalga boyu olduğu ifade edilmekte ve yetiştiricilikte kırmızı ışık oranının azalmasıyla bitki boylarının büyümesinin yavaşladığı rapor edilmiştir (Tanaka ve ark., 1998, Jin ve ark., 2023). Örneğin bir çalışmada, domates, turp, soya fasulyesi ve hıyar fideleriyle 200 ve 500  $\mu\text{mol m}^{-2} \text{s}^{-1}$  PPFD ışık yoğunluğunda yapılan yetiştiricilikte, %100 mavi ışığın bitki boy uzamasını engellediği ve kırmızı ışığın ise bitki boy uzamasını teşvik ettiği belirlenmiştir (Snowden ve ark., 2016).



Çizelge 2. Işık kalitesinin domates fidelerinin ve köklerin yaş (YA) ve kuru ağırlığı (KA) üzerine etkileri  
Table 2. The effects of light quality on the fresh and dry weight of tomato seedlings and roots.

Uygulamalar	Fide YA (g bitki <sup>-1</sup> )	Fide KA (g bitki <sup>-1</sup> )	Kök YA (g bitki <sup>-1</sup> )	Kök KA (g bitki <sup>-1</sup> )
Kontrol	14.62 <sup>b</sup> ± 0.72	0.92 <sup>b</sup> ± 0.05	1.94 ± 0.21	0.21 ± 0.03
Kırmızı	17.14 <sup>a</sup> ± 0.51	1.47 <sup>a</sup> ± 0.05	2.56 ± 0.15	0.22 ± 0.01
Mavi	10.90 <sup>c</sup> ± 0.24	0.77 <sup>c</sup> ± 0.04	1.99 ± 0.16	0.16 ± 0.01
Yeşil	14.62 <sup>b</sup> ± 0.72	0.96 <sup>b</sup> ± 0.05	1.94 ± 0.21	0.16 ± 0.02
p değeri	0.0001	0.0001	0.01	0.11
LSD <sub>0.05</sub>	1.80	0.14	-	-

\*: P<0.05 Aynı sütunda farklı harflerle gösterilen değerler istatistiksel olarak birbirinden farklıdır.

Diğer bir çalışmada, *Arabidopsis* fidelerinde kırmızı ışığın yaprak alanı büyümesini, biyokütleyi ve net fotosentez hızını teşvik ettiği, mavi ışığın ise karotenoid ve antosiyanin içeriğini artırdığı bildirilmiştir (Yavari ve ark., 2021). Mavi ışık fotoreseptörü cry1'in (kriptokrom) giberellin ve oksin seviyelerini baskılayarak bitkilerin boy uzamasını kısıtladığı bildirilmiştir (Cosgrove, 1994; Folta ve ark., 2003). Ayrıca, kriptokromların, hipokotil ve boy büyümesini baskılamak için biyoaktif giberellinlerin birikimini engelleyebileceği belirlenmiştir (Zhao ve ark., 2007). Yine kırmızı ışığa maruz kalan bitkilerde görülen boy uzamasının giberellin sentezinin teşvik edilmesinden (Quyang ve ark., 2015) ve mavi ışığın neden olduğu boy uzaması baskılanmasının da giberellin sentezini bloke eden genlerin teşvik edilmesinden kaynaklandığı bildirilmiştir (Matsuo ve ark., 2019). Ayrıca bu araştırma sonuçları yeşil ışığın domates fidelerinin vejetatif büyümesi üzerine olumsuz bir etkisinin olmadığını ortaya koymuştur.

Benzer şekilde Claypool ve Lieth (2020) yeşil ışığa maruz kalan biber fidelerinde büyümenin olumsuz etkilenmediği ve fidelerin boyunun kırmızı ışığa maruz kalan fidelerle aynı olduğu bildirmiştir.

Işık kalitesinin domates fidelerinin klorofil, MDA ve H<sub>2</sub>O<sub>2</sub> içerikleri üzerine etkileri incelendiğinde stres parametreleri olan MDA ve H<sub>2</sub>O<sub>2</sub> içeriklerinin önemli seviyede etkilendiği fakat klorofil içeriğinin ise değişmediği görülmüştür. Bitkilerin H<sub>2</sub>O<sub>2</sub> içeriği kontrol grubu fidelerine (0.26 ± 0.01 µmol g<sup>-1</sup> TA) kıyasla kırmızı ve mavi ve ışık altında daha yüksek (sırasıyla 0.36 ± 0.03 µmol g<sup>-1</sup> TA ve 0.36 ± 0.02 µmol g<sup>-1</sup> TA) bulunmuştur. Yine kırmızı ışığa maruz kalan fidelerin MDA içeriğinin diğer uygulamalardaki fidelerden önemli seviyede daha yüksek olduğu belirlenmiştir. Bu sonuçlar kırmızı ve mavi ışığın domates fidelerinde strese neden olduğunu ortaya koymuştur (Çizelge 3). Ayrıca, ışığın kalitesinin domates fidelerinin klorofil içeriğini etkilemediği belirlenmiştir.

Çizelge 3. Işık kalitesinin domates fidelerinin klorofil, MDA ve H<sub>2</sub>O<sub>2</sub> içerikleri üzerine etkisi  
Table 3. The effect of light quality on the chlorophyll, MDA, and H<sub>2</sub>O<sub>2</sub> contents of tomato seedlings.

Uygulamalar	Klorofil (mg g <sup>-1</sup> TA)	MDA (mg g <sup>-1</sup> TA)	H <sub>2</sub> O <sub>2</sub> (mg g <sup>-1</sup> TA)
Kontrol	0.094 ± 0.002	2.28 <sup>b</sup> ± 0.14 b	0.26 <sup>b</sup> ± 0.01
Kırmızı	0.089 ± 0.001	3.32 <sup>a</sup> ± 0.23 a	0.36 <sup>a</sup> ± 0.03
Mavi	0.086 ± 0.002	2.69 <sup>b</sup> ± 0.09 b	0.36 <sup>a</sup> ± 0.02
Yeşil	0.088 ± 0.003	2.31 <sup>b</sup> ± 0.19 b	0.31 <sup>ab</sup> ± 0.02
p değeri	0.1321	0.0039	0.0208
LSD <sub>0.05</sub>	-	0.53	0.07

\*: P<0.05 Aynı sütunda farklı harflerle gösterilen değerler istatistiksel olarak birbirinden farklıdır.

Bu sonuçlara göre %100 mavi ve %100 kırmızı ışık uygulamalarının fidelerde strese neden olduğu anlaşılmıştır. Araştırmalarda, tek renk ışık kaynağı olarak mavi ve kırmızı ışığın kullanılmasının bitkilerde strese neden olduğu bilinmektedir. Örneğin, ıspanak fidelerinde beyaz, mavi, kırmızı ve kırmızı-mavi ışık uygulamalarında yapılan bir çalışmada 300 µmol m<sup>-2</sup> s<sup>-1</sup> PPFD'de mavi, kırmızı ve kırmızı-mavi ışıklar altında bırakılan fidelerin klorofil içerikleri

beyaz ışık altında yetiştirilen fidelerden daha düşük seviyede olduğu bildirilmiştir (Ohashi-Kaneko ve ark., 2007). Yine, *Camptotheca acuminata* bitkilerinde yapılan bir çalışmada, 120 µmol m<sup>-2</sup> s<sup>-1</sup> PPFD'de tek renk kaynağı mavi, sarı, yeşil ve kırmızı ışığın kullanıldığı ortamlarda yetiştirilen bitkilerin MDA içeriği en yüksek mavi ışık altında bulunmuştur (Yu ve ark., 2017). Ayrıca, domates fidelerinde yapılan bir çalışmada ise 30 gün boyunca sadece kırmızı ışığa maruz kalan fidelerde klorofil içeriğinin ve yaprak

kalınlığının sadece mavi ve beyaz ışığa maruz kalanlara kıyasla önemli seviyede daha düşük olduğu dolayısıyla kırmızı ışığın fidelerde strese neden olduğu bildirilmiştir (Li ve ark., 2021).

### Farklı oranlarda mavi ışığın domates fideleri üzerindeki etkileri

Veriler incelendiğinde ilk gerçek yaprağa kadar olan uzunluğun %50 mavi, %25 mavi ve %12.5 mavi ışık ile aydınlatılmış fidelerde kontrol ve %100 mavi ışığa maruz kalan fidelere kıyasla daha büyük olduğu görülmüştür. Ayrıca %100 mavi ışığa maruz kalan fidelerin ( $6.38 \pm 0.06$  cm) en kısa boya sahip olduğu ve bu fideleri sırasıyla kontrol grubu fideleri ( $8.01 \pm 0.31$  cm) ile %50 mavi ( $10.55 \pm 0.10$  cm), %25 mavi ( $12.36 \pm 0.20$  cm) ve %12.5 mavi ışık ( $13.62 \pm 0.29$  cm) ile aydınlatılmış fidelerin takip ettiği görülmüştür. Bu sonuçlar %100 mavi ışığın fide boy uzunluğu üzerinde

baskılayıcı bir etkiye sahip olduğunu, ancak kontrol grubu ışıklandırmasına eklenen mavi ışığın miktarının artmasının boy uzamasını teşvik ettiğini ortaya koymuştur (Şekil 2). Yine kontrol grubu fidelerinin en küçük gövde çap kalınlığı değerlerine sahip olduğu görülmüş buna karşılık gövde çapı en yüksek olan fideler ise %12.5 oranındaki mavi ışık ile aydınlatılmış uygulamadan elde edilmiştir. Yaprak sayısı ait verilere bakıldığında kontrol grubu ışığa değişen oranlarda eklenen mavi ışığın yaprak sayısı üzerinde etkisinin önemli olduğu görülmüş ve en yüksek yaprak sayısına mavi ışık oranının %12.5 olması durumunda ulaşıldığı belirlenmiştir. Buna karşılık yapılan ışıklandırmanın içerisinde mavi ışığın oranının değiştirilmesinin yaprak alanı üzerine bir etkisinin olmadığı ve tüm uygulamalardaki fidelerin istatistiksel açıdan benzer yaprak alanına sahip olduğu görülmüştür (Çizelge 4).

Çizelge 4. Farklı oranlardaki mavi led lambalar ile yapılan aydınlatmanın domates fidelerin vejetatif büyümesi üzerine etkileri

Table 4. The effects of lighting with different ratios of blue LED lights on the vegetative growth of tomato seedlings

Uygulamalar	İlk gerçek yaprak boyu (cm)	Fide Boyu (cm)	Gövde çapı (mm)	Yaprak Sayısı (Adet)	Yaprak alanı (cm <sup>2</sup> )
Kontrol	$5.60^b \pm 0.36$	$8.01^d \pm 0.31$	$2.70^c \pm 0.10$	$2.98^b \pm 0.06$	$65.06 \pm 1.83$
%100 Mavi	$5.06^b \pm 0.06$	$6.38^e \pm 0.05$	$2.83^{bc} \pm 0.06$	$2.92^b \pm 0.07$	$64.87 \pm 2.25$
%50 Mavi	$6.67^a \pm 0.30$	$10.55^c \pm 0.10$	$2.91^b \pm 0.05$	$3.06^b \pm 0.06$	$72.45 \pm 5.01$
%25 Mavi	$7.57^a \pm 0.39$	$12.36^b \pm 0.20$	$2.79^{bc} \pm 0.05$	$3.08^b \pm 0.08$	$68.80 \pm 1.63$
%12.5 Mavi	$7.63^a \pm 0.29$	$13.62^a \pm 0.29$	$3.13^a \pm 0.05$	$3.37^a \pm 0.06$	$69.19 \pm 2.08$
p değeri	0.0006	0.0001	0.0081	0.0072	0.3546
LSD <sub>0.05</sub>	0.95	0.66	0.20	0.21	-

\*: P<0.05 Aynı sütunda farklı harflerle gösterilen değerler istatistiksel olarak birbirinden farklıdır



Şekil 2. Farklı oranlardaki mavi ışık altında yetiştirilen domates fideleri  
Figure 2. Tomato seedlings were grown under different ratios of blue light.

Ayrıca veriler incelendiğinde en yüksek yaş ağırlığa ( $10.04 \pm 0.17$  g) %12.5 oranında mavi ışık ile aydınlatılmış fidelerin sahip olduğu buna karşılık en düşük fide yaş ağırlığının ( $6.35 \pm 0.13$  g) ise %100 mavi ışık ile aydınlatılmış fidelerde elde edildiği görülmektedir. Yine benzer bir şekilde bitki kuru

ağırlık değerleri incelendiğinde en yüksek fide kuru ağırlığa kontrol ve %12.5 oranında mavi ışık ile aydınlatılmış fidelerin sahip olduğu belirlenirken en düşük kuru ağırlığın da yine %100 mavi ışık ile aydınlatılmış fidelerin sahip olduğu belirlenmiştir. Elde edilen bu sonuçlar, %100 mavi ışık altında

yapılan domates fidesi yetiştirildiğinde toprak üstü yaş ve kuru kütle birikiminin azaldığı ve %12.5 oranında kullanılan mavi ışığın ise bitki vejetatif gelişimi ve büyümeyi teşvik ettiği söylenebilir. Kök yaş ağırlık değerleri incelendiğinde %100 mavi ışık ile yapılan aydınlatmanın fide kök yaş ağırlığında ciddi

düşüşlere neden olduğu bulunmuştur. Kök kuru ağırlık değerleri en düşük %25 mavi ışık ( $0.08 \pm 0.002$  g) uygulamasında gözlenmiş ve en yüksek kök kuru ağırlığı ise %100 mavi ışık ( $0.18 \pm 0.003$  g) ile aydınlatılmış fidelerde elde edilmiştir (Çizelge 5).

Çizelge 5. Farklı oranlardaki mavi led lambalar ile yapılan aydınlatmanın domates fidelerinin toprak altı ve üstü yaş ve kuru ağırlıkları üzerine etkileri

Table 5. The effects of lighting with different ratios of blue LED lights on the above and below-ground fresh and dry weights of tomato seedlings

Uygulamalar	Fide YA (g bitki <sup>-1</sup> )	Fide KA (g bitki <sup>-1</sup> )	Kök YA (g bitki <sup>-1</sup> )	Kök KA (g bitki <sup>-1</sup> )
Kontrol	7.20 <sup>c</sup> ± 0.30	0.77 <sup>ab</sup> ± 0.05	2.40 <sup>a</sup> ± 0.13	0.13 <sup>ab</sup> ± 0.001
%100 Mavi	6.35 <sup>d</sup> ± 0.13	0.58 <sup>c</sup> ± 0.03	1.73 <sup>b</sup> ± 0.14	0.18 <sup>a</sup> ± 0.003
%50 Mavi	8.24 <sup>b</sup> ± 0.07	0.69 <sup>bc</sup> ± 0.01	2.16 <sup>a</sup> ± 0.14	0.13 <sup>ab</sup> ± 0.002
%25 Mavi	8.27 <sup>b</sup> ± 0.11	0.68 <sup>bc</sup> ± 0.06	2.44 <sup>a</sup> ± 0.18	0.08 <sup>b</sup> ± 0.002
%12.5 Mavi	10.04 <sup>a</sup> ± 0.17	0.82 <sup>a</sup> ± 0.02	2.40 <sup>a</sup> ± 0.08	0.14 <sup>ab</sup> ± 0.001
p değeri	0.0001	0.0086	0.0213	0.05
LSD <sub>0.05</sub>	0.55	0.12	0.43	0.05

\*: P<0.05 Aynı sütunda farklı harflerle gösterilen değerler istatistiksel olarak birbirinden farklıdır.

Farklı mavi ışık oranlarına maruz kalan fidelerde en yüksek klorofil içeriği kontrol grubu bitkiler ile %25 ve %12.5 oranında mavi ışık ile aydınlatılmış bitkilerde ölçülmüştür. Mavi ışık oranının artırılarak %50 seviyesine çıkarılması yaprakların klorofil içeriğinde düşüşe neden olmuş ve en düşük klorofil içeriği ise %100 mavi ışık ile aydınlatılmış fidelerde belirlenmiştir. Buna karşılık en yüksek yaprak MDA içeriği ise %100 oranında mavi ışık ile aydınlatılmış fidelerde belirlenmiş ve mavi ışık oranının azaltılması yaprak MDA içeriğinde önemli düşüşlere neden olmuştur. Benzer şekilde uygulamalar arasında en yüksek yaprak H<sub>2</sub>O<sub>2</sub> içeriği %100 oranında mavi ışık ile aydınlatılmış fidelerde ölçülmüş, diğer tüm uygulamalarda yaprak H<sub>2</sub>O<sub>2</sub> içeriği önemli seviyede daha düşük bulunmuştur (Çizelge 6).

Sonuçlar detaylı bir şekilde değerlendirildiğinde farklı oranlarda mavi ışık altında bırakılan domates fidelerinde mavi ışığın oranının azaltılmasıyla fide boyu arasında ters bir ilişki olduğu görülmüş ve en düşük mavi ışık oranı olan %12.5 oranında en uzun boylu fideler elde edildiği görülmüştür. Yine bu çalışmada mavi ışık oranının düşürülmesi ile domates fidelerinin yaprak klorofil içeriğinde artışlar, MDA ve H<sub>2</sub>O<sub>2</sub> içeriklerinde ise düşüşler gözlemlenmiştir. Benzer şekilde, bitki aydınlatmasında azalan oranda kullanılan mavi ışığın sardunya ve petunyalarda bitki boyunun uzamasını ve klorofil içeriğini teşvik ettiği bildirilmiştir (Park ve Runkle, 2019). Mavi ışığın azaltılması yaprak alanını ve gövde uzamasını arttırdığı bunun da verimin artırılmasını teşvik edebileceği bildirilmiştir (Gent, 1995). Marul fidelerinde yapılan bir çalışmada aydınlatmada kullanılan ışık içerisindeki mavi oranının düşürülmesiyle biyokütle veriminin arttığı

belirlenmiştir (Wheeler ve ark., 1994). Son zamanlarda tek renk kaynağı üzerine yapılan çalışmalarda mavi ışığın genellikle bitki boy uzamasının ve sürgün ağırlığını baskıladığı ortaya konmuştur (Son ve Oh, 2013; Kopsell ve ark., 2015; Wollaeger ve Runkle, 2015). Bu çalışmada %12.5 mavi oranına sahip ışık ile aydınlatılmış domates fidelerinin yaprak sayısının ve fide yaş ağırlığının kontrol uygulamasındaki fidelerden daha fazla olduğu, fide kuru ağırlığının ise kontrol bitkileri seviyesinde olduğu belirlenmiştir. Hıyar fidelerinde yapılan bir çalışmada, 100 µmol m<sup>-2</sup> s<sup>-1</sup>lik bir PPFD'de mavi ışığın oranının %75'den %10'a düşürülmesiyle birlikte, fide boyu ve yaprak alanı doğrusal olarak arttığı buna karşılık ise stoma iletkenliği ve klorofil içeriğinin ise azaldığı bildirilmiştir (Hernández ve Kubota, 2016).

### Farklı Oranlarda Mavi Işığın Fide Kalitesi Üzerine Kalıcı Etkilerinin Araştırılması

Farklı oranlarda mavi ışığa maruz kaldıktan sonra gün ışığı ve beyaz ışık karışımı (kontrol) koşullarında 10 gün süreyle yetiştirilen domates fidelerine ait veriler incelendiğinde farklı oranlarda mavi ışığa maruz kalmanın etkilerinin hala sürmekte olduğu ve en kısa ilk gerçek yaprağa kadar olan uzunluğa %100 mavi ( $5.14 \pm 0.29$  cm) ile aydınlatılmış fidelerin sahip olduğu görülmüştür. Bu uygulamayı sırasıyla kontrol grubu fideleri ( $6.14 \pm 0.19$  cm) ve %50 mavi ( $6.94 \pm 0.35$  cm) ışık ile aydınlatılmış fideler takip etmiş ve ilk gerçek yaprağa kadar en uzun olan fideler ise %25 ( $7.99 \pm 0.23$  cm) ve %12.5 ( $8.71 \pm 0.08$  cm) oranında mavi ile aydınlatılmış fideler olduğu belirlenmiştir.

Çizelge 6. Farklı oranlardaki mavi led lambalar ile yetiştirilen fidelerinin klorofil, MDA ve H<sub>2</sub>O<sub>2</sub> içeriklerine etkisi  
Table 6. The effect of different ratios of blue LED lights on the chlorophyll, MDA, and H<sub>2</sub>O<sub>2</sub> contents of the seedlings

Uygulamalar	Klorofil (mg g <sup>-1</sup> TA)	MDA (mg g <sup>-1</sup> TA)	H <sub>2</sub> O <sub>2</sub> (mg g <sup>-1</sup> TA)
Kontrol	0.078 <sup>a</sup> ± 0.004	1.40 <sup>b</sup> ± 0.27	0.53 <sup>b</sup> ± 0.01
%100 Mavi	0.057 <sup>c</sup> ± 0.001	2.26 <sup>a</sup> ± 0.34	0.80 <sup>a</sup> ± 0.02
%50 Mavi	0.067 <sup>b</sup> ± 0.004	2.10 <sup>ab</sup> ± 0.15	0.59 <sup>b</sup> ± 0.04
%25 Mavi	0.072 <sup>ab</sup> ± 0.002	1.99 <sup>ab</sup> ± 0.19	0.54 <sup>b</sup> ± 0.01
%12.5 Mavi	0.074 <sup>ab</sup> ± 0.001	1.44 <sup>b</sup> ± 0.20	0.54 <sup>b</sup> ± 0.01
p değeri	0.0025	0.05	0.0001
LSD <sub>0.05</sub>	0.009	0.75	0.07

\*: P<0.05 Aynı sütunda farklı harflerle gösterilen değerler istatistiksel olarak birbirinden farklıdır.

Benzer şekilde fide boyu dikkate alındığında en kısa boylu fidelerin %100 mavi (8.04 ± 0.40 cm) ile aydınlatılan fideler olduğu belirlenmiş en uzun boylu fidelerin ise %12.5 oranında mavi (16.12 ± 0.70 cm) ışık ile aydınlatılmış fidelerin olduğu görülmüştür (Şekil 3). Fidelerin gövde kalınlıkları dikkate alındığında %100 mavi ile aydınlatılmış fidelerin (2.73 ± 0.09 cm) diğer tüm uygulamalara kıyasla daha ince oldukları belirlenmiştir. Farklı oranlarda mavi ışık ile yapılan aydınlatmanın yaprak alanı üzerine önemli bir

etkisinin olduğu gözlemlenmiş fakat yaprak sayısı üzerinde etkisinin ise önemsiz olduğu bulunmuştur. Uygulamalar arasında en yüksek yaprak alanına %25 mavi, %50 mavi ve %12.5 mavi ışık (sırasıyla 90.75 ± 2.79 cm<sup>2</sup>, 79.87 ± 2.22 cm<sup>2</sup> ve 85.69 ± 0.85 cm<sup>2</sup>) ile aydınlatılmış fidelerin sahip olduğu görülmüş bu değerleri kontrol grubu takip etmiş ve en düşük yaprak alanı ise %100 mavi ışığa (54.80 ± 0.85 cm<sup>2</sup>) maruz kalan fidelerde belirlenmiştir (Çizelge 7).



Şekil 3. Farklı oranlardaki mavi led lambalar ile yapılan aydınlatma sonrası kontrol ışığı koşullarında yetiştirilen domates fideleri

Figure 3. Tomato seedlings grown under controlled light conditions after exposure to different ratios of blue LED lights

En yüksek fide yaş ağırlığına %12.5 mavi ve %25 mavi ışık (sırasıyla 11.98 ± 0.26 g ve 10.91 ± 0.44 g) ile aydınlatılmış fidelerin sahip olduğu bulunmuş ve bu değerleri sırasıyla %50 mavi ile kontrol ışıklandırmasına maruz kalan fideler takip etmiştir. Fide yaş ağırlığında en düşük değer ise %100 mavi ışığa maruz kalmış (6.47 ± 0.22 g) fidelerde ölçülmüştür. En düşük fide kuru ağırlığı %100 mavi ışık (0.69 ± 0.08 g) uygulaması altında bırakılan fidelerden elde edilmiş ve diğer tüm uygulamalardaki fideler daha yüksek kuru ağırlığa sahip olmuşlardır. Ayrıca, en yüksek kök yaş ağırlığı %12.5 mavi ışık (5.48 ± 0.34 g) altında yetişen fidelerde görülürken bu

fideleri kontrol, %25 ve %50 mavi ışık altında bırakılan fideler takip etmiş ve en düşük kök yaş ağırlığı ise %100 mavi (2.37 ± 0.12 g) ışığa maruz kalan fidelerde ölçülmüştür. Kök kuru ağırlığında ise yine benzer bir eğilimin olduğu ortaya çıkmış ve %100 mavi ışık ile aydınlatılmış fideler en düşük kök kuru ağırlığına sahip olurken %12.5 ve %25 mavi ışık ile kontrol grubu aydınlatmasına maruz kalan fidelerde en yüksek değerler elde edilmiştir (Çizelge 8).

Farklı oranlarda mavi ışık ile yapılan aydınlatmanın domates fidelerinin klorofil içeriği üzerine etkisi önemli bulunmuş ve en yüksek klorofil içeriği (0.085 ± 0.001 µmol g<sup>-1</sup> TA) %12.5 mavi ışık ile aydınlatılmış



fidelerde gözlenmiş olup bu grubu sırasıyla kontrol ( $0.078 \pm 0.001 \mu\text{mol g}^{-1} \text{TA}$ ), %25 mavi ışık ( $0.070 \pm 0.0008 \mu\text{mol g}^{-1} \text{TA}$ ) ve %50 mavi ışık ( $0.067 \pm 0.001 \mu\text{mol g}^{-1} \text{TA}$ ) uygulaması takip etmiştir. En düşük yaprak klorofil içeriği ise ( $0.063 \pm 0.001 \mu\text{mol g}^{-1} \text{TA}$ ) %100 mavi ışık altında yetiştirilen domates fidelerinde

saptanmıştır. Farklı oranlarda mavi ışığa maruz kaldıktan sonra 10 gün süreyle günışığı koşullarında yapılan yetiştiriciliğin yaprak MDA ve  $\text{H}_2\text{O}_2$  içerikleri üzerine etkileri ise istatistiksel olarak önemsiz bulunmuştur (Çizelge 9).

Çizelge 7. Farklı oranlardaki mavi led uygulaması sonrası kontrol ışığı koşullarında yetiştirilen fidelerin ilk gerçek yaprak uzunluğu, fide boyu, gövde çapı, yaprak sayısı ve yaprak alanı

Table 7. Distance to first true leaf, seedling height, stem diameter, leaf number, and leaf area of seedlings grown under control light conditions after blue LED light application

Uygulamalar	İlk gerçek yaprak boyu (cm)	Fide Boyu (cm)	Gövde çapı (mm)	Yaprak sayısı (adet)	Yaprak alanı (cm <sup>2</sup> )
Kontrol	$6.14^c \pm 0.19$	$9.98^d \pm 0.19$	$3.08^a \pm 0.05$	$3.48 \pm 0.07$	$72.33^b \pm 9.24$
%100 Mavi	$5.14^d \pm 0.29$	$8.04^e \pm 0.40$	$2.73^b \pm 0.09$	$3.42 \pm 0.04$	$54.80^c \pm 0.85$
%50 Mavi	$6.94^b \pm 0.35$	$12.57^c \pm 0.14$	$3.03^a \pm 0.03$	$3.35 \pm 0.04$	$79.87^{ba} \pm 2.22$
%25 Mavi	$7.99^a \pm 0.23$	$14.11^b \pm 0.21$	$3.21^a \pm 0.01$	$3.40 \pm 0.15$	$90.75^a \pm 2.79$
%12.5 Mavi	$8.71^a \pm 0.08$	$16.12^a \pm 0.70$	$3.14^a \pm 0.03$	$3.40 \pm 0.14$	$85.69^{ba} \pm 0.85$
p değeri	0.0001	0.0001	0.0056	0.9345	0.0017
LSD <sub>0.05</sub>	0.77	1.21	0.21	-	14.06

\*: P<0.05 Aynı sütunda farklı harflerle gösterilen değerler istatistiksel olarak birbirinden farklıdır

Çizelge 8. Farklı oranlardaki mavi led uygulaması sonrası kontrol ışığı koşullarında yetiştirilen fidelerin toprak altı ve üstü yaş ve kuru ağırlık değerleri

Table 8. The above and below ground fresh and dry weight values of seedlings grown under controlled light conditions after blue LED light exposure

Uygulamalar	Fide YA (g bitki <sup>-1</sup> )	Fide KA (g bitki <sup>-1</sup> )	Kök YA (g bitki <sup>-1</sup> )	Kök KA (g bitki <sup>-1</sup> )
Kontrol	$9.41^c \pm 0.61$	$1.17^a \pm 0.14$	$3.57^b \pm 0.39$	$0.27^{ba} \pm 0.03$
%100 Mavi	$6.47^d \pm 0.22$	$0.69^b \pm 0.08$	$2.37^c \pm 0.12$	$0.15^c \pm 0.00$
%50 Mavi	$9.90^{bc} \pm 0.24$	$1.13^a \pm 0.05$	$3.26^b \pm 0.18$	$0.23^b \pm 0.01$
%25 Mavi	$10.91^{ba} \pm 0.44$	$1.13^a \pm 0.05$	$3.70^b \pm 0.10$	$0.26^{ba} \pm 0.03$
%12.5 Mavi	$11.98^a \pm 0.26$	$1.37^a \pm 0.04$	$5.48^a \pm 0.34$	$0.34^a \pm 0.01$
p değeri	0.0001	0.0020	0.0001	0.0028
LSD <sub>0.05</sub>	1.21	0.25	0.81	0.07

\*: P<0.05 Aynı sütunda farklı harflerle gösterilen değerler istatistiksel olarak birbirinden farklıdır.

Çizelge 9. Farklı oranlardaki mavi led uygulaması sonrası kontrol ışığı koşullarında yetiştirilen fidelerin klorofil, MDA ve  $\text{H}_2\text{O}_2$  içerikleri

Table 9. The chlorophyll, MDA, and  $\text{H}_2\text{O}_2$  contents of seedlings grown under controlled light conditions after blue LED light exposure

Uygulamalar	Klorofil (mg g <sup>-1</sup> TA)	MDA (mg g <sup>-1</sup> TA)	$\text{H}_2\text{O}_2$ (mg g <sup>-1</sup> TA)
Kontrol	$0.078^b \pm 0.001$	$2.45 \pm 0.50$	$0.66 \pm 0.01$
%100 Mavi	$0.063^e \pm 0.001$	$3.42 \pm 0.11$	$0.79 \pm 0.09$
%50 Mavi	$0.067^d \pm 0.001$	$3.29 \pm 0.68$	$0.75 \pm 0.03$
%25 Mavi	$0.070^c \pm 0.001$	$3.10 \pm 0.46$	$0.71 \pm 0.02$
%12.5 Mavi	$0.085^a \pm 0.001$	$2.56 \pm 0.30$	$0.68 \pm 0.01$
p değeri	0.0001	0.4885	0.2721

\*: P<0.05 Aynı sütunda farklı harflerle gösterilen değerler istatistiksel olarak birbirinden farklıdır.

Elde edilen sonuçlar domates fidelerinde mavi ışık oranına göre değişmekle birlikte fide boyu ve ilk gerçek

yaprağa kadar olan uzunluk açısından uygulamalar arasındaki farklılıkların korunduğu ama tüm

uygulamalardaki fidelerin gelişerek boylarının arttığı gözlemlenmiştir. Bu sonuçlar mavi ışığın fide boyu üzerindeki baskılayıcı etkisinin kalıcı olmadığı ve fideler kontrol (beyaz ve günışığı karışımı) ışığı koşullarına alındığında büyümelerinin normal şekilde devam ettiğini göstermiştir. Literatürde giberellin biyosentez inhibitörü çeşitli kimyasal maddelerin kullanılması sonucu fidelerde boy kontrolünün sağlandığı fakat çoğu zaman kullanılan kimyasalın dozunun yüksek olması halinde fidelerin arazi koşullarına aktarılmaları sonrası hala büyümelerinin duraksadığı ve çiçeklenmenin geciktiği hatta ürünün de olumsuz etkilendiği bildirilmiştir (Kofidis ve ark., 2008; Özbay ve Ergun, 2015). Bu nedenle tek renk kaynağı olarak mavi ışık kullanılmasının domates fidelerinin boylarının baskılanmasında kullanılabilceği ve elde edilen etkinin ise fidelerin normal aydınlatma koşullarına döndüklerinde kalıcı olmadığını belirlenmesi fide üreticileri açısından kullanılabilcek bir yöntem olarak görülmektedir.

## SONUÇ ve ÖNERİLER

Bu çalışmada farklı dalga boyunda ışınım yapan led lambalar altında yapılan yetiştiricilik sonucunda tek renk ışık kaynağı olarak mavi dalga boyunun kullanılmasının fidelerin boy uzamasını ve fide ağırlığını büyük oranda baskıladı ve dolayısıyla vejetatif büyümeyi sınırladığı görülmüştür. Buna karşılık, kırmızı ışık altında yetiştirilen fidelerin boylarının ve gövde çap kalınlıklarının arttığı ve dolayısıyla mavi ışığın aksine fide yaş ağırlığını arttığı belirlenmiştir. Ayrıca mavi ve kırmızı ışık altında yapılan yetiştiriciliğin bitkilerde MDA ve H<sub>2</sub>O<sub>2</sub> içeriklerini yükselttiği dolayısıyla strese neden olduğu belirlenmiştir.

Yine hazır fide formuna gelmiş fideler farklı oranlardaki mavi ışık altında yetiştirildiğinde %100 mavi ışığın fidelerin boy uzamasını engellediği ve mevcut ışık yoğunluğu içerisindeki mavi ışık oranının azaltılmasıyla doğru orantılı olarak fide boy uzamasının teşvik edildiği belirlenmiştir. Kontrol uygulamasını oluşturan beyaz-günışığı karışımına en düşük oranda (%12.5) eklenen mavi ışık, beklenen aksine domates fidelerinin boylarının ve ağırlıklarının önemli seviyede artmasına neden olmuştur. Böylece araştırmanın amaçlarından biri olan doğal aydınlatmaya ya da güneş ışığına ilave olarak fide boy gelişimini baskılayan renkteki (mavi) led lambaların karıştırılmasının fide boy kontrolü amacına yönelik pratikte kullanılmasının mümkün olmayacağı belirlenmiştir. Ancak, mavi ışık uygulamalarından sonra fidelerin beyaz ve günışığı karışımından oluşan kontrol koşullarına transfer edilmesiyle mavi ışığın neden olduğu büyümedeki gerilemenin kalıcı olmadığı, dolayısıyla fidelerin boylarının uzamaya devam ettiği belirlenmiştir. Sonuç olarak, domates fidelerinde etkileri kalıcı olmayan ışık dalga boyunun mavi olduğu

ve %100 mavi ışığın fidelerde boy uzamasını baskılayıcı bir uygulama olarak kullanılabilceği belirlenmiştir. Ancak, mavi ışığın sebep olduğu vejetatif büyüme üzerindeki baskılayıcı etkilerinin giberellin sentezinin geçici olarak engellenmesi olduğu düşünüldüğü için bu konunun detaylı bir şekilde araştırılmasının gerekli olduğu düşünülmektedir.

## TEŞEKKÜR

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## Investigations on Feeding Preferences of Adult *Sitona* Weevils (Coleoptera: Curculionidae) in Some Host Plants

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### ABSTRACT

In this study, we determined the feeding preferences of *Sitona callosus* Gyllenhal, 1834, *S. cylindricollis* Fåhraeus, 1840, *S. humeralis* Stephens, 1831, *S. longulus* Gyllenhal, 1834, *S. macularius* (Marsham, 1802), *S. obsoletus* (Gmelin, 1790), and *S. puncticollis* Stephens, 1831 species belonging to the genus *Sitona* (Coleoptera, Curculionidae) in alfalfa (*Medicago sativa* L.), black medick (*M. lupina* L.), white clover (*Trifolium repens* L.), soybean (*Glycine max* (L.)), birdsfoot trefoil (*Lotus corniculatus* L.), honey clover (*Melilotus albus* Medik.), yellow sweet clover (*M. officinalis* (L.)), sainfoin milk vetch (*Astragalus onobrychis* L.), sainfoin (*Onobrychis viciifolia* Scop.), and liquorice (*Glycyrrhiza glabra* L.) (Fabaceae) plants at 25°C±5°C in the laboratory. Plant preference experiments were established with 10 replications and as a result of analysis of variance (P>0.01), plant preference of each species was found to be significant. As a result, *M. sativa* and *T. repens* (100%) were the most preferred by *Sitona* species. Whereas *L. corniculatus* (91.43%), *M. albus* (87.14%), *O. viciifolia* (81.43%), *M. lupina* (78.57%), *M. officinalis* (60.00%), *A. onobrychis* (31.43%) followed, respectively. The least preferred species were *G. glabra* (7.14%) and *G. max* (1.43%).

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## Bazı Konukçu Bitkilerde *Sitona* Erginlerinin (Coleoptera: Curculionidae) Beslenme Tercihleri Üzerine Araştırmalar

### ÖZET

Bu çalışmada, *Sitona* cinsine ait *Sitona callosus* Gyllenhal, 1834, *S. cylindricollis* Fåhraeus, 1840, *S. humeralis* Stephens, 1831, *S. longulus* Gyllenhal, 1834, *S. macularius* (Marsham, 1802), *S. obsoletus* (Gmelin, 1790), ve *S. puncticollis* Stephens, 1831 türlerinin (Coleoptera, Curculionidae), yonca (*Medicago sativa* L.), şerbetçi otu yoncası (*M. lupina* L.), ak çöğül (*Trifolium repens* L.), soya fasulyesi (*Glycine max* (L.)), Gazel boynuzu (*Lotus corniculatus* L.), ak taş yoncası (*Melilotus albus* Medik.), sarı taş yoncası (*M. officinalis* (L.)), korungamsı geven (*Astragalus onobrychis* L.), adi korunga (*Onobrychis viciifolia* Scop.) ve meyan (*Glycyrrhiza glabra* L.) (Fabaceae) bitkilerinde beslenme tercihleri laboratuvar ortamında 25°C±5°C'de belirlenmiştir. Bitki tercih denemeleri 10 tekrarlı olarak kurulmuş ve varyans analizi sonucunda (P>0.01) her *Sitona* türünün bitki tercihleri önemli bulunmuştur. Sonuç olarak, *M. sativa* ve *T. repens* (%100) *Sitona* türleri tarafından en çok tercih edilen türler olmuştur. Bunu sırasıyla *L. corniculatus* (%91.43), *M. albus* (%87.14), *O. viciifolia* (%81.43), *M. lupina* (%78.57), *M. officinalis* (%60.00), *A. onobrychis* (%31.43) takip etmiştir. En az tercih edilen türler ise *G. glabra* (%7,14) ve *G. max* (%1,43) olmuştur.

### Bitki Koruma

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## INTRODUCTION

Within Insecta, the Curculionoidea superfamily of the Coleoptera order is a very important agricultural group with its high species diversity. The genus *Sitona* Germar, 1817, which is very important in terms of agricultural pests belonging to Curculionidae, is represented by more than 100 species identified in the world (Velázquez de Castro et al., 2007). They naturally spread in the Nearctic and Palaearctic regions, but some species have also been distributed in South Africa, Australia, and New Zealand (Phillips & Barratt, 2004). Adults of *Sitona* species feed on shoots and leaves of leguminous (Fabaceae) and germinating plants, causing their death. On the other hand, its larvae, cause serious damage by feeding on both roots and nodules (Scherf, 1964; Danthanarayana, 1967; Plaut, 1976; El-Dessouki & El-Awady, 1978; Aeschlimann, 1980; Syrett, 1992; Murray, 1996; Cantot, 2001). Various studies have been conducted to determine the host plants of *Sitona* species and the damage they cause (Cantot, 1979; Aeschlimann, 1980; Minda-Lechowska, 1980; Cmoluch, 1980; Dieckmann, 1980; Palm, 1996; Blaeser-Dieckmann, 1982; Murray & Clements, 1994). The Leguminosae or Fabaceae family, food for *Sitona* species, is the second largest family of flowering plants in the world with 650 genera and approximately 18,000 species. They spread on almost all continents except Antarctica. Species from this family vary from small grasses of arctic and alpine vegetation to large trees of tropical forests. The most characteristic feature of the family is its unique legume-type fruit and pea-like or bean-like husk. Additionally, the flower structure is very characteristic of the family. It is one of the most economically important families among dicotyledons. Legume seeds are of high quality in terms of protein content and because they contain high nutritional value, legumes are also used for chewing gum, glue, timber, medicinal purposes, human food, animal feed, and green manure (Yılmaz, 2007). IRLC (Inverted Repeat-Lacking Clade) is one of the most derived branches in the Papilionoideae subfamily of the Fabaceae family and they are economically important plants (Duan et al., 2021). IRLC plants can both grow wild in natural habitats and are also cultivated. *Sitona* species usually feed on IRLC plants (Velázquez de Castro et al., 2007). Although adults make the host preference, larval development takes place in plant nodules and then in roots. Additionally, the flower structure is very characteristic of the species. This is why, the larvae are more destructive (Fisher & O'Keeffe, 1979). Cantot (2001) stated that the presence of different nodule types and bacterial species can create a barrier for the larvae. However, the feeding preferences of *Sitona* species in root nodules are important, and same way, the morphological structure and chemical constituents of the leaf may also be key factors (Velázquez de Castro

et al., 2007). *Sitona* species, which are agricultural pests by feeding on cultivated legumes, are oligophagous insects (Scherf, 1964). Many studies have been conducted to identify the host plants of *Sitona* species (Cantot, 1979; Aeschlimann, 1980; Minda-Lechowska, 1980; Blaeser-Dieckmann, 1982; Murray & Clements, 1994; Syrett & Emberson, 1997). Studies were carried out to determine the plant preferences of some species belonging to the *Sitona* genus, which are abundant in nature, under laboratory conditions and to reveal which *Sitona* species can cause damage to which cultivated plants.

## MATERIAL and METHOD

To determine the host feeding preferences of *Sitona* species in laboratory conditions; *Melilotus officinalis* (L.), *M. albus* Medik., *Medicago sativa*, *M. lupina* L., *Trifolium repens*, *Lotus corniculatus*, *Astragalus onobrychis* L., *Onobrychis viciifolia* Scop., *Glycyrrhiza glabra* L. and *Glycine max* (L.) were collected freshly from the nature. *Sitona obsoletus* Gmelin, *S. cylindricollis* Fähræus, *S. callosus* Gyllenhal, *S. humeralis* Stephens, *S. puncticollis* Stephens, *S. macularius* Marsham, and *S. longulus* Gyllenhal adults, which were collected from nature in September-October in sufficient numbers, were brought to the laboratory for the experiments. They were separated in terms of species and gender. After the selected adults were starved for 48 hours in 100 ml jars, 2 individuals (1♀ + 1♂) were released into 9 cm x 2 cm petri. Plants with 3-4 leaves, the stems of which were tightly wrapped with wet cotton, were placed in each petri dish, and moisture was maintained throughout the experiment (Wightman, 1986). The individuals included in the experiment were checked daily and the experiment was terminated after 72 hours. In the evaluations, if at least 3 bites or 1/3 of a leaf were eaten, it was accepted as the food of the insect (Figure 1). Experiments were set up in a randomized plot design with 10 replications for each species at 25°C ± 5°C under laboratory conditions. Variance analysis was performed by subjecting the obtained findings to angle transformation in the JMP Pro 13 statistical package program. A student's test (LSD0.05) was used to group the significant data.

## RESULTS AND DISCUSSION

In this study, 7 *Sitona* weevils, which are common in nature and in agricultural fields, the host feeding preferences were investigated on 10 plant species belonging to Papilionoideae (Fabaceae). According to these results, *M. sativa* and *T. repens* (Trifolieae tribe) (100%) were the most preferred by *Sitona* species. The other results include *L. corniculatus* (Loteae) (91.43%), *M. albus* (87.14%) (Trifolieae), *O. viciifolia* (81.43%) (Hedysareae), *M. lupina* (78.57%), *M. officinalis*

(60.00%), (Trifolieae), *A. onobrychis* (31.43%) (Galegeae). The least preferred species were *G. glabra* (7.14%) (Hedysareae) and *G. max* (1.43%) (Phaseoleae) (Figure 2h). As a result of variance analysis in the host

plant feeding preference experiments of *Sitona* (Coleoptera, Curculionidae) species, it was found to be important ( $P>0.01$ ) in terms of plant preference (Table 1).



Figure 1. Feeding behavior experiments of *Sitona* species on host plants in the laboratory.  
Şekil 1. Laboratuvarda *Sitona* türlerinin konukçu bitkide beslenme davranışı çalışmaları.

Table 1. The host plant feeding preferences of *Sitona* species in the laboratory condition.

Çizelge 1. Laboratuvar koşullarında *Sitona* türlerinin konukçu bitki besleme tercihleri.

Host species	<i>Sitona</i> species*							Mean
	<i>S.ob</i>	<i>S.cy</i>	<i>S.ca</i>	<i>S.hu</i>	<i>S.pu</i>	<i>S.ma</i>	<i>S.lo</i>	
<i>Melilotus officinalis</i>	30 df	100 a	20 eg	100 a	50 cd	40 ce	80 ab	60,00 e
<i>Melilotus albus</i>	60 bc	100 a	90 a	100 a	100 a	60 bc	100 a	87,14 bc
<i>Medicago sativa</i>	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100,00 a
<i>Medicago lupina</i>	100 a	0 g	100 a	100 a	100 a	50 cd	100 a	78,57 d
<i>Trifolium repens</i>	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100,00 a
<i>Lotus corniculatus</i>	100 a	100 a	100 a	40 ce	100 a	100 a	100 a	91,43 b
<i>Astragalus onobrychis</i>	20 eg	0 g	20 eg	30 df	100 a	40 ce	10 fg	31,43 f
<i>Onobrychis viciifoli</i>	20 eg	100 a	100 a	100 a	100 a	100 a	50 cd	81,43 cd
<i>Glycyrrhiza glabra</i>	10 fg	0 g	0 g	0 g	10 fg	20 eg	10 fg	7,14 g
<i>Glycine max</i>	0 g	0 g	0 g	0 g	0 g	10 fg	0 g	1,43 g
<b>Mean</b>	<b>54 d</b>	<b>60 cd</b>	<b>63 bc</b>	<b>67 b</b>	<b>76 a</b>	<b>62 bc</b>	<b>65 bc</b>	<b>63,86</b>

CV(%)13.78, LSD<sub>0,05</sub>=Plant 8,22\*\*, Species 6,88\*\*, Host plant feeding X *Sitona* species 21,76\*\*, \*\*=( $P<0.01$ ) level is important.

\**S.ob*: *S.obsoletus*, *S.cy*: *S.cylindricollis*, *S.ca*: *S.callosus*, *S.hu*: *S.humeralis*, *S.pu*: *S.puncticollis*, *S.ma*: *S.macularius*, *S.lo*: *S.longulus*

In the experiments, *S. Callosus* individuals were fed in *M. sativa*, *M. lupina*, *T. repens*, *L. corniculatus*, *O. viciifoli* (100%) and *M. albus* (90%). It was determined that *A. onobrychis* (20%) and *M. officinalis* (20%) species were less preferred, while *G. glabra* and *G. max* species were not preferred (Figure 2a). In nature Lodos et al. (1978), *S. callosus* *Astragalus* sp. while Velázquez de Castro et al. (2007) *Medicago* pointed out that it feeds on plants belonging to the genus *Onobrychis*, *Ononis*. *S. cylindricollis* individuals were fed on *M. sativa*, *M. albus*, *M. officinalis*, *T. repens*, *L. corniculatus*, *O. viciifoli* (100%). *M. lupina*, *A. onobrychis*, *G. glabra* and *G. max* were not fed on (Figure 2b). Although *S. cylindricollis* has been reported to feed on *Melilotus* (Bright & Bouchard, 2008), this genus has not been recorded as a host plant of *S. cylindricollis* (Rim et al., 2019). However, in our laboratory studies, it was observed that *S. cylindricollis* fed on *M. officinalis* and *M. albus*. Bird (1947) reported that *S. cylindricollis* caused serious damage by feeding on sweet clover (*M. officinalis*). *S. humeralis* individuals were found to be fully fed on

(100%) *M. sativa*, *M. lupina*, *M. officinalis*, *M. albus*, *T. repens* and *O. viciifolia* plants. It was observed that *L. corniculatus* (40%) and *A. onobrychis* (30%) species were less preferred, while *G. glabra* and *G. max* plants were not preferred (Figure 2c). Aeschlimann (1984) reported that *S. humeralis* is very abundant in perennial species of *Medicago* in the Mediterranean region, but not in annual species. *S. humeralis* has been reported to be found on *Trifolium* and *Melilotus* (Koch, 1992), *Ononis repens* L., *Lathyrus aphacca* L., *T. repens*, and *Pisum sativum* L. (Hoffmann, 1950; Scherf, 1964; Nasredinov, 1975; Koch, 1992). According to Velázquez de Castro et al. (2007), *Medicago* and *Trifolium* plants are the host plants of *S. humeralis*.

In this experiment, it was seen that *S. humeralis* adults were fed with the leaves of *L. corniculatus* and *A. onobrychis* among these plants. In addition, it is known that *S. humeralis* feeds on cultivated *M. sativa* and causes economic damage (Tanasijevic, 1974; Kıvan, 1995; Atanasova, 2012; Arbab & McNeill, 2014;



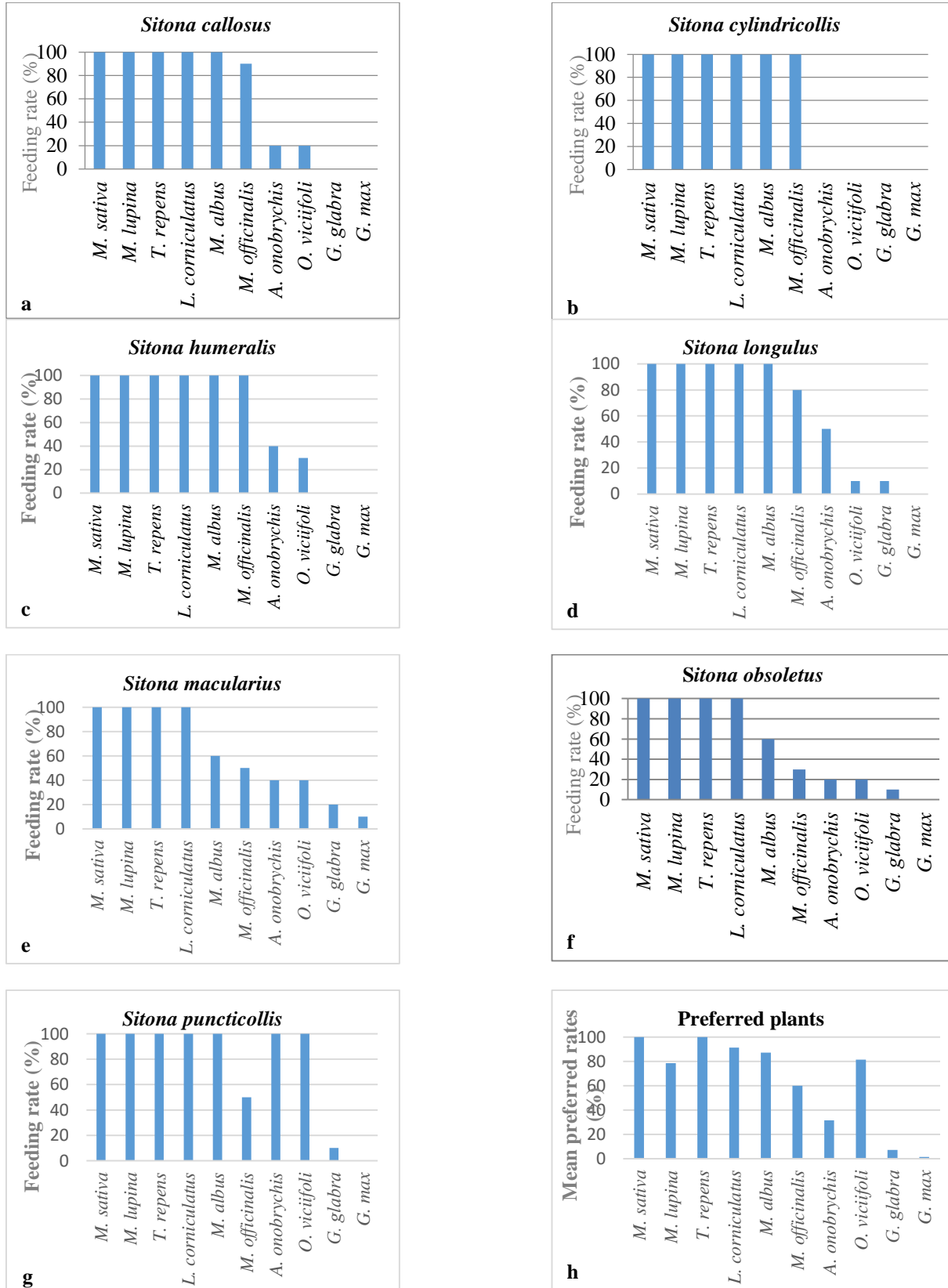


Figure 2. Host feeding preferences of *Sitona* species (a- *Sitona callosus* b- *Sitona cylindricollis*, c- *Sitona humeralis*, d-*Sitona longulus*, e-*Sitona macularius*, f-*Sitona obsoletus*, g-*Sitona puncticollis*, h-Preferred plants).

Şekil 2. *Sitona* türlerinin konukçu beslenme tercihleri (a- *Sitona callosus* b- *Sitona cylindricollis*, c- *Sitona humeralis*, d-*Sitona longulus*, e-*Sitona macularius*, f-*Sitona obsoletus*, g-*Sitona puncticollis*, h- Tercih edilen bitkiler).



Gözüaçık et al., 2021). *Sitona longulus* Gyllenhal individuals fully (100%) fed on *M. sativa*, *M. lupina*, *T. repens*, *L. corniculatus*, *M. albus* and *M. officinalis* plants. It was determined that *O. viciifoli* (50%) was moderate, *A. onobrychis* and *G. glabra* (10%) were very few and *G. max* was not preferred (Figure 2d). Lodos et al. (2003), the plants on which *S. longulus* is found are *M. sativa*, *Astragalus* sp. Velázquez de Castro et al. (2007) reported that it feeds on plants belonging to *Lathyrus*, *Medicago*, *Vicia* genus. *S. macularius* individuals preferred *M. sativa*, *T. repens*, *L. corniculatus*, and *O. viciifoli* (100%) much, *M. albus* (60%), *M. lupina* (50%), and *M. officinalis* (40%) moderate, and *G. glabra* (20%) and *G. max* (10%) very little (Figure 2e). Lodos et al. (1978), *M. sativa* and *Vicia* spp. plants may be hosts of *S. macularius* and, Velázquez de Castro et al. (2007) reported that it feeds on plants belonging to the genera *Lupinus*, *Lens*, *Medicago*, *Onobrychis*, *Trifolium*, *Pisum*, *Vicia*. Hariri (1981) and Solh et al. (1986) stated that *S. macularius* is the main pest of lentil plants in West Asia and North Africa. Tahhan and Hariri (1982) reported that this species is the most abundant *Sitona* species with a rate of 95% among the lentils found in northern Syria. *S. obsoletus* individuals preferred *M. sativa*, *M. lupina*, *T. repens*, and *L. corniculatus* species (100%) and *M. albus* (60%), while *M. officinalis* (30%), *A. onobrychis* (20%), *O. viciifoli* (20%) and *G. glabra* (10%) species were found to be less preferred, while they did not prefer *G. max* (Figure 2f). It has been reported that *S. obsoletus* feeds on white clover (*T. repens*) and red clover (*T. pratense*) in temperate meadows (Brudea, 1982; Murray & Clements, 1994; Gerard et al., 2005). Gerard et al. (2007) reported that *S. obsoletus* larvae are the main pest of *T. repens* in New Zealand, reducing yield by 34-35%, and doing the most damage in spring. *S. puncticollis* individuals prefer *M. sativa*, *M. lupina*, *M. albus*, *T. repens*, *A. onobrychis*, *O. Viciifoli* and *L. corniculatus* (100%), *M. officinalis* (50%) moderately, *G. glabra* at very low rates (10%), and did not prefer *G. max* (Figure 2g). Velázquez de Castro et al. (2007) expressed that *S. puncticollis*, feeds on species belonging to *Lotus*, *Lens*, *Medicago*, *Melilotus*, *Trifolium*, and *Vicia* genus as the hosts.

In this study, it was determined that *Sitona* species preferred some Fabaceae plant species that are abundant in nature and cultivated as their food. These plants were preferred by *Sitona* adults  $M. sativa \geq T. repens > L. corniculatus > M. albus > O. viciifoli > M. lupina > M. officinalis > A. onobrychis > G. glabra$  respectively. It has been determined that although the species is not the main host of most of the plants it feeds on, it can also feed on other alternative host plants from the same family in order to survive. The presence of several species on the same plant in nature was better understood by this study.

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## Researchers Contribution Rate Declaration Summary

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

## Conflicts of Interest Statement

The author declares no conflicts of interest.

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## Akdeniz Meyve Sineği [*Ceratitis capitata* Wied. (Diptera: Tephritidae)]'nin Şanlıurfa İli Nar Bahçelerindeki Popülasyon Gelişimi ve Bulaşıklık Oranı

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### ÖZET

Akdeniz meyve sineği, *Ceratitis capitata* Wied. (Diptera: Tephritidae) birçok meyve türünde ekonomik kayıplara sebep olan önemli bir zararlıdır. Bu çalışma, Akdeniz meyve sineğinin Şanlıurfa ilinin Akçakale, Haliliye, Karaköprü ve Suruç ilçelerindeki nar bahçelerinde popülasyon gelişiminin ve bulaşıklık oranının belirlenmesi amacıyla 2020-2021 yıllarında yürütülmüştür. Çalışmada zararlının popülasyon gelişimini belirlemek için her bahçeye üçer adet eşeysel çekici feromon tuzağı asılarak haftalık olarak takip edilmiştir. Zararlının nar bahçelerindeki bulaşıklık oranını belirlemek için hasat esnasında her bahçede 10'ar ağacın farklı yönlerinden alınan 100 meyvede zarar belirtisi ve larva kontrolü yapılmıştır. Çalışma sonucunda zararlının Şanlıurfa ilindeki nar bahçelerinde ilk olarak ağustos ayı itibariyle görülmeye başlandığı, 2020 yılında düşük olan popülasyonun 2021 yılında yükseldiği belirlenmiştir. Zararlının popülasyonunun yaz aylarında düşük iken özellikle Akçakale ve Suruç ilçelerindeki bahçelerde Ekim ve Kasım aylarında yüksek dalgalanma gösterdiği belirlenmiştir. Zararlı 2020 yılında en fazla Suruç ilçesindeki nar bahçesinde 16 Ekim tarihinde 17 ergin/tuzak ile yakalanırken 2021 yılında en yüksek ergin yakalanması Akçakale ilçesinde 19 Kasım tarihinde 565 ergin/tuzak, Suruç ilçesinde 25 Eylül tarihinde 216 ergin/tuzak ile gerçekleşmiştir. Zararlının yıllar ve lokasyonlara göre değişmekle beraber Ağustos ile Aralık ayları arasında iki tepe noktası oluşturarak doğada 1 ile 5 ay arasında aktif bulunduğu belirlenmiştir. Doğada aktif olduğu bu süre içerisinde tuzaklara yakalanan toplam ergin sayıları 2020 ve 2021 yıllarında sırasıyla Karaköprü ilçesinde 21 ve 312, Suruç ilçesinde 125 ve 1353, Akçakale ilçesinde 26 ve 1226 ve Haliliye ilçesinde 29 ve 259 ergin/tuzak olarak tespit edilmiştir. Çalışma sonucunda zararlının Haliliye ve Karaköprü ilçelerindeki nar meyvelerinde zarar meydana getirmediği tespit edilmiş olup Suruç ilçesindeki nar bahçesinde 2020 yılında bulaşıklık yok iken 2021 yılında %2 oranında meyve bulaşıklığı belirlenmiştir. Çalışmanın her iki yılında da en fazla bulaşıklık Akçakale ilçesinde ve sırasıyla %1 ve %4 olarak belirlenmiştir. Zararlının ilk ergin uçuşunun yaz aylarının sonuna doğru gerçekleşmesi ve popülasyonun sonbaharda yükselmesi Şanlıurfa koşullarında kışı geçiremediği sonucunu doğurmuştur. Sonuç olarak, zararlının Şanlıurfa ili nar bahçelerinde henüz ekonomik anlamda zarar meydana getirmediği ancak 2021 yılında popülasyon yoğunluğunda meydana gelen artışın gelecekte ekonomik anlamda bir zararlı konumuna gelebileceğini düşündürmektedir. Bu nedenle nar üreticilerinin, teknik elemanların ve ilgili paydaşların eğitim ve yayım faaliyetleri ile zararlı konusunda bilgilendirilmeleri mutlak önem arz etmektedir. Nar üreticilerine nar hasadının geciktirilmemesi ile ağaçta kalan ve yere düşen bulaşık nar meyvelerinin toplanarak imhası önerilmelidir.

### Bitki Koruma

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*Ceratitis capitata*

Popülasyon

Bulaşıklık

Feromon

Nar



## Population Development and Infestation Rate of Mediterranean Fruit Fly [*Ceratitis capitata* Wied. (Diptera: Tephritidae)] in Pomegranate Orchards in Şanlıurfa, Türkiye

### ABSTRACT

Mediterranean fruit fly [*Ceratitis capitata* Wied. (Diptera: Tephritidae)] is an important pest responsible for significant economic losses in several fruit species. This study determined the population development and infestation rate of Mediterranean fruit flies in pomegranate orchards situated in Akçakale, Haliliye, Karaköprü, and Suruç districts of Şanlıurfa province during the years 2020-2021. Three sexually attractive pheromone traps were hanged in each orchard and monitored weekly. A total of 100 fruits from randomly selected ten trees in each orchard were collected during harvest and examined to record the damage symptoms and larvae of the pest. The first flight of the pest was recorded in August and a lower population was noted during 2020 compared to 2021. The population was low in the summer, while it was high in the autumn months, particularly in Akçakale and Suruç districts. The highest population (17 adults/traps) during 2020 was recorded on October 16<sup>th</sup> in the Suruç orchard while it reached 565 adults/traps on November 19<sup>th</sup> in Akçakale and 216 adults/traps on September 25<sup>th</sup> in the Suruç district. Although varied across years and locations, two population peaks were recorded with an active period of 1-5 months between August and December. The total number of adults caught in the traps during the active period was 21 and 312 adults/trap in Karaköprü, 125 and 1353 adults/trap in Suruç, 26 and 1226 adults/trap in Akçakale, and 29 and 259 adults/trap in Haliliye during 2020 and 2021, respectively. The infestation rate was 0% and 1% in Suruç and 1% and 4% in Akçakale during 2020 and 2021, respectively. The pest did not exert any damage to the pomegranate orchard in the Haliliye and Karaköprü districts during both years. The adult flight of the pest was recorded at the end of summer and population increase during autumn indicated that the pest could not overwinter in Şanlıurfa. Hence, the pest did not exert any economic damage to the pomegranate orchards of Şanlıurfa, however, higher population density during 2021 suggests that it could become an economic pest in the future. For this reason, pomegranate producers must be informed about the pest through training and extension activities. Pomegranate producers should be advised to harvest pomegranate without delay and collect and destroy infested fruits on the trees and fallen on the ground.

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### Research Article

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### GİRİŞ

Dünyada ekonomik anlamda yetiştiriciliği yapılan önemli meyve türlerinden biri olan nar [(*Punica granatum* L. Myrtales: Punicaceae)], subtropik ve tropik iklim bölgelerine adapte olmuştur. Narın kültüre alınışının Pakistan ve Afganistan olduğu, daha sonra İran ve Mezopotamya'ya geçtiği bilinmektedir. Mezopotamya'da 4500 yıl öncesine ait eserlerde nar meyvesinden bahsedilmektedir (Onur, 1982; Glozer & Ferguson, 2008; Kaygısız, 2009; Oğuz

ve ark., 2011; Ünal, 2011). Günümüzde Avrupa'nın Güney kesimleri, Afrika'nın kuzey bölgelerinde, İran, Hindistan, Çin, Afganistan, Arjantin, Suudi Arabistan, Şili, Amerika ve Meksika'da yoğun olarak tarımı gerçekleştirilmektedir (Özbek, 1977; Dokuzoğuz & Mendilcioğlu, 1978; Onur, 1983).

Nar, taze tüketiminin yanında meyve suyu, nar ekşisi, nar pekmezi, nar konsantresi, marmelat, sirke, hayvan yemi, ilaç, boya ve sitrik asit gibi farklı alanlarda değerlendirilmektedir (Kingsly ve ark.,

2006). Potasyum, demir ve C vitamini açısından zengin olan narın güçlü bir antioksidan olduğu kanser ile kalp hastalıklarını önlediği bildirilmiştir (Lansky ve ark., 1998; Teniente ve ark., 2023). Bu üstün özelliklerinden dolayı narın dünyada en fazla tercih edilen on meyve türünden biri olduğu kabul edilmektedir (Çanakçı, 2010).

Türkiye, narın gen merkezlerinden biri olup en fazla Hicaznar, Wonderful, Alata, Oğuzeli, Devediş, Zivzik, Suruç, Katine ve Tirbey çeşitleri yetiştirilmektedir (Onur, 1982; Ercan ve ark., 1991; Yılmaz & Özgüven, 2009; Mamay, 2013). Türkiye’de en fazla Akdeniz, Güneydoğu Anadolu ve Ege bölgelerinde 1000 metre yüksekliğe sahip rakımlara kadar olan alanlarda yetiştiriciliği yapılmaktadır (Onur, 1988; Ünal ve ark., 1995; Şahin, 2006; Özgüven & Yılmaz 2000; Ünal, 2011). Türkiye’nin toplam nar üretiminin %56’sını Akdeniz Bölgesi (304 039 ton), %31’ini Ege Bölgesi (166 430 ton) ve % 11’ini ise Güneydoğu Anadolu Bölgesi (57 935 ton) karşılamaktadır (TÜİK, 2021). Türkiye nar üretiminde Antalya 160.621 ton ile ilk sırada yer alırken; Şanlıurfa, Türkiye genelinde 11.681 tonluk üretimi ile 9. sırada yer almaktadır. Ülkemizin önemli üretici illeri arasında bulunan Şanlıurfa’nın nar üretim alanı son on yılda 4.831 dekadardan, 15.708 dekara, meyve veren ağaç sayısı 48.655 adetten, 711.551 adede ve üretim miktarı 778 tondan, 11.681 tona yükselmiştir. (TÜİK, 2021). Şanlıurfa’da genel olarak nar bahçeleri Hicaz, Suruç ve Katine (Siverek) çeşitleri ile tesis edilmiştir (Mamay ve ark., 2014; İkinci & Dursun, 2021).

Nar üreticileri gerek üretim aşamasında gerekse depolama sırasında ciddi oranlarda verim ve kalite kaybına sebep olan çok sayıda zararlı ile mücadele etmek zorundadır. Nar yetiştiriciliğini olumsuz yönde etkileyen başlıca zararlılar, Harnup güvesi (*Ectomyelois ceratoniae* Zell.), Portakal güvesi (*Cryptoblabes gnidiella* Millièrè), Akdeniz meyve sineği (*Ceratitidis capitata* Wied.), Nar yaprakbiti (*Aphis punicae* Passerini), Turunçgil unlubiti (*Planococcus citri* Risso), Nar beyazsineği (*Siphoninus phillyreae* Haliday), Nar yaprakuyuzu (*Aceria (Eriophyes) granati* C.&M.) ve Ağaç sarıkurdu (*Zeuzera pyrina* L.)’dur (Öztürk ve ark., 2005; Öztürk & Ulusoy, 2009; Uluç & Demirel, 2009; Mamay ve ark., 2014; Mamay ve ark., 2016; Mamay, 2021).

Nardaki önemli zararlılardan biri olan *C. capitata*, Diptera takımının Tephritidae familyasına mensup olup vücut rengi sarımsı kahverengi, kanatlarında ise kahverengimsi-siyah bantlar mevcuttur (Bergsten ve ark., 1999; De Meyer, 2000; Thomas ve ark., 2004). Dişilerin abdomen sonunda 1-1.5 mm uzunluğunda ovipozitör bulunmaktadır. (De Meyer, 2000; Thomas ve ark., 2004). Yumurtaları silindirik, hafif kıvrık ve mekik şeklinde, yaklaşık olarak 1 mm boyunda ve parlak beyaz renkli, larvaları bacaksız, beyazımsı sarı renkte, 4-8 mm boylarında, caput kısmının abdomen

sonuna kıyasla daha ince ve hafif kıvrıktır. Pupaları ise kahve renkli olup fıçı tipinde ve 4-4.5 mm büyüklüğündedir (Elekçioğlu, 2009). *Ceratitidis capitata* larvalarının konukçu bitki meyvelerinin etli bölümlerinde tüneller açarak beslendiği ve üç larva dönemi geçirmesinin ardından çoğunlukla meyvelerden çıkarak pupa olmak amacıyla toprağa atladıkları ancak konukçu bitki meyvelerinde pupa döneminde kışlayabildikleri de kayıtlıdır (Bergsten ve ark., 1999; Thomas ve ark., 2004). Akdeniz iklimi hakim olan bölgelerde pupadan ergin bireylerin çıkması mart-nisan dönemlerinde olmakta ve çıkış yapan *C. capitata* ergin bireyleri çevredeki bitkilerin tatlımsı sıvı maddeleriyle beslenerek daha sonra cinsel olgunluğa ulaştığı belirtilmiştir (İleri, 1961; Elekçioğlu, 2009; Tiring, 2015; Satar ve ark., 2016). Dişi bireylerin çiftleşmenin ardından yumurtalarını olgunlaşmış meyvelere yumurta bırakma borusuyla açmış oldukları açıklığa 90-110 adet olmak üzere kümeler şeklinde bıraktıkları belirtilmiştir. Dişi bireylerin yaşam süresince 350-1250 adet yumurta bırakabildiği ve ergin ömrünün ortalama 40-50 gün olduğu ifade edilmiştir (Bergsten ve ark., 1999; Thomas ve ark., 2004; USDA, 2024). Zararlının Akdeniz Bölgesi’nde yılda 6-8 döl, Ege Bölgesi’nde ise 4-5 nesil verebildiği ifade edilmiştir (Öztop, 2008; Başpınar ve ark., 2009; Tiring ve ark., 2016).

Akdeniz meyve sineği, turunçgiller ile sert ve yumuşak çekirdekli meyveler gibi birçok önemli türde ürün kayıplarına yol açmakta olup 300 civarında konukçusu bulunan ve çok geniş alanlarda yayılım gösteren önemli bir zararlıdır. Nar, turunçgiller, nektarin, şeftali, incir, kayısı, Trabzon hurması ve elma gibi birçok meyve tür ve çeşitlerinde zarara sebebiyet vermesi, *C. capitata*’yı önemli seviyede ekonomik değere haiz bir organizma konumuna oturtmuştur (Demirdere, 1961; Elekçioğlu, 2009). Zararlının dünyada farklı meyvelerde verim ve kaliteyi olumsuz etkileyen en önemli zararlılardan biri olduğu kabul edilmektedir (Fimiani, 1989; White & Elson-Haris, 1992). Akdeniz meyve sineğinin asıl zararını larvaları yapmakta olup ihraç edilen ürünlerdeki toleransı sıfır olan önemli bir dış karantina zararlısıdır (Liquidò ve ark., 1991; Thomas ve ark., 2004; Kasap & Aslan, 2016).; Tiftikçi, 2020).

Zararlının konukçu ağaçların altındaki toprakta pupa olarak kışı geçirebildiği (White & Elson-Haris, 1992), kayısı ve şeftalide haziran ve temmuzda (Christenson & Foote, 1960) narda ise bu tarihin ağustosa denk geldiği (Demirel, 2014; Demirel ve ark., 2018) kayıtlıdır. Larvaların nar meyvelerinde beslenmesi sonucu, meyvelerin bozulduğu, sekonder zararlılar ile saprofit bakteri ve fungusların bulaşarak pazar değerini tamamen düşürdüğü bildirilmiştir (White & Elson-Haris, 1992; Bergsten ve ark., 1999).

Akdeniz meyve sineğinin biyolojisi, popülasyonu, zarar oranı ve mücadelesi ile ilgili farklı konukçular

üzerinde Türkiye'nin birçok bölgesinde çok sayıda çalışma yürütülmüş olmasına rağmen Şanlıurfa'da zararlının popülasyon gelişimi ve bulaşıklık oranı ile ilgili herhangi bir çalışma yürütülmemiştir (Başpınar & ark., 2007; Elekçioğlu, 2009; Yıldırım & Başpınar, 2011; Elekçioğlu, 2013; Demirel, 2014; Çardak, 2015; Kasap & Aslan, 2016; Tülü, 2019; Üçpınar & Ünlü, 2019; Buğday & Keçeci, 2020; Gülcüoğlu & Başpınar, 2020; Tiftikçi, 2020; Elitaş, 2022; Tamer & Yıldırım, 2023). Bu nedenle, Şanlıurfa ilinde yetiştiriciliği yapılan meyve türleri içerisinde ön sıralarda yer alan nar alanlarında zararlı ile ilgili en temel çalışmalardan olan popülasyon gelişimi ve bulaşıklık oranının belirlenmesi mutlak önemlidir.

Şanlıurfa'nın Akçakale, Haliliye, Karaköprü ve Suruç ilçelerinde nar alanlarında Akdeniz meyve sineğinin popülasyon gelişimi ve bulaşıklık oranının

belirlenmesi bu çalışmada amaçlanmıştır. Bu kapsamda zararlının ilk ergin uçuşu, popülasyonun yoğun olduğu dönemleri ifade eden tepe noktaları, doğada aktif olduğu süre, son ergin uçuşu ve nar meyvelerindeki bulaşıklık oranı gibi temel veriler elde edilerek nar üreticilerine zararlının mücadelesine yönelik yol gösterici bilgiler sağlanmıştır.

## MATERYAL ve METOD

Araştırmanın ana materyalini; *C. capitata*, Şanlıurfa ili nar bahçeleri, delta tipi eşeyssel çekici feromon tuzakları ve bulaşık nar meyveleri oluşturmaktadır. Çalışma, Şanlıurfa ilinin Akçakale, Haliliye, Karaköprü ve Suruç ilçelerindeki birer adet nar bahçesinde yürütülmüştür. Çalışmaların yürütüldüğü nar bahçelerine ait bilgiler çizelgede verilmiştir (Çizelge 1).

Çizelge 1. Çalışmanın yürütüldüğü nar bahçeleri ve özellikleri

Table 1. Characteristics of the pomegranate orchards where the studies were carried out

İlçe (District)	Köy (Village)	Çeşit (Variete)	Yaş (Age)	Alan (da) (Area) (decar)	Ağaç sayısı (adet) (Tree number)	Koordinatlar (Coordinates)	Rakım (m) (Altitude)
Akçakale	Koruklu	Hicaz	35	8	330	36°54' 08"K 38°55' 19"D	379
Haliliye	Sırrın	Hicaz	20	25	1000	37°09' 44"K 38°51' 07"D	485
Karaköprü	Akıncı	Hicaz	20	13	1000	37°04' 01"K 38°30' 11"D	647
Suruç	Aligör	Suruç	30	6	400	37°01' 25"K 38°26' 19"D	513

### *Ceratitis capitata*'nın Popülasyon Gelişiminin Belirlenmesi

Zararlının popülasyon gelişiminin belirlenmesi, 2020 ve 2021 yıllarında Şanlıurfa ilinin Akçakale, Haliliye, Karaköprü ve Suruç ilçelerindeki birer adet nar bahçesinde yürütülmüştür. *Ceratitis capitata*'nın popülasyon gelişiminin belirlenmesi amacıyla her bir bahçeye üçer adet delta tipi eşeyssel çekici feromon (2 g Trimedlure,) tuzağı (Russel IPM, Flintshire, İngiltere), mart ayının ortalarında 50'şer metre ara ile yerden 1.5-2 metre yüksekliğinde, ağaçların güney yönüne asılmıştır (Şekil 1).

Tuzaklar, ilk ergin uçuşu belirleninceye kadar üç günde bir, ilk erginin yakalanmasından sonra son ergin uçuşuna kadar haftada bir kez ve aynı gün takip edilmiştir. Feromon kapsülleri aylık olarak yenilenmiştir. Yapışkan tablalar yapışkanlık özelliğini kaybedince yenisi ile değiştirilmiştir. Feromon tuzaklarında yakalanan *C. capitata*'nın ergin (erkek) sayıları haftalık olarak kaydedilerek tuzak başına ortalama şeklinde grafiklere işlenmiştir. Çalışmada her bir bahçeye asılan üç tuzakta yakalanan ergin bireylerin ortalama sayıları kullanılarak zararlının popülasyon eğrileri elde edilmiştir. Sonuç olarak, zararlının önemli popülasyon parametrelerinden; ilk

ergin uçuşu, popülasyonun yoğun olduğu dönemler, tepe noktaları, popülasyon gelişimi, doğal şartlarda tahmini döl sayısı, son ergin uçuşu gibi popülasyon dinamikleri belirlenmiştir.

### *Ceratitis capitata*'nın Nar Bahçelerindeki Bulaşıklık Oranının Belirlenmesi

*Ceratitis capitata*'nın nar meyvelerindeki bulaşıklık oranını belirlemek için, hasat esnasında her bahçede en az 10'ar ağacın farklı yönlerinden alınan 100 meyve zarar belirtisi ve larva açısından kontrol edilmiştir. Meyveler sağlam ve bulaşık şeklinde kaydedilerek bulaşıklık oranı % olarak aşağıdaki formül ile hesaplanmıştır

$$\text{Bulaşıklık oranı} = \frac{\text{Bulaşık meyve sayısı}}{\text{Kontrol edilen meyve sayısı}} * 100$$

### BULGULAR ve TARTIŞMA

Çalışma, 2020-2021 yıllarında Şanlıurfa ilinin Akçakale, Haliliye, Karaköprü ve Suruç ilçelerindeki nar bahçelerinde yürütülerek zararlının popülasyon gelişimi ve bulaşıklık oranı belirlenmiştir.





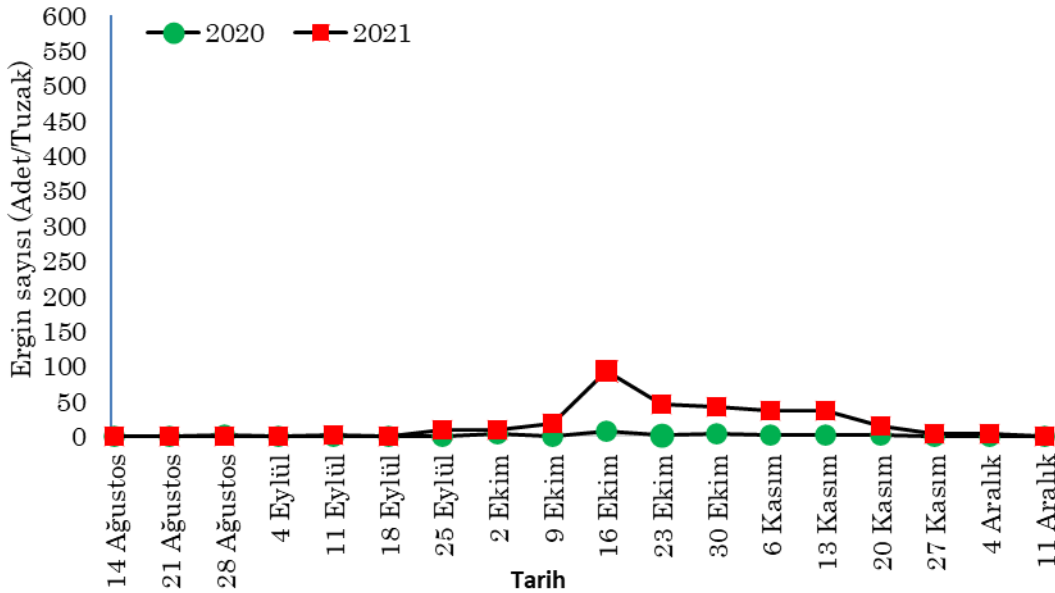
Şekil 1. Çalışmalarda *Ceratitis capitata*'nın popülasyon takibinde kullanılan delta tipi eşeyssel çekici feromon tuzakları

Figure 2. Delta-type sexual attractant pheromone traps are used in the studies to determine the population development of *Ceratitis capitata*

### *Ceratitis capitata*'nın Popülasyon Gelişimi

Şanlıurfa İli Karaköprü ilçesindeki nar bahçesinde *C.*

*capitata*'nın 2020 ve 2021 yıllarındaki popülasyon gelişimi Şekil 2'de verilmiştir.

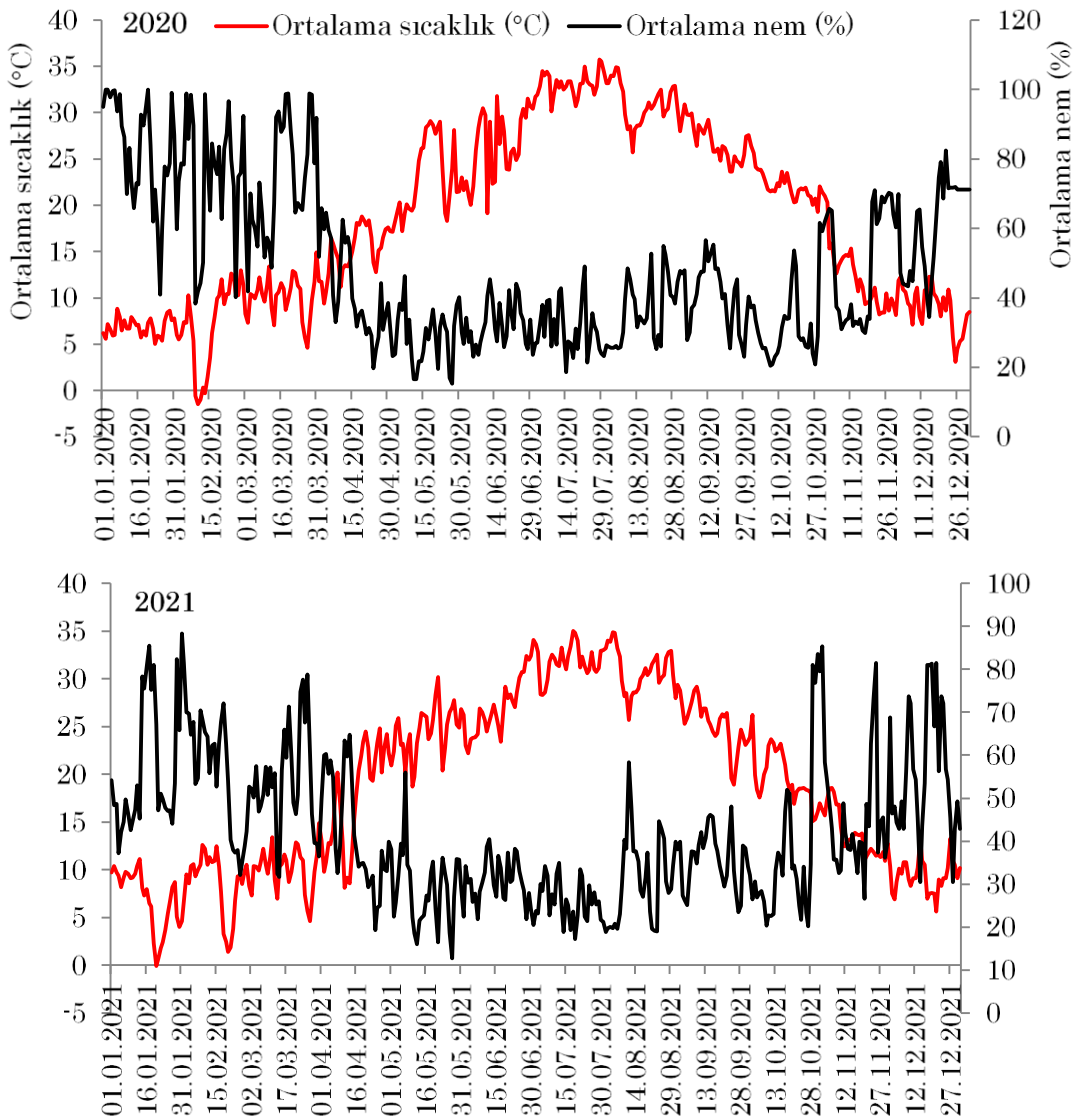


Şekil 2. Karaköprü ilçesindeki nar bahçesinde *Ceratitis capitata*'nın 2020 ve 2021 yıllarındaki popülasyon gelişimi  
Figure 2. Population development of *Ceratitis capitata* in pomegranate orchards in Karaköprü district in 2020 and 2021 years



Karaköprü ilçesindeki nar bahçesinde ilk ergin uçuşu 2020 yılında 28 Ağustos tarihinde, 2021 yılında ise 10 Eylül tarihinde gerçekleşmiştir. Bu tarihlerde ortalama sıcaklığın yıllara göre sırasıyla 32.8°C ve 28.3°C, nem değerlerinin ise sırasıyla %40.1 ve %39.2 olarak ölçüldüğü kaydedilmiştir (Şekil 3). Zararının her iki yılda da birer tepe noktası oluşturduğu belirlenmiştir. Zararının 2020 ve 2021 yıllarında oluşturduğu tepe noktaları sırasıyla 16 Ekim tarihinde sırasıyla 8 ve 94 ergin/tuzak ile gerçekleşmiştir (Şekil 2). Tepe noktasının oluştuğu tarihte sıcaklık ortalaması 11.2°C ve nem ortalaması ise %30.6 olarak ölçülmüştür. Yıl boyunca tuzak başına 2020 yılında

ortalama 21 ergin yakalanırken 2021 yılında 312 birey yakalanmıştır (Şekil 10). Bu durum zararının yıllara göre popülasyonunda artış göstereceği işareti olarak algılanmıştır. Zararının son ergin uçuşu 2020 ve 2021 yıllarında sırasıyla 20 Kasım (ortalama sıcaklık: 10.2°C, ortalama nem: %67.6) ve 3 Aralık (ortalama sıcaklık: 6.9°C, ortalama nem: %48.1) tarihlerinde gerçekleşmiştir. Bu verilere göre zararının doğada yaklaşık 3 ay aktif olarak uçtuğu belirlenmiştir. Şanlıurfa İlinin Karaköprü ilçesinin 2020 ve 2021 yıllarına ait ortalama sıcaklık ve nem verileri Şekil 3'te verilmiştir.



Şekil 3.Karaköprü ilçesinin 2020-2021 yıllarındaki ortalama sıcaklık ve nem değerleri

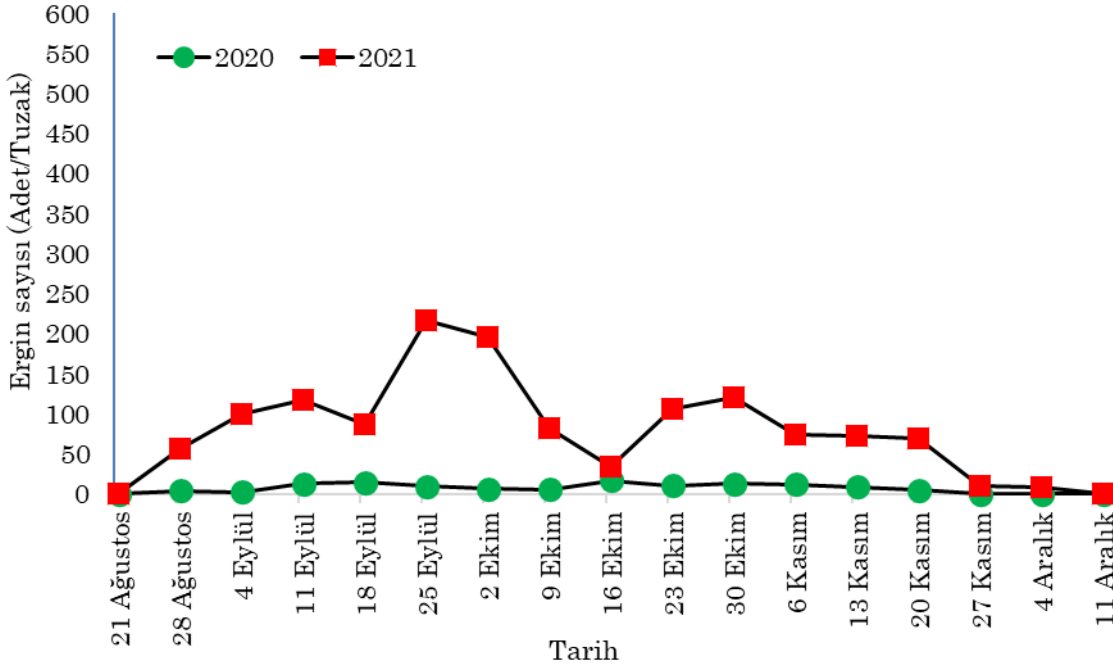
Figure 3. Average temperature and relative humidity values of Karaköprü district for 2020 and 2021 years

Suruç ilçesindeki nar bahçesinde *C. capitata* 'nın ilk ergin uçuşu her iki yılda da 28 Ağustos tarihinde gerçekleşmiştir (Şekil 4). Bu tarihte sıcaklık ve nem değerlerinin 2020 ve 2021 yıllarında sırasıyla 27.8°C ve

%47.9 ile 30.5°C ve %41.6 olduğu görülmüştür (Şekil 5). Zararlı 2020 yılında belirgin bir tepe noktası oluşturmazken 2021 yılında ilk ergin uçuşundan sonra zararının popülasyonu yükselişe geçerek mevsim

sonuna kadar dalgalı bir seyir izlemiş ve belirgin iki tepe noktası oluşturmuştur. Zararının tuzak başına en fazla ergin yakalanması 2020 yılında 16 Ekim tarihinde 17 ergin/tuzak ile gerçekleşirken bu tarihte ölçülen sıcaklık ve nem değerlerinin sırasıyla 21.7°C ve %45.5 olduğu belirlenmiştir. Çalışmanın ikinci yılında

tepe noktalarını temsil eden 25 Eylül ve 30 Ekim tarihlerinde sırasıyla 216 ve 121 ergin/tuzak ile *C. capitata* ergin bireyleri yakalanmıştır (Şekil 4). Tepe noktalarının olduğu bu tarihlerde ölçülen ortalama sıcaklık ve nem değerlerinin 18.8°C-%35 ve 16.1°C-%60.7 olduğu kaydedilmiştir (Şekil 5).



Şekil 4. Suruç ilçesindeki nar bahçesinde *Ceratitis capitata*'nın 2020 ve 2021 yıllarındaki popülasyon gelişimi  
Figure 4. Population development of *Ceratitis capitata* in pomegranate orchards in Suruç district in 2020 and 2021 years

Suruç ilçesindeki nar bahçesinde son ergin uçuşunun ilk yıl kasım ayının üçüncü haftasında olduğu (ortalama sıcaklık: 8.6°C, ortalama nem: %61.3), ikinci yıl ise aralık ayının ilk haftasında (ortalama sıcaklık: 5.7°C ve ortalama nem: %58.9) gerçekleştiği belirlenmiştir. Bu verilere göre zararlının doğada 4 ay aktif olarak uçtuğu belirlenmiştir. Suruç ilçesinde nar bahçesinde yürütülen bu çalışmada feromon tuzakları tarafından yıllara göre toplam 125 ve 1353 adet Akdeniz meyve sineği ergini yakalanmıştır (Şekil 10). Bu veriler, zararlının popülasyonunun çalışmanın ikinci yılında oldukça yükseldiğini göstermiştir. Şanlıurfa ilinin Suruç ilçesinin 2020 ve 2021 yıllarına ait ortalama sıcaklık ve nem verileri Şekil 5'te verilmiştir.

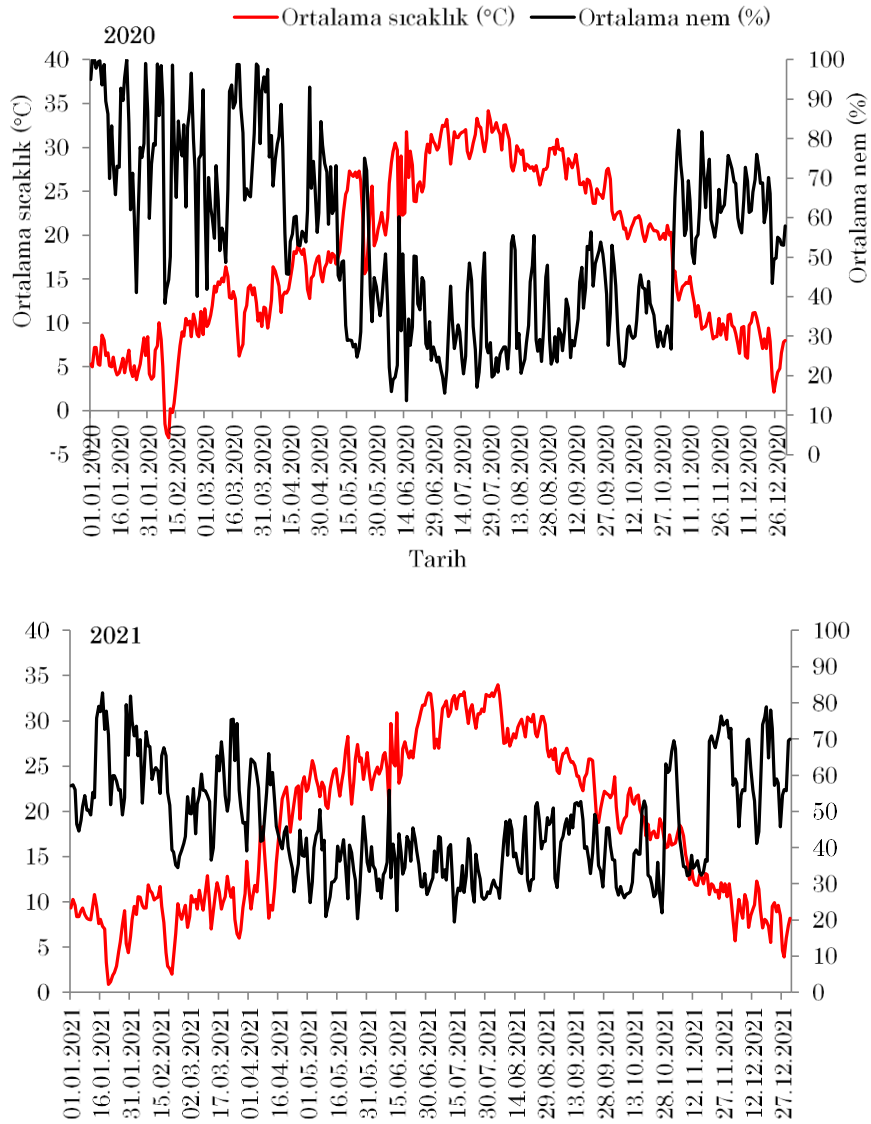
Şanlıurfa ilinin Akçakale ilçesindeki nar bahçelerinde *C. capitata*'nın 2020 ve 2021 yıllarına ait popülasyon gelişimi Şekil 6'da verilmiştir.

Akçakale ilçesindeki nar bahçesinde 2020 yılında ilk ergin uçuşu 8 Ekim tarihinde görülürken (sıcaklık:16.5°C; nem: %50.9) 2021 yılında ise 30 Temmuz tarihinde (sıcaklık: 33.7°C; nem: %30.7) gerçekleşmiştir. Tuzaklara ilk ergin yakalandıktan sonra zararlının popülasyonu 2020 yılında önemli bir artış göstermezken, 2021 yılında yükselişe geçerek

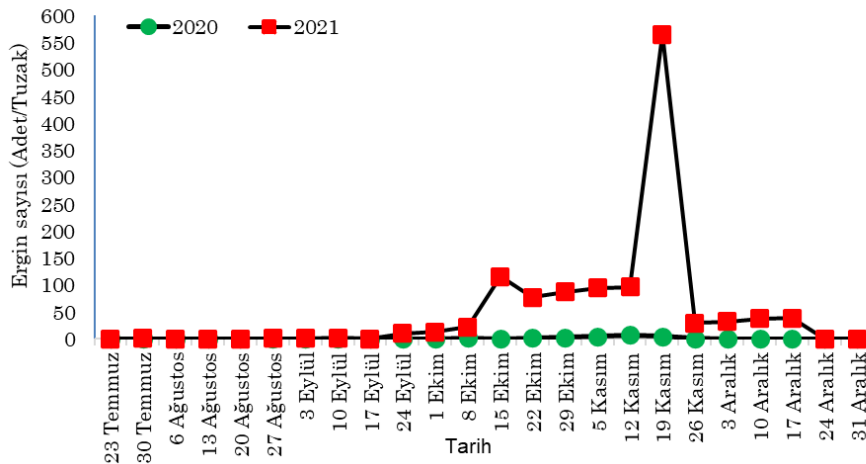
dalgalı bir seyir izlemiş olup 2 tepe noktası oluşturmuştur. Zararlının 2021 yılında oluşturduğu tepe noktaları 15 Ekim (sıcaklık: 23.2°C; nem: % 42.2) ve 19 Kasım (sıcaklık: 10.1 °C; nem: % 79) tarihlerinde sırasıyla 116 ve 565 ergin/tuzak yakalanması ile gerçekleşmiştir. Son ergin uçuşunun yıllara sırasıyla 26 Kasım (sıcaklık: 10.8°C; nem: %85.8) ve 17 Aralık tarihinde (sıcaklık: 10.3°C; nem: %65.5) olduğu tespit edilmiştir. Bu veriler doğrultusunda zararlının 2020 yılında 1.5 ay kadar aktif olurken 2021 yılında ise farklılık göstererek doğada yaklaşık 5 ay aktif olarak uçtuğu belirlenmiştir. Akçakale ilçesinde feromon tuzaklarında 2020 ve 2021 yıllarında sırasıyla toplam 26 ve 1226 adet Akdeniz meyve sineği ergini yakalanmıştır (Şekil 10).

Şanlıurfa ilinin Akçakale ilçesine ait 2020 ve 2021 yıllarına ait ortalama sıcaklık ve nem verileri Şekil 7'de verilmiştir.

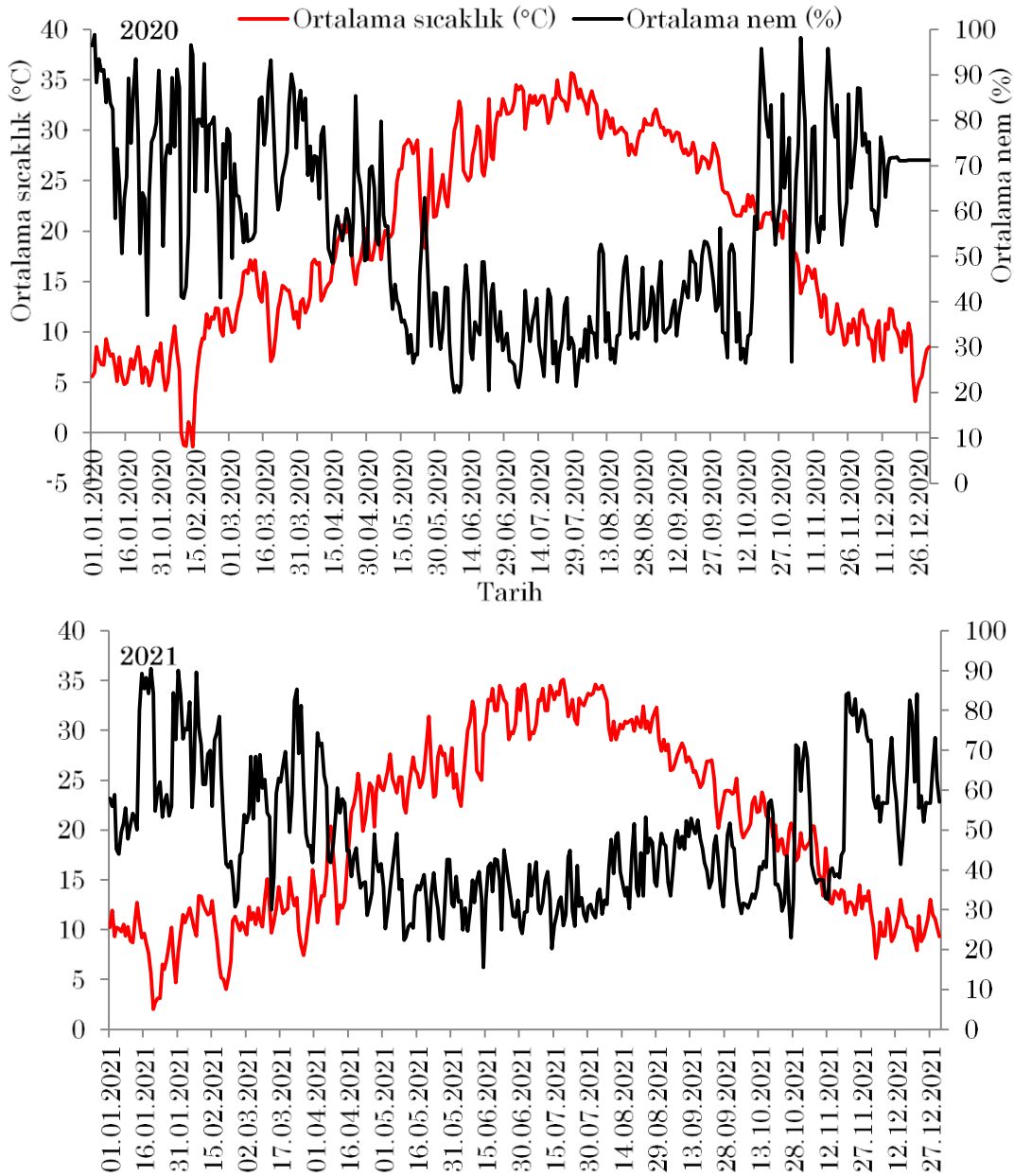
Şanlıurfa ilinin Haliliye ilçesindeki nar bahçesinde 2020 ve 2021 yıllarına ait *C. capitata*'nın eşeysel çekici feromon tuzaklarıyla belirlenen popülasyon gelişimi Şekil 8'de verilmiştir. Haliliye ilçesindeki nar bahçelerinde Akdeniz meyve sineğinin ilk ergin uçuşu 2020 yılında 24 Eylül tarihinde görülürken, 2021 yılında ise 10 Eylül tarihinde görülmüştür.



Şekil 5. Suruç ilçesinin 2020-2021 yıllarındaki ortalama sıcaklık ve nem değerleri  
Figure 5. Average temperature and relative humidity values of Suruç district for 2020 and 2021 years



Şekil 6. Akçakale ilçesindeki nar bahçesinde *Ceratitıs capitata*'nın 2020 ve 2021 yıllarındaki popülasyon gelişimi  
Figure 6. Population development of *Ceratitıs capitata* in pomegranate orchards in Akçakale district in 2020 and 2021 years



Şekil 7. Akçakale ilçesinin 2020-2021 yıllarındaki ortalama sıcaklık ve nem değerleri

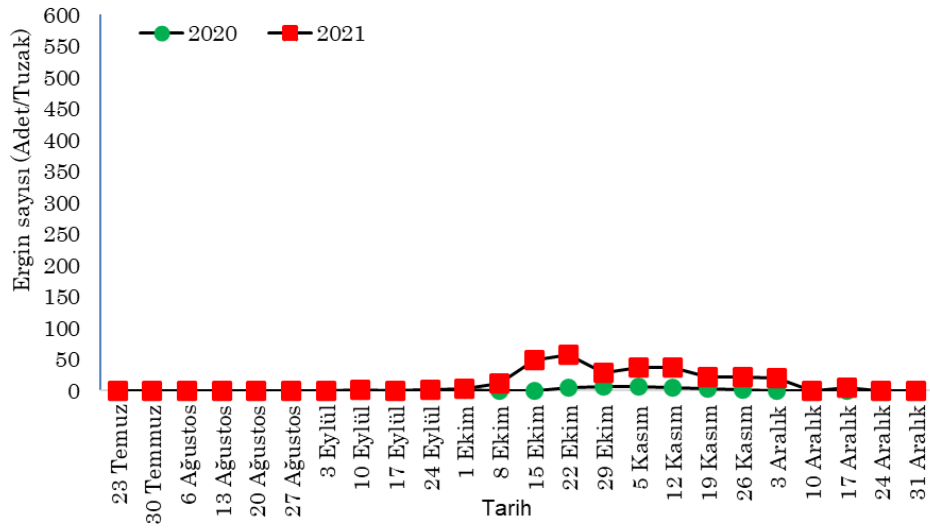
Figure 7. Average temperature and relative humidity values of Akçakale district for 2020 and 2021 years

Bu tarihlerde sıcaklık ve nem değerleri sırasıyla 19.6°C ve %49.3 ile 26.8°C ve %47.5 olarak kaydedilmiştir. Tuzaklara ilk ergin yakalandıktan sonra 2020 yılında popülasyon belirgin olmasa da 1 tepe noktası, 2021 yılında dalgalı bir seyir izlemiş olup 2 belirgin tepe noktası oluşturmuştur. Zararlıının 2020 yılında oluşturduğu tepe noktası 30 Ekim tarihinde 7 ergin/tuzak ile gerçekleşirken 2021 yılında ise 22 Ekim ve 5 Kasım tarihlerinde sırasıyla 52 ve 31 ergin/tuzak ile gerçekleşmiştir. Çalışmanın ikinci yılında bu tepe noktalarının oluştuğu tarihlerde ölçülen sıcaklık ve nem değerleri sırasıyla 20.2°C-%28.4 ve 21.3°C-% 37.4 olarak kaydedilmiştir (Şekil 9).

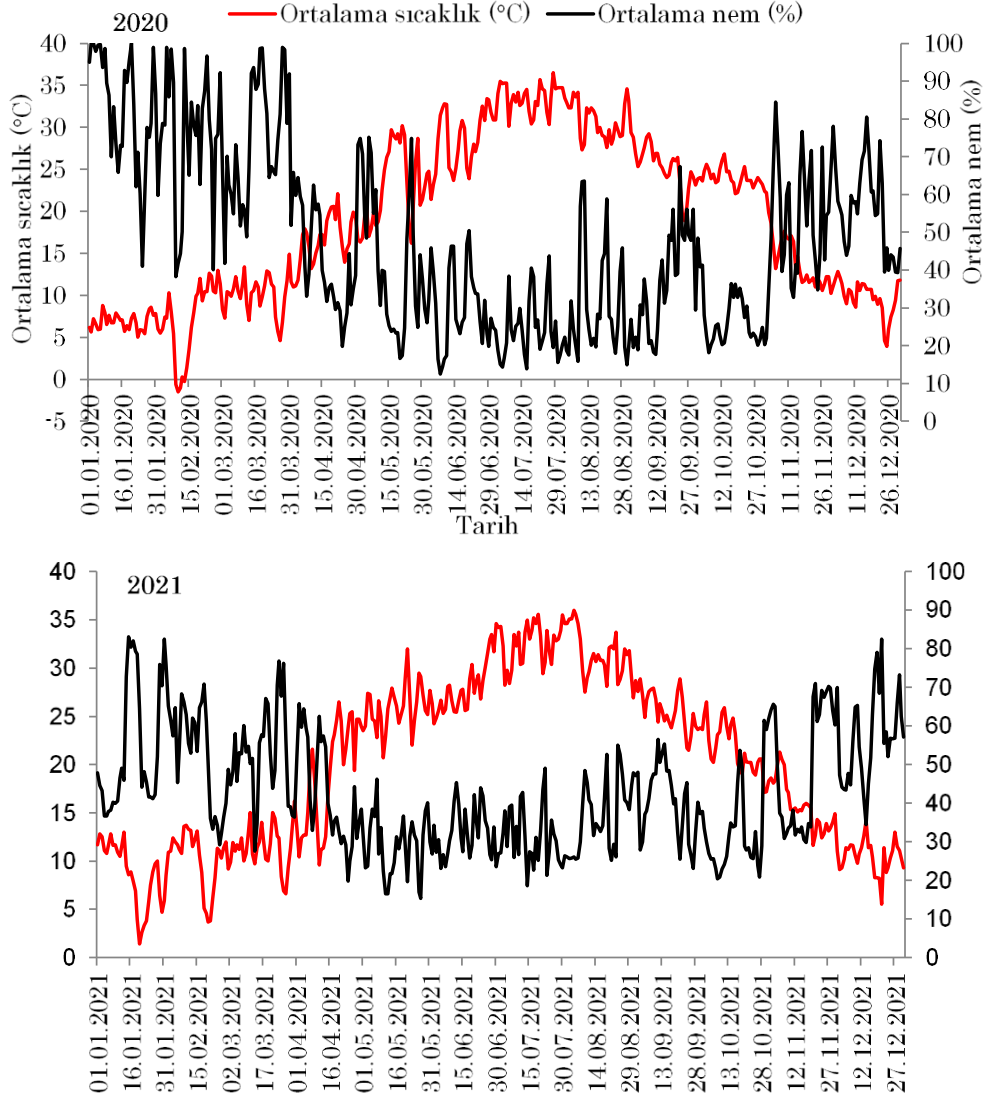
Zararlıının Haliliye ilçesi nar bahçesindeki son ergin uçuşu 2020 yılında Kasım ayının son haftası (sıcaklık: 10.6°C; nem: %72.5) 2021 yılında ise Aralık ayının ortalarına (sıcaklık: 10.4°C; nem: %63) kadar devam etmiştir. Bu sonuçlar değerlendirildiğinde zararlıının yıllara göre doğada aktif olarak 2-3 ay uçtuğu tespit edilmiştir. Çalışma sonucunda feromon tuzakları tarafından toplam 2020 yılında 29 adet, 2021 yılında ise 259 adet Akdeniz meyve sineği ergini yakalanmıştır.

Şanlıurfa İlinin Haliliye ilçesinin 2020-2021 yıllarına ait ortalama sıcaklık ve nem verileri Şekil 9'da verilmiştir.





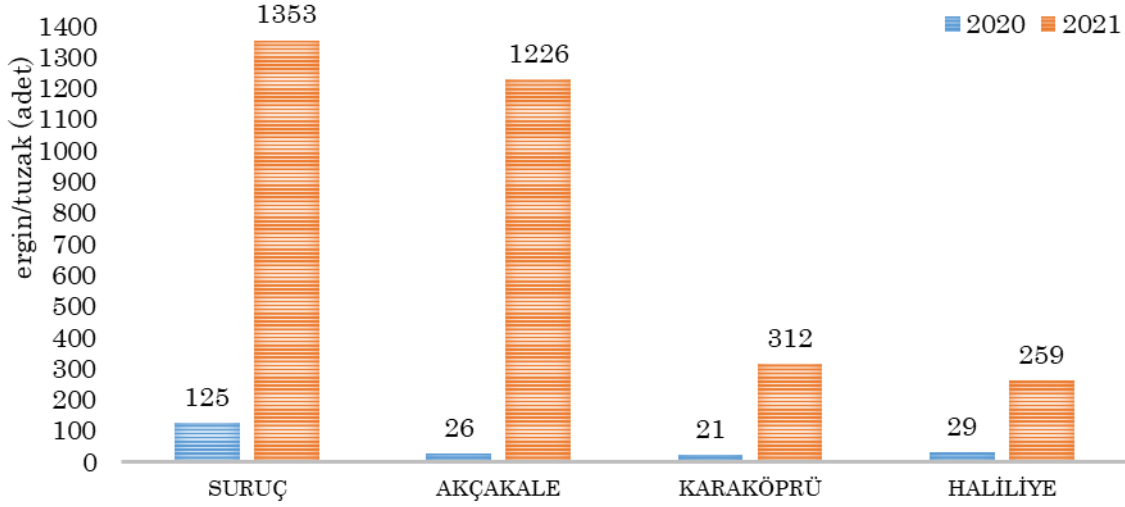
Şekil 8. Haliliye ilçesindeki nar bahçesinde *Ceratitıs capitata*'nın 2020 ve 2021 yıllarındaki popülasyon gelişimi  
Figure 8. Population development of *Ceratitıs capitata* in pomegranate orchards in Haliliye district in 2020 and 2021 years



Şekil 9. Haliliye ilçesinin 2020-2021 yıllarındaki ortalama sıcaklık ve nem değerleri  
Figure 9. Average temperature and relative humidity values of Haliliye district for 2020 and 2021 years

Her bir ilçeye ait popülasyon verilerinin ayrı ayrı verildiği yukarıdaki grafikler birlikte

değerlendirildiğinde ilçelere göre yıllık bazda yakalanan ergin sayıları Şekil 10'da verilmiştir.



Şekil 10. Şanlıurfa ilinde 2020 ve 2021 yıllarında nar bahçelerinde feromon tuzak başına yakalanan *Ceratitis capitata*'nın toplam ergin sayıları

Figure 10. Total number of *Ceratitis capitata* adults caught per pheromone trap in pomegranate orchards in Şanlıurfa Province in 2020 and 2021 years

Şekil 10 incelendiğinde tuzaklara yakalanan toplam ergin sayıları Karaköprü ilçesinde 2020 yılında 21 iken 2021 yılında yaklaşık 14 kat artarak 312, Suruç ilçesinde 2020 yılında 125 iken 2021 yılında yaklaşık 10 kat artışla 1353 ergin olmuştur. Benzer şekilde, Akçakale ilçesinde 2020 yılında toplam 26 ergin yakalanırken 2021 yılında yaklaşık 47 kat artışla 1226 ve Haliliye ilçesinde ise 2020 yılında yakalanan toplam ergin sayısı 29 iken 2021 yılında 8 kat artışla 259 ergin olarak tespit edilmiştir.

Şekil 10'daki veriler ilçeler bazında aylara göre değerlendirilerek zararlının popülasyonunun hangi aylarda yoğunluk gösterdiğini incelemek amacıyla bir Chord Diagram oluşturulmuştur (Şekil 11).

Çalışmanın yürütüldüğü lokasyonlarda zararlının 2020 ve 2021 yıllarındaki popülasyonları bağımsız t-testine tabi tutulduğunda bütün ilçelerde zararlının iki yıllık popülasyonu arasında fark olduğu görülmüştür (Çizelge 2).

Çizelge 2. İlçelere göre *Ceratitis capitata*' *Ceratitis capitata*' sayıları

Table 2. *Ceratitis capitata*' *Ceratitis capitata*' numbers by districts

ilçe	SD	t değeri	p değeri*
Akçakale	24	2.089	0.02
Haliliye	24	2.678	0.006
Karaköprü	24	2.487	0.01
Suruç	24	3.668	0.0006

\*p değeri 0.05'ten küçük olan satırdaki lokasyonda zararlının 2020 ve 2021 yıllarındaki popülasyonları arasında fark vardır.

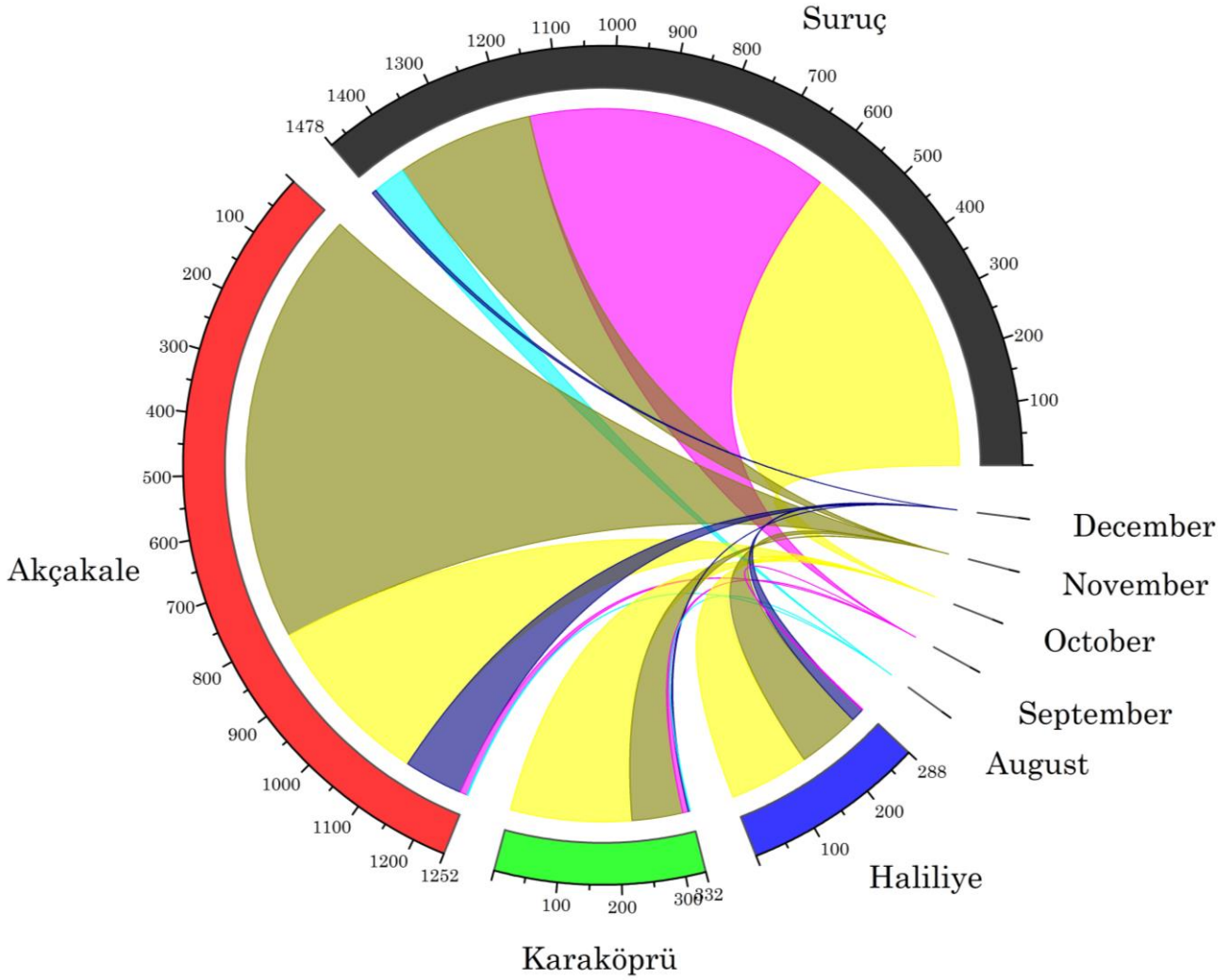
Çalışmanın yürütüldüğü ilçelere ait popülasyon verileri birlikte değerlendirildiğinde zararlının en fazla Ekim ayında tuzaklarda yakalandığı ve onu Kasım ayının takip ettiği anlaşılmaktadır (Şekil 11). Akdeniz meyve sineği erginleri aylara göre en fazla sırasıyla 1288, 1268, 586, 144 ve 64 ergin/tuzak ile Ekim, Kasım, Eylül, Aralık ve Ağustos aylarında yakalandığı belirlenmiştir. Bu sonuçlara göre zararlının Ağustos ayından önce Şanlıurfa ilindeki nar bahçelerinde önemli bir popülasyon oluşturmadığı anlaşılmıştır.

### *Ceratitis capitata*'nın Nar Bahçelerindeki Bulaşıklık Oranı

Şanlıurfa ilinin farklı ilçelerindeki nar bahçelerinde 2020 ve 2021 yıllarında belirlenen Akdeniz meyve sineği bulaşıklık oranı Çizelge 3'de verilmiştir.

Çizelge 3. Şanlıurfa ilinde nar bahçelerinde *Ceratitis capitata*'nın 2020 ve 2021 yıllarındaki bulaşıklık oranı  
Table 3. Infestation rate of *Ceratitis capitata* in pomegranate orchards in Şanlıurfa in 2020 and 2021 years

İlçe (District)	Köy (Village)	Bulaşıklık Oranı (%) (Infestation Rate)	
		2020	2021
Suruç	Aligör	0	2
Akçakale	Koruklu	1	4
Karaköprü	Akıncı	0	0
Haliliye	Sırrın	0	0



Şekil 11. Şanlıurfa İlinde nar bahçelerinde ilçeler bazında 2020-2021 yıllarında aylara göre feromon tuzaklarında yakalanan toplam *Ceratitıs capitata* ergin sayısı

Figure 11. Total number of *Ceratitıs capitata* adults caught by pheromone traps in pomegranate orchards based on counties in Şanlıurfa by months in 2020-2021 years

Akdeniz meyve sineğinin nar meyvelerindeki bulaşıklık oranını belirlemek amacıyla yapılan çalışmalar neticesinde 2020 ve 2021 yıllarındaki bulaşıklık oranlarının sırasıyla Suruç ilçesinde %0 ve %2, Akçakale ilçesinde %1 ve %4 olarak belirlenirken Karaköprü ve Haliliye ilçelerinde her iki yılda da bulaşıklık saptanmamıştır. Bu veriler zararlının henüz ekonomik anlamda zarar meydana getirmediği sonucunu doğurmuştur. Ancak yıllar itibariyle gerek popülasyon yoğunluğunda gerekse bulaşıklık oranında bir artış olduğu ve gelecekte Şanlıurfa ili nar bahçelerinde ekonomik anlamda bir zararlı olabileceğini düşündürmektedir.

Şanlıurfa ilinin Akçakale, Haliliye, Karaköprü ve Suruç ilçelerindeki nar bahçelerinde *C. capitata*'nın popülasyon gelişimi ile ilgili elde edilen iki yıllık veriler birlikte değerlendirildiğinde; zararlının Şanlıurfa koşullarında ilk ergin uçuşunun Akdeniz ve Ege bölgelerimiz ile kıyaslandığında oldukça geç bir

tarih sayılan Ağustos ayı itibariyle başladığı belirlenmiştir. Nitekim Tiring ve Satar (2017), Çukurova bölgesinde *C. capitata*'nın ilk erginlerinin şeftali ve incir bahçelerinde Mayıs ayı başlarında yakalandığını bildirmişlerdir. Yapılan başka bir çalışmada zararlının kayısı bahçelerinde ilk ergin uçuşunun Malatya ilinde 2017 yılında Ağustos ayı sonunda, 2018 yılında ise Haziran sonunda görüldüğü ancak Elazığ İl merkezinde 2018 yılında 28 Haziran tarihinde çıkış yaptığı bildirilmiştir (Buğday & Keçeci, 2020). Benzer şekilde Gülcüoğlu ve Başpınar (2020), Aydın İlinde hünnap bahçelerinde *C. capitata*'nın ilk olarak 3 Haziran tarihinde görüldüğünü bildirmişlerdir. Aynı şekilde Papadopoulos ve ark. (2001), Yunanistan'da en erken olgunlaşan kayısı bahçelerinde ilk ergin uçuşunun 24 Haziran tarihinde gerçekleştiğini bildirmişlerdir. Atıfta bulunulan bu çalışmalara göre zararlının Şanlıurfa ilinde nispeten geç ortaya çıktığı anlaşılmaktadır. Ancak Şanlıurfa ili

nar bahçelerinde elde edilen verileri destekleyen çalışmalar da yapılmıştır. Nitekim Elitaş (2022) Bursa İlinde Trabzon hurması, şeftali ve armut bahçelerinde zararlının ilk ergin uçuşunun 23 Ağustos tarihinde gerçekleştiğini, Yıldırım ve Başpınar (2011) Aydın İlinde nar bahçelerinde *C. capitata*'nın ilk ergin uçuşunun 2009 yılında Ekim ayında, 2010 yılında ise Eylül ayının ilk haftasında gerçekleştiğini bildirmişlerdir. Yapılan çalışmalarda zararlının ilk ergin uçuşunun farklı tarihlerde gerçekleşmesinin farklı ekolojik koşullar ve farklı konukçulardan kaynaklandığı düşünülmektedir. Yukarıdaki araştırmalar farklı iklim şartlarının haiz olduğu ve farklı ürün desenlerine sahip bölgelerde farklı meyve türlerinde yürütüldüğü için elde edilen sonuçları bu yargımızı güçlendirmektedir.

Yürütülen bu çalışma sonucunda *C. capitata*'nın ilk ergin uçuşundan sonra düşük bir popülasyonda seyrettiği ve ancak Eylül ayından sonra tepe noktaları oluşturmaya başladığı, en yüksek popülasyonun ise Ekim ve Kasım aylarında meydana geldiği belirlenmiştir. Dört ilçenin popülasyon eğrisi göz önünde bulundurulduğunda Şanlıurfa şartlarında 2 tepe noktası oluşturduğu için teorik olarak 2 döl verebileceği düşünülmektedir. Ancak zararlının ilk ergin uçuşunun yaz aylarının ortalarından sonra hatta sonuna doğru gerçekleşmesi Şanlıurfa koşullarında kışı geçiremediği fikrini akla getirmektedir. Bu çalışmanın aksine; Elekçioğlu (2013), zararlının popülasyonunun Türkiye turuncgil alanlarında yüksek olduğunu, ihracatımız açısından önemli problemlere sebep olduğunu ve yılda 4-8 döl verdiğini bildirmiştir. Benzer şekilde Kasap (2016), Adana ilinin Seyhan ilçesinde farklı nar çeşitlerindeki Akdeniz meyvesineğinin popülasyon gelişimi ve nar meyvesindeki zarar oranını belirlemiş olup Wonderful ve Hicaz nar çeşitlerinde 7-8 döl ve Acco nar çeşidinde 6-7 döl verdiğini bildirmiştir. Yürütülen bu çalışmadan farklı sonuçların elde edilmesi Akdeniz bölgesinin subtropik ikliminden dolayı zararlının rahatlıkla kışı geçirmesi, erken kışlamadan çıkması, ara konukçuların yoğunluğu ve uygun yaz mevsiminden kaynaklandığı düşünülmektedir. Çünkü, bu çalışmanın yürütüldüğü Şanlıurfa'nın farklı ilçelerindeki kış iklimi verilen sıcaklık ve nem grafiklerinden de anlaşıldığı üzere, Akdeniz Bölgesine göre daha soğuk ve uzun, yaz mevsiminin ise hem çok sıcak ve uzun hem de nispi nem çok düşük olduğundan kurak geçmektedir. Döl sayısı ile ilgili farklılığın diğer bir sebebi de bu çalışmanın yürütüldüğü bölgede ürün deseninde tarla bitkileri yetiştiriciliğinin yaygın olması ve *C. capitata*'nın konukçusu olabilecek meyve bahçelerinin bulunmaması olduğu düşünülmektedir.

Akdeniz meyve sineği popülasyon yoğunluğunun takip edildiği 4 farklı ilçede görülen en fazla ergin yakalanması 2020 yılında Karaköprü ilçesinde 16 Ekim (8 ergin/tuzak), Suruç ilçesinde 16 Ekim (17

ergin/tuzak), Akçakale ilçesinde 13 Kasım (8 ergin/tuzak) ve Haliliye ilçesinde 30 Ekim (7 ergin/tuzak) tarihinde gerçekleşmiştir. 2021 yılında ise Karaköprü ilçesinde 15 Ekim (94 ergin/tuzak), Suruç ilçesinde 25 Eylül (216 ergin/tuzak), Akçakale ilçesinde 19 Kasım (565 ergin/tuzak) ve Haliliye ilçesinde 22 Ekim (52 ergin/tuzak) tarihinde en yüksek popülasyon yoğunluğu görülmüştür. Tuzaklara yakalanan toplam ergin sayıları 2020 ve 2021 yıllarında sırasıyla Karaköprü ilçesinde 21 ve 312, Suruç ilçesinde 125 ve 1353, Akçakale ilçesinde 26 ve 1226 ve Haliliye 29 ve 259 ergin/tuzak olarak tespit edilmiştir. Bu verilere göre zararlının popülasyonunun çalışmanın ikinci yılında oldukça yükseldiği belirlenmiştir. Tiftikçi (2021), kayısı bahçesinde zararlının ergin bireylerinin 10 Ekim 2016 tarihinde tuzak başına 630 ergin birey, 24 Ekim 2017 tarihinde tuzak başına 3 ergin birey, 12 Eylül 2018 tarihinde tuzak başına 581 ergin birey, 24 Ekim 2019 tarihinde ise tuzak başına 20 ergin birey ile tepe noktalarını oluşturduğunu tespit etmiştir. Diğer bir çalışmada, Yıldırım ve Başpınar (2011), nar bahçelerinde Akdeniz meyve sineğinin hem 2009 hem de 2010 yılında en yüksek popülasyonunun Ekim-Kasım aylarında görüldüğünün, 2009 yılında en yüksek belirlenen popülasyonun 185 ergin/tuzak iken 2010 yılında ise 380 ergin/tuzak olduğunu belirtmişlerdir. Aynı şekilde Çardak (2015), Osmaniye ilinin Hicaznar bahçelerinde zararlının en fazla ergin bireyinin 2012 yılında 26 Ekim tarihinde 168 ergin/tuzak olup 2013 yılında ise 10 Kasım tarihinde 274 ergin/tuzak olarak gerçekleştiğini bildirmiştir. Yine kayısı bahçelerinde yürütülen bir çalışmada 2017 yılında tuzaklara yakalanan en fazla ergin sayısının Malatya ilinde 17 Kasım tarihinde 365 ergin/tuzak olduğu, 2018 yılında ise 31 Ağustos tarihinde 804 ergin/tuzak yakalandığı kaydedilmiştir. Aynı çalışmada, Elazığ ili merkez ilçesinde 2018 yılında 2 Ağustos tarihinde 250 ergin/tuzak olduğu, Baskil ilçesinde ise 13 Eylül tarihinde 486 ergin/tuzak ile en yüksek yoğunluğa ulaştığı bildirilmiştir (Buğday & Keçeci, 2020). Ayrıca Akyol (2014), Hatay İlinde Satsuma çeşidi mandarin bahçesinde 2011 yılında toplam 8968 adet Akdeniz meyve sineği ergininin yakalandığını, en fazla ergin bireyin Ekim ayında 6396 adet olduğunu belirlemiştir. İkinci yılında ise tuzaklara toplam 1307 ergin birey yakalandığını, tuzaklara en fazla erginin Eylül ayında 420 adet olduğu bildirilmiştir. Aynı şekilde Elitaş (2022) şeftali, armut ve Trabzon hurması bahçelerinde tuzaklara yakalanan toplam ergin sayısının sırasıyla 660, 678 ve 1402 adet olduğunu gözlemlemiştir. Görüldüğü üzere yaptığımız çalışmaya paralel olarak farklı lokasyonlarda ve farklı konukçularda zararlı yıllara ve aylara göre farklı popülasyon gelişimi gösterebilmekte ve farklı popülasyon yoğunluklarında tepe noktaları oluşturabilmektedir. Bu çalışmada, yıllar itibarıyla zararlının popülasyonunun mevsim başında düşük kalması ve sonbahara doğru yükselişe



geçerek artırması ise bölgede ara konukçu yetersizliği ile aşırı sıcak ve kurak yaz sıcaklarından kaynaklandığı düşünülmektedir. Bu durum şüphesiz zararlarının döl sayısını da etkilemektedir.

Yapılan bu çalışma neticesinde *C. capitata*'nın yıllar ve lokasyonlara göre Ağustos-Aralık ayları arasında olmak üzere 1-5 ay arasında farklı sürelerle doğada aktif bulunduğu belirlenmiştir. Gülcüoğlu ve Başpınar (2020) bu çalışmayı destekleyen sonuçlar elde etmiş ve *C. capitata*'nın Aydın ili Çine ilçesinde bulunan hünnap bahçelerinde 2017-2018 yıllarında yaklaşık 5 ay aktif olduğunu bildirmişlerdir.

Zararlıların nar bahçelerindeki bulaşıklık oranı ile ilgili yapılan çalışmalar neticesinde zararlıların Şanlıurfa ve ilçelerinde yıllara ve lokasyonlara göre %0-4 arasında bulaşıklığa sebep olduğu belirlenmiştir. Yıldırım ve Başpınar (2011), nar bahçelerinde *C. capitata*'nın meyvedeki zarar oranının 2009 yılında %1.9-2.20, 2010 yılında ise %0.77-%0.43 oranında olduğunu bildirmiştir. Benzer olarak Martinez Ferrer ve ark. (2012), klemantin bahçelerinde Akdeniz meyve sineğinin meyvedeki zararının %0.5 seviyelerinde olduğunu belirtmişlerdir. Gülcüoğlu ve Başpınar (2020) ise Akdeniz meyve sineğinin Hünnap meyvesindeki en yüksek zararının %45.7 en düşük zararının ise %16.7 olduğunu belirlemişlerdir. Zararlı, aynı meyve türüne ait farklı çeşitlerde farklı oranlarda zarar meydana getirebilmektedir. Bunu teyit eder şekilde farklı nar çeşitlerindeki meyve kabuk kalınlığı ve çatlama oranının Akdeniz meyve sineğinin bulaşıklığını doğrudan etkilediği kaydedilmiştir (Kasap, 2016; Güler, 2022).

## SONUÇ ve ÖNERİLER

Bu çalışma, Şanlıurfa ilinin nar bahçelerinde zararlı olan *C. capitata*'nın popülasyon gelişimi ve bulaşıklık oranının belirlenmesi amacıyla 2020-2021 yıllarında Akçakale, Haliliye, Karaköprü ve Suruç ilçelerinde yürütülmüştür. Akdeniz meyve sineğinin bütün lokasyonlardaki popülasyon gelişimi ile ilgili veriler değerlendirildiğinde zararlıların nar bahçelerinde ilk olarak ağustos ayı itibarıyla görülmeye başladığı, 2020 yılında düşük olan popülasyonun 2021 yılında yükseldiği belirlenmiştir. Zararlıların popülasyonunun yaz aylarında düşük iken özellikle Akçakale ve Suruç ilçelerindeki bahçelerde sonbahar aylarında yüksek dalgalanma gösterdiği belirlenmiştir. Zararlıların popülasyon seyri incelendiğinde 2020 yılında ergin popülasyonunun en fazla görüldüğü ilçe Suruç'ta 16 Ekim tarihinde 17 ergin/tuzak olduğu buna karşın 2021 yılında en yüksek ergin yakalanması Akçakale ilçesinde 19 Kasım tarihinde 565 ergin/tuzak olarak gerçekleşmiştir. Zararlıların yıllar ve lokasyonlara göre değişmekle beraber Aralık ayına kadar tuzaklara yakalandığı ve 1 ile 5 ay arasında farklı sürelerle doğada aktif bulunduğu belirlenmiştir.

Çalışma sonucunda zararlıların nar meyvelerindeki

bulaşıklık oranının iki yıllık ortalamaya göre Suruç ilçesinde %1 ve Akçakale ilçesinde %2.5 olduğu tespit edilmiş olup her iki yılda da Haliliye ve Karaköprü ilçelerinde meyvede bulaşıklık olmadığı belirlenmiştir.

Çalışmanın yürütüldüğü dört ilçenin popülasyon eğrisi göz önünde bulundurulduğunda Şanlıurfa şartlarında 2 tepe noktası oluşturduğu için teorik olarak 2 döl verebileceğini düşündürmektedir. Ancak zararlıların ilk ergin uçuşunun yaz aylarının ortalarından sonra hatta sonuna doğru gerçekleşmesi Şanlıurfa koşullarında kışı geçiremediği fikrini akla getirmektedir. Bu nedenle zararlıların Şanlıurfa koşullarında kışlama biyolojisi başta olmak üzere biyoeolojisinin araştırılması gerekmektedir.

Çalışma kapsamında elde edilen tüm veriler birlikte değerlendirildiğinde zararlıların henüz ekonomik anlamda zarar meydana getirmediği sonucunu doğurmuştur. Ancak yıllar itibarıyla gerek popülasyon yoğunluğunda gerekse bulaşıklık oranında bir artış olduğu ve gelecekte Şanlıurfa ili nar bahçelerinde ekonomik anlamda bir zararlı olabileceğini düşündürmektedir. Bu nedenle nar üreticilerinin teknik elemanlar tarafından zararlı ile ilgili eğitim yayım faaliyetleriyle bilgilendirilmeleri mutlak önem arz etmektedir. Zararlıdan kaynaklı kayıpların azaltılması için nar hasadının geciktirilmemesi, ağaçta kalan ve yere düşen bulaşık nar meyvelerinin toplanarak imhası konusunda üreticiler uyarılmalıdır.

## TEŞEKKÜR

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MM çalışmayı tasarlamış, CG çalışmayı yürütmüş, CS arazi çalışmalarında görev almıştır. Tüm yazarlar makale yazımında katkı sağlamış olduklarını beyan ederler.

## Çıkar Çatışması Beyanı

Makale yazarları aralarında herhangi bir çıkar çatışması olmadığını beyan ederler.

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## Malatya İli Kayısı Bahçelerinde Erik Koşnili *Sphaerolecanium prunastri* (Boyer De Fonscolombe) (Hemiptera: Coccidae)'nin Yayılış Alanları, Bulaşma Oranları ve Kimyasal Mücadelenin Zararlıının Popülasyon yoğunluğuna Etkileri

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### ÖZET

Malatya ilinde kayısı yetiştiriciliğinin yoğun olarak yapıldığı ilçelerde, *Sphaerolecanium prunastri* (Boyer de Fonscolombe) (Hemiptera: Coccidae)'nin yayılışı ve bulaşma oranlarının belirlenmesi amacıyla 2018 ve 2019 yıllarında sörvey çalışması yapılmıştır. Araştırmanın sonucunda Malatya ili Akçadağ, Battalgazi, Darende, Doğanşehir, Hekimhan, Kale, Merkez, Yazıhan ve Yeşilyurt ilçeleri kayısı alanlarında *S. prunastri*'nin yayılış gösterdiği belirlenmiştir. Yapılan örnekleme alanlarına göre bulaşıklığın en yüksek Battalgazi ilçesinde olduğu belirlenmiştir. Malatya ili Akçadağ ilçesinde üç farklı köydeki insektisit kullanılan ve kullanılmayan üçer adet kayısı bahçesinde nisan ve kasım aylarında düzensiz aralıklarla yapılan gözlemler ile *S. prunastri*'nin popülasyon yoğunluğu araştırılmıştır. Çalışma sonucunda insektisit kullanılmayan bahçelerde zararlıının popülasyon yoğunluğunun insektisit kullanılan bahçelere göre daha yüksek olduğu belirlenmiştir.

### Entomoloji

### Araştırma Makalesi

### Makale Tarihçesi

Geliş Tarihi :

Kabul Tarihi :

### Anahtar Kelimeler

*Sphaerolecanium prunastri*

Coccidae

Kayısı

Malatya

## Distribution and infestation rates of the Plum scale, *Sphaerolecanium prunastri* (Boyer De Fonscolombe) (Hemiptera: Coccidae) in apricot orchards of Malatya province and the effects of chemical control on the population density of the pest

### ABSTRACT

A survey study was conducted in 2018 and 2019 to determine the distribution and infestation rates of *Sphaerolecanium prunastri* (Boyer de Fonscolombe) (Hemiptera: Coccidae) in the districts where apricot cultivation is intensively carried out in Malatya province. As a result of the survey, it was determined that *S. prunastri* is widespread in apricot areas of Akçadağ, Battalgazi, Darende, Doğanşehir, Hekimhan, Kale, Merkez, Yazıhan and Yeşilyurt districts of Malatya province. According to the sampling areas, the highest level of infestation was determined in the Battalgazi district. Population density of *S. prunastri* was investigated in three apricot orchards with and without insecticides in three different villages in Akçadağ district of Malatya province with observations made at irregular intervals between April and November. As a result of the study, it was determined that the population density of the pest was higher in orchards where insecticides were not used than in orchards where insecticides were used.

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## GİRİŞ

Anavatanı Orta Asya, Batı Çin ve İran-Kafkasya olan kayısı (*Prunus armeniaca*)'nın, başta Akdeniz ülkeleri olmak üzere, birçok ülkede yetiştiriciliği yapılmaktadır (Anonim, 2024). Birleşmiş Milletler Gıda ve Tarım Örgütü (FAO) verilerine göre, 2022 yılında yaklaşık 56 bin hektar alanda 3,9 milyon ton kayısı üretimi gerçekleştirilmiştir (FAO, 2024). Türkiye hem kayısı üretim alanı hem de üretim miktarı bakımından dünyada birinci sırada yer almakta olup, Türkiye İstatistik Kurumu'nun verilerine göre (TÜİK), Türkiye'de 2022 yılında 141851 hektar alanda, 803 bin ton kayısı üretimi gerçekleştirilmiştir (TÜİK, 2024). Malatya ili yaklaşık 878 bin dekar alanda, 304 bin ton kayısı üretimi ile Türkiye'de en çok kayısı üretilen ildir (Anonim, 2024). Türkiye tarımsal üretiminde en önemli ürünlerden biri olan kayısı bitkisi üzerinde farklı takım ve familyalardan çok sayıda zararlı bulunmaktadır. Malatya ilinde kayısı yetiştiriciliği yapılan alanlarda 63 zararlı türü tespit edilmiştir (Öztürk ve ark., 2004). Bu zararlıların en önemlilerinden biri de erik koşnili olarak bilinen *Sphaerolecanium prunastri* (Boyder de Fonscolombe) (Hemiptera: Coccidae)'dir. *S. prunastri* Türkiye'nin de içinde bulunduğu Palearctic ve Nearctic Zoo-coğrafi bölgelerde bulunan 38 ülkede yayılış gösterdiği bildirilmektedir (Garcia Morales ve ark., 2016). *S. prunastri* Türkiye'nin tüm coğrafik bölgelerinde saptanmıştır (Bodenheimer, 1953; Soydanbay, 1976; Öncüer, 1977; Ülgentürk & Toros, 1999; Karsavuran ve ark., 2001; Ülgentürk ve ark., 2001; Öztürk ve ark., 2004; Kaydan ve ark., 2007; Hazır & Ulusoy, 2009; Özgen & Bolu, 2009; Akşit & Kaya Apak, 2013; Yiğit & Tunaz, 2015; Develioğlu ve ark., 2018). Zararlı başta Rosaceae familyası ve *Prunus* cinsi olmak üzere, 5 familya ve 9 cinse ait en az 20 konukçu bitkide beslenmektedir (Garcia Morales ve ark., 2016). *S. prunastri* konukçu bitkilerin dallarında ve gövdesinde koloniler oluşturup, hem bitkilerde özsuyu ile beslenmekte, hem de yoğun bir

şekilde ballı madde salgılaması nedeniyle fumajin zararına neden olmakta, sonuç olarak da konukçularında önemli verim kayıpları meydana gelmekte, hatta önlem alınmayan ağaçlarda kurumalara neden olmaktadır.

Bu çalışmayla, *S. prunastri*'nin Malatya ilindeki yayılış alanları, bulaşma oranları belirlenmiştir. Çalışmada ayrıca, pestisit uygulamalarının zararlının popülasyon yoğunluğuna olan etkileri araştırılmış, bu amaçla Malatya ili Akçadağ ilçesindeki üç köyde pestisit uygulanan ve uygulanmayan birer adet kayısı bahçesinde *S. prunastri*'nin bulaşma oranları karşılaştırılmıştır.

## MATERYAL ve METOD

### Malatya İli Kayısı Bahçelerinde *Sphaerolecanium prunastri*'nin Yayılış Alanları ve Bulaşma Oranları

*Sphaerolecanium prunastri*'nin yayılış ve bulaşma oranlarının belirlenmesi amacı ile, 2018 ve 2019 yıllarında Malatya'da kayısı yetiştiriciliğinin yaygın olarak yapıldığı Akçadağ, Battalgazi, Darende, Hekimhan, Kale, Merkez, Yazıhan ve Yeşilyurt ilçelerine sörveyler düzenlenmiş, zararlının yayılış ve bulaşma oranları belirlenmiştir. Çalışma, bölgenin iklim koşullarını da göz önüne alarak nisan-ekim aylarında genellikle hafta sonları olmak üzere, düzensiz arazi çıkışları şeklinde yapılmıştır (Çizelge 1). Sörvey çalışmalarında örneklemeler Erkam (1981)'in Lazarov ve Grigorov (1961)'u esas alarak düzenlediği sörvey metoduna göre yapılmıştır.

- 1-20 ağaç olan bahçenin tamamı,
- 21-70 ağaç olan bahçede 10-30 ağaç,
- 71-150 ağaç olan bahçeden 31-40 ağaç,
- 151-500 ağaç olan bahçeden 41-80 ağaç,
- 501-1000 ağaç olan bahçenin % 15'i ve
- 1000'den fazla ağaç olan bahçenin ise % 5'i incelenmiştir.

Çizelge 1. Malatya ili 2018 ve 2019 yılı meteorolojik verileri

Table 1. Meteorological data of Malatya province for 2018 and 2019

Yıl	İklim* Verisi	Ocak	Şubat	Mart	Nisan	Mayıs	Haziran	Temmuz	Ağustos	Eylül	Ekim	Kasım	Aralık
2018	OS	0.2	1.8	6.2	15.1	18.4	23.9	26.2	26.3	19	13.1	7.5	3.2
	OMMS	4.6	6.9	11.5	25.1	26.8	33.1	35.3	35.1	26	20	12.5	7
	OMNS	-3.8	-2.6	0.6	5.1	11.1	14.4	17.1	17.5	12	11.9	2.5	-0.6
	TY	32.4	33.3	28.8	5.4	67.1	25.2	5.4	2.9	18	27.8	18.8	34.2
2019	OS	0.5	3.4	7	11.4	19.9	25.4	25.5	26.4	20	15.9	6.68	
	OMMS	4.4	9	12.9	17.9	28.5	34.7	34.2	35.1	29	24.1	14.7	
	OMNS	-3.4	-2.1	1.1	5.1	10.3	15.5	16.9	17.7	11	7.8	-1.4	
	TY	55.3	46.1	44.1	76.7	5.9	12.3	0	1.4	3.1	26.2	1.9	

\*OS: ortalama sıcaklık, OMMS: ortalama maksimum sıcaklık, OMNS: ortalama minimum sıcaklık, TY: toplam yağış

Bulaşıklık yoğunluğunun saptanmasında Özgen ve Bolu (2009)'nun uyguladığı yöntemle göre, bahçelerin her iki köşegeni boyunca yürünerek, yukarıda verilen ağaç sayısına uygun olarak her ağaç 4 yönden incelenmiş ve genel durum dikkate alınarak *S. prunastri* popülasyon yoğunluğu "az", "orta" ve "yüksek" olarak değerlendirilmiştir. Bir ağacın gövde kalın dallarının herhangi birinde zararlı tek tek görülüyorsa "az", kümeleşme görülüyorsa "orta", ağacın tek bir dalında veya gövdenin sadece bir yönünde bile sıvama bulaşıklık görülüyorsa "yüksek" olarak kabul edilmiştir.

### Akçadağ İlçesine Bağlı Üç Köyde Pestisit Uygulanan ve Uygulanmayan Kayısı Bahçelerinde *Sphaerolecanium prunastri*'nin Bulaşma Oranları

Çalışma, Malatya'nın kayısı yetiştiriciliğinin yaygın olarak yapıldığı Akçadağ ilçesinde organik ve konvansiyonel üretim yapılan üçer adet bahçede yürütülmüştür. Bu amaçla Akçadağ ilçesine bağlı Eğin, Yağmurlu ve Sahil köylerinde, pestisit uygulanan ve uygulanmayan birer bahçede, nisan-ekim aylarında ve genellikle hafta sonları olmak üzere, düzensiz arazi çıkışları düzenlenerek örneklemeler yapılmıştır. Seçilen bu bahçeler yukarıda belirtilen yöntemle, Erkam (1981)'in Lazarov ve Grigorov (1961)'u esas alarak düzenlediği sörvey metodu ve Özgen ve Bolu (2009)'nun uyguladığı skala yöntemine göre yapılmıştır.

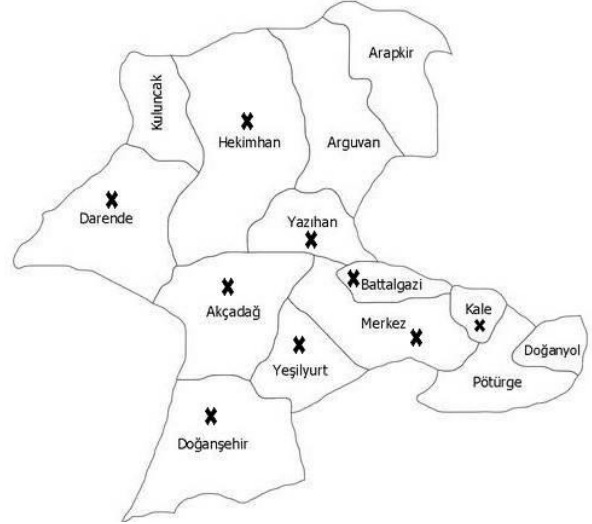
### BULGULAR ve TARTIŞMA

#### Malatya İli Kayısı Bahçelerinde *Sphaerolecanium prunastri*'nin Yayılış Alanları ve Bulaşma Oranları

Malatya ilinde gerek 2018 ve gerekse 2019 yıllarında yapılan sörvey çalışmaları kapsamında, zararlının, Malatya'nın yaygın olarak kayısı üretimi yapılan Akçadağ, Battalgazi, Darende, Doğanşehir, Hekimhan, Kale, Merkez, Yazıhan ve Yeşilyurt ilçelerinde bulunan kayısı bahçelerinde yayılış gösterdiği tespit edilmiştir (Şekil 1).

2018 yılında yapılan sörvey çalışmalarında, en yüksek bulaşıklık oranının Battalgazi (% 82.12) ve % Akçadağ (80.40) ilçelerinde olduğu, en düşük bulaşıklık oranlarının ise Hekimhan (2.09) ve Doğanşehir (4.67) ilçelerinde olduğu tespit edilmiştir (Çizelge 2). Zararlının genel olarak organik bahçelerle bitişik ve orantılı nemin yüksek olduğu bahçelerde yoğun olduğu gözlemlenmiştir. Özellikle, Battalgazi ilçesindeki bu yoğunluğun bahçelerin genellikle baraja yakın olması ve bölgenin ortalamasının üzerinde bir düzeyde nemli olmasından kaynaklanabileceği düşünülmektedir. Düşük bulaşıklık durumunun, Hekimhan (1.122 m) ve Doğanşehir (1.225 m) ilçelerinin rakımının yüksekliğinden dolayı olabileceği, ayrıca zararlının bu ilçelerdeki kayısı

ağaçlarına son yıllarda bulaşmış olabileceği ile ilgili olduğu düşünülmektedir. Bununla birlikte, zararlının popülasyon yoğunluğunun izlenen skala'ya göre, "yüksek" olarak gözlemlendiği ilçenin Darende (%36.58) olduğu kaydedilmiştir.



Şekil 1. *Sphaerolecanium prunastri*'nin Malatya ilindeki yayılışı (İşaretili alanlar).

Figure 1. Distribution of *Sphaerolecanium prunastri* in Malatya province (Marked areas)

2019 yılında yapılan sörvey çalışmaları sonucunda, sörvey yapılan hiç bir ilçede zararlının yüksek popülasyonuna rastlanmazken, en yüksek toplam bulaşıklığın Battalgazi (%13.00) ilçesinde, en düşük toplam bulaşıklığın ise Doğanşehir (%3.24) ilçesinde olduğu belirlenmiştir (Çizelge 3).

Türkiye'de doğrudan *S. prunastri*'nin yaygınlık ve bulaşıklığı ile ilişkili sadece birkaç çalışma yapılmıştır. Bunlardan birinde zararlının Afyon, Burdur ve Isparta illerinde çeşitli konukçu bitkiler üzerindeki yayılışları ve popülasyon yoğunlukları hakkında bilgiler verilmiştir (Ülgentürk ve ark., 2001).

Bir diğer çalışma Aydın'da, zararlının erik ağaçlarında ki yaygınlığı ile ilgilidir (Akşit & Kaya Apak, 2013). Diğer iki çalışma ise Malatya'da yürütülmüştür. Bu çalışmalardan Özgen ve Bolu (2009) tarafından 2003-2004 yıllarında Malatya ilinde kayısı bahçelerinde yürütülen çalışmada, *S. prunastri*'nin Malatya ilinde; Battalgazi, Kale, Merkez, Yazıhan ve Yeşilyurt ilçelerinde yayılış gösterdiği bildirilmiştir. Araştırmacılar, Yazıhan ilçesinde bulaşıklılığın diğer örnekleme alanlarına göre daha yüksek düzeyde olduğunu, Akçadağ, Darende ve Hekimhan örnekleme alanlarının ise temiz olduğunu bildirmişlerdir. Malatya'da daha sonraki yıllarda Yiğit ve Tunaz (2015) tarafından yapılan bir başka sörvey çalışmasında ise, çalışma alanı Akçadağ, Battalgazi, Hekimhan, Kale, Merkez, Yazıhan ilçeleri ile sınırlandırılmış, zararlının sörvey yapılan bölgedeki kayısı bahçelerinde farklı oranlarda (az, orta, yüksek)

bulunduğu bildirmiştir. Araştırmacılar ayrıca, erik koşnili'nin en yüksek %55.4'lük oranla Merkez ilçede, en düşük ise %1.29'lük oranla Hekimhan ilçesinde görüldüğünü ve zararının Malatya geneli yaygınlık oranının %23.5 olduğunu saptamışlardır (Yiğit & Tunaz, 2015). Yapılan bu çalışmada ise, erik koşnili'nin Malatya ilinde dokuz ilçede yayılış gösterdiği, yayılış alanların yıllar ilerledikçe genişlediği ve zararının kayısı bahçelerindeki bulaşıklılığının arttığı belirlenmiştir. Ayrıca, yapılan sorvey çalışmalarında, 2018 ve 2019 yıllarında zararının popülasyon yoğunluğunda önemli

farklılıklar saptanmıştır. 2019 yılında yapılan çalışmada elde edilen verilere göre zararlı popülasyonunun 2018 yılına göre az olmasının sebebi, 2019 yılında kış-bahar aylarının sürekli yağışlı olması nedeniyle (Tablo 1), gerek şiddetli yağışın ve gerekse ortaya çıkan sürekli ve yüksek nemin zararlı popülasyonu üzerinde olumsuz bir etki yapmış olabileceği düşünülmektedir. Sonuç olarak elde edilen veriler, zararının popülasyon yoğunluklarının farklı yıllarda ortaya çıkan iklimsel şartlara göre önemli derecede değişiklikler gösterebileceğini ortaya koymuştur.

Çizelge 2. 2018 yılında Malatya ili kayısı alanlarında *Sphaerolecanium prunastrı*'nin bulaşma oranları (%)  
Table 2. In 2018, infestation rates of *Sphaerolecanium prunastrı* in apricot fields in Malatya province (%)

İncelenen ilçeler	İncelenen ağaç sayısı	Bulaşıklılık Oranı (%)			
		Temiz	Az	Orta	Yüksek
Akçadağ	102	19.60	30.39	30.39	19.60
Battalgazi	123	17.88	31.70	33.33	17.07
Darende	164	38.41	9.75	15.24	36.58
Doğanşehir	150	95.33	3.33	1.33	0.00
Hekimhan	144	97.91	1.38	0.69	0.00
Kale	154	85.71	9.74	3.89	0.64
Merkez	81	65.43	29.62	4.93	0.00
Yazıhan	109	67.88	6.42	11.01	14.67
Yeşilyurt	112	27.67	22.32	29.46	20.53

Çizelge 3. 2019 yılında Malatya ili kayısı alanlarında *Sphaerolecanium prunastrı*'nin bulaşma oranları (%)  
Table 3. In 2019, infestation rates of *Sphaerolecanium prunastrı* in apricot fields in Malatya province (%)

İncelenen ilçeler	İncelenen ağaç sayısı	Bulaşıklılık Oranı (%)			
		Temiz	Az	Orta	Yüksek
Akçadağ	81	90.12	7.40	2.46	0.00
Battalgazi	123	86.99	9.75	3.25	0.00
Darende	102	90.19	6.86	2.94	0.00
Doğanşehir	123	96.74	2.43	0.81	0.00
Hekimhan	123	95.94	3.25	0.81	0.00
Kale	123	91.06	6.50	2.44	0.00
Merkez	81	90.12	7.40	2.47	0.00
Yazıhan	112	91.07	7.14	1.79	0.00
Yeşilyurt	102	93.13	4.90	1.97	0.00

### Akçadağ İlçesine Bağlı Üç Köyde Pestisit Uygulanan ve Uygulanmayan Kayısı Bahçelerinde *Sphaerolecanium prunastrı*'nin Bulaşma Oranları

2018 yılında Malatya ili Akçadağ ilçesinde yürütülen çalışmaların sonucunda *S. prunastrı*'nin yoğunluğu, seçili 6 bahçede yapılan incelemelerle belirlenmiştir. Yapılan gözlemlerde genel olarak insektisit uygulanmayan bahçelerdeki zararlı yoğunluğunun insektisit uygulanan bahçelere göre daha yüksek olduğu tespit edilmiştir. En yüksek zararlı yoğunluğunun % 85.46 oran ile Eğin köyünde kimyasal uygulanmayan bahçede olduğu, en düşük zararlı yoğunluğunun ise % 14.37 oran ile kimyasal uygulanan Yağmurlu köyünde olduğu saptanmıştır

(Çizelge 4).

2019 yılında bahçelerde yapılan gözlemlerde de genel olarak bir önceki yıla benzer sonuçlar elde edilmiş, kimyasal uygulanmayan bahçelerdeki zararlı yoğunluğunun kimyasal uygulanan bahçelere göre daha yüksek olduğu belirlenmiştir. En düşük zararlı yoğunluğunun yine Yağmurlu köyündeki kimyasal uygulanan bahçede (%11.94) olduğu, her üç köydeki kimyasal uygulanmayan bahçelerdeki zararlı yoğunlukları birbirine yakın olsa da, en yüksek zararlı yoğunluğunun Sahil köyündeki kimyasal uygulanmayan bahçede (%47.00) olduğu tespit edilmiştir (Çizelge 5).



Çizelge 1. 2018 yılında Malatya'nın Akçadağ ilçesinin köylerinde, kimyasal uygulanan ve uygulanmayan bahçelerde *Sphaerolecanium prunastrî*'nin yoğunluğu (%)

Table 4. Density (%) of *Sphaerolecanium prunastrî* in chemical treated and non-chemical treated orchards in villages of Akçadağ district of Malatya in 2018

Akçadağ İlçesinin Köyleri	Kimyasal uygulaması	İncelenen Ağaç sayısı	Bulaşıklık Oranı (%)			
			Temiz	Az	Orta	Yüksek
EğİN	Uygulanan	110	60.90	29.09	8.18	2.72
	Uygulanmayan	165	14.54	39.39	26.66	18.78
Yağmurlu	Uygulanan	341	85.63	12.90	1.46	0.00
	Uygulanmayan	110	32.72	28.18	20.00	19.09
Sahil	Uygulanan	110	58.18	31.81	9.09	0.90
	Uygulanmayan	110	41.81	29.09	18.18	11.81

Çizelge 5. 2019 yılında Malatya'nın Akçadağ ilçesinin köylerinde, kimyasal uygulanan ve uygulanmayan bahçelerde *Sphaerolecanium prunastrî*'nin yoğunluğu (%)

Table 5. Density (%) of *Sphaerolecanium prunastrî* in chemical treated and non-chemical treated orchards in villages of Akçadağ district of Malatya in 2019

Akçadağ İlçesinin Köyleri	Kimyasal uygulaması	İncelenen Ağaç sayısı	Bulaşıklık Oranı (%)			
			Temiz	Az	Orta	Yüksek
EğİN	Uygulanan	100	76.00	23.00	1.00	0.00
	Uygulanmayan	150	58.00	28.66	9.33	4.00
Yağmurlu	Uygulanan	310	88.06	10.96	0.96	0.00
	Uygulanmayan	100	56.00	34.00	9.00	1.00
Sahil	Uygulanan	100	79.00	21.00	0.00	0.00
	Uygulanmayan	100	53.00	32.00	10.00	5.00

Malatya'da üreticiler tarafından erik koşnili mücadelesinde farklı etken maddeleri içeren çok sayıda kimyasal preparat kullanılmaktadır. Üreticilerle yapılan görüşmelerde, *S. prunastrî*'nin mayıs ayının ilk haftası ve haziran ayının ortasına kadar ki süreçte ballı madde salgısının yüksek olması nedeniyle, üreticilerin haftada bir insektisit kullanarak ballı madde durdurmaya yönelik yoğun ilaçlamalar yaptıkları görülmüştür. Ayrıca, üreticilerin bu ilaçları genellikle uygulama dozundan daha yüksek ve birçok farklı pestisit ve yaprak gübresini karıştırıp kullandıkları saptanmıştır. Yapılan yoğun ilaçlamalar nedeniyle pestisit uygulanan bahçelerde zararlıların popülasyonunun kimyasal uygulanmayan bahçelere göre daha düşük olduğu tespit edilmiştir. Bununla birlikte, yoğun kimyasal uygulanan bahçelerde doğal düşman aktivitesinin az olduğu, ilaçlama yapılmayan bahçelerde koşnil popülasyonları üzerindeki doğal düşman aktivitesinin daha yoğun olduğu gözlemlenmiştir. Akşit ve Kaya Apak (2013), *S. prunastrî*'ye karşı uyguladıkları farklı preparatlardan, günümüzde yasaklı carbosulfan içeren preparatın yüksek öldürücü etkiye sahip olduğunu (2008'de etki % 91.90 ve 2010'da % 88.09), diğer etkili maddelerde

etkinin % 53.46'nın altında kaldığını bildirmişlerdir.

## SONUÇ ve ÖNERİLER

Malatya ili kayısı alanlarında bulunan *S. prunastrî*'nin kayısılarda daha önce yapılan çalışmalar da göz önüne alındığında, il genelinde ki yayılımını arttırdığı ve ilçelere göre bulaşıklık oranının değişmekle birlikte, il genelinde önemli derecede zarar yaptığı gözlemlenmiştir. Gerek bu çalışma ve gerekse bölgede önceki yıllarda yapılan çalışmalar bir arada düşünüldüğünde, bu eğilimin gelecek yıllarda da süreceği öngörülmektedir. Bu nedenle, Malatya kayısı alanlarında en önemli zararlılardan biri haline gelen erik koşnilinin devamlı gözlem altında tutulması, yeni yayılan ilçelerde yayılmasının engellenmesi için üreticilerin bilinçlendirilmesi, bulaşma yollarının engellenmesi, iklimsel değişikliklerin zararlıların popülasyon üzerinde etkilerinin sürekli takip edilmesi ve doğal düşman aktivitesi de göz önüne alınarak, zararlıya karşı yapılacak mücadelenin zamanında ve entegre mücadele yaklaşımı çerçevesinde ele alınması gerektiği düşünülmektedir. Bölgede üreticiler zararlıya karşı ilaçlamaları genellikle diğer ilaçlarla karıştırarak ve şubat-mart (kayısının tomurcuklanma dönemi), nisan sonu-mayıs başı (3. nimf-dişi dönemi)

ve mayıs sonu-haziran ortası (yumurta açılımı ve 1. nimf dönemi) olmak üzere üç farklı dönemde yapılmaktadır. Bununla birlikte, erken ilk bahar ilaçlamalarının yerine özellikle kış mücadelesinin yapılmadığı bahçelerde, 1. dönem nimf popülasyonunun görüldüğü mayıs sonu-haziran ortası döneminde yapılacak doğru bir ilaçlama ile zararlı popülasyonunun etkili bir şekilde baskılanabileceği düşünülmektedir.

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## Curculionoidea (Insecta: Coleoptera) Species Detected on Some Weeds in Kahramanmaraş Province, Türkiye Part II

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### ABSTRACT

This study was conducted to determine the Curculionoidea species on weeds found in non-agricultural areas in Kahramanmaraş Province, Türkiye between 2021 and 2022, weekly from late March-early April until the end of September after the weeds begin to germinate, and at fifteen-day intervals when the weeds start to dry towards the end of September. As a result of this study, one genus belonging to the subfamily Brentidae of the family Apionidae of the superfamily Curculionoidea, eleven genera belonging to the family Curculionidae, Lixinae Schoenherr, 1823, Hyperinae Marseul, 1863, Curculioninae Latreille, 1802, Baridinae Schoenher, 1836, Ceutorhynchinae Bedel, 1881, Entiminae Schoenherr, 1823 subfamilies and fifteen species belonging to these genera were identified. A total of eight species, including *Hypera striata* (Boheman, 1834), *Rhinusa (Gymnaetron) bipustulata* (Rossi, 1792), *Smicronyx jungermanniae* (G. C. Reich, 1797), *Aulacobaris picicornis* (Marsham, 1802), *Baris analis* (Olivier, 1790) Gyllenhal, 1837, *Glocianus distinctus* (C. Brisout, 1870) and *Rhopalapion longirostre* (Olivier, 1807), are new records for Kahramanmaraş Province. In addition, fifteen weed species were identified as new host plants for the identified Curculionoidea species.

### Entomology

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## Kahramanmaraş İlindeki Bazı Yabancı Otlar Üzerinde Saptanan Curculionoidea (Insecta: Coleoptera) Türleri Kısım II

### ÖZET

Bu çalışma Kahramanmaraş ilinde 2021-2022 yılları arasında tarım dışı alanlarda bulunan yabancı otlar üzerindeki Curculionoidea türlerini belirlemek amacıyla yabancı otların çimlenmeye başlamasını takiben mart sonu-nisan ayı başlarından eylül ayının sonuna kadar haftalık olarak, eylül ayının sonuna doğru yabancı otların kurumaya başlamasıyla 15 günlük aralıklarla arazi çalışmaları yürütülmüştür. Yürütülen bu çalışma sonucunda Curculionoidea üst familyasının Brentidae familyası Apioninae alt familyasına bağlı 1 cins, Curculionidae familyası, Lixinae Schoenherr, 1823, Hyperinae Marseul, 1863, Curculioninae Latreille, 1802, Baridinae Schoenher, 1836, Ceutorhynchinae Bedel, 1881, Entiminae Schoenherr, 1823 alt familyalarına bağlı 11 cins ve bu cinslere ait 15 tür tespit edilmiştir. Tespit edilen türlerden *Hypera striata* (Boheman, 1834), *Rhinusa (Gymnaetron) bipustulata* (Rossi, 1792), *Smicronyx jungermanniae* (G. C. Reich, 1797), *Aulacobaris picicornis* (Marsham, 1802), *Baris analis* (Olivier, 1790), *Ceutorhynchus picitarsis* Gyllenhal, 1837, *Glocianus distinctus* (C. Brisout, 1870) ve *Rhopalapion longirostre* (Olivier, 1807) olmak üzere toplam 8 tür Kahramanmaraş İli için yeni kayıt niteliğindedir. Ayrıca belirlenen Curculionoidea türleri için 15 yabancı ot türü yeni konukçu bitki olarak belirlenmiştir.

### Entomoloji

### Araştırma Makalesi

### Makale Tarihçesi

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### Anahtar Kelimeler

Curculionoidea

Brentidae

Yabancı ot

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## INTRODUCTION

One of the most important factors affecting crop production is weeds. Weeds survive in the same environment as crops and compete with them for water, nutrients, and light, negatively affecting the qualities of the agricultural products, and also causing a loss of approximately \$ 7.6 billion worldwide (Pacanoski, 2007).

The order Coleoptera occupies an important place among the insects used for biological weed control (Kısmalı & Madanlar, 1990). Within this order, the superfamily Curculionoidea has a higher proportion of weed hosts than other species (Oberprieler et al., 2007). Larvae mostly prefer the root collar and roots of plants as feeding habitat, and they feed on the root parts by forming galls (Volovnik, 2010) within the plant tissue (Trnka et al., 2015) or by moving freely in the soil. This group of insects is also important for biological control of weeds, depending on their specific nutritional characteristics (Stinson et al., 1994; Story et al., 2006; Gültekin et al., 2019).

Studies on the species of the superfamily Curculionoidea began in the 1700s, and many foreign and local researchers contributed by carrying out systematic, taxonomic, and faunistic studies. Some of them; Winkler (1924-1932), Emden (1944), Lodos (1960; 1971; 1972), Voss (1962), Altay et al. (1972), Lodos et al. (1978; 2003), Dieckmann (1980), Alonso-Zarazaga & Lyal (1999), Gültekin (2001), Marvaldi & Lanteri (2005), Pehlivan et al. (2005a; 2005b), Keskin (2005), Gültekin (2006c), Davidian & Gültekin (2006), Wanat (2007), Bolu & Legalov (2008), Erbey (2010), Uzun & Tezcan (2011), Avgın & Colonnelli (2011), Gültekin & Podlussany (2012), Aydın (2013), Talamelli (2014), Gürler (2014), Yılmaz (2015), Aydın & Hacet (2016), Özgen et al. (2016), Erdem (2016), Güven (2019), Kapucu (2019), Hacet & Colonnelli (2019), Erbey & Bolu (2021).

In this study, weeds are plants that are adapted to different climatic conditions and soil structures and serve as intermediate hosts for many living organisms due to their ability to withstand difficult ecological conditions and create their own population diversity in the ecosystem. For this reason, one genus belonging to the subfamily Apioninae of the family Brentidae of the superfamily Curculionoidea, which has a very important place among weeds in non-agricultural areas of Kahramanmaraş Province, Curculionidae family, Lixinae Schoenherr, 1823, Hyperinae Marseul, 1863, Curculioninae Latreille, 1802, Baridinae Schoenher, 1836, eleven genera belonging to the subfamilies Ceutorhynchinae Bedel, 1881, Entiminae Schoenherr, 1823 and fifteen species

belonging to these genera were identified.

## MATERIAL AND METHOD

This study was conducted on the weeds found in non-agricultural areas in Kahramanmaraş Province during 2021-2022.

### Material

The main material of this study consists of species belonging to the superfamily Curculionoidea found in Kahramanmaraş Province, Türkiye, and the weeds that these species feed on. In the study, a trap, killing jar, culture containers, sample containers, polyethylene bags, suction tube, 70% ethyl alcohol, forceps, insect needle, petri dish, cotton, soft-tipped brush, tulle, rubber, and GPS device were used.

### Method

#### Field and Laboratory Studies

The study was carried out in weekly intervals, starting from the end of March-early April and till the end of September, after the germination of the weeds in the non-agricultural areas of Kahramanmaraş Province, and at fifteen-day intervals when the weeds started to dry up towards the end of September. In the samples, all the weeds were examined because the species of Curculionoidea were found in the weeds' roots, stems, leaves, and generative organs. The phenological period of each weed in which the species was found, the part where the insect feeds, and the locations of the weed species were determined by GPS.

Weeds belonging to the superfamily Curculionoidea were observed in the wild, with large species collected by hand, small species collected with a suction tube, and a trap used for collection. Some life stages of Curculionoidea species such as egg, larva, pupa, and adult from the collected samples were brought to the laboratory conditions and cultured with the plant whether they fed on. To maintain the humidity of the cultured samples, water was sprayed at regular intervals, and nutrients were replaced as necessary. Cultured weedy plant samples were checked at regular intervals, and egg, larva, pupa, and adult emergence dates were recorded.

Adult insect species collected during field works were killed with the help of the killing jars or directly brought to the laboratory in separate sample containers with their label information. The location where the samples were collected, latitude and longitude, was recorded using GPS. With this information, the samples were labeled and prepared for expert identification. Herbaria of weed species

belonging to the superfamily Curculionoidea were also designed and made available for identification.

Dieckman (1977), Gültekin (2008a), Morris (2008), Erbey (2010), Caldara (2014), and Erbey & Bolu (2021) were used to determine the morphological characteristics of the species belonging to the superfamily Curculionoidea. Identifications of insect specimens belonging to the superfamily Curculionoidea. Associate. Prof. Dr. Mahmut ERBEY (Kırşehir Ahi Evran University, Faculty of Science and Letters, Department of Molecular Biology and Genetics) and Dr. Sci. Andrei Aleksandrovich Legalov (Institute of Systematics and Ecology of Animals, Laboratory of Phylogeny and Faunogenesis) and identifications of weeds by Associate. Prof. Dr. Tamer ÜSTÜNER (Kahramanmaraş Sütçü İmam University Faculty of Agriculture, Department of Plant Protection) was done.

## RESULT and DISCUSSION

In this study conducted in Kahramanmaraş Province, one genus belonging to the Apioninae subfamily of the family Brentidae of the superfamily Curculionoidea, 11 genera belonging to the family Curculionidae, subfamilies Lixinae Schoenherr, 1823, Hyperinae Marseul, 1863, Curculioninae Latreille, 1802, Baridinae Schoenherr, 1836, Ceutorhynchinae Bedel, 1881, Entiminae Schoenherr, 1823 and 15 species belonging to these genera were identified (Figure 1). A total of 8 species, including *Hypera striata* (Boheman, 1834), *Rhinusa (Gymnaetron) bipustulata* (Rossi, 1792), *Smicronyx jungermanniae* (G. C. Reich, 1797), *Aulacobaris picicornis* (Marsham, 1802), *Baris analis* (Olivier, 1790), *Ceutorhynchus picitarsis* Gyllenhal, 1837, *Glocianus distinctus* (C. Brisout, 1870), *Rhopalapion longirostre* (Olivier, 1807), are new records for Kahramanmaraş Province. Furthermore, 15 weed species were identified for the first time as new host plants for the identified Curculionoidea species (Table 1).



*Bangasternus orientalis* Capiomont, 1873



*Hypera postica* (Gyllenhal, 1813)





*Hypera striata* (Boheman, 1834)



*Cionus olivieri* Rosenschold, 1838



*Rhinusa acifer* Caldara



*Rhinusa asellus* (Gravenhorst, 1807)



*Rhinusa tetra* (Fabricius, 1792)



*Smicronyx jungermanniae* (G. C. Reich, 1797)



*Aulacobaris picicornis* (Marshall, 1802)



*Baris analis* (Olivier, 1790)



*Ceutorhynchus picitarsis* Gyllenhal, 1837



*Sitona puncticollis* Stephens, 1831



*Rhinusa (Gymnaetron) bipustulata* (Rossi,  
1792)



*Glorianus distinctus* (C.Brisout, 1870)





*Rhopalapion longirostre* (Olivier, 1807)

Figure 1. Curculionoidea (Insecta: Coleoptera) species detected on the weeds in Kahramanmaraş Province

Şekil 1. Kahramanmaraş İli yabancı otlar üzerinde tespit edilen Curculionoidea (Insecta: Coleoptera) türleri

Table 1. Curculionoidea (Insecta: Coleoptera) species and their host plant detected in Kahramanmaraş Province  
Çizelge 1. Kahramanmaraş İlinde tespit edilen Curculionoidea (Insecta: Coleoptera) türleri ve konukçu bitkileri

Species	Host Plant	References
<i>Bangasternus orientalis</i>	<i>Amygdalus communis</i>	Ter-Minassian, 1978
	<i>Carduus acanthoides</i> *	Maddox et al., 1991
	<i>Carduus nutans</i> *	Bolu & Legalov, 2008
	<i>Centaurea calcitrapa</i>	Gültekin, 2008b
	<i>Centaurea iberica</i>	Anonymous, 2022b
	<i>Centaurea solstitialis</i>	
	<i>Centaurea virgata</i>	
	<i>Pistacia vera</i>	
<i>Hypera postica</i>	<i>Amygdalus communis</i>	Hoffman, 1963
	<i>Centaurea</i> sp.	Fick, 1976
	<i>Cirsium arvense</i> *	Anay & Kornoşor, 2000
	<i>Cirsium</i> sp.	Lodos et al., 2003
	<i>Medicago maritimus</i>	Bolu & Legalov, 2008
	<i>Medicago sativa</i>	Moradi-Vajargah et al., 2011
	<i>Melilotus</i> sp.	Bolu, 2016
	<i>Onopordum</i> sp.	Shrestha et al., 2020; François et al., 2021
	<i>Quercus</i> sp.	
	<i>Trifolium</i> sp.	
<i>Trigonella</i> sp.		
<i>Verbascum</i> sp.		
<i>Vicia</i> sp.		
<i>Hypera striata</i>	<i>Lens esculenta</i>	Hoffman, 1954
	<i>Plantago coronopus</i>	Smreczynski, 1968
	<i>Vicia</i> sp.	Angelov, 1978
	<i>Vicia sativa</i>	Tempere & Pericart, 1989
	<i>Quercus macranthera</i>	Koch, 1992
	<i>Trifolium repens</i> *	Pehlivan et al., 2005a; Skuhrovec, 2003a; 2006 Ghahari et al., 2009
<i>Cionus olivieri</i>	<i>Buddleje</i> sp.	Hoffman, 1958
	<i>Limosella</i> sp.	Smreczynski, 1976
	<i>Verbascum densiflorum</i>	Read, 1977
	<i>Verbascum longiflorum</i>	Rather, 1989
	<i>Verbascum nigrum</i> <i>Verbascum</i>	Balalaikins et al., 2011; Akrawi & Mahmoud, 2019
	<i>phlomoides</i> <i>Verbascum sinuatum</i>	
	<i>Verbascum songaricum</i>	Kostal & Caldara, 2019; Baviera & Caldara, 2020; Jiang et al., 2020
	<i>Scrophularia</i> sp.	
	<i>Verbascum</i> sp.	
	<i>Verbascum speciosum</i> <i>Verbascum</i>	

<i>thaplus</i>		
<i>Rhinusa acifer</i>	<i>Verbascum sinuatum</i>	Aslan & Candan, 2018
<i>Rhinusa (Gymnaetron) asellus</i>	<i>Alcea calvardis*</i> <i>Verbascum gaillardotii</i> <i>Verbascum nigrum</i> <i>Verbascum phlomoides</i> <i>Verbascum pulverulentum</i> <i>Verbascum sinuatum</i> <i>Verbascum sp.</i> <i>Verbascum speciosum</i> <i>Verbascum thapsoides</i> <i>Verbascum thapsus</i> <i>Verbascum virgatum</i>	Hoffman, 1958 Halperin & Fremuth, 2003; Caldara et al., 2010; Fernández, 2012 Vinolas et al., 2012 Caldara, 2014 Abad et al., 2015, 2016; Akrawi & Mahmoud, 2019; Digirolomo et al., 2019 Bolu et al., 2023
<i>Rhinusa (Gymnaetron) bipustulata</i>	<i>Alcea calvardis*</i> <i>Verbascum sinuatum*</i> <i>Althaea officinalis</i> <i>Scrophularia aquatica</i> <i>Scrophularia canina</i> <i>Scrophularia canina</i> <i>Scrophularia nodosa</i> <i>Scrophularia olympica</i> <i>Scrophularia sp.</i> <i>Scrophularia striata</i> <i>Scrophularia variegata</i> <i>Scrophularia nervosa</i>	Lodos et al., 2003 Skuhrovec, 2004 Gosik, 2010 Abad et al., 2015 Forbicioni et al., 2019
<i>Rhinusa tetra</i>	<i>Alcea calvardis*</i> <i>Haplophyllum perforatum</i> <i>Juniperus sp.</i> <i>Medicago sativa</i> <i>Mentha sp.</i> <i>Onopordum sp.</i> <i>Phlomis sp.</i> <i>Pinus sp.</i> <i>Prunus domestica</i> <i>Verbascum sp.</i> <i>Quercus sp.</i> <i>Rosa sp.</i> <i>Salvia sp.</i> <i>Scrophularia auriculata</i> <i>Scrophularia canina</i> <i>Scrophularia sp.</i> <i>Silene spergulifolia</i> <i>Sinapis sp.</i> <i>Styrax sp.</i> <i>Triticum sp.</i> <i>Verbascum blattaria</i> <i>Verbascum boerhavii</i> <i>Verbascum creticum</i> <i>Verbascum gaillardoti</i> <i>Verbascum sinuatum*</i> <i>Verbascum lychnitis</i> <i>Verbascum nigrum</i> <i>Verbascum phlomoides</i> <i>Verbascum phoeniceum</i> <i>Verbascum pulverulentum</i> <i>Verbascum speciosum</i> <i>Verbascum thapsiforme</i>	Hoffmann, 1958 Anderson, 1973 O'Brien & Wibmer, 1982 Lodos et al., 2003 Karaca et al., 2006 Caldara et al., 2010; 2012; Legalov et al., 2010 Bosmans, 2012 Balalaikins, 2012 Pesic, 2012 Caldara, 2014 Abad et al., 2015 Doğanlar & Üremiş, 2014; Özgen et al., 2016 Forbicioni et al., 2019 Bolu et al., 2023

	<i>Verbascum thapsus</i>	
	<i>Vicia ervilia</i>	
<i>Smicronyx jungermanniae</i>	<i>Amygdalus communis</i>	Gertz, 1928
	<i>Carduus nutans</i> *	Porta, 1932
	<i>Citrus unshiu</i>	Hoffman, 1951-1958
	<i>Cuscuta campestris</i>	Hoffman, 1958
	<i>Cuscuta epithymum</i>	Marikovskiy & Ivannikov, 1968
	<i>Cuscuta europaea</i>	Tyurebaev, 1977
	<i>Cuscuta</i> sp.	Lodos et al., 1978
	<i>Triticum</i> sp.	Bargagli, 1883
		Anonymous, 1987
		Tempere & Pericart, 1989
		Bayer & Winkelmann, 2005
		Teodosie et al., 2004
		Colonnelli, 2016
		Erbey & Bolu, 2021
<i>Aulacobaris picicornis</i>	<i>Crambe orientalis</i> *	Colonnelli, 2004
	<i>Reseda lutea</i>	Dedyukhin, 2014
<i>Baris analis</i>	<i>Pulicaria dysenterica</i>	<i>Crambe</i>
	<i>orientalis</i> *	Lodos et al., 2003
	<i>Rubus</i> sp.	Forbicioni et al., 2019
	<i>Verbascum</i> sp.	
<i>Ceutorhynchus picitarsis</i>	<i>Alnus glutinosa</i>	<i>Sisymbrium</i>
	<i>loeselii</i>	Hoffmann, 1954
	<i>Brassica napus</i>	Scherf, 1964
	<i>Anchusa arvensis</i> *	Dieckmann, 1972
	<i>Barbarea vulgaris</i>	Gültekin, 2001
	<i>Brassica oleifera</i>	Lodos et al., 2003
	<i>Brassica oleracea</i>	Yoshitake et al., 2017
	<i>Brassica rapa</i>	Anita, 2018
	<i>Crambe orientalis</i> *	Keyhanian et al., 2020
	<i>Diplotaxis tenifolia</i>	Özder & Altın, 2020
	<i>Eruca pinmatifida</i>	Gültekin, 2020
	<i>Isatis tinctoria</i>	
	<i>Lonchophora capiomontana</i>	
	<i>Medicago sativa</i>	
	<i>Quercus</i> sp.	
	<i>Rapistrum rugosum</i>	
	<i>Rubus</i> sp.	
	<i>Sinapis</i> sp.	
	<i>Triticum</i> sp.	
	<i>Verbascum</i> sp.	
<i>Glocianus distinctus</i>	<i>Calendula arvensis</i> *	Hoffman, 1954
	<i>Crepis</i> sp.	Teodor, 2011
	<i>Crepis virens</i>	Ryaskin, 2019
	<i>Hieracium</i> sp.	
	<i>Hypochoeris maculata</i>	
	<i>Hypochoeris</i> sp.	
	<i>Lactuca serriola</i>	
	<i>Lactuca</i> sp.	
	<i>Picris</i> sp.	
	<i>Taraxacum officinale</i>	
	<i>Taraxacum</i> sp.	
<i>Sitona puncticollis</i>	<i>Amygdalus communis</i>	Meyer, 1941
	<i>Astragalus</i> sp.	Lodos et al., 2003
	<i>Hypericum perforatum</i>	<i>Rubia</i>
	<i>tinctorum</i>	Phillips & Barratt, 2004
	<i>Trifolium</i> sp.	Bagheri & İsfahani, 2008; Castro et al., 2010

	<i>T. repens</i>	Delbol & Lempereur, 2014; Gözüaçık et al., 2021a; 2021b
	<i>T. pratense</i>	
	<i>Melilotus albus</i>	Bolu & Legalov, 2008
	<i>Medicago</i> sp.	
	<i>M. sativa</i>	
	<i>Melilotus</i> sp.	
	<i>Lotus corniculatus</i>	
	<i>Lens culinaris</i>	
	<i>Pinus</i> sp.	
	<i>Vicia</i> sp.	
	<i>V. villosa</i>	
	<i>V. faba</i>	
	<i>Quercus</i> sp.	
<i>Rhopalapion longirostre</i>	<i>Alcea rosea</i>	Ter-Minnasyan, 1972
	<i>Alcea</i> sp.	Ehret, 1990
	<i>Althaea officinalis</i>	Reavey & Lawton, 1991; Schmitz & Maczey, 1993
	<i>Althaea rosea</i>	
	<i>Amygdalus communis</i>	Pupier, 1997
	<i>Gossypium</i> sp.	Mazur, 2007
	<i>Malva</i> sp.	Bolu & Legalov, 2008
	<i>Malva sylvestris</i> ssp. <i>mauritanica</i>	Krivan & Stejskal, 2009; Wilhelm et al., 2011
		Valentin et al., 2011
		Bolu, 2016
		Wanat et al., 2016
		Alekseev et al., 2021

\*New host plant

**Superfamily:** Curculionoidea

**Family:** Curculionidae

**Subfamily:** Lixinae Schoenherr, 1823

**Tribus:** Lixini Schoenherr, 1823

**Genus:** *Bangasternus* Gozis, 1886

**Species:** *Bangasternus orientalis* Capiomont, 1873

**Material examined:** Kahramanmaraş, Onikisubat, Kahramanmaraş Sütçü Imam University Avşar Campus, N37°35'14,400/E36°48'42,179, May 9, 2022 (number of insect samples: 7); May 17, 2019 (number of insect samples: 15); May 24, 2022 (number of insect samples: 11), on *Centaurea solstitialis*; May 9, 2022 (number of insect samples: 2), on *Carduus nutans* (new host plant), Kahramanmaraş, Pazarcık, Sarierik Village, N37°20'50,503/E37°6'16,540, May 11, 2022 (number of insect samples: 2), on *Carduus acanthoides* (new host plant).

**Distribution in Türkiye:** Adana, Ankara, Antalya, Aydın, Batman, Bilecik, Bitlis, Çankırı, Diyarbakır, Elazığ, Eskişehir, Gaziantep, Hatay, Içel, İzmir, Kahramanmaraş, Karabük, Karaman, Kayseri, Kilis, Konya, Kırşehir, Manisa, Mardin, Muğla, Niğde, Osmaniye, Sivas, Trabzon, Yozgat (Lodos et al., 1978, 2003; Sert, 1995; Pehlivan et al., 2005a; Bolu & Legalov, 2008; Erbey, 2010; Yılmaz, 2015).

**Subfamily:** Hyperinae Marseul, 1863

**Tribus:** Hyperini Marseul, 1863

**Genus:** *Hypera* Germar, 1817

**Species:** *Hypera postica* (Gyllenhal, 1813)

**Material examined:** Kahramanmaraş, Onikişubat, Kahramanmaraş Sütçü Imam University Avşar Campus, N37°35'14,400/E36°48'42,179, May 9, 2022 (number of insect samples: 2), on *Cirsium arvense* (new host plant).

**Distribution in Türkiye:** Adana, Afyonkarahisar, Antalya, Ağrı, Aksaray, Amasya, Ankara, Artvin, Aydın, Balıkesir, Bartın, Bilecik, Bitlis, Bolu, Bursa, Çanakkale, Çorum, Denizli, Diyarbakır, Edirne, Elazığ, Erzincan, Erzurum, Eskişehir, Gaziantep, Hakkari, Hatay, Isparta, Iğdır, Içel, İzmir, Kahramanmaraş, Kars, Kayseri, Kırıkkale, Kırklareli, Kırşehir, Kilis, Kocaeli, Konya, Kütahya, Malatya, Manisa, Mardin, Mersin, Muğla, Muş, Nevşehir, Niğde, Osmaniye, Sakarya, Samsun, Siirt, Sinop, Sivas, Şanlıurfa, Şırnak, Tekirdağ, Tokat, Trabzon, Uşak, Van, Yozgat, Zonguldak (Lodos et al., 1978, 2003; Sert, 1995; Anay & Kornoşor, 2000; Pehlivan et al., 2005a; Bolu & Legalov, 2008; Erbey, 2010; Gürler, 2014; Yılmaz, 2015; Bolu, 2016; İreç, 2017).

**Species:** *Hypera striata* (Boheman, 1834)

**Material examined:** Kahramanmaraş, Onikişubat, Kahramanmaraş Sütçü Imam University Avşar Campus, N37°35'14,400/E36°48'42,179, May 9, 2022 (number of insect samples: 2), on *Trifolium repens* (new host plant).



**Distribution in Türkiye:** Ankara, İzmir, Kilis, Mersin, Niğde (Pehlivan et al., 2005a; Erbey, 2010; Gürler, 2014), Kahramanmaraş (new record).

**Subfamily:** Curculioninae Latreille, 1802

**Tribus:** Cionini Schoenherr, 1825

**Genus:** *Cionus* Clarville, 1798

**Species:** *Cionus olivieri* Rosenschold, 1838

**Material examined:** Kahramanmaraş, Dulkadiroğlu, Sekamer, N37°35'28,975/E37°3'30,066, May 11, 2022 (number of insect samples: 1), on *Verbascum sinuatum*.

**Distribution in Türkiye:** Adana, Afyonkarahisar, Ankara, Antalya, Bitlis, Çankırı, Eskişehir, Gaziantep, Hatay, İçel, Kahramanmaraş, Kastamonu, Kayseri, Kırıkkale, Kırşehir, Konya, Mersin, Muğla, Nevşehir, Niğde (Lodos et al., 1978; 2003; Erbey, 2010; Gürler, 2014; Yılmaz, 2015; Bolu, 2016; Kapucu, 2019).

**Tribus:** Mecinini Germar, 1824

**Genus:** *Rhinusa* Stephens, 1829 (*Gymnaetron* Schoenherr, 1825)

**Species:** *Rhinusa acifer* Caldara

**Material examined:** Kahramanmaraş, Onikişubat, Kahramanmaraş Sütçü Imam University Avşar Campus, N37°35'14,400/E36°48'42,179, June 24, 2016 (number of insect samples: 18), on *Verbascum sinuatum*.

**Distribution in Türkiye:** Adıyaman, Antalya, Denizli, Elazığ, Gaziantep, Kahramanmaraş, Kayseri (Caldara, 2014; Aslan & Candan, 2018).

**Species:** *Rhinusa (Gymnaetron) asellus* (Gravenhorst, 1807)

**Material examined:** Kahramanmaraş, Türkoğlu, Uzunsöğüt Village, N37°23'36,403/E36°58'54,046, May 11, 2022 (number of insect samples: 1), on *Alcea calvardis* (new host plant); Kahramanmaraş, Dulkadiroğlu, Kozludere Village, N37°36'51,871/E37°6'27,905, April 28, 2022 (number of insect samples: 1), on *Verbascum sinuatum*; Kahramanmaraş, Dulkadiroğlu, Sekamer, N37°35'28,975/E37°3'30,066, April 11, 2022 (number of insect samples: 2), on *Verbascum sinuatum*; Kahramanmaraş, Onikişubat, Kahramanmaraş Sütçü Imam University Avşar Campus, N37°35'14,400/E36°48'42,179, May 16, 2022 (number of insect samples: 1); May 9, 2022 (number of insect samples: 2); May 24, 2022 (number of insect samples: 2), on *Verbascum sinuatum*.

**Distribution in Türkiye:** Adana, Afyonkarahisar, Aksaray, Ankara, Antalya, Aydın, Bitlis, Bolu, Çankırı, Denizli, Diyarbakır, Edirne, Eskişehir, Gaziantep, Isparta, İçel, İzmir, Kahramanmaraş, Karabük, Karaman, Kayseri, Kırıkkale, Kırklareli, Kırşehir, Kilis, Konya, Kütahya, Manisa, Mersin,

Muğla, Nevşehir, Niğde, Osmaniye, Uşak, Yozgat (Lodos et al., 1978, 2003; Sert, 1995; Erbey, 2010; Sert et al., 2013; Gürler, 2014; Yılmaz, 2015; Kapucu, 2019; Bolu et al., 2023).

**Species:** *Rhinusa (Gymnaetron) bipustulata* (Rossi, 1792)

**Material examined:** Kahramanmaraş, Türkoğlu, Uzunsöğüt Village, N37°23'36,403/E36°58'54,046, May 11, 2022 (number of insect samples: 1), on *Alcea calvardis* (new host plant); Kahramanmaraş, Dulkadiroğlu, Kozludere Village, N37°36'51,871/E37°6'27,905, April 28, 2022 (number of insect samples: 1), on *Verbascum sinuatum* (new host plant).

**Distribution in Türkiye:** Afyonkarahisar, Düzce, Karabük, Kırıkkale, Mersin, Niğde, Tekirdağ (Lodos et al., 1978; 2003; Erbey, 2010; Sert et al., 2013), Kahramanmaraş (new record).

**Species:** *Rhinusa tetra* (Fabricius, 1792)

**Material examined:** Kahramanmaraş, Türkoğlu, Uzunsöğüt Village, N37°23'36,403/E36°58'54,046, May 11, 2022 (number of insect samples: 4), on *Alcea calvardis* (new host plant); Kahramanmaraş, Dulkadiroğlu, Sekamer, N37°35'28,975/E37°3'30,066, May 2, 2022 (number of insect samples: 2), on *Verbascum sinuatum* (new host plant); Kahramanmaraş, Onikişubat, Kahramanmaraş Sütçü Imam University Avşar Campus, N37°35'14,400/E36°48'42,179, May 24, 2022 (number of insect samples: 1), on *Alcea calvardis*.

**Distribution in Türkiye:** Adana, Afyonkarahisar, Ankara, Antalya, Aydın, Balıkesir, Bilecik, Bitlis, Bolu, Çanakkale, Çankırı, Çorum, Denizli, Diyarbakır, Edirne, Eskişehir, Elazığ, Gaziantep, Hatay, Isparta, İçel, İzmir, Kahramanmaraş, Karabük, Karaman, Kayseri, Kırıkkale, Kırklareli, Kırşehir, Kütahya, Konya, Manisa, Mersin, Muğla, Nevşehir, Niğde, Osmaniye, Tekirdağ, Uşak, Zonguldak (Lodos et al., 1978; 2003; Karaca et al., 2006; Erbey, 2010; Avgın & Colonnelli, 2011; Vera, 2011; Doğanlar & Üremiş, 2014; Gürler, 2014; Yılmaz, 2015; Özgen et al., 2016; Kapucu, 2019; Bolu et al., 2023).

**Tribus:** Smicronychini Seidlitz

**Genus:** *Smicronyx* Schoenherr, 1843

**Species:** *Smicronyx jungermanniae* (G.C.Reich, 1797)

**Material examined:** Kahramanmaraş, Onikişubat, Kahramanmaraş Sütçü Imam University Avşar Campus, N37°35'14,400/E36°48'42,179, May 9, 2022 (number of insect samples: 2), on *Carduus nutans* (new host plant).

**Distribution in Türkiye:** Burdur, Edirne, Elazığ, Konya, Muğla, Nevşehir (Lodos et al., 1978; 2003; Kaplan & Yücel, 2014; Tolga & Yoldaş, 2020; Erbey & Bolu, 2021), Kahramanmaraş (new record).

**Subfamily:** Baridinae Schoenher, 1836  
**Tribus:** Baridini Schoenherr, 1836  
**Genus:** Aulacobaris Desbrochers, 1892  
**Species:** *Aulacobaris picicornis* (Marsham, 1802)  
**Material examined:** Kahramanmaraş, Onikişubat, Kahramanmaraş Sütçü Imam University Avşar Campus, N37°35'14,400/E36°48'42,179, May 15, 2019 (number of insect samples: 2), on *Crambe orientalis* L. (new host plant).  
**Distribution in Türkiye:** Ankara (Gürler, 2014), Kahramanmaraş (new record).  
**Genus:** Baris Germar, 1817  
**Species:** *Baris analis* (Olivier, 1790)  
**Material examined:** Kahramanmaraş, Onikişubat, Kahramanmaraş Sütçü Imam University Avşar Campus, N37°35'14,400/E36°48'42,179, May 9, 2022 (number of insect samples: 2), on *Crambe orientalis* L. (new host plant).  
**Distribution in Türkiye:** Düzce, Gaziantep (Lodos et al., 2003), Kahramanmaraş (new record).  
**Subfamily:** Ceutorhynchinae Price, 1881  
**Tribus:** Ceutorhynchini Germar, 1824  
**Genus:** *Ceutorhynchus* Germar, 1823  
**Species:** *Ceutorhynchus picitarsis* Gyllenhal, 1837  
**Material examined:** Kahramanmaraş, Onikişubat, Kahramanmaraş Sütçü Imam University Avşar Campus, N37°35'14,400/E36°48'42,179, May 23, 2019 (number of insect samples: 1); May 15, 2019 (number of insect samples: 3), on *Crambe orientalis* (new host plant); April 27, 2022 (number of insect samples: 2), on *Anchusa arvensis* (new host plant).  
**Distribution in Türkiye:** Ankara, Antalya, Artvin, Bartın, Çanakkale, Edirne, Erzurum, Içel, İstanbul, İzmir, Karaman, Kastamonu, Kayseri, Kars, Kırıkkale, Kırşehir, Konya, Mersin, Niğde, Sivas, Tekirdağ, Trabzon, Yozgat (Lodos et al., 1978; 2003; Sert, 1995; Sert & Çağatay, 1999; Gültekin, 2001; Erbey, 2010; Aydın, 2013; Gürler, 2014; Yılmaz, 2015; Hacet & Colonnelli, 2019; Özder & Altın, 2020; Gültekin, 2020), Kahramanmaraş (new record).  
**Genus:** *Glocianus* Reitter, 1916  
**Species:** *Glocianus distinctus* (C.Brisout, 1870)  
**Material examined:** Kahramanmaraş, Onikişubat, Kahramanmaraş Sütçü Imam University Avşar Campus, N37°35'14,400/E36°48'42,179, April 27, 2019 (number of insect samples: 3), on *Calendula arvensis* L. (new host plant).  
**Distribution in Türkiye:** Edirne, İstanbul, Tekirdağ, (Aydın, 2013; Hacet & Colonnelli, 2019), Kahramanmaraş (new record).  
**Subfamily:** Entiminae Schoenherr, 1823  
**Tribus:** Sitonini Gistel, 1856

**Genus:** *Sitona* Germar, 1824  
**Species:** *Sitona puncticollis* Stephens, 1831  
**Material examined:** Kahramanmaraş, Onikişubat, Kahramanmaraş Sütçü Imam University Avşar Campus, N37°35'14,400/E36°48'42,179, May 9, 2022 (number of insect samples: 6); May 24, 2022 (number of insect samples: 4), on *Trifolium repens*.  
**Distribution in Türkiye:** Adana, Afyon, Ankara, Antalya, Bartın, Bolu, Çanakkale, Çorum, Denizli, Edirne, Elazığ, Erzurum, Hatay, Iğdır, İzmir, Kahramanmaraş, Kayseri, Kırklareli, Kırşehir, Manisa, Muğla, Niğde, Sinop, Usak, Zonguldak (Lodos et al., 1978; 2003; Özbek, 1986; Bolu & Legalov, 2008; Erbey, 2010; Gürler, 2014; Yılmaz, 2015; Tolga & Yoldaş, 2020; Gözüaçık et al., 2021a; 2021b).  
**Family:** Brentidae  
**Subfamily:** Apioninae  
**Tribus:** Malvapiini Alonso-Zarazaga, 1990  
**Genus:** *Rhopalapion* Schilsky, 1906  
**Species:** *Rhopalapion longirostre* (Olivier, 1807)  
**Material examined:** Kahramanmaraş, Onikişubat, Kahramanmaraş Sütçü Imam University Avşar Campus, N37°35'14,400/E36°48'42,179, May 24, 2022 (number of insect samples: 3), on *Alcea* sp..  
**Distribution in Türkiye:** Ağrı, Bingöl, Denizli, Diyarbakır, Elazığ, İzmir, Mardin, Siirt, Zonguldak (Bolu & Legalov, 2008; Tezcan et al., 2011; Bolu, 2016), Kahramanmaraş (new record).

## CONCLUSION

As a result of this study, it was found that the Curculionoidea fauna feeding on weeds in non-agricultural areas of Kahramanmaraş Province is quite rich. In addition to investigating the relationship between Curculionoidea and weeds, this study has provided new data that will form the basis for future biological control and other studies in the region.

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## Contribution of the Authors as Summary

Authors declares the contribution of the authors is

equal.

### Statement of Conflict of Interest

Authors have declared no conflict of interest.

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## Yonca Hortumlu Böceği, *Hypera postica* (Gyllenhal 1813) (Coleoptera: Curculionidae)'nın Zararı ve Larva, Pupa ve Ergin Gelişim Morfolojisi

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### ÖZET

Bu çalışmada, yonca bitkisinde zararlı bir tür olan *Hypera postica* (Gyllenhal 1813) (Coleoptera: Curculionidae)'nın zararı, larva, pupa ve ergin gelişim morfolojisi, stereomikroskop ve taramalı elektron mikroskobu kullanılarak incelenmiştir. *H. postica* yumurtaları sarımsı renkte ve nispeten küçüktür. *H. postica* larvalarının başı ovaldir. Larvalar sarımsı-yeşilimsi renktedir. Larvaların sırtında uzunlamasına beyaz bir şerit vardır. Larvaların vücudu segmentlidir ve üzerinde setalar vardır. Larvalar, larva aşamasından prepupal aşamaya kadar sarımsı-yeşilimsi renkten kahverengiye dönüşür. Bu aşamada C şeklinde bir görünüme sahiptir. Baş ve vücudun diğer kısımlarında çok sayıda seta ayırt edilir. *H. postica* pupaları sarı renklidir. Kokon yapısı şeffaf ve ağsıdır ve farklı kalınlıklarda iç içe geçmiş delikli bantlar şeklinde görünür. *H. postica* erginlerinin vücut rengi parlak ve kontrastlı görünür. Ergin *H. postica*'nın vücut yapısı setalarla kaplı olup iki farklı seta yapısı bulunmaktadır. *H. postica* dünyanın birçok bölgesinde yoncanın verimini, kalitesini düşürmekte olup ekonomik kayıplara neden olmaktadır. Bu çalışma ile yonca zararlısı olan ve ekonomik öneme sahip bu böceğin gelişim morfolojisinin belirlenmesi ve bu zararlıya karşı daha etkin mücadele yöntemlerinin geliştirilmesine katkı sağlanması amaçlanmıştır.

### Entomoloji

### Araştırma Makalesi

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### Anahtar Kelimeler

Ergin

Larva

Pupa

Stereomikroskop

Taramalı elektron mikroskobu

## Adult development morphology and pupae, larval and damage of Alfalfa Weevil *Hypera postica* (Gyllenhal 1813) (Coleoptera: Curculionidae)

### ABSTRACT

In this study, adult developmental morphology and the pupal, and larval of *Hypera postica* (Gyllenhal 1813) (Coleoptera: Curculionidae), which is a harmful species in alfalfa, and its damage in alfalfa were investigated using stereomicroscope and scanning electron microscope. *H. postica* eggs are yellowish in color and relatively small. The head of *H. postica* larvae is oval. Larvae are yellowish-greenish in color. The larvae have a longitudinal white stripe on the back. The body of the larvae is segmented and has setae on it. Larvae change from yellowish-greenish to brown in the larval to the prepupal stage. At this stage, it has a C-shaped appearance. Numerous setae are distinguished on the head and other parts of the body. *H. postica* pupae are yellow. The cocoon structure is transparent and reticulate, appearing as intertwined perforated bands of different thicknesses. The body color of *H. postica* adults appears bright and contrasting. The body structure of adult *H. postica* is covered with scales, and two different scales were found. *H. postica* reduces the yield and quality of alfalfa in many parts of the world and causes economic losses. This study aimed to determine the developmental morphology of this economically important alfalfa pest and contribute to the development of more effective control methods against this pest.

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## INTRODUCTION

Curculionidae family is a group of insects, commonly known as the chamber bugs, and are widespread throughout the world. The family is one of the largest families of insects, with approximately 83,000 described species (Oberprieler et al., 2007). In addition to causing structural and nutritional damage to various plants, some species of Curculionidae also play a role in spreading plant diseases (Parker et al., 2016). The alfalfa weevil, *Hypera postica* (Gyllenhal, 1813) (Curculionidae), is a destructive pest of alfalfa (*Medicago sativa* L.), the world's most important cultivated plant (Hoffmann, 1963). Damage caused by larvae consists of defoliation, which reduces the yield and quality of forages and causes economic losses in many regions of the world (Goosey, 2012; Saeidi & Moharrampour 2017; Soroka et al., 2020). *H. postica* is an oligophagous insect that feeds exclusively on leguminous plants of the genus *Medicago*. However, it can feed on several species of related genera, such as *Melilotus*, *Trifolium*, and *Trigonella*, in most alfalfa-growing regions worldwide. Heavily infested fields may appear silver or white as most leaves are destroyed or completely consumed. Severe pest pressure can destroy crops (Fick, 1976). The alfalfa weevil, *H. postica* (Coleoptera, Curculionidae), was studied to determine some biological characteristics in nature. It was found that females lay eggs on alfalfa stems in November, spend the winter in both egg and adult stages, and from mid-February, when the average temperature is above 9 °C, the adults migrate back into the field, mate, and start laying eggs. At the same time, it was determined that larvae emerged in early March from the eggs laid in the fall (Gözüaçık & İreç, 2019).

The family Curculionidae has a legless (apod) larva type. Larvae are generally seen in a "C" shape. The free pupal type is seen in the Curculionidae family. There are distinct wing marks on the pupa. The pupal period is spent in the soil or on the host plant (Lodos, 1960).

Studies on the morphology of larvae, pupae, and adults of Curculionidae are as follows. The morphology of immature larvae, pupae, and adults of *Hypera temperei* (Curculionidae) and *Phloeosinus tacubayae* (Curculionidae) were studied (Germann, 2021; Cervantes-Espinoza et al., 2023). The larvae and pupae were examined in *Phelypera supply* (Boheman, 1834) (Curculionidae) *Gymnetron species* (Curculionidae), *Smicronyx smreczynskii* Solari, 1952 (Curculionidae), (Vanin et al., 2012; Skuhrovec et al., 2022; Sprick, & Gosik, 2023). Skuhrovec (2006), *H. postica* explained its periods using chaetotaxy. Skuhrovec (2004) also described mature larvae of the

subgenus *Hypera*.

Studies on this subject are quite limited and no study has been conducted on *H. postica*, an important species. Therefore, in this study, larval, pupal, and adult developmental morphology of *H. postica* and the damage caused by it on alfalfa plants were investigated.

## MATERIAL and METOD

### Stereomicroscopy (SM)

In this study, *H. postica* was collected from alfalfa fields (*Medicago sativa* L.) from Bingöl, Turkey, in May 2022. Adult, pupa, and larvae of *H. postica* were photographed under an Olympus SZX7 SM.

### Scanning electron microscopy (SEM)

For scanning electron microscopy (SEM), adult, larva, and pupae specimens were fixed in 2.5% glutaraldehyde (pH 7.2, phosphate buffered), rinsed three times with phosphate buffer, and separately dehydrated 15 min by using an ethanol progressive series (70%, 80%, 90%, and 100%). The tissues were transferred to hexamethyldisilazane, and then samples were dried with air. They were mounted on SEM stubs and coated with a gold sputter coater (Polaron SC 502). The samples were then photographed SEM (JEOL JSM 6060 LV).

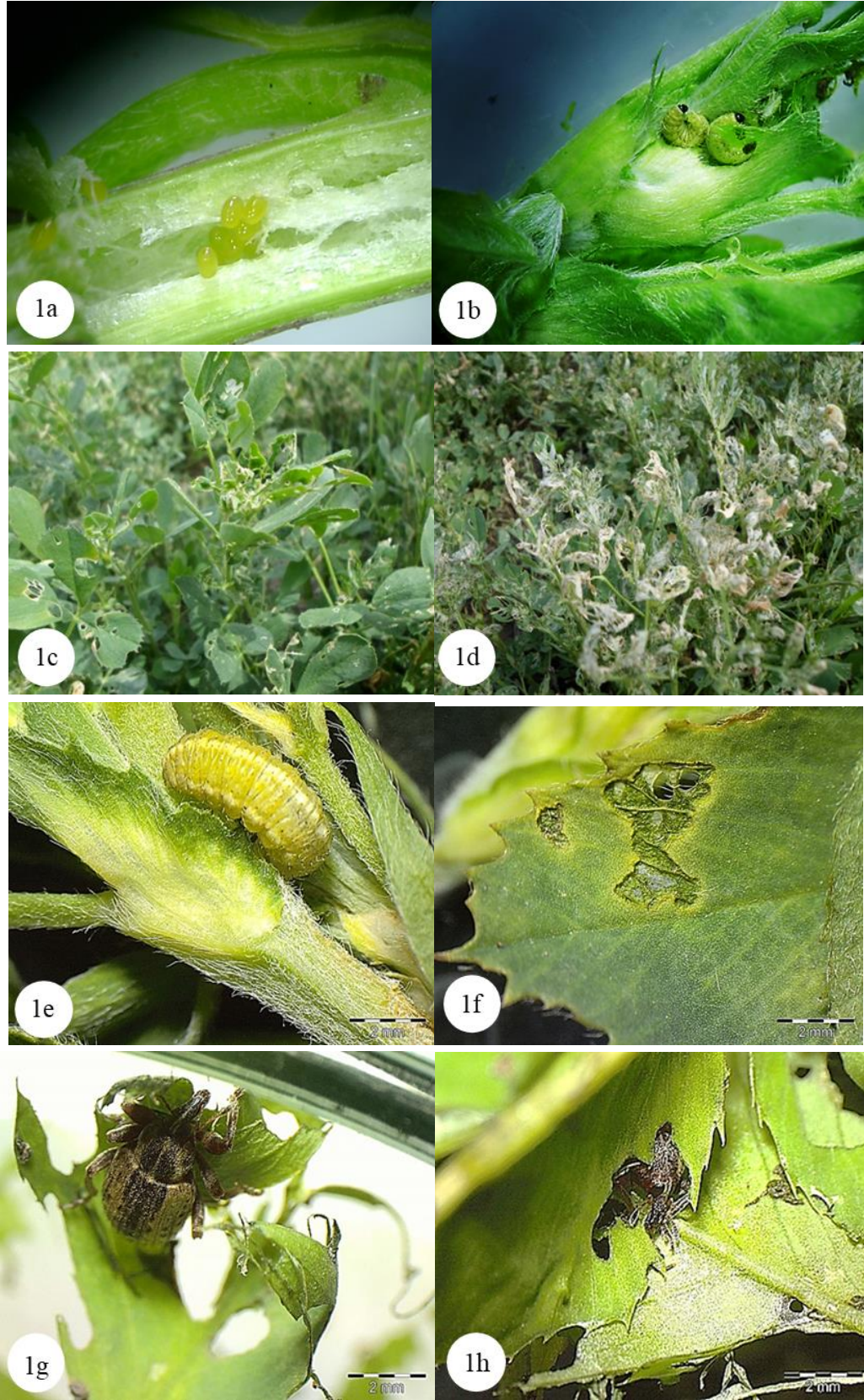
## RESULTS and DISCUSSION

*H. postica* adult females lay their eggs in groups on the stem parts of the clover plant. The eggs are yellowish and quite small (Figure 1a). Figure 1b shows newly hatched greenish and C-shaped larvae. The newly hatched larvae move to the terminal leaves and feed, causing small holes as they feed. Larvae and adults cause the plant to dry out (Figures 1c-h).

### Larvae

Larvae when they first hatch, their heads are dark black and their bodies are off-white. In the second period of the larvae, the body color is light yellow-greenish. Larvae (L); 1st (L1) and 2nd (L2) stages are similar to each other. 3rd (L3) period. In larvae, the body is greenish. The 4th (L4) instar is also light green. From the third instar onwards, the dorsal and lateral parts of the larvae are white lines (Anonim, 2008). The fourth instar (mature) larva is 8 mm long the body is greenish, with a distinct white line running along the dorsal area present. Larvae go through 4 stages (Gözüaçık, 2022).



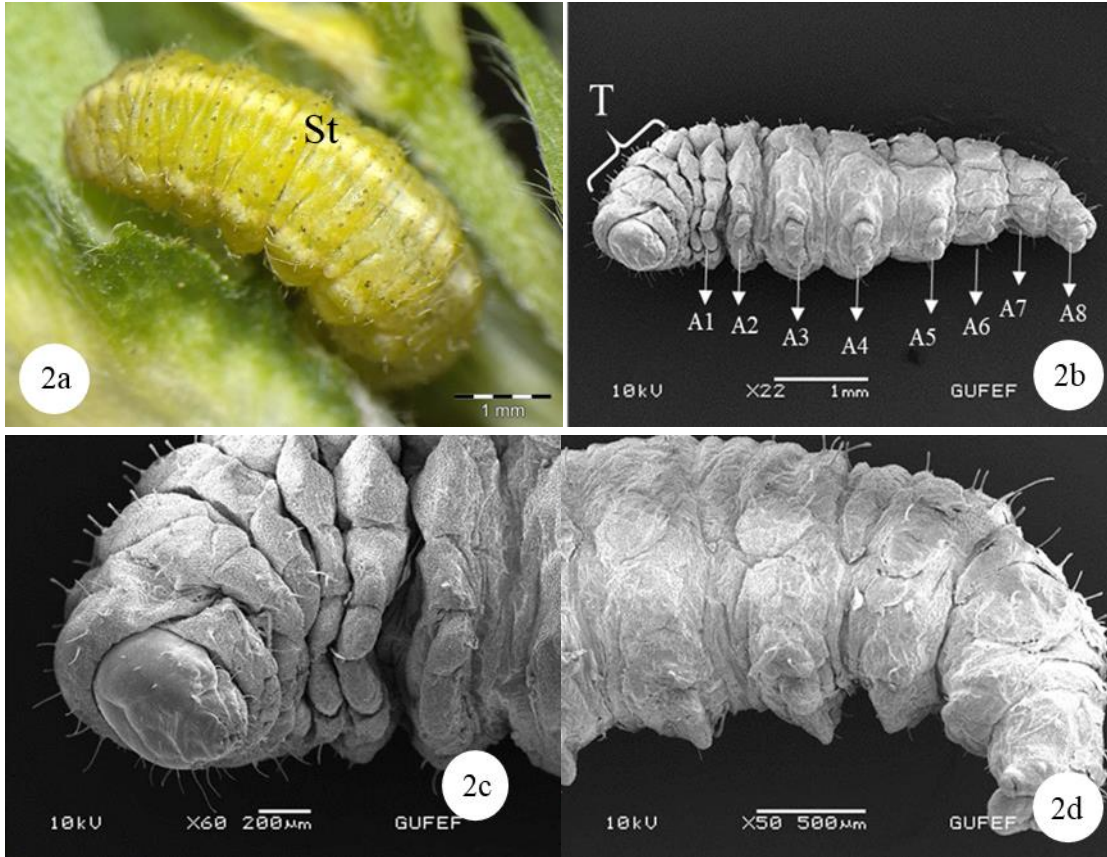


Şekil 1. (a).Yonca zararlısının yumurta kümesi. (b). Yonca zararlısının ikinci dönem larvaları. (c-f). Larvaların *Medicago sativa* üzerindeki zararı. (g, h). Yonca zararlısı ergin bireyleri ve *Medicago sativa* üzerindeki zararı.  
Figure 1. (a). Egg mass of Alfalfa weevil. (b). Second instars larvae of Alfalfa weevil. (c-f). Damage of Alfalfa weevil larvae on *Medicago sativa*. (g, h). The adult individual and damage of Alfalfa weevil on *Medicago sativa*.



The head of *H. postica* larvae is oval. 4th instar larvae of *H. postica*, yellowish-greenish in color (Figure 2a). The larvae have a longitudinal white stripe on their back (Figure 2a). The larvae body is segmented (Figures 2b-d) and has setae on it (Figures 2c, d). The body surface is soft and flexible. Larvae move worm-like by contracting and relaxing. The head, thorax, and abdomen gradually become prominent in the developing larvae (Figure 2b). *H. postica*, spiraculum on abdominal segments I–VIII positioned above the dorsopleural lobe (Skuhrovec, 2004). In the transition

from larva to prepupa, the larvae change color from yellowish-greenish to brown (Figure 3a). After this stage, they molt and begin to form the outline of the adult form. At this stage, it has a C-shaped appearance (Figure 3b). Two different setae, long and short, were found on the head of *H. postica* larvae. When SEM photographs are examined, we see the posterolateral and distal setae (Figure 3c). Numerous setae are distinguished on the head and other parts of the body (Figures 3b-f). Mouthparts on the head are distinguishable (Figure 3e).



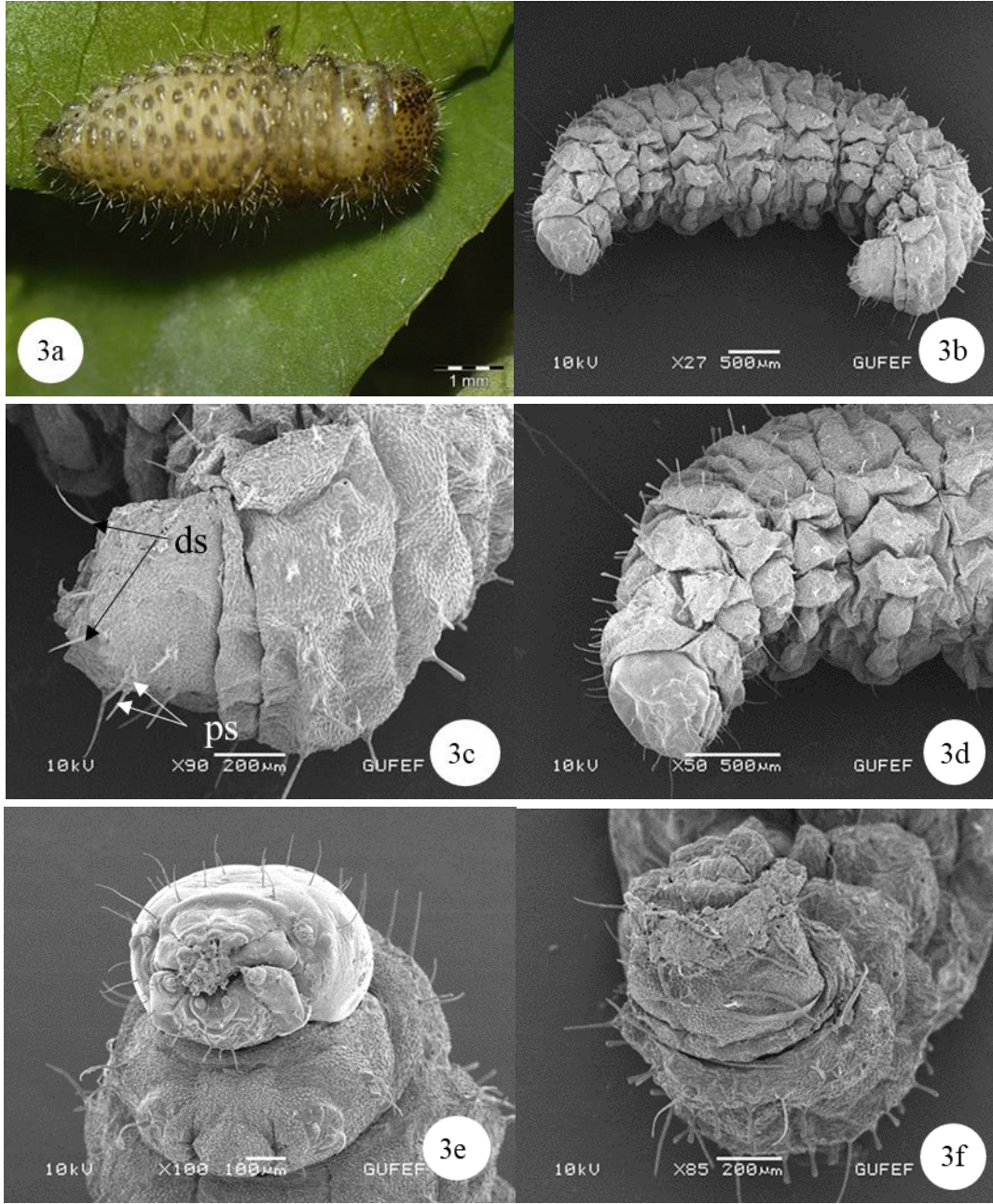
Şekil 2. (a). Sırtta beyaz şeritli (St) ve sarımsı-yeşilimsi renli 4. dönem larva *H. postica*'nın genel görünümü (b). 4. İnstar olgun larvanın lateral görünümünün SEM görüntüleri. T-toraks, A1-A8-abdominal segmentler (c). *H. postica* larvasının baş kısmının SEM görüntüsü. (d) Larvaların son abdominal segmentleri (SEM).  
Figure 2. (a). General view of *H. postica* 4th instar larva yellowish-greenish in color with a white stripe on the back (St) (b). SEM images of lateral view of mature larvae at 4th instar. T-thorax, A1-A8-abdominal segments (c). SEM images of the head of *H. postica* larvae. (d) The last abdominal segments of larvae (SEM).

Larvae of another species, *Lignyodes bischoffi* Blatchley, 1916 (Curculionidae) and *L. enucleator* (Curculionidae), unlike planned *H. postica* larvae, have white to whitish-yellow thorax and abdominal segments. The setae on the body are thin, orange, and distinct (Gosik et al., 2017).

*Metadonus vuillefroyanus* (Curculionidae), as for the larvae, the head changes from dark brown to black. All thorax and abdominal segments are greenish with longitudinal white stripes on both parts of the body. However, it has a thick long yellow stripe on its back and pink and purple stripes with small black stripes

inside. All hairs are thorn-like and are located on distinct black protrusions in skinny white transverse stripes (Skuhrovec & Bogusch, 2016).

While the caudal spines in the larvae of *H. postica* show growth in the first stage towards later stages, there is a decrease in *P. tacubayae* (Curculionidae) (Cervantes-Espinoza, et al., 2023). *Adosomus roridus* (Curculionidae) has a brown or dark brown head when mature larvae are examined. All thoracic and abdominal segments are white, with a long light brown stripe only on the dorsal part of the protonum (Trnka et al., 2015).



Şekil 3. (a-f). Larvadan prepupaya geçiş. (a) Larvadan prepupaya geçişin genel görünümü (SM). (b-f) *H. postica* larvasının abdominal segmentlerin SEM görüntüleri (ds: distal setae, ps: postero-lateral setae).  
Figure 3.(a-f). Transition from larva to prepupa. (a) General view of transition from larva to prepupa (SM). (b-f) SEM images of the abdominal segments of *H. postica* larva (ds: distal setae, ps: postero-lateral setae).

We observed that the abdominal segments in *H. postica* consist of eight segments. Similarly, *H. arator* (Linné, 1758), *H. denominanda* (Capiomont, 1868), *H. jucunda* (Capiomont, 1868), *H. nigrirostris* (Fabricius, 1775), *H. plantaginis* (De Geer, 1775), In *H. suspiciosa* (Herbst, 1795), *H. venusta* (Fabricius, 1781) and *H. viciae* (Gyllenhal, 1813), the abdominal segments were reported to consist of eight segments (Skuhrovec, 2004).

Skuhrovec (2006), stated that *H. postica* larvae have

one seta in the first instar, 3 in the second instar, and 3 setae in the abdominal regions in the third and fourth instars.

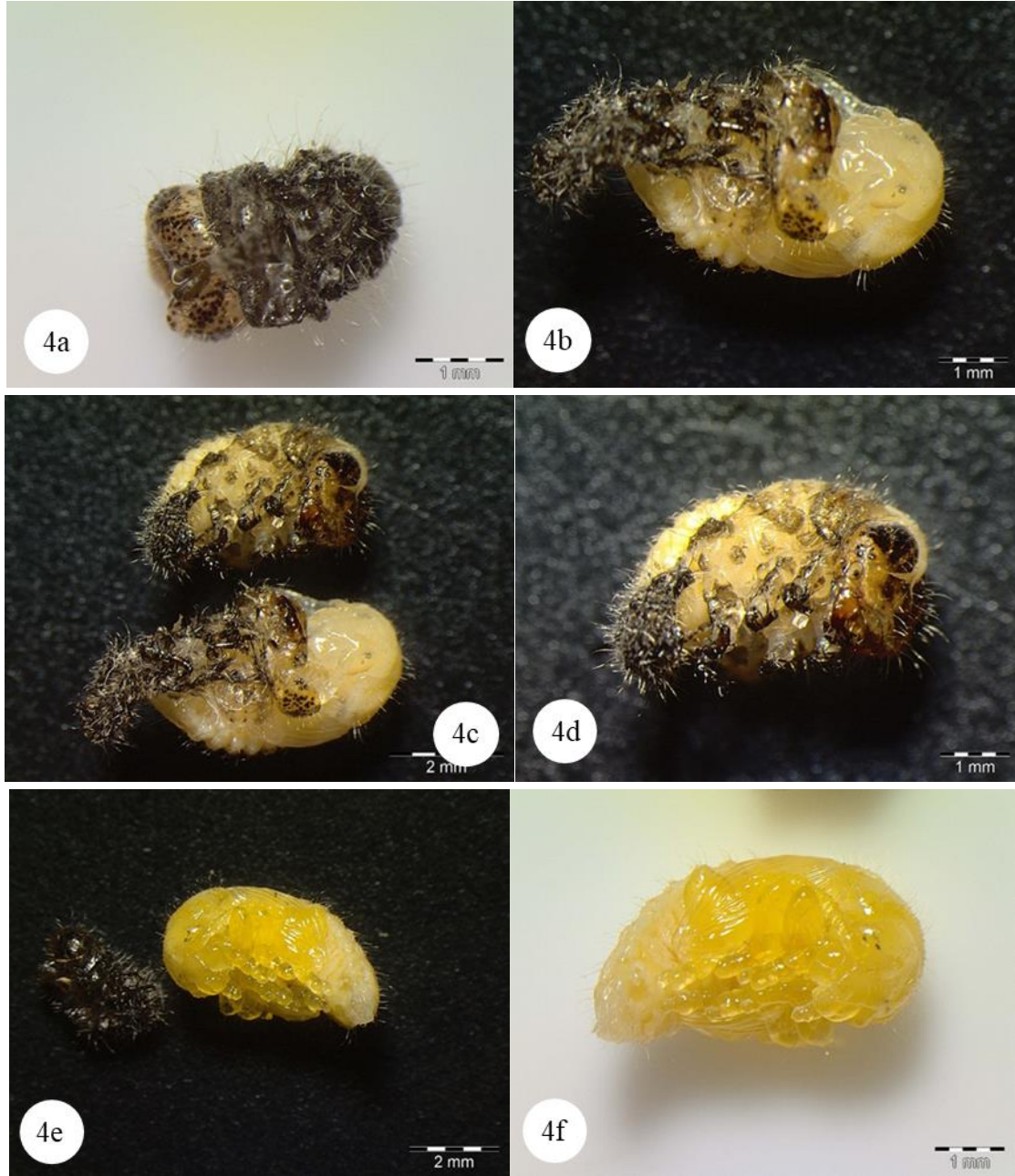
#### Pupae

Figures 4a-d show the skin change of *H. postica* as it transitions from the prepupa stage to the pupa stage. Pupae is the period known as dormancy. *H. postica* pupae are yellow (Figures 4e, f). Pupae of another species, *A. roridus* (Curculionidae), have a different color from whitish to yellow (Trnka et al., 2015). *P.*



*tacubayae* (Curculionidae) pupae (Cervantes-Espinoza et al., 2023) are similar to *H. postica* pupae. The color of the pupae of *M. vuillefroyanus* (Curculionidae) is

yellow on the body and greenish on the abdomen (Skuhrovec, & Bogusch, 2016).



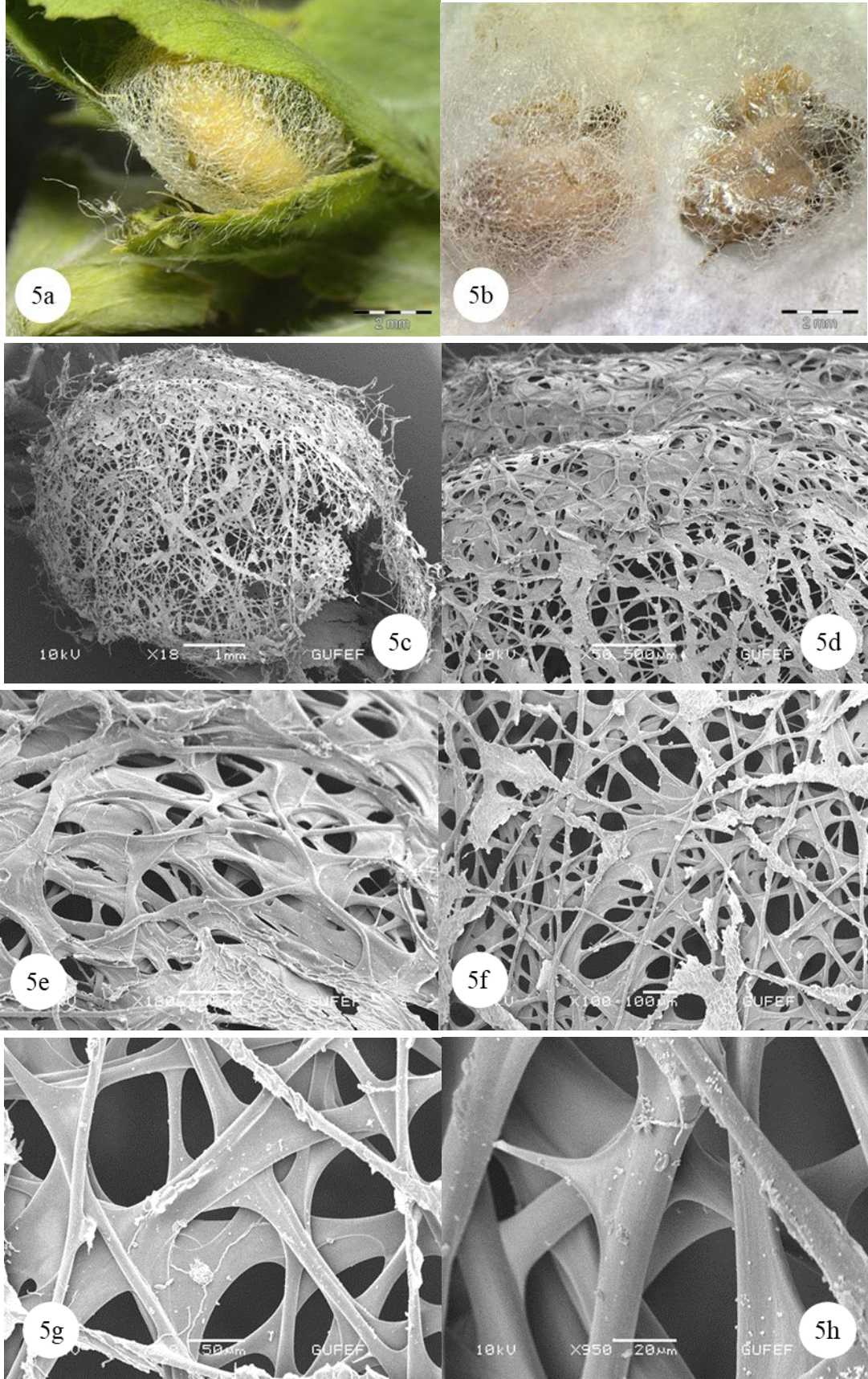
Şekil 4. (a-f). *H. postica*'da prepupadan pupaya geçişin SM fotoğrafları.  
Figure 4. (a-f). SM photographs of the transition from prepupa to pupa in *H. postica*.

### Adult

The cocoon structure is a transparent and reticulated layer and appears as intertwined perforated bands of different thicknesses (Figures 5a-h). The pupal cocoons in *P. schuppeli* (Curculionidae) are spherical, cage-like, and translucent white (Vanin et al., 2012). The stage

of the last larval instar of *H. temperei* (Curculionidae) built a net cocoon, whose colour reached from transparent-whitish to slightly brownish (Germann, 2021). As the prepupa inside the cocoon matures, its color changes from yellow to brown (Figures 5a, b). SEM photographs show the structure of the cocoon fibers woven by the last-stage larvae (Figures 5c-h).





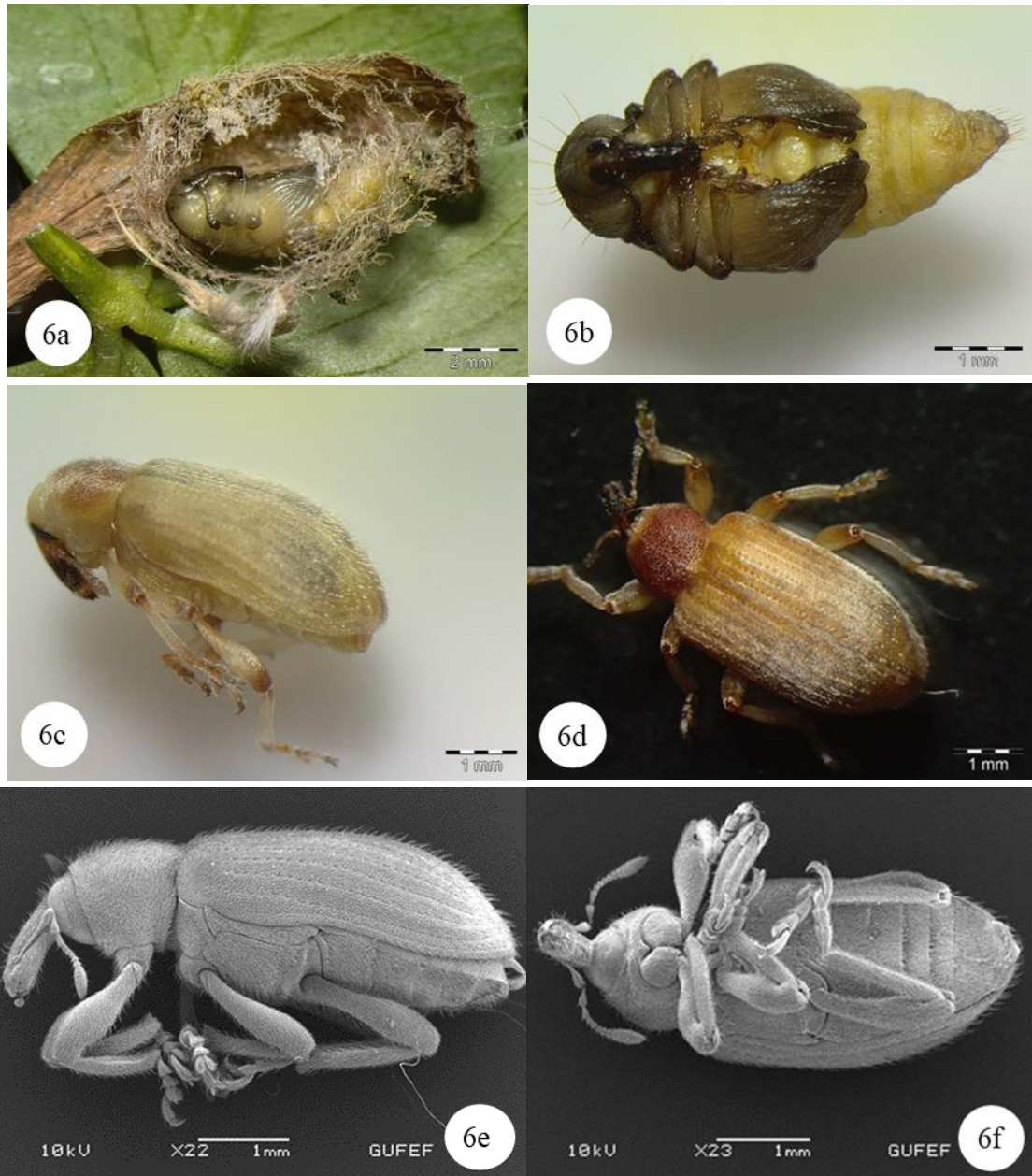
Şekil 5. (a,b). *H. postica*'nın kokon içindeki prepupası (SM). (c). Kokonun genel görünümü (SEM). (d-h). Son dönem larvası tarafından örülen kokon liflerinin mikro yapısı (SEM).  
Figure 5. (a,b). Prepupa of *H. postica* in a cocoon (SM). (c). Genel view of cocoon (SEM). (d-h). A microstructure of the cocoon fibers spun by a last instar larva (SEM).

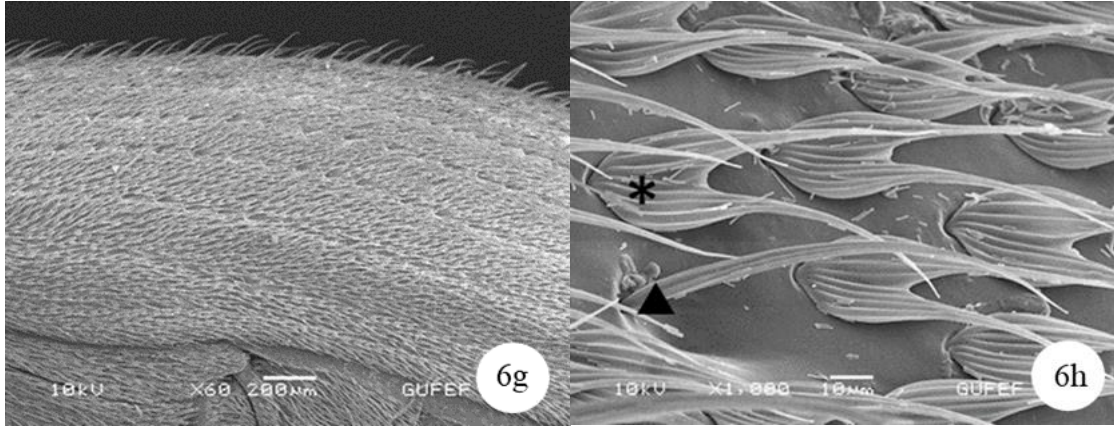


In Figure 6a, a pupa is seen inside the cocoon. The free pupa is seen in *H. postica* (Figure 6b). Figure 6c shows the transition from prepupa to adult. At this stage, white setae are distinguished on the elytra (Figure 6c). The body of *H. postica* adults ultimately acquires a bright brownish color. The instrestium in the dorsal part of the elytra also consists of a series of points (Figure 6d). In SEM photographs of adults of *H. postica*, the lateral and ventral parts can be seen, and their body lines are clear. Adults have a rostrum on their heads that starts from the eyes (Figures 6e, f). *H. postica* rostrum is “short and robust”, but *H. temperei* (Curculionidae) rostrum is “long and slender” (Germann, 2021). The body structure of *H. postica* is covered with scales, and two different scales were found (Figures 6g, h). The first one shows a thick

sword-like structure at the base and becomes thinner towards the tip. The second one resembles a tulip and is forked. Both flake types have parallel lines on their surfaces (Figure 6h).

The shape and patterning of the scales on the elytra varies from species to species. In *Cionus hortulanus* (Curculionidae), the scales are elongated and parallel-sided, the apical part is serrated, the lateral side is smooth, and has large holes on the surface (Erbey, & Candan, 2015). In the *Cionus opens* (Curculionidae), the scales are long and elliptical; the apical part is sharpened; the surface of the scales has two or three longitudinal protrusions; the serrated part of the interstrain on the lateral side near the apical part has a series of clearly erect scales (Erbey, & Candan, 2015).





Şekil 6. (a). Pupanın kokon içindeki genel görünümü. (b). Serbest pupanın genel görünümü (SM). (c). Prepupadan ergine geçiş (SM). Elytra üzerinde beyaz setalar. (d). Gelişimini tamamlamış ergin (SM). (e, f) Yetişkinlerin SEM görüntüleri yan ve ventral wiew. (g, h). Elytrasındaki pulların SEM görüntüleri (\*:lale benzeri pullar, ►:kılıç benzeri pullar).

Figure 6. (a). General view of the pupa inside the cocoon. (b). General view of the free pupa (SM). (c). Transition from prepupa to adult (SM). White setae on elytra. (d). Fully developed adult (SM). (e, f) SEM images lateral and ventral view of adults. (g, h). SEM images of scales in the elytra (\*: tulip-like scales, sword-like scales).

The larva, pupa, and adult forms of this alfalfa pest, *H. postica*, have been described in detail, and the similarities and differences with other species are explained and contribute to taxonomic methods. It also forms the basis for studies on biological control against this species. Chemical residues in the plant, serious damage to non-target organisms, and the risks posed to the environment and human and animal health have very serious consequences. As a result, the pest biology, economic damage threshold natural enemies existence, alternative methods of struggle and all these should be handled within the framework of 'Integrated Struggle' is considered useful to be taken (Gözüaçık, 2022).

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## Contribution Rate Statement Summary of Researchers

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

## Conflict of Interest

The authors have declared no conflict of interest.

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## Çukurova Bölgesi Turunçgil Üretim Alanlarında Citrus Yellow Vein Clearing Virus (CYVCV; *Potexvirus citriflavivenae*)'ünün Araştırılması ve İzolatlar Arası Benzerlik Oranlarının Belirlenmesi

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### ÖZET

Citrus yellow vein clearing virus (CYVCV; *Potexvirus citriflavivenae*) dünyada ilk kez Pakistan'da ardından Hindistan'da tespit edilmiştir. Türkiye'de ise 2000'li yıllarda bu hastalığın varlığı yeni bir turunçgil viral etmeni olarak bildirilmiştir. Bu dönemde virüsün ilk belirlendiği il olan Adana ilinde eradikasyon işlemi gerçekleştirilerek enfekteli ağaçlar kesilmiştir. CYVCV'nin tek sarmal pozitif RNA yapısında olduğu 2012 yılında ortaya konmuştur. CYVCV'nin Çukurova Bölgesi'ndeki durumunu araştırmak, etmenin enfeksiyon durumunu belirlemek ve izolatlar arasındaki farklılığı moleküler olarak saptamak amacıyla bu çalışma yürütülmüştür. Sörvey çalışmaları 2016-2022 yılları arasında Hatay, Adana ve Mersin illerini kapsayacak şekilde güdümlü sörvey çalışması olarak gerçekleştirilmiştir. Çalışma başta limon olmak üzere portakal, greyfurt ve turunç çeşitlerini kapsamıştır. Toplamda 150 turunçgil bahçesi ve 10 farklı fidan üretim alanında CYVCV'nin varlığına bakılmış ve 100 örnek RT-PCR yöntemiyle testlenmiştir. Örneklerin toplanması etmenin karakteristik yaprak belirtileri göz önüne alınarak yapılmıştır. Alınan örneklerden TNA ekstraksiyonu CTAB tampon kullanılarak gerçekleştirilmiştir. RT-PCR çalışmaları sonucunda elde edilen amplifikonlar sekans analizine gönderilmiş, elde edilen baz dizimleri NCBI veri tabanında BLAST metoduyla seçilen kayıtlı CYVCV izolatları ile karşılaştırılmıştır. Toplamda 35 örneğin CYVCV ile enfekteli olduğu belirlenmiştir. Enfekteli olarak belirlenen bu örnekler eradikasyon işlemi uygulanmıştır. Çalışma sonucunda izolatların benzerlik oranlarının yüksek olduğu (%97-99.8) tespit edilmiştir.

### Fitopatoloji

### Araştırma Makalesi

### Makale Tarihçesi

Geliş Tarihi : 12.08.2024

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### Anahtar Kelimeler

CYVCV  
Türkiye  
Turunçgil  
RT-PCR

## Investigating the Citrus Yellow Vein Clearing Virus (CYVCV; *Potexvirus citriflavivenae*) in the Çukurova Region's Citrus Production Areas and Analyzing the Identity Rates Between Isolates

### ABSTRACT

Citrus yellow vein clearing virus (CYVCV; *Potexvirus citriflavivenae*) was first detected in Pakistan and then in India. In Türkiye, this disease was reported as a new citrus viral disease in the 2000s. In Adana province, where the virus was first detected, eradication was carried out and infected trees were cut down. In 2012, the single-stranded positive RNA structure of CYVCV was identified. The purpose of this investigation was to assess the current state of CYVCV in the Çukurova Region, ascertain the agent's current infection status, and identify molecular differences between the isolates. The survey studies were conducted between 2016 and 2022, including the provinces of Hatay, Adana, and Mersin. The study focused on lemon and included grapefruit, orange, and sour orange species. CYVCV was investigated using the RT-PCR method in 150 citrus parcels and 10 different seedling production locations, as well as 100 different samples. Samples were collected by taking into account the characteristic leaf symptoms of the agent. CTAB buffer was used to extract total nucleic acids from the samples. The sequence analysis of the amplicons obtained from RT-PCR analyses was conducted and the resulting base sequences were compared with the

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registered CYVCVs chosen using the BLAST method in the NCBI database. A total of 35 samples were revealed infected with CYVCV. These samples that were found to be contaminated underwent an eradication procedure. As a result of the study, it was determined that there was a high similarity between the isolates (97-99.8%).

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## GİRİŞ

Turunçgil sarı damar açılması hastalığı, virüs tarafından oluşturulan ve ilk olarak 1988 yılında Pakistan'da bildirilen bir hastalıktır (Catara ve ark., 1993). Daha sonra hastalığın varlığı Hindistan, Türkiye ve Çin'de turunçgil yetiştiricilik alanlarında belirlenmiştir (Önelge 2002; Alshami ve ark., 2003; Chen ve ark., 2014). Virüsün varlığı en son İran'da ve Amerika'nın Kaliforniya eyaletinde bildirilmiştir (Hashmian & Aghajanzadeh, 2017; EPPO, 2022). Loconsole ve ark., (2012) yapmış oldukları çalışmada hastalık etmeninin Alphaflexiviridae familyası içinde yer alan Mandarivirüs cinsine ait citrus yellow vein clearing virus (CYVCV; *Potexvirus citriflavivenae*) olduğunu belirlemişlerdir. Etmen pozitif yapıda tek sarmal RNA (+ssRNA) virüsü olup 7.5 kb büyüklüğünde ve 6 adet açık okuma bölgesine (ORFs) sahiptir (Loconsole ve ark., 2012). Etmen tüm turunçgil çeşitlerini enfekte edebilmekte ancak bunların pek çoğu etmeni semptomsuz taşımaktadır. Özellikle limon (*Citrus limon*) ve anaç olarak kullanılan turunç (*C. aurantium*) çeşitleri etmenin en belirgin semptomları olan; bitki yaprak damarlarının sarı renkte açılması, yaprak deformasyonları, yaprak arka yüzeyinde damarlarda su emgisi lekelerinin oluşumunu göstermektedir. Genç yapraklarda oldukça belirgin olan hastalık semptomları olgun yapraklar üzerinde de kalıcı olmakta, yapraklar ışığa tutulduğunda semptomlar belirgin şekilde gözlenmektedir (Önelge, 2002). CYVCV, enfekteli üretim materyali, mekanik olarak ve vektör böceklerle taşınabilmektedir. CYVCV, otsu indikatör bitkilerden fasulye (*Phaseolus vulgaris*), börülce (*Vigna unguiculata*), kazayağı (*Chenopodium quinoa*) bitkilerine mekanik olarak (Catara ve ark., 1993; Önelge ve ark., 2011; Zhou ve ark., 2016), enfekteli limondan fasulye bitkilerine *Aphis craccivora* ve *A. spiraecola* (Önelge, 2002) ile ayrıca enfekteli limondan limona *A. spiraecola* ve *Dialeurodes citri* (Zhang ve ark., 2018) ile taşınabilmektedir. Zhou ve ark., (2016) Çin'de yapmış oldukları çalışmada Türkiye'deki izolatlarla benzer olarak etmenin farklı RNA izolatlarını RT-PCR yöntemi ile incelemişler ve Türkiye izolatı ile %97.2 oranında benzer olduğu

bildirmişlerdir. Çukurova Bölgesi'nde CYVCV tespit edildikten ve etmen 2012 yılında karakterize edildikten sonra bölgede eradikasyon çalışması başlatılmış ve enfekteli bahçelerin kontrollü olarak sökülmesine 2022 yılına kadar gidilmiştir. Ancak 2020-2022 yıllarında özellikle Zagara Bianca limon çeşidi ve farklı mandarin çeşitlerinden gelen örneklerde CYVCV'nin makroskobik semptomları gözlenmiştir. Bu nedenle etmenin bölgedeki durumunu güncellemek amacıyla turunçgil bahçelerinde ve fidanlıklarda sorvey çalışmaları gerçekleştirilerek; hastalığın durumunun belirlenmesi ve etmenin örtü proteini baz alınarak genetik çeşitliliğin araştırılması amaçlanmıştır.

## MATERYAL ve METOD

### Sorvey Çalışması ve Arazi Gözlemleri

Sorvey çalışması Çukurova Bölgesi'nde önemli fidanlık ve turunçgil yetiştiricilik alanlarını kapsayan Hatay, Adana ve Mersin illerinde 2016-2022 yılları arasında yürütülmüştür. Toplamda 150 turunçgil bahçesi ve 10 farklı fidanlıkta bulunan portakal (*C. sinensis*), greyfurt (*C. paradisi*) turunç (*C. aurantium*), limon (*C. limon*) türlerini kapsayacak şekilde gerçekleştirilmiştir (Çizelge 1). Sorvey çalışması güdümlü örnek alımı şeklinde yürütülmüştür (Hewitt & Gifford, 1956; Bovey 1965). Örneklerin toplanmasında CYVCV etmeninin yapraklarda oluşturduğu sarı renkli damar açılması ve yaprak deformasyonları, yaprak arka yüzeyinde damarlarda su emgisi lekelerinin oluşumu esas alınmıştır.

Çizelge 1. Sorvey çalışması yapılan iller ve toplanan örnek sayıları

Table 1. The field survey and number of samples collected.

	Bahçe			Fidanlık		
	Adana	Mersin	Hatay	Adana	Mersin	Hatay
Mandarin	10	-	-	-	-	-
Portakal	10	-	-	-	-	-
Turunç	10	10	10	-	-	-
Greyfurt	10	-	-	-	-	-
Limon	15	5	10	-	5	5
Toplam	55	15	20	-	5	5

## Total Nükleik Asit Ekstraksiyonu (TNA) ve RT-PCR Çalışmaları

CTAB temelli total nükleik asit ekstraksiyonu Murray ve Thompson (1980)'a göre gerçekleştirilmiştir. Elde edilen TNA'lar Çizelge 2'de yer alan CYVCV'nin

spesifik örtü protein primerleri kullanılarak Ters Transkripsiyon Polimeraz Zincir Reaksiyonu (RT-PCR) ile taranmıştır (Loconsole ve ark., 2012). Adana ilinden 55, Mersin ilinden 20 ve Hatay ilinden 25 örnek etmen açısından incelenmiştir.

Çizelge 2. RT-PCR çalışmasında kullanılan primerler  
Table 2. Primers used in the RT-PCR study

Primer	Primer dizilimi (5'-3')	Ürün büyüklüğü (bp)
7081 forward 7560 reverse (Loconsole ve ark., 2012)	5'- ACCTCACGATGGACCACGTT-3' 5'- CAGAAAATGGAAACTGAAAGCCTG -3'	479 bp
1 forward 921 reverse (Loconsole ve ark., 2012)	5'- GAAAAGCAAACAGTAACAAACACACCC -3' 5'- GGGCAAGAGCATTGGGTATCT -3'	921 bp

## Elektroforez Çalışmaları ve Örneklerin Görüntülenmesi

RT-PCR çalışmaları sonucunda elde edilen DNA örnekleri elektroforez cihazında, %1.5'lik agaroz jelde, 110 miliamperde yürütülmüş ve UV ışık altında amplikonların var olup olmadığına bakılmıştır.

## Sekans Analizleri

Agaroz jel görüntüleme sonrası pozitif bulunan izolatlar sekans analizi için hizmet alımına gönderilmiştir. Sekans analizi sonucu elde edilen baz dizilimleri, NCBI veri tabanında BLASTn metoduyla seçilen kayıtlı CYVCV izolatları ile karşılaştırılmıştır. Soy ağacı Mega X programı ve Neighbour Joining yöntemi ile 1000 bootstrap tekrarlı olarak gerçekleştirilmiştir. Sequence Demarcation Tool Version 1.2 (SDTv1.2) yöntemi kullanılarak örtü proteinine ait nükleotid benzerlik oranları belirlenmiştir.

## BULGULAR ve TARTIŞMA

### Sörvey Çalışmaları ve Fidanlık Gözlemleri

CYVCV etmeninin Adana ilinde ilk bildiriminden sonra eradikasyon işlemi gerçekleştirilerek ticari bahçelerin sökülmesine gidilmiş ve Tarım ve Orman Bakanlığı bu etmeni karantinaya tabi etmen olarak bildirmiştir (EPPO, 2022). Bu çalışmada Mersin ve Hatay illerinde yer alan turuncgil fidanlıklarında sörvey çalışması gerçekleştirilerek fidanlıkta bulunan bitkiler CYVCV hastalığının makroskobik belirtilerini açısından değerlendirilmiştir. Hastalığın başlıca karakteristik belirtisi olan yaprak damarlarında sarı renkli açılmaları ve klorotik lekeler gösteren bitkilerin enfekteli olabileceği düşünülerek örnek alınmaya gidilmiştir. CYVCV etmeninin moleküler tanısı yapıldıktan sonra fidanlık sahiplerine durum bildirilmiş ve bu fidanların imha edilmesi gerektiği belirtilmiştir ve enfekteli bitkilerin imhası

gerçekleştirilmiştir. Fidanlıklarda en fazla simptom gözlenen türler limon ve turunc çeşitleri olmuştur. Özellikle Hatay ve Mersin ili fidanlıklarında Kütdiken ve Zagara Bianca limon çeşidinde hastalık etmeninin makroskobik belirtilerini belirgin şekilde gözlenmiştir (Şekil 1). Fidanlıklarda gözlenen başlıca belirtiler daha önce Bozdoğan ve Önelge (2016)'nin bildirdiği belirtilerle benzerdir. Limon yapraklarının kenarlarında defomasyonlar, yaprak damarlarında sarı renkli çizgi formunda renk açılmaları, yaprak yüzeyinde ceplenmeler ve yaprağın arka yüzeyinde damarlarda su emmiş gibi lekelerin oluşumu gözlenen belirtilerdir. Bu belirtiler hastalığın bildirildiği Hindistan ve Pakistan'da belirtilen belirtilerle de benzerdir (Catara ark., 1993; Alshami ve ark., 2003). Ayrıca Çin'de Zhou ve ark., (2017) CYVCV etmeni için benzer belirtiler bildirmişlerdir.

### Turuncgil Bahçesi Gözlemleri

Bu çalışmada Adana, Mersin ve Hatay illerinde toplam 150 turuncgil bahçesi görsel olarak incelenmiştir. CYVCV etmeni ile enfekteli olabilecek 90 örnek toplanmıştır. Limon bahçelerinde de fidanlıklarda gözlenen belirtilere benzer şekilde yapraklarda sarı renkli açılmalar, yaprak defomasyonları, gondol yaprak oluşumları, yaprağın normalden daha küçük gelişmesi ve yaprak arka yüzeyinde su emgisi lekelerin bulunması belirlenen belirtiler olmuştur (Şekil 2).

Sörvey yapılan bu bahçelerin bitişiğinde ve yakınında bulunan bahçeler gözlenerek benzer belirtilerin varlığına bakılmıştır. Bazılarında CYVCV'nin makroskobik belirtilerini gözlenirken bazı bahçelerde yine turuncgil virüs hastalığı olan citrus chlorotic dwarf virus (CCDaV; *Citlodavirus citri*)'ünün makroskobik belirtilerini gözlenmiştir. Bu iki virüs farklı genetik yapıya sahiptir. Ancak bu virüsün oluşturduğu belirtilerin pek çoğu birbirine oldukça benzerdir (Önelge ve ark., 2011).





Şekil 1. a) Sörvey yapılan ve RT-PCR çalışması sonucu CYVCV etmeni ile enfekteli bulunan turunçgil fidanlıđı, b) fidanlıkta bulunan limon yapraklarında deformasyonlar, yaprak arka yüzeyinde damarlarda su emgisi lekelerinin oluşumu, c) yaprak yüzeyinde sarı renk açılmaları.

*Figure 1. a) Citrus nursery that was surveyed and found to be infected with CYVCV as a result of RT-PCR study, b) deformations on lemon leaves in the nursery, formation of water soaked spots on the veins on the back surface of the leaf, c) yellow discoloration on the leaf surface.*



Şekil 2. Sörvey yapılan ve RT-PCR çalışması sonucu CYVCV etmeni ile enfekteli bulunan limon ağacı yapraklarında gözlenen simptomlar, a) yapraklarda sarı renk açılmaları, b) yaprak arka yüzeyinde su emgisi leke oluşumu, c) yaprak deformasyonları, gondol yaprak oluşumu, yaprağın normalden daha küçük gelişmesi.

*Figure 2. RT-PCR investigations revealed symptoms on lemon tree leaves that were checked and confirmed to be CYVCV-infected, a) yellow discoloration on leaves, b) water-soaked spot formation on the back surface of the leaf, c) leaf malformations, smaller-than-normal leaf development, and gondola growth of leaves.*

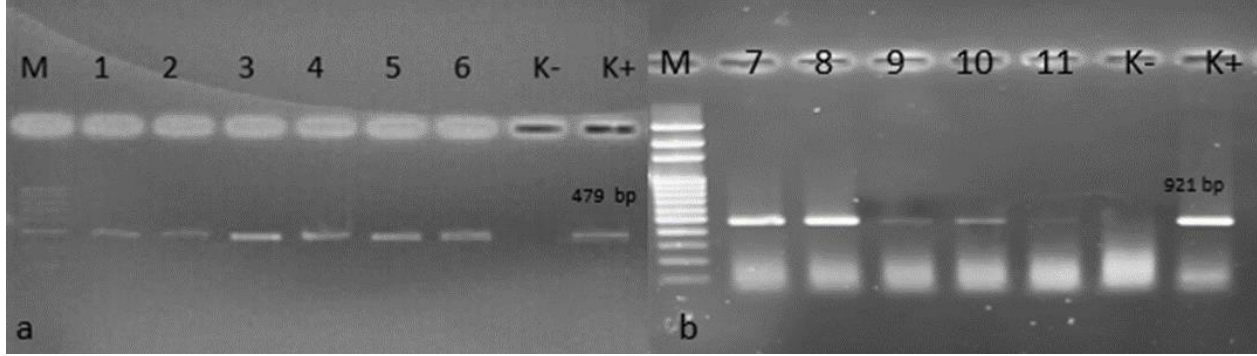
CYVCV etmeni ile enfekteli limon ağaçlarının yakınında yer alan mandarin ve greyfurt ağaçlarından da örneklemeler yapılmıştır. CYVCV etmeni ülkemizde mandarin ve greyfurtlarda simptom oluşturmamakta ancak latent olarak bulunabilmektedir (Önelge, 2002). Çin'de yürütülen bir çalışmada da etmenin portakallarda latent olarak

kaldığı belirtilirken (Zhang ve ark., 2019), Pakistan'da bazı portakal çeşitlerinde etmenin hafif yaprak beneklenmesi simptomu oluşturduğu bildirilmiştir (Catara ve ark., 1993). Ancak bu çalışma kapsamında RT-PCR çalışmalarıyla enfekteli olarak belirlenen portakal ve greyfurt bahçelerinde herhangi bir makroskobik simptoma rastlanmamıştır.

### RT-PCR Çalışmaları

Arazi gözlemleri sonucunda Adana, Mersin ve Hatay illerinden toplanan 100 bitki örneğinden TNA'lar bitkisel dokulardan ayrıştırılmıştır. Elde edilen TNA'lar RT-PCR çalışmalarında kullanılmıştır. Örtü protein bölgesine ait iki farklı primer çiftinin kullanıldığı çalışmada araştırılan 100 örnekten 35

tanenin CYVCV ile enfekteli olduğu belirlenmiştir. Enfekteli bulunan izolatlardan 8 tanesi fidanlık, 27 tanesi ise bahçeden alınan izolatlardır. Adana ilinden toplanan 55 örneğin 20 tanesi 479 nükleotid seviyesinde bant oluşturmuştur. Mersin ilinde 10 örnek, Hatay ilinde ise 5 örnek hastalıkla enfekteli olarak belirlenmiştir (Şekil 3).



Şekil 3. Test edilen örneklerin %1.5 agaroz jel görüntüleri, a) M; 100 bp DNA markör, RT-PCR çalışması sonucu pozitif bulunan ve sekans analizi için seçilen CYVCV izolatları (1-2: Mersin, 3-4: Adana, 5-6: Hatay), K-: Negatif kontrol, K+: Pozitif kontrol, b) M: 1 kb DNA markör, 7-8: Hatay ili Zagara Bianca limon izolatları, 9-10: Mersin ili Kütdiken limonu fidanlık izolatları, 11: Mersin ili Zagara Bianca limonu fidanlık izolatu, K-: Negatif kontrol, K+: Pozitif kontrol.

Figure 3. 1.5% agarose gel pictures of the examined samples. a) M; 100 bp DNA ladder, CYVCV isolates found positive as a result of RT-PCR study and selected for sequence analysis (1-2: Mersin, 3-4: Adana, 5-6: Hatay), K-: Negative control, K+: Positive control, b) M: 1 kb DNA ladder, 7-8: Hatay province Zagara Bianca lemon isolates, 9-10: Mersin province Kütdiken lemon nursery isolates, 11: Mersin province Zagara Bianca lemon nursery isolate, K-: Negative control, K+: Positive control.

RT-PCR çalışmaları sonucunda makroskobik simptom göstermeyen portakal ve greyfurt ağaçlarında da virüs etmeninin latent olarak kalabildiği görülmüştür. Bu durum enfekteli ağaçların inokulum kaynağı olabileceğini ve özellikle vektör böceklerle taşınan bir etmen olan CYVCV etmeninin yayılmasında etkili olabileceği sonucunu ortaya koymaktadır. Nitekim Çukurova Bölgesi'nde 1980'li yıllarda ortaya çıkan CCDaV etmenin vektörü olan *Parabemisia myricae* on yıl gibi kısa bir sürede tüm Mersin ve Adana ili yetiştiricilik alanlarına yayılmıştır (Korkmaz ve ark., 1994; Bozan & Önelge 2018). CYVCV etmeninin taşıyıcı vektörlerinden olan *A. gossypii* ve *A. craccivora* bölgemizde oldukça yaygın olarak bulunmaktadır. Bu durum etmenin yayılmasında önemli bir rol oynamakta ve eradikasyon işlemlerine rağmen etmenin varlığının tehlikesini göstermektedir. Her üç ilde de enfekteli bulunan limon çeşidi Zagara Bianca limonu olmuştur. Bu çeşit hem arazide hem de fidanlıkta enfekteli olarak belirlenmiş ve hemen imhası gerçekleştirilmiştir. Enfekteli olarak belirlenen 35 izolattan 6 tanesi 479 bp'da amplifikon oluşturan primer çifti kullanılarak RT-PCR yöntemiyle çoğaltılarak sekans analizine gönderilmiştir.

### Sekans Analizleri

Sekans sonuçları elde edilip baz dizilimleri BLAST analizi ile National Center for Biotechnology

Information (NCBI) veri tabanında kayıtlı izolatlarla karşılaştırılmıştır. Bu çalışmada elde edilen örtü proteinine ait nükleotid dizilimleri Çizelge 3'de belirtilen farklı CYVCV etmenleri ile karşılaştırıldığında %97'nin üzerinde bir oranla benzerlik gösterdiği belirlenmiştir.

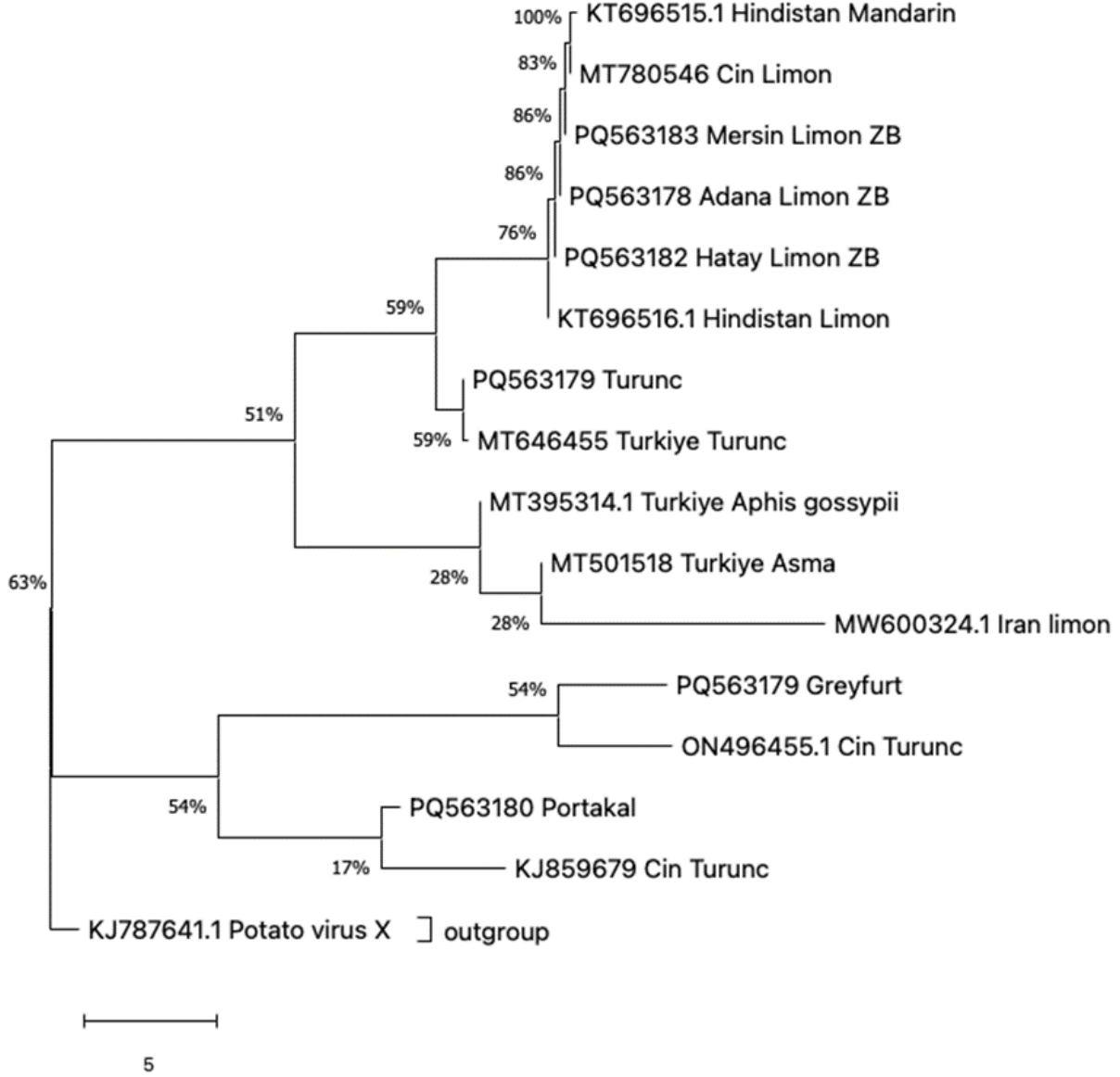
Neighbor-joining metodu ile 1000 tekrarlı bootstrap değeri uygulanarak oluşturulan soy ağacında Adana, Mersin ve Hatay illerinden elde edilen Zagara Bianca limon çeşidine ait örneklerin oldukça birbirine benzer olduğu, aynı kümede Hindistan'dan izole edilen CYVCV etmenine ait limon ve mandarin izolatlarıyla bir arada bulunduğu ortaya çıkmıştır. Adana ilinden alınan turunc izolatı NCBI verilerinde kayıtlı olan turunc izolatı (MT646455) ile aynı kümede yer almıştır. Bu çalışmada greyfurt ve portakaldan elde edilen CYVCV izolatları soy ağacında Çin'in iki farklı turunc izolatıyla aynı küme içinde gruplanmıştır (Şekil 4).

Sequence Demarcation Tool Version 1.2 (SDTv1.2) yöntemi kullanılarak, bu çalışmada elde edilen izolatlar ile NCBI'dan seçilen CYVCV izolatları arasındaki örtü proteinine ait nükleotid benzerliği yaklaşık %97 üzerinde olduğu belirlenmiştir (Şekil 5). Genel olarak değerlendirdiğimizde Hindistan, Pakistan ve sonrasında ülkemizde belirlenen CYVCV etmeni enfekteli aşı gözü yanında vektör olarak afidlerle (*A. craccivora* ve *A. Spiraecola*) ve beyaz



sinikle (*D. citri*) taşınabilen turunçgil ve yabancı otları enfekte edebilen bir hastalık etmenidir (Zhou ve ark.,

2017; Önelge ve ark., 2016).



Şekil 4. CYVCV izolatlarının genom nükleotid dizilerinin MEGAX programında Neighbor-joining metodu ile 1000 tekrarlı bootstrap değeri uygulanarak oluşturulan filogenetik ağaç.

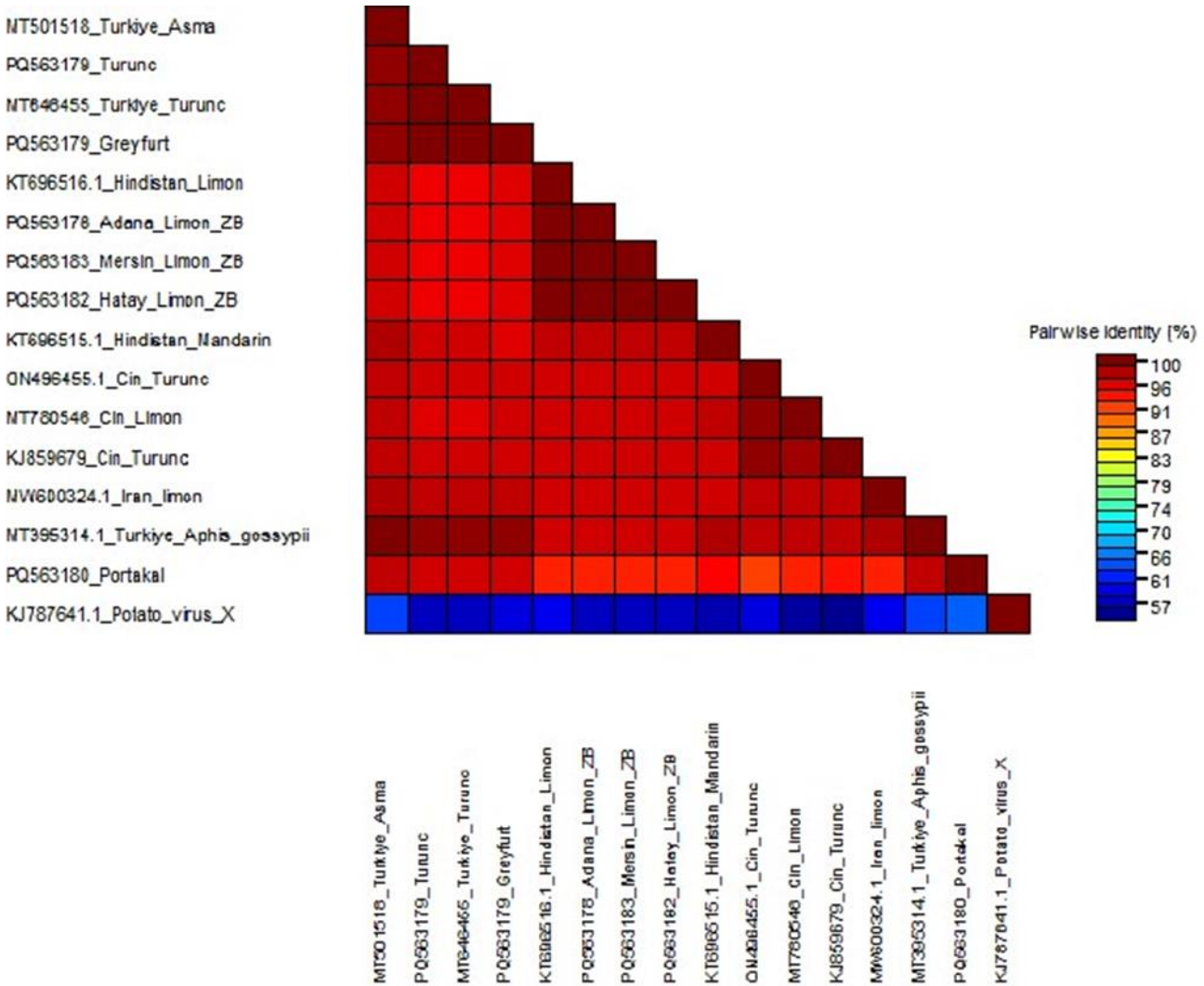
Figure 4. Phylogenetic tree constructed from genome nucleotide sequences of CYVCV isolates using the Neighbor-joining method using MEGAX. Bootstrap values for 1,000 replicates are indicated at the main branches.

Bu çalışmada ele alınan izolatlar farklı turunçgil türlerine ait olup dört farklı ülkeyi kapsamaktadır. Türkiye’de CYVCV vektörü olarak afidlerden elde edilen ve NCBI’ya kaydedilen MT395314 no’lu izolat İran limon izolatıyla aynı kümede yer alıp %97 benzerlik göstermiştir (Hashmian ve ark., 2017). Tüm izolatların birbiri ile olan benzerlikleri yaklaşık %97’nin üzerindedir. Elde edilen bu sonuç etmenin araştırılan kısmi gen bölgesi bakımından çok büyük bir farklılık göstermediğini ortaya koymaktadır. Benzer şekilde Zhou ve ark., (2017)’ları CYVCV

etmeninin sekans analizlerini değerlendirdikleri bir çalışmada kendi izolatları ve Adana ilinden elde edilen izolatın (JX040635.1) aynı kümede yer aldığı belirtilmiştir ve %97 oranında birbiri ile benzer olduğunu bildirmişlerdir (Zou ve ark., 2017). Bu çalışmada da Çin izolatı (ON496455) Adana ilinden elde edilen ve nükleotid dizilimi belirlenen greyfurt izolatıyla %98 benzerlik göstermiştir. Bu sonuçlarda CYVCV etmeninin örtü protein açısından çalışılan bölgede büyük farklılıklar göstermediğini ortaya koymaktadır.

Çizelge 3. Çalışmada kullanılan NCBI'da kayıtlı farklı CYVCV ve dış grup olarak seçilen izolatlar ve erişim numaraları.  
Table 3. Different CYVCV isolates and outgroup isolate accession numbers registered in NCBI in this study.

NCBI Erişim Numarası	İzolat
KT696516.1	Hindistan (limon)
MW600324.1	İran (limon)
MT780546.1	Çin (limon)
MT646455.1	Türkiye (turunç)
ON496455.1	Çin (turunç)
KJ859679.1	Çin (turunç)
KT696515.1	Hindistan (mandarin)
MT501518.1	Türkiye (asma)
MT395314.1	Türkiye ( <i>Aphis gossypii</i> )
KJ787641.1	Potato virus X (dış grup)
PQ563183	Mersin limon ZB (bu çalışma)
PQ563182	Hatay limon ZB (bu çalışma)
PQ563178	Adana limon ZB (bu çalışma)
PQ563179	Adana turunç (bu çalışma)
PQ563179	Adana greyfurt (bu çalışma)
PQ563180	Adana portakal (bu çalışma)



Şekil 5. Sequence Demarcation Tool Version 1.2 (SDTv1.2) yöntemi kullanılarak, bu çalışmada elde edilen izolatlar ile NCBI'dan seçilen CYVCV izolatları arasındaki örtü protein gen bölgesine ait nükleotid dizilim benzerlikleri.  
Figure 5. Nucleotide sequence similarities of the coat protein gene region between the isolates obtained in this study and CYVCV isolates selected from NCBI using the Sequence Demarcation Tool Version 1.2 (SDTv1.2) method.

## SONUÇ ve ÖNERİLER

CYVCV ülkemizde yaklaşık 20 yıl önce bildirilmiş ve hastalıkla enfekteli ağaçlara eradikasyon işlemleri gerçekleştirilmiştir. Ancak günümüzde çok yoğun olmasa da bazı turuncgil yetiştiricilik alanlarında ve fidanlıklarda hastalığın varlığı gözlenmektedir. Yürütülen bu çalışma sonucunda etmenin limon, turunc ve latent olarak portakal ve greycfurt çeşitlerinde varlığı belirlenmiştir. CYVCV'nin örtü proteinine ait nükleotid dizilimleri ve NCBI kayıtlarında yer alan diğer ülkelere ait örtü protein dizilimleri doğrultusunda yürütülen SDT 1.2 analiz sonucunda etmenin kendi arasında ve diğer ülkelere bildirilen izolatlarla nükleotid benzerliğinin %97 ve üzerinde olduğu ve yüksek bir benzerlik oranına sahip olduğu belirlenmiştir. Virüs hastalıklarında erken tanılama oldukça önemlidir. Özellikle eradikasyon programına alınmış etmenlerin rutin sorveylerinin gerçekleştirilmesi, RT-PCR gibi hızlı tanılama yöntemlerinin kullanılması ve saptanan enfekteli örneklerin hızla eradike edilmesi etmenlerin yayılmasını önlemede başarı sağlamaktadır. Ayrıca CYVCV gibi turuncgillerde vektör ile taşınan virüsler açısından vektör mücadelesi yanında mekanik de taşınabilen bu gibi etmenlerde alet ve ekipmanların sterilizasyonu da oldukça önemlidir. Bu çalışma 2016-2022 yılları arasında toplanan izolatlarla gerçekleştirilmiştir. Örnek alınan ticari bahçe ve fidanlıklarda RT-PCR sonucunda enfekteli bulunan ağaçlar ve fidanlar eradike edilmiştir. Belirli aralıklarla fidanlık ve bahçe gözlemleri sonucunda enfekteli olabilecek bitki materyalleri incelenmekte Tarım ve Orman Bakanlığı'nda konu ile ilgili birimler bilgilendirilerek, eradikasyon işlemleri rutin olarak sağlanmaktadır. Sağlıklı ve sertifikalı üretim materyali ile yeni turuncgil bahçelerinin tesis edilmesi ve karantina önlemlerinin yeterince alınması vektör ile taşınan bu etmenin epidemisi açısından oldukça 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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## Tokat Kazova'da Sürdürülebilir Üretim İçin Toprak İşleme ve Doğrudan Ekim Sistemlerinin Ekim Kalitesi Yönünden Değerlendirilmesi

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### ÖZET

Geçit iklim kuşağında yer alan Tokat şartlarında yürütülen çalışmada ikinci üründe ekim kalitesi belirlenmiştir. Geleneksel toprak işleme sistemi (M1), azaltılmış toprak işleme sistemi-1 (M2), azaltılmış toprak işleme sistemi-2 (M3) ve doğrudan ekim (M4) sistemleri karşılaştırılmıştır. Ekim kalitesi tarla filiz çıkış derecesi, kabul edilebilir bitki aralığı, ikizlenme oranı, boşluk oranı, ekim indeksi, verim ve ekim derinliğine göre belirlenmiştir. Toprak işleme sistemlerinin önemli bir etkisinin olmadığı tarla filiz çıkış derecesinin en düşük ve en yüksek değerleri sırasıyla M4 (%64.53) ve M1 (%76.53) sistemlerinde elde edilmiştir. Toprak işleme sistemleri kabul edilebilir bitki aralığı ile ikizlenme oranı değerlerini  $P<0.01$  ve boşluk oranı değerlerini  $P<0.05$  seviyesinde istatistiksel olarak önemli bir şekilde etkilemiştir. Kabul edilebilir bitki aralığı, ikizlenme ve boşluk oranları değerleri bakımından M1, M2 ve M3 toprak işleme sistemleri arasında istatistiksel olarak önemli bir farklılık görülmemektedir. Toprak işleme sistemleri ikizlenme oranı bakımından değerlendirildiğinde M1 sisteminin iyi, M3 sisteminin orta ve M2 ile M4 sisteminin yetersiz olduğu görülmüştür. Ekim kalitesinin bir diğer göstergesi olan boşluk oranı değerlerine göre M1 ile M2 sistemlerinin orta ve M3 ile M4 sistemlerinin ise yetersiz olduğu belirlenmiştir. Çalışmanın sonuçları bölgedeki tarımsal üretimin sürdürülebilirliğine önemli katkı sağlayacaktır.

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*Yeşil ot verimi*

## Evaluation of Soil Tillage and Direct Sowing Systems for Sustainable Production in Tokat Kazova in Terms of Sowing Quality

### ABSTRACT

In the study carried out in Tokat conditions, located in the transition climate zone, the sowing quality of the second crop was determined. Conventional tillage system (M1), reduced tillage system-1 (M2), reduced tillage system-2 (M3), and direct seeding (M4) systems were compared. The sowing quality was determined according to the percentage of emerged seedlings, the quality of feed index, multiple index, mass index, planting index, yield, and sowing depth. The lowest and highest values of the percentage of emerged seedlings where soil tillage systems did not have a significant effect, were obtained in the M4 (64.53%) and M1 (76.53%) systems, respectively. Soil tillage systems significantly affected the quality of feed index and multiple index values at  $P<0.01$  and miss index values at  $P<0.05$  levels. There was no statistically significant difference between the M1, M2, and M3 tillage systems regarding the miss index, multiple index, and quality of feed index values. When soil tillage systems were evaluated in terms of multiple index, it was observed that the M1 system was good, the M3 system was medium, and the M2 and M4 systems were inadequate. According to the miss index values, which is another indicator of sowing quality, it was determined that M1 and M2 systems were medium and M3 and M4 systems were inadequate. The study's results will significantly contribute to the sustainability of agricultural production in the region.

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## INTRODUCTION

Sowing is placing and closing the seeds in the seedbed at a certain depth to the requirements of the seed with a proper distribution in the horizontal and vertical plane (Karayel and Özmerzi, 2005). The main purpose of the precision planter is to plant the seeds in a way that provides the most suitable environment for their homogeneous emergence. Proper placement of the seed is essential to provide sufficient moisture, which is one of the most important criteria for germination and improving conditions that affect germination (Grassbaugh and Bennett, 1998). The benefits of sowing maize seeds with precision planter seed savings include fewer working hours, sowing depth, homogeneous row spacing, and, as a result, a more consistent yield (Turan et al., 2014). The planting process should result in the expected seed distribution and homogeneous emergence in the unit area. The most common methods used to assess planter performance include uniformity of plant distribution and emergence rate (Staggenborg et al., 2004). Precision maize sowing is aimed to achieve high efficiency by ensuring a certain plant output per unit area. For this reason, uniform seed sowing should be done to reduce the competition of plants for soil moisture and nutrients (Yazgi, 2016).

The most critical factor in obtaining maximum efficiency from the unit area is precision planting technology. By distributing the seeds evenly in the seedbed, the competition of the plants with each other for water, light, nutrients, and air is reduced, and the yield obtained from the unit area is increased (Liu et al., 2012; Wang et al., 2012). Sowing maize seeds at irregular intervals reduces the yield by 5% to 10% (Searle et al., 2008). In 2023, approximately 524860.9 hectares of silage maize was planted in Turkey. The production amount is 28653531 tons. The yield is 54670 kg/ha (TÜİK, 2023). Using the 2023 average sales price of 0.15 US Dollars per kilogram (TOBB, 2023), a 10% decrease in yield means 829.57 US Dollars per hectare and a 434824283.56 US Dollars income decrease in the production amount in Turkey. Doerge et al. (2002) stated that the yield could increase by 84 kg ha<sup>-1</sup> with every centimeter decrease in the standard deviation of the row distance. Nielsen (2001) found that when the standard deviation was greater than 5 cm, corn grain yield decreased by an average of 62 kg ha<sup>-1</sup> for every cm increase in the standard deviation of plant spacing.

Precision Planting of the United States developed the 20/20 SeedSense monitoring system using WaveVision

sensors (Anonymous, 2024a). The Sistema Full Semina precision planting system, developed by MC in Italy, can electronically monitor the sowing of medium-sized and large seeds (Anonymous, 2024b). Özgöz et al. (2020) used the statistical process control approach when determining sowing quality, and Dursun and Dursun (2000) used the sticky bant system made in the laboratory environment to assess the uniformity of distribution on the row in sowing machines. Mean and standard deviation of seed or plant row spacing (Parish et al., 1991; Hollowell, 1992), miss index, multiple index (Brooks and Church, 1987), and coefficient of variation (Jasa and Dickey, 1982; Hofman, 1988) are used to determine the performance of the planter (Singh et al., 2005; Kuş, 2014).

The general objective of this study was to evaluate the sowing quality depending on the seedbed characteristics created by different soil tillage systems compared to utilizing the vegetation period effectively in the transition climate zone. In this study, the effect of different tillage and sowing systems on sowing quality was investigated for sustainable production during the silage maize vegetation period (July 2022-October 2022) following a five-year rotation (winter wheat + second crop silage maize, triticale-vetch mixture + second crop silage maize) on a clay loam soil under transition climate zone conditions. For this purpose, the percentage of emerged seedlings, the quality of feed index, multiple index, miss index, planting index, green grass yield, and sowing depth were determined.

## MATERIAL and METHOD

### Experimental Area

The study was conducted as a continuation of the TAGEM project titled "Comparison of Soil Properties, Yield, and Energy Efficiencies in Main and Second Crop Rotations of Different Soil Processing Methods" conducted by Afacan et al. (2023) between 2017 and 2021.

The soil tillage, crop rotations, and cultural operations of the project continued. In this study, primary crop silage triticale-vetch mixture + second crop silage maize rotation was carried out in the same experimental plots.

The study was carried out in Tokat-Kazova, located in the transition climate zone between the Eastern Black Sea and Central Anatolia regions, on the land of the Middle Black Sea Transitional Zone Agricultural Research Institute (Figure 1). The soil of the

experimental area is clay loam and homogeneous. Some chemical properties of the experimental area

soils before the experiment are given in Table 1.



Figure 1. Experimental area

Şekil 1. Çalışma alanı

Table 1. Some chemical properties of the experimental area soils before the experiment

Çizelge 1. Çalışma alanı topraklarının çalışma öncesi bazı kimyasal özellikleri

Soil tillage system	Depth (cm)	Electrical Conductivity (mmhos/cm)	Total Salt (%)	pH	Lime (%)	Available P <sub>2</sub> O <sub>5</sub> (kg ha <sup>-1</sup> )	Available K <sub>2</sub> O (kg ha <sup>-1</sup> )	Organic matter (%)
M1	0-10	1.14	0.04	7.69	10.85	48.8	733.6	2.02
	10-20	1.13	0.04	7.70	10.59	44.4	754.8	2.05
M2	0-10	0.95	0.04	7.87	10.98	52.0	803.6	2.24
	10-20	1.05	0.04	7.77	10.85	40.2	674.8	2.06
M3	0-10	0.85	0.03	7.90	10.59	55.7	832.0	2.32
	10-20	1.04	0.04	7.89	11.11	60.9	684.5	2.06
M4	0-10	0.86	0.04	7.84	10.98	86.4	915.1	2.60
	10-20	0.81	0.03	7.81	10.98	18.7	665.2	2.11

M1: Conventional soil tillage, M2: Reduced tillage-1, M3: Reduced tillage-2, M4: Direct sowing.

### Climate Characteristics

According to the climate data of the province for many years (1929-2023), the maximum temperature is 18.8 °C, the minimum temperature is 7.2 °C, the average temperature is 12.5 °C, the average monthly total rainfall is 435 mm, and the average sunshine duration is 5.8 h (MGM, 2024). Climate data for the silage maize vegetation period (July 2022-October 2022) are shown in Table 2.

### Experimental Design, Soil Tillage Systems, and Cultural Treatments

The experiment was established with three replications according to the randomized block design and the plots were 50 m x 5.6 m in size. The space around the study plots is 2 meters. Some technical details of the soil tillage and sowing machines used in the experiment are given in Table 3. Four different tillage and sowing systems were carried out in the study:



Table 2. Climate data for the silage maize vegetation period (July 2022- October 2022) (MGM, 2022)

Çizelge 2. Silajlık mısır vejetasyon süresine ait iklim verileri (Temmuz 2022- Ekim 2022) (MGM, 2022)

Climate data						
Months	Total precipitation (mm)	Average temperature (°C)	Maximum temperature (°C)	Minimum temperature (°C)	Average relative humidity (%)	
July	0.0	20.7	34.1	8.5	69.5	
August	4.7	24.9	38.2	13.4	68.4	
September	27.4	19.4	38.8	3.5	67.9	
October	35.3	13.3	30.4	-1.1	76.2	

Table 3. Some technical details of the soil tillage and sowing machines used in the experiment (Afacan et al., 2023)

Çizelge 3. Denemede kullanılan toprak işleme ve ekim makinalarının bazı teknik özellikleri (Afacan et al., 2023)

Machines	Number of units	Working width (cm)	Working Depth (cm)	Weight (kg)
Moldboard plough	4	120	20-25	470
Chisel plow	7	210	20-25	400
Vertical axis rotary cultivator	16 blades	200	15-20	900
Disc harrow	18	200	10-15	930
Spring tine cultivator and rolling harrow combination	29	300	10-15	900
Pneumatic precision seeder	4 rows with axe	290	5	845
Pneumatic precision seeder for direct seeding	4 rows with disc	210	5	1000

M1- Conventional soil tillage: Moldboard plough + disc harrow + spring tine cultivator and rolling harrow combination,

M2- Reduced tillage-1: Chisel plow + disc harrow,

M3- Reduced tillage-2: Vertical axis rotary cultivator and

M4- Direct sowing: No tillage is done on the plots in this application. Sowing was performed with a direct seeder.

First crop triticale-vetch mixture + second crop silage maize rotation were carried out. The data on the second crop silage maize rotation was used in this study. Soil tillage was done on July 05, 2022, sowing on July 06, 2022, and harvest on October 27, 2022. Sowing was done at 70 cm between rows and 16 cm in-row at a depth of 5 cm. With the sowing, 200 kg ha<sup>-1</sup> DAP was applied, and on August 10, 2022, Nitropower (33N%) was used at 220 kg ha<sup>-1</sup> (Upper). Before sowing, 3000 ml ha<sup>-1</sup> of total herbicide was applied directly to the sowing plots. When the plant height is 20-25 cm, Ghibli (220 g l<sup>-1</sup> Dicamba + 50 g l<sup>-1</sup> Nicosulfuron) Mospilan 20 SP (20%) Acetamiprid) was administered

### Determination of Sowing Quality

The percentage of emerged seedlings (PES), an important parameter used to evaluate sowing success, was determined according to Equation 1 (Önal, 2006).

$$PES = \left( \frac{N_x - N_0}{N_i} \right) * 100 \quad (1)$$

Where Nx is the total number of all plant spacing at a given row length, No is the total number of intervals

less than 0.5\*Z, Ni is the theoretical total number of plants, and Z is the seed distance within the row.

To determine the seed distribution uniformity in the rows of silage maize, the distances between plants were measured in 3 rows of 10 m in length, randomly chosen over the rows immediately after germination. The in-row plant distribution homogeneity was determined using the values obtained from these measurements (Karayel and Özmerzi, 2005). The multiple index, the miss index, and the quality of the feed index, which indicate the evenness of distribution over the row were determined according to Table 4. The uniformity of plant distribution on the row was evaluated by considering the criteria (Anonymous, 1999) shown in Table 5.

Table 4. Evaluation of plant distribution evenness on rows (Aykas et al., 2013)

Çizelge 4. Sıra üzeri bitki dağılım düzgünlüğünün değerlendirilmesi (Aykas ve ark., 2013)

Plant spacing in rows	Definition
<0.5 Z/PES	Multiple index
(0.5-1.5) Z/PES	Quality of feed index (QFI)
>1.5 Z/PES	Miss index

PES: Percentage of emerged seedling

The planter index developed by Jasa and Dickey (1982) was used to evaluate seed spacing uniformity. The percentage error value was assigned an intermediate value from 0 to 5. 5 indicates less than 10 percent error. More than 50 percent of errors were assigned a value of 0. Both multiple and miss indexes have a value of 0. The index assignment is summarized in Table 6.



Table 5. Evaluation of quality of feed index, multiple index, miss index (Aykas et al., 2013)

Çizelge 5. Kabul edilebilir bitki aralığı, ikizlenme oranları ve boşluk oranlarının değerlendirilmesi (Aykas ve ark., 2013)

QFI (%)	Multiple index (%)	Miss index (%)	Evaluation
>98.6	<0.7	<0.7	Very good
>90.4 ≤98.6	≥0.7 - <4.8	≥0.7 - <4.8	Good
≥82.3 - 90.4	≥4.8 - ≤7.7	≥4.8 - ≤10	Middle
<82.3	>7.7	>10	Insufficient

QFI: Quality of feed index

$$\text{Percent miss from ideal spacing (İe)} = \left( \frac{\text{Actual distance} - \text{Ideal spacing}}{\text{Ideal spacing}} \right) * 100 \quad (2)$$

Table 6. Evaluation of planter index (Jasa and Dickey, 1982)

Çizelge 6. Ekim indeksinin değerlendirilmesi (Jasa ve Dickey, 1982)

Percent error in seed placement	Planting index
0-10	5
10.1-20.0	4
20.1-30.0	3
30.1-40.0	2
40.1-50.0	1
>50	0

The sowing depth was observed with 20 seedlings in each plot, which seedlings were pulled out carefully and their root depths remained in the soil and were close to white measured. According to Önal (2006), the coefficient of variation (%) in the sowing depth distribution was accepted as a reference value as not being more than 20%. During the sowing process, the tractor's forward speed was determined as 6.69km h<sup>-1</sup>.

For silage maize yield, plants in 3 strips of 6 m length, randomly selected from the experimental area, were cut 5 cm above the soil surface, weighed, and calculated as green grass yield per hectare.

### Statistical Analysis

Before starting statistical analyses, a normal distribution test (Shapiro-Wilk) was applied to the data sets. Appropriate transformation procedures were performed to transform non-normally distributed data sets into normal distributions (Webster, 2001). Analysis of variance (ANOVA) and DUNCAN, a multiple comparison test, were used to compare the data obtained for sowing quality and to determine similar tillage systems. Statistical analyses of the relevant parameters were performed using the SPSS 17.0 software (SPSS, 2017).

## RESULTS and DISCUSSION

### Determination of Second Crop Silage Maize Sowing Quality

In the study, descriptive statistics were performed on the data sets obtained by using the measurements made in the field. The maximum, minimum, and mean values, standard deviation, coefficient of variation,

variance, skewness, and kurtosis values, which are accepted as indicators of normal distribution, are given in Table 7.

After germination, the distance between plants in 3 rows of 10 m length randomly determined in each plot was evaluated to determine the homogeneity of seed distribution in each row. The evaluation of in-row plant distribution uniformity, quality of feed index, miss index, multiple index, and were made according to Tables 4 and 5. The percentage of emerged seedling, quality of feed index, multiple index, miss index, planter index, and yield values for silage maize are given in Table 8; percentage of emerged seedling, quality of feed index, miss index, multiple index, planter index and yield values for second crop silage maize are given in Table 9.

The soil tillage systems did not show a statistically significant effect on the percentage of emerged seedlings. The minimum and maximum values of the percentage of emerged seedlings were obtained in the M4 (64.53%) and M1 (76.53%) systems, respectively (Table 8). Korucu and Arslan (2009) received the minimum percentage of emerged seedling rate of 80.3% in direct sowing and the maximum percentage of emerged seedling rate of 88.0% in modified direct sowing (2) for second crop silage maize. As a result of their study, they reported that tillage alone did not affect the degree percentage of emerged seedlings; they pointed out the similar performances of conventional tillage and direct sowing systems. Karaağaç and Barut (2007) determined that the effect of different tillage and sowing methods on emergence percentage in second crop silage maize was statistically significant at P<0.01 level, and the maximum percentage of emerged seedlings was 100% in the conventional tillage system and the minimum percentage of emerged seedling was 72% in direct sowing system. The results found in this study support this study.

Soil tillage systems significantly affected the multiple index and quality of feed index values at p<0.01 and miss index values at P<0.05 levels. There is no statistically significant difference between M1, M2, and M3 tillage systems regarding the multiple index, quality of feed index, and miss index values (Table 8).

Table 7. Descriptive statistics of sowing quality parameters

Çizelge 7. Ekim kalitesi parametrelerine ait tanımlayıcı istatistik bilgiler

STS	SQP	N	Minimum	Maximum	Mean	Standard deviation	Coefficient of variation	Skewness	Kurtosis
M1	PES	6	70.40	81.60	76.53	4.22	5.51	-0.32	-1.17
	QFI	6	78.26	98.04	88.24	6.84	7.75	-0.07	0.10
	MUI	6	0.00	6.52	3.09	2.91	94.17	0.010	-2.25
	MI	6	1.96	15.22	8.67	4.56	52.60	-0.10	0.17
	PI	6	2.38	3.24	2.70	0.37	13.70	0.84	-1.56
GGY	6	59000	112500	96250	21127.59	21.95	-1.40	1.11	
M2	PES	9	51.20	78.40	68.09	9.16	13.45	-0.78	-0.38
	QFI	9	64.71	91.67	80.77	8.50	10.52	-0.50	0.22
	MUI	9	2.08	20.59	9.30	5.54	59.57	0.86	1.13
	MI	9	3.85	15.38	9.92	3.71	37.40	-0.02	-0.35
	PI	9	1.18	3.26	2.34	0.63	26.92	-0.26	0.60
GGY	9	63000	132500	89444.44	19687.31	22.01	1.24	2.61	
M3	PES	9	56.00	81.60	69.16	10.30	14.89	-0.35	-1.75
	QFI	9	69.44	92.31	83.62	7.17	8.57	-0.93	0.54
	MUI	9	1.92	16.67	6.09	4.96	81.44	1.37	1.50
	MI	9	5.77	14.29	10.29	3.28	31.88	-0.11	-1.41
	PI	9	1.29	2.79	2.21	0.54	24.43	-0.54	-1.05
GGY	5	69000	104000	94300	14294.23	15.16	-2.12	4.61	
M4	PES	6	56.00	75.20	64.53	8.51	13.19	0.15	-2.53
	QFI	6	51.43	80.00	63.88	12.22	19.13	0.32	-2.33
	MUI	6	8.00	27.03	17.34	8.28	47.75	0.13	-2.59
	MI	6	10.53	22.86	15.16	4.73	31.20	1.03	-0.22
	PI	6	1.65	2.91	2.29	0.51	22.27	-0.17	-2.00
GGY	9	67000	122000	98111	15551.88	15.85	-0.52	1.56	

STS: Soil tillage systems, M1: Conventional Soil Tillage, M2: Reduced tillage-1, M3: Reduced tillage-2, M4: Direct sowing, SQP: Sowing quality parameters, PES: Percentage of emerged seedling (%), QFI: Quality of feed index (%), MUI: Multiple index (%), MI: Miss index (%), PI: Planter index, GGY: Green grass yield (kg ha<sup>-1</sup>)

Table 8. Percentage of emerged seedling, quality of feed index, miss index, multiple index, planter index, and yield values for second crop silage maize

Çizelge 8. İkinci ürün silajlık mısır için tarla filiz çıkış, kabul edilebilir bitki aralığı, boşluk oranı, ikizlenme oranı, ekim indeksi ve yeşil ot verimi değerleri

STS	Percentage of emerged seedling (%)	Quality of feed index (%)	Multiple index (%)	Miss index (%)	Planter Index	Green grass yield (kg ha <sup>-1</sup> )
M1	76.53±4.22	88.24±6.84a	3.09±2.91b	8.67±4.56b	2.70±0.37	96250±21127.6
M2	68.09±9.16	80.77±8.50a	9.30±5.54b	9.92±3.71b	2.34±0.63	89444±19687.3
M3	69.16±10.30	83.62±7.17a	6.09±4.96b	10.29±3.28b	2.21±0.54	94300±14294.2
M4	64.53±8.51	63.88±12.22b	17.34±8.28a	15.16±4.73a	2.29±0.51	98111±15551.9
<b>F value</b>	2.04 <sup>ns</sup>	9.19 <sup>**</sup>	7.34 <sup>**</sup>	3.17 <sup>*</sup>	1.08 <sup>ns</sup>	0.38 <sup>ns</sup>

STS: Soil tillage systems, M1: Conventional Soil Tillage, M2: Reduced tillage-1, M3: Reduced tillage-2, M4: Direct sowing. <sup>\*\*</sup> Significant at P<0.01 level, <sup>\*</sup> Significant at P<0.05 level, <sup>ns</sup>: Insignificant value. There is no statistical difference between the values shown with the same letter in the columns.

Table 9. Evaluation of quality of feed index, miss index, and multiple index

Çizelge 9. Kabul edilebilir bitki aralığı, ikizlenme ve boşluk oranlarının değerlendirilmesi

STS	Quality of feed index (%)	Multiple index (%)	Miss index (%)
M1	Middle	Good	Middle
M2	Insufficient	Insufficient	Middle
M3	Middle	Middle	Insufficient
M4	Insufficient	Insufficient	Insufficient

STS: Soil tillage systems, M1: Conventional soil tillage, M2: Reduced tillage-1, M3: Reduced tillage-2, M4: Direct sowing.

In respect of the quality of the feed index, M1 and M3 systems are moderate, while M2 and M4 systems are insufficient. When the tillage systems were evaluated

in terms of multiple index, it was observed that the M1 system was good, the M3 system was average, and the M2 and M4 systems were inadequate. According to the

miss index values, another indicator of sowing quality, it was determined that M1 and M2 systems were moderate and M3 and M4 systems were inadequate (Table 9). These results indicate that precautions should be taken regarding the sowing process. Karaağaç and Barut (2007) compared different tillage and sowing methods in second-crop silage maize. They determined the miss index as 2.37%, 5.84%, and 37.73% in conventional, reduced tillage system and direct sowing, respectively, and the multiple index as 1.59%, 5.84%, and 0.00%, and the quality of feed index as 96.04%, 94.17% and 62.07%, respectively. Karayel and Özmerzi (2002), in their study on the effect of tillage systems on the sowing quality (uniformity) of maize, stated that the multiple index and miss index rates were not significantly affected by tillage systems. It was determined that the effect of tillage systems on green grass yield was statistically insignificant (Table 8). Tillage systems were ranked as M4>M1>M3>M2 regarding silage maize yield. Some researchers have stated that equal plant spacing does not increase grain yield (Ehrbach et al., 1972; Muldoon and Daynard, 1981). Liu et al. (2004) determined in their experiments in different locations that maize grain yield was not affected by the variability in plant spacing. The results found by the researchers support this view. Buehring et al. (2002) reported that when the inter-row distance is lower than the appropriate value, the seeds compete with each other; that is,

multiple index occurs, and when the distance is increased, the number of foreign plants increases due to the miss index between the rows. Korucu and Arslan (2009) reported that the yield of second-crop silage maize was the lowest (8590 kg ha<sup>-1</sup>) in direct sowing and the highest (9016 kg ha<sup>-1</sup>) in the conventional tillage system. Karaağaç and Barut (2007) reported that the highest second-crop silage maize green grass yield was obtained in a reduced tillage system, while the minimum yield was obtained in a banded tillage system. These results show the importance of determining the appropriate soil tillage system for sustainable production. Due to the protection and development of natural resources and economic advantages, sufficient effort and sensitivity should be shown to be successful in the application of direct sowing (Gültekin et al., 2017).

The four different systems applied do not affect the planter index values statistically significantly. The minimum and maximum values of the planter index were obtained in the M3 (2.21) and M1 (2.70) systems, respectively (Table 8). In the planter index developed by Jasa and Dickey (1982) to evaluate plant spacing for different tillage systems; they reported that even the best planters make 20% to 30% errors in seed placement and that the effect of the tillage system on the seeding index is quite small. They determined that the average measured planter index was 2.41, and sowing index values ranged from 1.21 to 4.22.

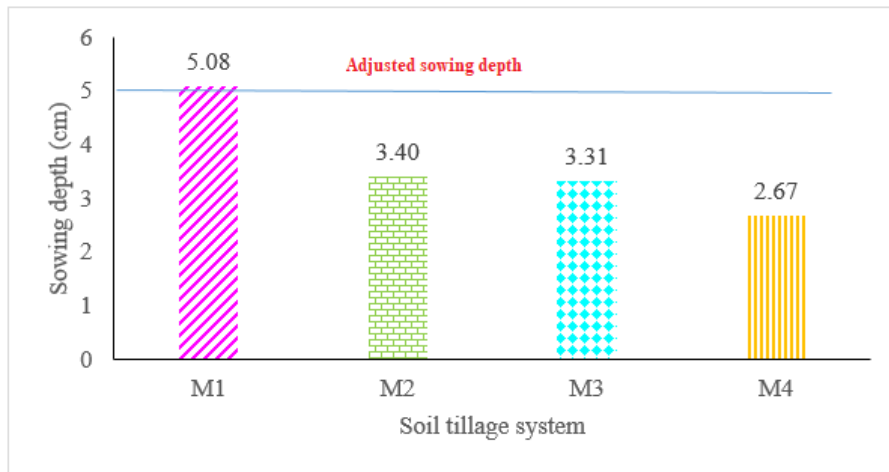


Figure 2. Adjusted and measured average sowing depth values

Şekil 2. Ayarlanan ve ölçülen ortalama ekim derinliği değerleri

(M1: Conventional soil tillage. M2: Reduced tillage-1, M3: Reduced tillage-2, M4: Direct sowing)

The treatments did not affect the measured sowing depth values at a statistically significant level. The measured sowing depth values were 5.08 cm, 3.40 cm, 3.31 cm, and 2.67 cm for M1, M2, M3, and M4 treatments, respectively (Figure 2). When the average sowing depth values measured in the plots are compared with the sowing depth values set on the

seeder before sowing, it is seen that the actual sowing depth is lower. The conventional tillage system was the only system in which the measured value was close to the set value. Da Silva et al. (2004) stated that sowing depth is one of the vital factors affecting vegetative development and emergence homogeneity in maize (*Zea mays* L.). Korucu and Arslan (2009) reported that

soil tillage systems had an effect of  $P < 0.01$  on sowing depth in second-crop silage maize (*Zea mays* L.) and that the best sowing depth was realized in conventional tillage (5.99 cm) and the lowest sowing depth was realized in direct sowing system (2.90 cm). This result supports the sowing depth values determined in this study.

The coefficient of variation values of sowing depth for M1, M2, M3, and M4 treatments were 35.89%, 25.64%, 36.42%, and 34.03%, respectively. According to Önal (2006), the coefficient of variation in the sowing depth distribution was accepted as a reference value as not being more than 20%. According to these reference values, all soil tillage systems are above the reference values. Karayel and Özmerzi (2001) reported that seed distribution in the vertical plane was related to sowing depth.

## CONCLUSION

Depending on the characteristics of the seedbed created with different tillage systems, the systems were also evaluated in terms of sowing quality to use the vegetation period effectively in the second crop, especially in the study area located in the transitional climate zone. In respect of the quality of the feed index, M1 and M3 systems are moderate, while M2 and M4 systems are insufficient. When the tillage systems were evaluated in terms of multiple index, it was observed that the M1 system was good, the M3 system was average, and the M2 and M4 systems were inadequate. According to the miss index values, another indicator of sowing quality, it was determined that M1 and M2 systems were moderate and M3 and M4 systems were inadequate. These results indicate that measures should be taken regarding the sowing process. On the other hand, it was determined that the data on the values of the sowing quality parameters were better in the conventional tillage system compared to the different systems. It was concluded that tillage practices should be continued, and the changes that will occur in the long term should be monitored, along with taking necessary measures to improve the quality of cultivation. It is known that if the plant spacing on the row is smaller than the needs of the plants, the competition between plants for nutrients in the soil, water, and sunlight increases. In addition, this situation can limit the root system of the plants and negatively affect growth. On the other hand, if the plant spacing on the row is wide, it can make land use inefficient and cause soil erosion. It also causes a decrease in yield. Considering all these, attention should be paid to issues such as the homogeneity of the plant spacing on the row (missing index and multiple index), seeder working speed, feed quality index, percentage of emerging seedlings, and slippage. This study will contribute significantly to the sustainability of agricultural production in the region.

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## Contribution Rate Statement Summary of Researchers

The authors declare the contribution of the authors is equal.

## Conflicts of Interest

The authors declare that there are no conflicts of interest.

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## The Effect of Biological Secondary Treated Domestic Wastewater on Agronomic Properties and Element Content of Some Forage Plants

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### ABSTRACT

This study was carried out to determine the effect of advanced biological treated domestic wastewater on the agronomic properties and nutritional content of some forage crops, as a result of the increasing interest in using treated wastewater in agriculture. The study of diluting biologically treated domestic wastewater with varying amounts of pure water 25%, 50%, and 75% pure water, and applied to crested wheatgrass (*Agropyron cristatum* Geartn), orchard grass (*Dactylis glomerata* L.) and alfalfa (*Medicago sativa* L.) at field capacity. The trial plants used were alfalfa, orchard grass, and crested wheatgrass. The application of the diluted wastewater influenced the height of all three plants. After the first cutting, both alfalfa and orchard grass had increased green and dry weights per pot, with the most significant increase observed in alfalfa. The application of wastewater resulted in an increase in nutrient and metal concentrations in all three plants. Additionally, the concentration of selenium decreased in alfalfa and crested wheatgrass. The diluted wastewater did not affect the calcium concentration in alfalfa. However, it had a positive effect on the relationship between orchard grass and all elements, except for selenium which had a negative effect. Moreover, the application of wastewater led to an increase in soil organic matter and electrical conductivity, while decreasing the pH with increasing wastewater ratio. Moreover, the application of wastewater led to an increase in soil organic matter and electrical conductivity, while decreasing the pH with increasing wastewater ratio. The concentration of nutrients and metals was significantly affected by the application of diluted domestic water. This was due to the increased uptake of plant elements, which in turn was associated with responses in soil organic matter and electrical conductivity. It may be advisable to conduct long-term field studies to determine the dilution rate at which the salinity risk threshold can be established.

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## İleri Biyolojik Evsel Atık Suyunun Bazı Yem Bitkilerinin Tarımsal Özellikleri ve Element İçeriklerine Etkisi

### ÖZET

Bu çalışma, arıtılmış atık suların tarımda kullanımına olan ilginin artmasına sonucunda, evsel ileri biyolojik atık suyunun bazı yem bitkilerinin tarımsal ve besin içeriğine etkisini belirlemek amacıyla yürütülmüştür. Biyolojik işlemlerle arıtılan evsel atık su, saf su ile seyreltilerek otlak ayrığı (*Agropyron cristatum* Geartn), domuz ayrığı (*Dactylis glomerata* L.) ve yoncanın (*Medicago sativa* L.) bazı tarımsal özellikleri ve element içerikleri üzerine etkileri araştırılmıştır. Atık su %25, %50 ve %75 oranlarında saf su ile seyreltilerek tarla kapasitesine göre saksılara uygulanmıştır. Seyreltilmiş atık su uygulamasının otlak ayrığı, domuz ayrığı ve yoncanın bitki boyuna etkisi önemli olmuştur. İlk biçmeden itibaren yonca ve domuz ayrığının saksı başına yeşil ve kuru ağırlıkları artmış, en belirgin artış ise yoncada belirlenmiştir. Seyreltik arıtma suyu oranları üç bitkide de besin elementi ve metal

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konsantrasyonlarının artmasına neden olurken, yonca ve otlak ayrığına Se konsantrasyonunun azalmasına neden olmuştur. Her iki buğdaygil türünden farklı olarak atıksu oranı yonca Ca konsantrasyonunu önemli ölçüde etkilememiştir. Seyreltik atık su domuz ayrığının tüm elementlerle ilişkisini önemli ve pozitif olarak etkilerken, Se ile negatif yönde etkilemiştir. Atık su seyreltme oranlarının artmasıyla toprağın organik maddesi ve elektriksel iletkenlik ile toprak pH'sı düşmüştür. Seyreltilmiş evsel su uygulaması, toprağın organik maddesi ve elektriksel iletkenlik tepkileriyle ilişkili olarak bitki elementi alımının artmasına aracılık ederek besin ve metal konsantrasyonlarında önemli rol oynamıştır. Tuzluluk risk eşiğinin belirlenebileceği sulandırma oranı için, uzun süreli arazi çalışmalarının yapılması gerekmektedir.

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## INTRODUCTION

Due to the rapid development and population growth in Turkey, there has been an increase in industrial, urban, and agricultural activities. As a result, pollutants from these activities have contaminated water sources (Khan & Avari, 2014). The country's population exceeded 85 million in 2023 (TUİK, 2023), which presents a significant challenge in meeting the demand for drinking, utility, and agricultural water. According to recent research conducted by Phasinam et al. (2022) and Wu et al. (2022), it has been found that more than 70% of the world's water resources are utilized for irrigation purposes. As agricultural activity continues to grow, globally water resources is also increasing, both on a global scale and within our country. The World Economic Forum has identified the water crisis as one of the five most significant global risks. Currently, it is a fact that a quarter of the world's population faces significant water stress (WRI, 2019). If water consumption continues at the current rate, it is predicted by Du et al. (2022) that two-thirds of the global population will live in regions with severe water stress by 2050. Therefore, the use of treated wastewater for agricultural irrigation has become increasingly important, as highlighted by Perez et al. (2015) and Aşık & Özsoy (2016). Local administrators and policymakers have made efforts to use recycled water for irrigation instead of discharging it into natural receiving environments, as noted by Rahman et al. (2016). This approach can contribute to the health of freshwater ecosystems. According to FAO (2017), the annual amount of N, P, and K used in agriculture globally was 186.67 million tons in 2015, and it is expected to reach 201.66 million tons by the end of 2020. The impact of treated wastewater on plant growth is influenced by the chemical properties of the water and the type of plant (Erdogan et al., 2009; Shahrivar et al., 2019). Additionally, it is worth noting that treated

wastewater can serve as a viable alternative to chemical fertilizers, particularly in terms of macronutrients such as nitrogen, phosphorus, potassium, calcium, magnesium, and sodium, which have both economic and ecological importance (Arvas & Çelebi, 2023). However, it is worth noting that domestic wastewater treatment may result in higher soil salt and Na concentrations compared to other cations, which could potentially have a negative impact on plant growth and yield (Becerra-Castro et al., 2015; Arvas et al., 2022; Ngara et al., 2012; Levy et al., 2013).

To investigate the impact of the environmental pollutants of wastewater treatment facility water mixed with certain proportions of distilled water, as an alternative to chemical fertilizers with high cost, this study examined various dilute wastewater rates (25%, 50%, and 75%) on crested wheatgrass, orchard grass and alfalfa.

## MATERIAL and METHOD

### Trial Plants

Alfalfa (*Medicago sativa* L. 'Bilensoy 80' cultiv.) is a perennial forage legume plant that is resistant to winter, drought, and lodging except in the year of planting, and can be harvested in the first week of June under Anatolian conditions. Having the ability to grow after mowing increases the number of mowings. (TIGEM, 2024)

Orchard grass (*Dactylis glomerata* L. 'Amba' cultiv.) is a long-life, drought-resistant cool seasonal grass forage plant and the best in terms of vegetative and generative shoots length, number of shoots/bush, and foliar area/shoot. (Corches & Moissuc, 2010).

Crested Wheatgrass (*Agropyron cristatum* L. (gaertn) 'Fairway' cultiv.) is a long-lived with a deep root system, drought-resistant cool seasonal grass forage plant. It is used extensively for reclamation,



stabilization and erosion control. introduced/released 1983, from Ankara Turkey. (Great Basin Seed, 2024).

### Biological Treated Domestic Wastewater

The wastewater used in the research was taken from the Biologically Treated Domestic Wastewater

(BTDW) discharge point after removal of carbon, nitrogen, and phosphorus at the Ministry of Environment and Urbanization, Housing Development Administration (TOKİ) housing located at 34° 59' 14.30" E and 42° 53' 265.20" N (Table 1).

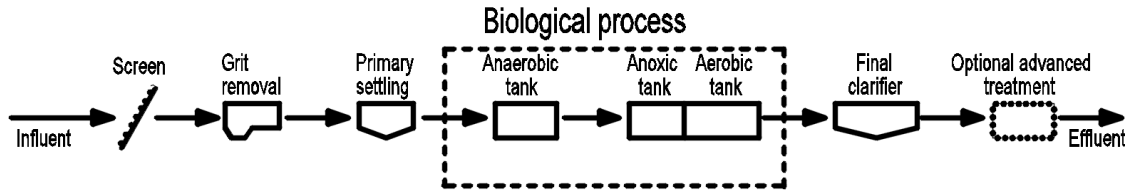


Figure 1. The flow of chart biologically treated process wastewater treatment (Van Metropolitan Municipality, VASKİ, Arvas et al., 2022)

Şekil1. Atıksu arıtma tesisinin biyolojik arıtım işlemi akış şeması (Van Büyük Şehir Belediyesi, VASKİ, Arvas et al., 2022).

Table 1. Some characteristics and limit values for wastewater and soil in the research

Çizelge 1. Çalışmada kullanılan toprak ve atıksuyun bazı özellikler ve sınır değerler

Measurement	Soil	Wastewater	Limit value*
Texture class	Loamy	-	-
Sand (%)	29.20	-	-
Clay (%)	42.80	-	-
Silt (%)	28.0	-	-
pH	8.45	7.81	6-9
EC (dS/m)	0.285	0.654	0.250-3
Organic matter (%)	1.27	-	-
Nitrate (ppm)	-	226.27	5-20
Ca (%)	0.627	0.013	75-200
Mg (%)	0.147	0.006	50-150
Na (%)	0.045	0.013	0.025
Fe (ppm)	102.2	0.004	0.3-1
Cu (ppm)	0.113	4.41	0.2-5
Zn (ppm)	0.213	8.84	2-10
Mn (ppm)	2.471	9.02	0.2-10
Mo (ppm)	nd**	nd**	0.01-0.05
Se (ppm)	nd**	nd**	0.02-0.2
As (ppm)	0.116	0.891	0.1-2
Pb (ppm)	0.026	0.159	5-10

\*Regulation Amending the Regulation on Surface Water Quality Management, April 15, 2015, and Official Gazette No. 29327 of Turkish Republic, converted from the European Union legislations.

\*\* nd: non detected

### Plant Growth Condition

The experiment was carried out in three repetitions in pots with a height of 22.5 cm, a base diameter of 7.5 cm, and a top diameter of 10 cm, according to the randomized plot design. The soil used for the pots was obtained from a field that had previously been used for alfalfa production, at a depth of 0-30 cm. Wastewater (WW) was diluted by 75%, 50%, and 25% with distilled water (DW). 30 kg N ha<sup>-1</sup> and 80 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> were applied to the control pot (100% pure

water) according to the volume calculation (Kacar & Katkat, 2009). Clover, crested wheatgrass, and orchard grass were sowed separately in each pot and thinned to five plants per pot after emergence. The pots were subsequently moved to a climate-controlled room, where the temperature was set to 22/18 °C (day/night), the humidity was maintained at 65%, and a 16/8-hour day/night photoperiod was established. After the wastewater taken from the discharge point was diluted with distilled water, it was applied to the potting soil with 20% field capacity

with 400 ml at 8-day intervals. Since the aim of the study was accumulating nutrients and the vegetative development of the species, the vernalization process has been neglected. The buds formed in the root crown of grasses form more vegetative parts without being vernalized. Plant morphology was affected by vernalization via a decrease in biomass resulting from a reduced tiller number, the time to panicle emergence and the number of leaves on the main stem were reduced by the chilling treatment in the field and greenhouse experiments (Chauvel et al, 2002).

The mowing was started one month after sowing and then repeated at 15-day intervals. Plant height was measured, and then plants were cut to a height of 3 cm to simulate grazing by sheep. The total green weight of the plant was measured in the pot, and then the plant was dried at 65 °C until a fixed weight was reached. The dry weight was calculated as g/pot (Zhao et al., 2020). The trial lasted for four months, during which nutrient and heavy metal concentrations in the potting soil were identified before and after the trial.

#### Physical and chemical analysis of plants and soil

After soil samples were dried in the open air and passed through 2 mm sieves, the soil reaction (McLean., 1983), electrical conductivity (EC) (Richard, 1954), and composition (Bouyoucos, 1951) were identified. The total macro and microelements and metals were extracted from soil and plant samples using a mixture of 67-70% nitric acid (HNO<sub>3</sub>) and 70% HNO<sub>3</sub>/4HClO<sub>4</sub> perchloric acid (HClO<sub>4</sub>) (Khan et al., 1983). Due to the low concentration of molybdenum (Mo) in filters, it was converted to ppm after being detected with ICP-MS (X II SERIES). The concentrations of other elements such as phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), copper (Cu), zinc (Zn), manganese (Mn), selenium (Se), arsenic (As) and lead (Pb) were detected using ICP-OES (ICAP6300 DUO).

#### Wastewater analysis

Wastewater samples were collected separately for each irrigation and stored at a temperature of -18°C for analysis. The pH and electrical conductivity of the wastewater were measured using the methods described by McLean (1983) and Richard (1954), respectively. At the end of the trial, the collected wastewater samples were mixed and diluted 8 times and element content was determined via ICP-MS and ICP-OES. Due to low concentration, Mo was converted to ppm after being detected with ICP-MS (X II SERIES). The concentrations of nitrate (NO<sub>3</sub>), Ca, Mg, sodium (Na), Fe, Cu, Zn, Mn, Se, As, and Pb in solution were determined with ICP-OES (ICAP6300 DUO).

#### Statistical Analysis

Statistical analyses were conducted using IBM SPSS Statistics Version 22.0 software (IBM corp.). The data obtained was presented as mean ± standard deviation and analyzed using analysis of variance (ANOVA). Differences between means were determined using the Duncan Multiple Range Test. Pearson correlation coefficients were used to identify the correlation between wastewater ratio and element contents.

## RESULTS AND DISCUSSION

#### Plant Height

The study found that the impact of dilute wastewater and the number of cuts, as well as their interaction, had a significant effect ( $P < 0.01$ ) on the plant height of alfalfa, crested wheatgrass, and orchard grass. Crested wheatgrass had the highest plant height measured with 50%DW+50%WW irrigation in the first cutting period (22.0 cm). According to the experiment results, orchard grass had the highest height when irrigated with a combination of 50%DW+50%WW, measuring 20.00 cm during the eighth cut. The control group, which was irrigated with 100%DW, had the lowest plant height at the first cut. As for alfalfa, the highest plant height was observed during the 2<sup>nd</sup> and 7<sup>th</sup> cuttings when irrigated with 50%DW+50%WW, measuring 16.67 cm and 15.33 cm, respectively. On the other hand, the lowest plant height was observed when irrigated with 25%DW+75%WW, measuring 5.00 cm at the first cut (Table 3). According to Arvas et al. (2022) and Lv et al. (2023), it was found that high nitrate content in wastewater had a positive effect on the plant height of alfalfa and orchard grass. Additionally, Altın et al. (2009) suggested that the lack of difference in crested wheatgrass for cuts after the 1<sup>st</sup> and 2<sup>nd</sup> cuts was due to slower emergence, emplacement, and development periods after planting.

#### Green Weight

The dilute wastewater significantly increased the green weight of the crested wheatgrass, orchard grass, and alfalfa plants ( $P < 0.01$ ). Additionally, the interaction between the number of cuts and wastewater proportions was also found to be very significant ( $P < 0.01$ ). For the control irrigation (chemical fertilizer + distilled water) and later cuts of crested wheatgrass, the green weight significantly reduced, while the weight increased with increasing wastewater proportions. The highest green weight for crested wheatgrass was observed during the 2<sup>nd</sup> cut with the use of 50% DW+50%WW and 25% DW+75%WW applications (Table 3).

Green weight increased significantly at all irrigation rates and in subsequent cuttings of orchard grass. The highest mean weight was observed for the 25%

DW+75%WW irrigation at the 3rd and 8th cuts (0.130 and 0.126 g/pot, respectively).

The dilute wastewater had a more significant effect on increasing the alfalfa green weight compared to the other two plants. Despite frequent cutting, there was a progressive increase in green weight with progressive cuts, with the highest increase observed for all irrigation applications at the 8th cut (0.100, 0.270, 0.361, and 0.334 g/pot, respectively). This increase is attributed to the variable cations (K, Ca, Mg, Na) found in wastewater which encourage the development of alfalfa (Arvas et al., 2022). In general, frequent mowing and irrigation are the two main factors that cause low yield and withdrawal of alfalfa. Repeated mows once a month led to a stepped increase in the yield of the alfalfa in the planting year, while subsequent cutting in the second year caused a reduction in yield (Erbeyi, 2022). This result supports our findings. In the interaction of mowing numbers and wastewater rates, the highest alfalfa green weight was at the 8th mowing of 50% DW + 50% WW application (0.361 g/pot) and the average highest green weight (0.185 g/pot) was 75% WW + 25%DW application.

Significant weight increases were determined in orchard grass and alfalfa plants with diluted wastewater ratios in subsequent mowing (Table 2). The increase in plant height increased the weight per plant and height is accepted as a measure of yield and weight of pasture and forage (Avcioğlu, 1996). The findings of this study are consistent with previous research on orchard grass. Koç and Gökkuş (1996) found that good care conditions and abundant leaves led to good development. Similarly, Avcı and Doğrusöz (2012) observed that frequent and deep cuts resulted in abundant spikelets and increased weight with height.

### Dry Weight

The effects of wastewater and mowing on the dry weight of crested wheatgrass, orchard grass, and alfalfa plants were significant ( $P < 0.01$ ).

The results show that crested wheatgrass reached its highest dry weight during the 2nd cut when irrigated the 50% DW+50%WW irrigation (0.013 g/pot). Conversely, the lowest mean weight was observed for the control group (100%DW) and 25% WW+75%DW irrigations. For orchard grass, all cuts had increases in dry weights, with the highest dry weight observed during the 4th and 8th mows when irrigated with 25%DW+75%WW irrigation (0.043 and 0.042 g/pot, respectively). The lowest weight (0.05 g/pot) was obtained for the 1st cut with 75%DW+25%WW irrigation. The effect of irrigation on the dry weight of orchard grass was significant, with the highest average dry weight (0.028 g/pot) obtained with 25%DW+75%WW irrigation.

The effect on alfalfa was more pronounced compared to the other two grass plants, with all irrigations having weight increases in the 8th mows compared to previous mows. As the number of mowing increased in the alfalfa, more Duncan groupings formed. The highest dry weight was obtained from the 8th mow with 50%DW+50%WW irrigation (0.120 g/pot), with the lowest dry weight at the 1st mow with 25%WW+75%DW irrigation. Municipal wastewater was reported to increase dry matter significantly for alfalfa, corn, elephant grass, and oxtail millet (Aghtape et al., 2011; Matheyarasu et al., 2017).

### Nutrients and Heavy Metal Content of Species

The concentrations of nutrients and metals examined were significant in terms of plants and irrigation water ratios ( $P < 0.01$ ). The highest P, Fe, Cu, Mn, and Pb concentrations in alfalfa were obtained with 25%DW+75%WW irrigation, and the highest Mg, Zn, Mo, and As concentrations were obtained with 50%DW+50%WW and 25%DW+75%WW applications, respectively (Table 3). However, the highest Se concentrations were determined in the control (100%DW+chemical NP). The lowest P, Mg, Fe, Mn, and As concentrations were obtained with control and 75%DW+25%WW irrigations. The lowest Zn, Cu, Mo, and Pb were obtained with the control irrigation. Diluted wastewater ratios did not affect the Ca concentration in alfalfa, but numerically.

For crested wheatgrass, the highest P, Mg, Cu, Zn, Cu, and Pb concentrations were identified with 25%DW+75%WW irrigation. There were no differences between 50%DW+50%WW and 25%DW+75%WW irrigations for Ca, Fe, Mn, and As concentrations. The highest Se concentration was detected with the control irrigation and 75%DW+25%WW. The lowest P and Mg were with the control and 75%DW+25%WW irrigations. The lowest Ca, Fe, Zn, Cu, Mo, Mn, As, and Pb concentrations were obtained from control and 75%DW+25%WW application. The wastewater did not affect the Se concentration of crested wheatgrass.

Diluted wastewater and chemical fertilizer (control) applications to orchard grass affected the concentration of all elements in the plant by significant levels, apart from selenium. The highest Mg, Fe, Zn, Cu, Mn, As, and Pb concentrations were determined with 25%DW+75%WW irrigation, with the highest P, Ca, and Mo concentrations determined with 25%DW+75%WW and 50%DW+50%WW irrigations.

The lowest P and Mg concentrations were determined with control and 75%DW+25%WW irrigations, with the lowest Ca, Fe, Zn, Cu, Mo, Mn, As, and Pb from the control.

Table 2. The effects of wastewater rates and mowing numbers on some agronomic properties of crested wheatgrass, orchard grass and alfalfa (mean ± sd)\*.

Çizelge 2. Arıtma suyu oranları ve biçim sayılarının otlak ayırığı, domuz ayırığı ve yoncanın bazı tarımsal özellikleri üzerine etkisi (mean ± sd)\*.

WR	MN	Plant height (cm) Bitki Boyu (cm)			Green weight (g pot <sup>-1</sup> ) Yaş ağırlık (g saksı <sup>-1</sup> )			Dry weight (g pot <sup>-1</sup> ) Kuru ağırlık (g saksı <sup>-1</sup> )		
		<i>A. cristatum</i>	<i>D. glomerata</i>	<i>M. sativa</i>	<i>A. cristatum</i>	<i>D. glomerata</i>	<i>M. sativa</i>	<i>A. cristatum</i>	<i>D. glomerata</i>	<i>M. sativa</i>
W <sub>1</sub>	1	20.66±1.53 <sup>a</sup>	10.00±4.58 <sup>c</sup>	6.00±1.73 <sup>b</sup>	0.016±0.00 <sup>c</sup>	0.023±0.00 <sup>d</sup>	0.036±0.01 <sup>e</sup>	0.005±0.000 <sup>c</sup>	0.008±0.00 <sup>d</sup>	0.012±0.00 <sup>e</sup>
	2	20.33±3.06 <sup>a</sup>	13.67±2.89 <sup>bc</sup>	11.33±1.53 <sup>ab</sup>	0.018±0.00 <sup>b</sup>	0.022±0.00 <sup>d</sup>	0.048±0.11 <sup>c</sup>	0.006±0.000 <sup>b</sup>	0.007±0.00 <sup>d</sup>	0.016±0.00 <sup>c</sup>
	3	13.00±3.00 <sup>b</sup>	16.00±1.73 <sup>ab</sup>	7.67±0.58 <sup>ab</sup>	0.022±0.00 <sup>a</sup>	0.045±0.00 <sup>b</sup>	0.039±0.00 <sup>e</sup>	0.007±0.000 <sup>a</sup>	0.015±0.00 <sup>b</sup>	0.013±0.00 <sup>e</sup>
	4	10.33±2.31 <sup>b</sup>	13.67±0.58 <sup>bc</sup>	8.00±2.65 <sup>ab</sup>	0.007±0.00 <sup>d</sup>	0.023±0.00 <sup>d</sup>	0.059±0.00 <sup>b</sup>	0.002±0.000 <sup>d</sup>	0.008±0.00 <sup>d</sup>	0.020±0.00 <sup>b</sup>
	5	9.00±2.00 <sup>b</sup>	14.33±0.58 <sup>bc</sup>	8.00±4.36 <sup>ab</sup>	0.007±0.00 <sup>d</sup>	0.032±0.00 <sup>c</sup>	0.047±0.00 <sup>cd</sup>	0.002±0.000 <sup>d</sup>	0.011±0.00 <sup>c</sup>	0.016±0.00 <sup>cd</sup>
	6	10.33±0.58 <sup>b</sup>	16.67±1.16 <sup>ab</sup>	7.67±2.08 <sup>ab</sup>	0.006±0.00 <sup>d</sup>	0.041±0.00 <sup>b</sup>	0.045±0.01 <sup>cd</sup>	0.002±0.000 <sup>d</sup>	0.014±0.00 <sup>b</sup>	0.015±0.00 <sup>cd</sup>
	7	9.67±1.15 <sup>b</sup>	19.00±2.00 <sup>a</sup>	10.00±4.36 <sup>ab</sup>	0.005±0.00 <sup>d</sup>	0.043±0.00 <sup>b</sup>	0.057±0.01 <sup>b</sup>	0.002±0.000 <sup>d</sup>	0.015±0.00 <sup>b</sup>	0.019±0.00 <sup>b</sup>
	8	9.00±2.00 <sup>b</sup>	15.33±2.31 <sup>ab</sup>	9.00±1.73 <sup>ab</sup>	0.006±0.00 <sup>d</sup>	0.069±0.01 <sup>a</sup>	0.100±0.01 <sup>a</sup>	0.002±0.000 <sup>d</sup>	0.023±0.00 <sup>a</sup>	0.033±0.00 <sup>a</sup>
<b>Mean</b>		13.71±4.56 <sup>c</sup>	12.79±1.35 <sup>d</sup>	8.25±2.07 <sup>c</sup>	0.011±0.00 <sup>c</sup>	0.037±0.02 <sup>c</sup>	0.054±0.02 <sup>d</sup>	0.003±0.00 <sup>c</sup>	0.013±0.01 <sup>c</sup>	0.018±0.01 <sup>d</sup>
W <sub>2</sub>	1	21.67±4.51 <sup>a</sup>	13.00±1.00 <sup>bcd</sup>	5.67±1.53 <sup>d</sup>	0.022±0.00 <sup>a</sup>	0.016±0.00 <sup>d</sup>	0.026±0.00 <sup>e</sup>	0.007±0.00 <sup>a</sup>	0.005±0.00 <sup>d</sup>	0.009±0.00 <sup>e</sup>
	2	18.33±0.58 <sup>a</sup>	11.33±1.16 <sup>d</sup>	12.67±1.53 <sup>a</sup>	0.022±0.00 <sup>a</sup>	0.021±0.00 <sup>d</sup>	0.084±0.01 <sup>bc</sup>	0.007±0.00 <sup>a</sup>	0.007±0.00 <sup>d</sup>	0.028±0.00 <sup>c</sup>
	3	12.00±2.00 <sup>b</sup>	15.00±1.00 <sup>ab</sup>	7.67±1.53 <sup>cd</sup>	0.019±0.00 <sup>a</sup>	0.041±0.00 <sup>bc</sup>	0.068±0.00 <sup>cd</sup>	0.006±0.00 <sup>a</sup>	0.014±0.00 <sup>bc</sup>	0.023±0.00 <sup>cd</sup>
	4	9.67±1.16 <sup>b</sup>	13.33±1.16 <sup>bcd</sup>	8.67±3.06 <sup>bcd</sup>	0.011±0.00 <sup>bc</sup>	0.037±0.00 <sup>bc</sup>	0.082±0.00 <sup>cd</sup>	0.004±0.00 <sup>bc</sup>	0.012±0.00 <sup>bc</sup>	0.027±0.00 <sup>dc</sup>
	5	9.33±1.16 <sup>b</sup>	12.67±1.53 <sup>cd</sup>	7.33±2.52 <sup>d</sup>	0.008±0.00 <sup>c</sup>	0.036±0.00 <sup>bc</sup>	0.054±0.00 <sup>d</sup>	0.003±0.00 <sup>c</sup>	0.012±0.00 <sup>bc</sup>	0.018±0.00 <sup>d</sup>
	6	8.67±2.08 <sup>b</sup>	13.33±0.58 <sup>bcd</sup>	9.00±3.00 <sup>abcd</sup>	0.005±0.00 <sup>d</sup>	0.035±0.00 <sup>c</sup>	0.068±0.00 <sup>cd</sup>	0.002±0.00 <sup>d</sup>	0.012±0.00 <sup>b</sup>	0.023±0.00 <sup>dc</sup>
	7	12.00±1.00 <sup>b</sup>	13.67±1.16 <sup>bc</sup>	11.33±1.53 <sup>abc</sup>	0.007±0.00 <sup>c</sup>	0.047±0.00 <sup>b</sup>	0.144±0.02 <sup>b</sup>	0.002±0.00 <sup>d</sup>	0.016±0.00 <sup>b</sup>	0.048±0.01 <sup>b</sup>
	8	11.67±0.58 <sup>b</sup>	16.67±1.53 <sup>a</sup>	12.00±1.00 <sup>ab</sup>	0.013±0.01 <sup>b</sup>	0.070±0.02 <sup>a</sup>	0.270±0.03 <sup>a</sup>	0.004±0.00 <sup>b</sup>	0.023±0.01 <sup>a</sup>	0.090±0.01 <sup>a</sup>
<b>Mean</b>		13.04±3.88 <sup>d</sup>	13.63±1.56 <sup>c</sup>	9.50±2.52 <sup>b</sup>	0.013±0.01 <sup>b</sup>	0.038±0.02 <sup>c</sup>	0.100±0.07 <sup>c</sup>	0.004±0.00 <sup>c</sup>	0.013±0.01 <sup>c</sup>	0.033±0.02 <sup>c</sup>



Table 2. Continuation of the table

Çizelge 2. Çizelgenin devamı

W <sub>3</sub>	1	22.00±1.73 <sup>a</sup>	10.33±2.52 <sup>c</sup>	5.33±0.58 <sup>c</sup>	0.025±0.01 <sup>b</sup>	0.030±0.00 <sup>e</sup>	0.079±0.00 <sup>d</sup>	0.008±0.00 <sup>b</sup>	0.010±0.00 <sup>e</sup>	0.026±0.00 <sup>d</sup>
	2	20.67±1.53 <sup>a</sup>	15.33±4.16 <sup>abc</sup>	16.67±3.06 <sup>a</sup>	0.039±0.00 <sup>a</sup>	0.031±0.00 <sup>e</sup>	0.066±0.01 <sup>d</sup>	0.013±0.00 <sup>a</sup>	0.010±0.00 <sup>e</sup>	0.022±0.00 <sup>d</sup>
	3	12.67±2.08 <sup>b</sup>	17.33±4.04 <sup>ab</sup>	9.67±2.08 <sup>b</sup>	0.025±0.00 <sup>b</sup>	0.066±0.00 <sup>b</sup>	0.118±0.01 <sup>c</sup>	0.008±0.00 <sup>b</sup>	0.022±0.00 <sup>b</sup>	0.039±0.00 <sup>c</sup>
	4	11.33±0.58 <sup>b</sup>	14.00±4.58 <sup>abc</sup>	9.67±2.08 <sup>b</sup>	0.017±0.00 <sup>c</sup>	0.048±0.00 <sup>cd</sup>	0.080±0.00 <sup>d</sup>	0.006±0.00 <sup>c</sup>	0.016±0.00 <sup>cd</sup>	0.027±0.00 <sup>d</sup>
	5	10.00±3.46 <sup>b</sup>	13.33±2.8 <sup>bc</sup>	9.33±3.51 <sup>b</sup>	0.008±0.00 <sup>d</sup>	0.040±0.00 <sup>de</sup>	0.083±0.02 <sup>d</sup>	0.003±0.00 <sup>d</sup>	0.014±0.00 <sup>de</sup>	0.028±0.01 <sup>d</sup>
	6	14.00±9.53 <sup>b</sup>	17.33±0.58 <sup>ab</sup>	12.67±0.58 <sup>ab</sup>	0.009±0.00 <sup>d</sup>	0.046±0.02 <sup>cd</sup>	0.133±0.02 <sup>c</sup>	0.003±0.00 <sup>d</sup>	0.015±0.01 <sup>cd</sup>	0.044±0.01 <sup>c</sup>
	7	12.00±1.00 <sup>b</sup>	19.00±2.65 <sup>ab</sup>	15.33±2.52 <sup>a</sup>	0.011±0.00 <sup>cd</sup>	0.059±0.00 <sup>bc</sup>	0.181±0.02 <sup>b</sup>	0.004±0.00 <sup>cd</sup>	0.020±0.00 <sup>bc</sup>	0.060±0.01 <sup>b</sup>
	8	11.67±1.16 <sup>b</sup>	20.00±3.61 <sup>a</sup>	15.00±1.00 <sup>a</sup>	0.013±0.00 <sup>cd</sup>	0.100±0.01 <sup>a</sup>	0.361±0.02 <sup>a</sup>	0.004±0.00 <sup>cd</sup>	0.032±0.00 <sup>a</sup>	0.120±0.01 <sup>a</sup>
Mean	14.63±4.52 <sup>a</sup>	14.46±2.40 <sup>b</sup>	11.38±3.17 <sup>a</sup>	0.019±0.01 <sup>a</sup>	0.052±0.02 <sup>b</sup>	0.138±0.09 <sup>b</sup>	0.006±0.00 <sup>b</sup>	0.017±0.01 <sup>b</sup>	0.046±0.03 <sup>b</sup>	
W <sub>4</sub>	1	20.00±6.00 <sup>a</sup>	10.67±4.04 <sup>b</sup>	5.00±1.00 <sup>b</sup>	0.019±0.00 <sup>cd</sup>	0.045±0.02 <sup>d</sup>	0.091±0.00 <sup>e</sup>	0.007±0.00 <sup>cd</sup>	0.015±0.01 <sup>d</sup>	0.030±0.00 <sup>e</sup>
	2	20.00±6.00 <sup>a</sup>	13.33±3.51 <sup>ab</sup>	13.33±2.08 <sup>a</sup>	0.032±0.00 <sup>a</sup>	0.086±0.02 <sup>c</sup>	0.133±0.02 <sup>d</sup>	0.011±0.00 <sup>a</sup>	0.029±0.01 <sup>c</sup>	0.044±0.01 <sup>d</sup>
	3	17.00±1.00 <sup>ab</sup>	16.67±1.53 <sup>ab</sup>	11.33±0.58 <sup>a</sup>	0.021±0.00 <sup>bc</sup>	0.130±0.00 <sup>a</sup>	0.170±0.01 <sup>c</sup>	0.007±0.00 <sup>bc</sup>	0.043±0.00 <sup>a</sup>	0.057±0.00 <sup>c</sup>
	4	10.67±3.21 <sup>c</sup>	13.67±1.53 <sup>ab</sup>	9.67±5.51 <sup>ab</sup>	0.018±0.00 <sup>cd</sup>	0.081±0.00 <sup>c</sup>	0.131±0.01 <sup>d</sup>	0.006±0.00 <sup>cd</sup>	0.027±0.00 <sup>c</sup>	0.044±0.00 <sup>d</sup>
	5	9.67±2.88 <sup>c</sup>	15.33±3.22 <sup>ab</sup>	11.00±2.65 <sup>ab</sup>	0.009±0.00 <sup>f</sup>	0.050±0.01 <sup>d</sup>	0.190±0.02 <sup>c</sup>	0.003±0.00 <sup>f</sup>	0.017±0.00 <sup>d</sup>	0.063±0.01 <sup>c</sup>
	6	9.33±2.08 <sup>c</sup>	17.67±3.06 <sup>a</sup>	9.00±3.46 <sup>ab</sup>	0.014±0.00 <sup>e</sup>	0.056±0.01 <sup>d</sup>	0.138±0.01 <sup>cd</sup>	0.005±0.00 <sup>e</sup>	0.019±0.00 <sup>d</sup>	0.046±0.00 <sup>d</sup>
	7	12.00±3.61 <sup>bc</sup>	17.67±3.06 <sup>a</sup>	14.33±4.93 <sup>a</sup>	0.017±0.00 <sup>d</sup>	0.104±0.01 <sup>b</sup>	0.291±0.02 <sup>b</sup>	0.006±0.00 <sup>d</sup>	0.035±0.00 <sup>b</sup>	0.097±0.01 <sup>b</sup>
	8	10.00±2.65 <sup>c</sup>	17.67±4.16 <sup>a</sup>	13.67±2.08 <sup>a</sup>	0.022±0.00 <sup>a</sup>	0.126±0.00 <sup>a</sup>	0.334±0.02 <sup>a</sup>	0.007±0.00 <sup>a</sup>	0.042±0.00 <sup>a</sup>	0.111±0.00 <sup>a</sup>
Mean	14.13±4.58 <sup>b</sup>	15.75±1.62 <sup>a</sup>	11.67±2.99 <sup>a</sup>	0.019±0.01 <sup>a</sup>	0.085±0.03 <sup>a</sup>	0.185±0.08 <sup>a</sup>	0.006±0.00 <sup>a</sup>	0.028±0.01 <sup>a</sup>	0.062±0.03 <sup>a</sup>	

\* Means with different letters in the same column are significantly different (P <0.05), based on Duncan's multiple range test.

\*\*W: Water ratios; W<sub>1</sub>: Control; W<sub>2</sub>: 25 % WW +75 % DW; W<sub>3</sub>: 50 % WW + 50 % DW; W<sub>4</sub>: 75 % WW + 25 % DW

\*\*\*WW: Wastewater; DW: Diluted Wastewater

\*\*\*\*MN: Mowing Number

Table 3. The effects of wastewater rates on the some nutrient and metal (ppm) contents of crested wheatgrass, orchard grass and alfalfa (mean ± sd)\*.

Çizelge 3. Arıtma suyu oranlarının otlak ayrığı, domuz ayrığı ve yoncanın bazı besin elementleri ve metal içeriğine etkisi (mean ± sd)\*.

Factors	P	Ca	Mg	Fe	Zn	Cu	Mo	Se	Mn	As	Pb	
A	W1	18589.00±166.1 <sup>c</sup>	44587.67±279.8	21927.67±364.1 <sup>b</sup>	0.28±0.01 <sup>c</sup>	35.78±0.78 <sup>c</sup>	56.86±0.17 <sup>d</sup>	0.01±0.00 <sup>c</sup>	0.73±0.05 <sup>a</sup>	318.10±4.49 <sup>c</sup>	2.39±0.14 <sup>b</sup>	0.01±0.00 <sup>d</sup>
	W2	18846.33±113.5 <sup>c</sup>	44958.67±328.9	21918.33±60.0 <sup>b</sup>	0.29±0.00 <sup>c</sup>	50.19±0.81 <sup>b</sup>	62.89±0.19 <sup>c</sup>	0.02±0.00 <sup>a</sup>	0.64±0.01 <sup>b</sup>	311.50±1.44 <sup>c</sup>	2.51±0.16 <sup>b</sup>	0.39±0.01 <sup>c</sup>
	W3	19405.67±178.4 <sup>b</sup>	44848.00±120.8	22276.33±356.8 <sup>ab</sup>	0.32±0.01 <sup>b</sup>	59.58±0.72 <sup>a</sup>	67.62±0.33 <sup>b</sup>	0.01±0.00 <sup>b</sup>	0.62±0.01 <sup>b</sup>	338.80±5.91 <sup>b</sup>	2.92±0.12 <sup>a</sup>	0.44±0.01 <sup>b</sup>
	W4	19877.00±190.5 <sup>a</sup>	44921.67±57.5	22810.00±58.4 <sup>a</sup>	0.39±0.01 <sup>a</sup>	60.95±0.89 <sup>a</sup>	71.67±0.30 <sup>a</sup>	0.02±0.00 <sup>a</sup>	0.65±0.01 <sup>b</sup>	363.57±5.13 <sup>a</sup>	2.87±0.11 <sup>a</sup>	0.49±0.01 <sup>a</sup>
<b>Mean</b>	<b>19179.50±536.0<sup>a</sup></b>	<b>44829.00±245.1<sup>a</sup></b>	<b>22233.08±435.5<sup>a</sup></b>	<b>0.321±0.04<sup>b</sup></b>	<b>51.62±10.5<sup>a</sup></b>	<b>64.76±5.8<sup>c</sup></b>	<b>0.01±0.00<sup>c</sup></b>	<b>0.66±0.05<sup>a</sup></b>	<b>332.99±21.6</b>	<b>2.67±0.26<sup>a</sup></b>	<b>0.33±0.19<sup>b</sup></b>	
CW	W1	15708.00±327.1 <sup>d</sup>	31374.33±155.1 <sup>c</sup>	15734.00±77.8 <sup>c</sup>	0.29±0.01 <sup>c</sup>	31.43±0.41 <sup>d</sup>	61.66±0.33 <sup>d</sup>	0.01±0.00	0.47±0.03 <sup>a</sup>	310.50±1.00 <sup>c</sup>	2.12±0.05 <sup>b</sup>	0.01±0.00 <sup>d</sup>
	W2	16451.67±315.5 <sup>c</sup>	32618.33±104.2 <sup>b</sup>	15854.00±148.3 <sup>c</sup>	0.34±0.01 <sup>b</sup>	33.20±0.68 <sup>c</sup>	69.58±0.17 <sup>c</sup>	0.02±0.00	0.47±0.01 <sup>a</sup>	317.17±0.55 <sup>b</sup>	2.26±0.05 <sup>b</sup>	0.46±0.02 <sup>c</sup>
	W3	17477.33±338.2 <sup>b</sup>	32987.67±11.0 <sup>a</sup>	17585.00±270.5 <sup>b</sup>	0.74±0.03 <sup>a</sup>	45.28±0.39 <sup>b</sup>	73.44±0.47 <sup>b</sup>	0.02±0.00	0.43±0.00 <sup>b</sup>	366.20±1.57 <sup>a</sup>	2.69±0.06 <sup>a</sup>	0.54±0.01 <sup>b</sup>
	W4	18410.33±455.2 <sup>a</sup>	33198.67±67.8 <sup>a</sup>	18969.67±334.9 <sup>a</sup>	0.71±0.01 <sup>a</sup>	48.72±0.34 <sup>a</sup>	78.53±0.13 <sup>a</sup>	0.02±0.00	0.45±0.01 <sup>b</sup>	363.43±3.37 <sup>a</sup>	2.74±0.11 <sup>a</sup>	0.67±0.012 <sup>a</sup>
<b>Mean</b>	<b>17011.83±1112.5<sup>c</sup></b>	<b>32544.75±743.2<sup>b</sup></b>	<b>17035.67±1408.5<sup>c</sup></b>	<b>0.519±0.21<sup>a</sup></b>	<b>39.66±7.8<sup>c</sup></b>	<b>70.80±6.4<sup>a</sup></b>	<b>0.02±0.00<sup>b</sup></b>	<b>0.45±0.02<sup>b</sup></b>	<b>339.33±26.8</b>	<b>2.46±0.28<sup>b</sup></b>	<b>0.42±0.26<sup>a</sup></b>	
OG	W1	16966.33±168.0 <sup>b</sup>	30364.67±203.4 <sup>c</sup>	18521.00±161.0 <sup>c</sup>	0.30±0.00 <sup>d</sup>	32.32±0.87 <sup>d</sup>	59.61±0.13 <sup>d</sup>	0.01±0.00 <sup>c</sup>	0.42±0.01	321.90±1.00 <sup>d</sup>	2.37±0.05 <sup>d</sup>	0.01±0.00 <sup>d</sup>
	W2	17186.67±168.3 <sup>b</sup>	31228.67±82.7 <sup>b</sup>	18707.00±323.8 <sup>c</sup>	0.37±0.02 <sup>c</sup>	38.65±0.11 <sup>c</sup>	62.66±0.11 <sup>c</sup>	0.02±0.00 <sup>b</sup>	0.39±0.01	327.53±2.51 <sup>c</sup>	2.47±0.05 <sup>c</sup>	0.44±0.01 <sup>c</sup>
	W3	18323.33±176.9 <sup>a</sup>	32596.33±122.2 <sup>a</sup>	19530.00±231.1 <sup>b</sup>	0.57±0.03 <sup>b</sup>	50.21±0.79 <sup>b</sup>	68.80±0.27 <sup>b</sup>	0.02±0.00 <sup>a</sup>	0.41±0.01	337.83±1.14 <sup>b</sup>	2.66±0.01 <sup>b</sup>	0.57±0.02 <sup>b</sup>
	W4	18325.67±587.7 <sup>a</sup>	32684.67±90.7 <sup>a</sup>	21743.67±158.9 <sup>a</sup>	0.67±0.02 <sup>a</sup>	58.99±0.97 <sup>a</sup>	74.77±0.22 <sup>a</sup>	0.02±0.00 <sup>a</sup>	0.40±0.01	348.30±1.22 <sup>a</sup>	2.91±0.06 <sup>a</sup>	0.66±0.02 <sup>a</sup>
<b>Mean</b>	<b>17700.50±714.3<sup>b</sup></b>	<b>31718.58±1021.3<sup>c</sup></b>	<b>19625.42±1351.9<sup>b</sup></b>	<b>0.479±0.16<sup>a</sup></b>	<b>45.04±10.7<sup>b</sup></b>	<b>66.460±6.1<sup>b</sup></b>	<b>0.02±0.00<sup>a</sup></b>	<b>0.41±0.01<sup>c</sup></b>	<b>333.89±10.6</b>	<b>2.60±0.22<sup>a</sup></b>	<b>0.42±0.26<sup>a</sup></b>	

\*Means with different letters in the same column are significantly different (P < 0.05), based on Duncan's multiple range test.

\*\*W: Water ratios; W<sub>1</sub>: Control; W<sub>2</sub>: 25 % WW + 75 % DW; W<sub>3</sub>: 50 % WW + 50 % DW; W<sub>4</sub>: 75 % WW + 25 % DW

\*\*\*WW: Wastewater; DW: Diluted wastewater

\*\*\*\* A: Alfalfa; CW: Crested Wheatgrass; OC: Orchard Grass

Table 4. Pearson's Correlation Coefficients (r) for diluted wastewater nutrients and metal concentration in crested wheatgrass, orchard grass and alfalfa \*\*,\*

Çizelge 4. Arıtma suyunun otlak ayrığı, domuz ayrığı ve yoncadaki besin maddeleri ve metal konsantrasyonu için Pearson Korelasyon Katsayıları (r) \*\*,\*

Plants	P	Ca	Mg	Fe	Zn	Cu	Mo	Se	Mn	As	Pb
Agropyron cristatum	.959**	.918**	.948**	.896**	.956**	.988**	.602*	-.132**	.905**	.926**	.926**
Dactylis glomerata	.853**	.952**	.906**	.976**	.993**	.990**	.877**	.132**	.983**	.964**	.928**
Medicago sativa	.964**	.425	.800**	.943**	.943**	.995**	.680**	-.570	.886**	.825**	.883**

\*Correlation is significant at the P<0.05 level, \*\*Correlation is significant at the 0,01 level.

When the species are assessed together, the highest P, Ca, Mg, Zn, Se, and As concentrations were in alfalfa, with the highest Cu concentration in crested wheatgrass and the highest Mo and As in orchard grass. The highest Fe and Pb concentrations were identified in crested wheatgrass and orchard grass. Findings related to the content of calcium, magnesium, zinc, and selenium in alfalfa are consistent with results of the study by researchers reported that two-year wastewater irrigation of barley increased the macro and micronutrient element and heavy metal contents (Frame, 2005 & Rusan et al., 2007). Wastewater increases the nutrient and metal contents of alfalfa and grasses (Osman, 2017).

The insignificant correlation for different proportions of dilute wastewater with the Ca content of alfalfa, with high cation exchange capacity, is attributed to the high Na concentration of wastewater limiting the uptake of Ca (Siebe, 2017). There was a negative and insignificant relationship of wastewater with Se concentration ( $r=-0.507$ ), but positive with Ca concentration ( $r=-0.425$ ). However, the relationship of wastewater with other elements was significant for alfalfa ( $P<0.01$ ). The relationship between dilute wastewater with crested wheatgrass Se concentration ( $r=-0.132$ ) was negative and significant, but with orchard grass was positive and significant ( $P<0.01$ ). However, for crested wheatgrass and orchard grass, the relationship between the concentration of other

elements and wastewater was positive and very significant ( $P<0.01$ ), whereas for crested wheatgrass Mo concentration was significant ( $P<0.05$ ). It is estimated that the positive response of orchard grass to irrigation is higher than the other two plants, causing the relationship of the studied nutrient concentration with wastewater to be positive and very important (Table 4).

### Some Physical and Chemical Features of Soil After Trial

In the potting soil after the experiment, the highest EC, organic matter, Fe, Cu, As was determined in 25%DW+ 75%WW irrigation, the highest Mn in three ratios of diluted wastewater, and the highest pH in the control pot. While the concentrations of Mo and Se could not be detected, the effect of irrigation on Pb concentration was insignificant.

Researchers reported that the use of wastewater for irrigation increased soil salinity (Gao et al., 2021; Yerli, 2023). It has been reported that soil pH changes towards neutral after 20 days of wastewater irrigation (Agrawal et al., 2014). Some researchers also found that wastewater reduced soil pH and increased EC and organic matter (Mojiri, 2011). The Fe, Cu, Zn, Mn, and As concentrations increased with the increasing wastewater proportions, while the Pb concentration was not affected (Table 5).

Table 5. Effect of different rates of wastewater on nutrients, heavy metals, pH, organic matter and EC concentration in pot soil after trial\*.

*Çizelge 5. Arıtma suyunun deneme sonrası saksı toprağının besin maddeleri, ağır metaller, pH, organik madde ve EC konsantrasyonu üzerine etkisi\*.*

Property	Control	75%DW+25%WW	50%DW+50%WW	25%DW+75%WW
pH	8.43 <sup>a</sup>	8.36 <sup>b</sup>	8.10 <sup>c</sup>	8.07 <sup>c</sup>
EC ((dS/m)	0.229 <sup>d</sup>	0.362 <sup>c</sup>	0.441 <sup>b</sup>	0.505 <sup>a</sup>
Organic Madde (%)	1.613 <sup>b</sup>	1.62 <sup>b</sup>	1.62 <sup>b</sup>	1.64 <sup>a</sup>
Iron (Fe)	88.76 <sup>d</sup>	96.73 <sup>c</sup>	102.16 <sup>b</sup>	103.80 <sup>a</sup>
Copper (Cu)	0.106 <sup>d</sup>	0.113 <sup>c</sup>	0.116 <sup>b</sup>	0.121 <sup>a</sup>
Zinc (Zn)	0.18 <sup>c</sup>	0.21 <sup>b</sup>	0.22 <sup>a</sup>	0.21 <sup>b</sup>
Manganese (Mn)	2.24 <sup>b</sup>	2.37 <sup>a</sup>	2.36 <sup>a</sup>	2.39 <sup>a</sup>
Molybdenum (Mo)	nd <sup>**</sup>	nd <sup>**</sup>	nd <sup>**</sup>	nd <sup>**</sup>
Selenium (Se)	nd <sup>**</sup>	nd <sup>**</sup>	nd <sup>**</sup>	nd <sup>**</sup>
Arsenic (As)	0.10 <sup>c</sup>	0.11 <sup>b</sup>	0.11 <sup>a</sup>	0.11 <sup>a</sup>
Lead (Pb)	0.02	0.02	0.02	0.02

\* Different letters in the same row are significantly different ( $P<0.05$ ), \*\* nd: non detected

### CONCLUSION

Diluted biologically treated domestic wastewater caused an increase in the height of alfalfa and the two grasses. After the first mow, there was a reduction in the height of crested wheatgrass in the subsequent

mows, which is due to the slow seedling development of crested wheatgrass. Having a higher plant height of orchard grass than the other two plants is since it is more resistant to frequent irrigation and rapid development after mowing. In addition, the

significance of the wastewater relationship with all the examined elements can be attributed to the water consumption and rapid development of the orchard grass feature. The wastewater ratio increased the green and dry weight of alfalfa gradually compared to the grass. However, the increasing number of mows at all wastewater ratios and in control irrigation reduced the green and dry weights of crested wheatgrass. Based on the wastewater ratio, apart from selenium, there were increases in nutrients and heavy metals in all three plants. The exchangeable cation of Ca significantly increased in both grasses depending on the wastewater ratio, while it did not change in alfalfa. While wastewater provides a positive result by reducing alkaline soil pH and increasing its organic matter, it also causes an important risk by increasing salinity.

In conclusion, the development of treatment systems that remove or at least reduce will resolve salinity risk. If biologically treated domestic wastewater is used for irrigation purposes, it must be diluted by 75%. However, to obtain more reliable results, it is recommended to investigate dilute wastewater irrigation under field conditions for many years.

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#### Researchers' Contribution Rate Statement

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

#### Conflict of Interest Statement

The authors declare no conflict of interest.

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## Estimating the Poverty Status Among Ginger Women Farmers in Kaduna State, Nigeria

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### ABSTRACT

This research estimated the poverty status among ginger women farmers in Kaduna State, Nigeria. Primary data were used based on a well-designed questionnaire. Data were analyzed using descriptive statistics, Foster, Greer and Thorbecke (FGT) poverty index, Tobit regression model, and principal component model. The outcome shows that the average age, years of farming experience, and educational level approximate 48 years, 9 years, and 11 years respectively. The poverty line is approximately 4, 172.06 Naira (1 USD = 1 104 Naira). The poverty incidence ( $P_0$ ) approximately 0.5298, 95% CI [0.4575, 0.6021] this corresponds to 52.98% of ginger women farmers are poor, from the sampling population, this adds up to the fact that 63 ginger women farmers are poor, while 57 ginger women farmers were non-poor. Approximately 4.7 million of the entire population were poor, while 4.2 million were non-poor. The poverty depth ( $P_1$ ) which explains that the gap between the poor ginger women farmers and the poverty line is calculated as 0.2876, 95% CI [0.2465, 0.3287] this implies that it will take the poor ginger women farmers ( $28.76\% \times 4, 172.06$  Naira) the amount of 1, 199.88 Naira to cover up or make up for the poverty gap. The severity of poverty ( $P_2$ ) which measures the squares of the poverty gap relative to the poverty line was calculated at 0.1967, 95% CI [0.1575, 0.2359], this means that 19.67% of the ginger women farmers were severely poor. The educational level, years of ginger farming, income from ginger farming, off-farm income, and membership of cooperative were different significantly from zero in influencing poverty status among ginger women producers. The major limitations were a lack of credit facilities, a lack of access to land, and a lack of farm inputs. The ginger women farmers should join cooperative associations as this will enable them to share ideas, and information on new farm techniques and access credit at single digit interest rates to increase productivity and reduce poverty.

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### INTRODUCTION

Ginger (*Zingiber officinale*) has a medicinal value and significant valuable export commodity in Nigeria with 90% of what is produced being exported and 10% is consumed locally (Nextzon, 2018). Ginger is produced in 6 states in Nigeria, but Kaduna State stands out as the main ginger-producing state (Nextzon, 2018). Ginger is cultivated for its rhizome, and dried ginger is more desirable for export. Ginger from Nigeria is progressively sought because of its oil and pungency.

Ginger is useful in the following industries: food processing, pharmaceuticals, confectionaries, and beverages. The most ginger producing-countries are India, China, Nepal, Indonesia, and Nigeria(Nextzon, 2018). The main importing countries include the United States Saudi Arabia, Russia, Netherlands, Germany, France, Italy, Denmark, Belgium, Switzerland, and Greece (Nextzon, 2018). According to FAO (2024), in 2021 and 2022, the production of ginger in Nigeria approximates 707,100 tonnes and

743,527,47 tonnes respectively. In 2021 and 2022, the area harvested for ginger approximates 102320 ha and 96120 ha respectively (FAO, 2024). In 2021 and 2022 the world production of ginger approximates 4855031.84 tonnes and 4874216.17 tonnes respectively (FAO, 2024). This estimate corresponds that Nigeria produces 14.56% and 15.25% of world ginger production in 2021 and 2022 respectively. The world area harvested for ginger in 2021 and 2022 approximates 465,821ha and 450,647ha respectively.

In sub-Saharan Africa, Nigeria inclusive, women accounted for 70% of agricultural labour, 60% of agricultural production, and 80% of food production (Alabi et al., 2021). There are no quantitative and qualitative data on the role of women in sub-Saharan agriculture and rural development (Lawal et al., 2017). There is an absence of statistical information on the performance and status of all farmers in Africa including women farmers (Lawal et al., 2017). All farmers including women farmers in Africa are poor, food insecure, no adequate nutrition, have no access to clean water, and lack basic needs of well-being. The illiteracy of women in Africa is two times higher than men which confirms their disadvantaged position (Lawal et al., 2017). Rural women in Nigeria faced poverty, food insecurity, and the right to survive was not assured. Food poverty is the inability to have access to food or afford food that makes up a healthy diet. Food insecurity is rooted in poverty and has long-term measures on the country's communities to develop prosper and grow or on the ability to farm (FAO, 2004). In rural areas in Nigeria, poverty is severe and this affects the farming households where social services and infrastructures are limited or non-existent. When discussing nutrition and food security in sub-Saharan Africa, rural women farmers are very significant (Lawal et al., 2017). Rural women producers had difficulty of access to agricultural information even with the advent of ICTs (Information and Communication Technologies). Agricultural information is needed by rural women producers on market prices, credit, early warning systems on flood, drought, pest infestations and diseases, new farming technologies, storage, marketing, processing, and financing (Ofuoku et al., 2008). Agricultural information is needed by rural women producers to obtain the highest agricultural yields, increase income, and reduce poverty. Women's performance is very vital to the overall success of work directed towards remote area development for enhanced agricultural productivity (Ali et al., 2017). If the income of women is enhanced they will have more access to resources and invest in healthcare, children's education, and nutrition (Ogunlela and Mukhtar, 2009). In Africa, women are excluded from inheriting farmland like their male counterparts due to traditional and cultural practices (Moore et al., 2015). Women are prone to have

lesser access to agricultural productive factors than men counterparts due to gender-specific limitations, they have less power, less control, and less decision-making power over productive agricultural resources than men (FAO, 2011). Women in Africa also have less access to extension services and lower access to credit, they use fewer inputs such as improved seeds, fertilizers, and mechanical equipment (Lawal et al., 2017). This disparity negatively influences the productivity of women farmers, researches have shown that women are efficient as men and significantly contribute to agricultural productivity (FAO 2010; World Bank, 2008; Deere and Doss, 2006). Conflict and insecurity have continued to be the drivers of food insecurity with an estimated 11% rise in poverty in 2018 (World Food Program, 2018). Farmersherdsmen conflict has remained the most significant resource – use conflict in Nigeria (Ajuwon, 2004., Fasona and Omojola, 2005). The conflicts have demonstrated a high potential to increase the insecurity, food crisis, and poverty, particularly in rural communities where most of the conflicts are localized with devastating consequences nationwide (Adisa, 2012). Farmersherdsmen have contributed serious threats to the means of survival and livelihood which have resulted in large-scale destruction of lives and properties (Solomon, 2021). According to Ogunyemi (2019), the farmersherdsmen conflict has not only heightened the level of insecurity but has also demonstrated high potential to increase the food crisis in Nigeria due to the loss of farmers' lives, animals, crops, and valuable properties.

Folorunso et al. (2018) analyzed food security and poverty status among agro-pastoralists in Barkin Ladi local government area, Plateau State, Nigeria using the Foster, Greer, and Thorbecke (FGT) food security index. The poverty line was estimated at 7, 599.26 Naira. The poverty incidence ( $P_0$ ), poverty depth ( $P_1$ ), and poverty severity ( $P_2$ ), were estimated at 0.50, 0.39, and 0.15, respectively.

Omonona et al. (2006) in their report titled Urban People's Perception and Causes of Poverty in Nigeria documented that farming experience, off-farm income are significant factors influencing poverty status, while household size was not a significant factor influencing poverty status.

Omonona and Agoi (2007) analyzed the food security situation among Nigerian urban households, evidence from Lagos State, Nigeria. The food security index was employed. The food insecurity line was defined as two-thirds of the mean per capita food expenditure of the total households. The households whose per capita expenditure falls below 7,967.19 Naira are designated food insecure, while the households whose mean per capita food expenditure equals or is greater than 7, 967.19 Naira are food secure. The food insecurity incidence for the household is found to be 0.49.



Igbalajobi et al. (2013) determined poverty incidence among rural farmers in Ondo State, Nigeria. The data were analyzed using the Foster–Greer–Thorbecke (FGT) poverty measure. Approximately 59.3% of the respondents were actually poor, while 27.6% of the poverty line (580.42 USD) was needed to get out of poverty.

The objective of the study is to estimate the poverty status among ginger women farmers in Kaduna State, Nigeria. Specifically, the objectives include:

- (i) estimate the poverty status of ginger women farmers,
- (ii) evaluate the factors influencing the poverty status of ginger women farmers,
- (iii) determine the constraints faced by ginger women farmers.

### EMPIRICAL and LITERATURE REVIEWS

Oyekale et al. (2021) determined the poverty status among farming households in the Odogbolu local government area, Ogun State, Nigeria. The data were analyzed using descriptive statistics, Foster–Greer and Thorbecke (FGT), and the Tobit regression model. The outcome shows that 26.7% of the households were poor. The female-headed households were poorer (42%) than the male-headed households (21%). The Tobit regression model result shows that age, household size, and land size were significant factors influencing the poverty status of farming households.

Aguibiade and Oke (2019) examined the poverty status and factors influencing the poverty profile of cassava farming households in Osun State, Nigeria. Data were analyzed using the Foster–Greer–Thorbecke index and Tobit regression model. The result of FGT analysis reveals that poverty incidence was 28.9%, poverty depth was 5.3%, and poverty severity was 1.5%. The Tobit regression model result shows that household size, farming experience, and revenue generated from cassava farms were factors influencing the poverty profile of the farming households.

Ahmed et al. (2019) examined the poverty status of women in rural farming households in the Iwo local government area, Osun state, Nigeria. Data were analyzed using descriptive statistics, Foster–Greer–Thorbecke, and Logit regression analysis. The FGT analysis showed that the headcount index, poverty gap index, and poverty severity index were 0.580, 0.331 and 0.132, respectively. The Logit regression analysis revealed that age and household size had a negative influence on poverty status, while household expenditure and farming experience had a positive influence on poverty status.

Olorunsanya et al. (2012) evaluated the poverty status of rural farming households in Osun State, Southwestern, Nigeria. Data were analyzed using descriptive statistics, the Foster–Greer–Thorbecke

(FGT) index, and the Tobit regression model. The result of the poverty indices shows that 35% of the farmers’ beneficiaries of the empowerment program were poor, while 55% of the non-beneficiaries of the program were non-poor. The Tobit regression result shows that household size, amount of credit utilized, and annual farm income were factors influencing the poverty status of rural farming households.

Alabi et al. (2021) evaluated smallholder rural women rice farmers’ decision-making process, agricultural intensification, and poverty status in Abuja, Nigeria. Data were analyzed using descriptive statistics, Heckman–2–stage model, Foster–Greer–Thorbecke (FGT) poverty model, and Logit regression model. The FGT result shows that poverty incidence, poverty depth, and poverty severity were 0.5178, 0.2866, and 0.1956, respectively. The significant factors influencing poverty status include educational level, access to credit, membership of cooperatives, and farm income.

Hussaini et al. (2020) examined investment in rice-value addition activities among farmers in Kebbi state, Nigeria. Data were analyzed using the Foster–Greer–Thorbecke (FGT) index and Logit regression model. The result revealed that 58.3% of rice farmers were poor, while 42.7% were non-poor. The marginal effect of income from parboiling, winnowing, drying, destoning, and bagging value addition was negative and statistically significant in influencing poverty status.

### MATERIALS and METHOD

This study was conducted in Kaduna State, Nigeria. The population of the entire state as of 2021 is 8.9 million (NPC, 2022). A multi-stage sampling design was used to select 120 ginger women producers. In the first stage, the purposive sampling method was used to select Kaduna State, the major producer of ginger in Nigeria. Second stage, two local government areas were randomly chosen. In the third stage, three villages in each local government area were randomly chosen making a total of six villages. In the fourth stage, a proportionate-random sampling method was used to select 120 ginger women farmers. The total sample frame of ginger women farmers was 171. Primary data were used based on a well-designed questionnaire. The sample number of ginger women producers was based on the established Yamane (1967) formula given as:

$$n = \frac{N}{1 + N(e^2)} = \frac{171}{1 + 171(0.05)^2} = 120 \dots \dots \dots (1)$$

Where,

$n$  = The Sample Number

$N$  = The Sample Frame of Ginger Women Producers

The data were analyzed using descriptive and inferential statistics as follows:

### FGT (Foster, Greer and Thorbecke)

This follows Adekoya (2014) and it is expressed as:

$$P = \frac{1}{N} \sum_{i=1}^q \left[ \frac{(Z - Y_i)}{Z} \right]^\alpha \dots \dots \dots (2)$$

Where,

$P$  = Foster, Greer, and Thorbecke Index ( $0 \leq P \leq 1$ )

$N$  = Total Number of Ginger Women Farmers (Number)

$q$  = Number of Ginger Women Farmers below the Poverty Line

$Z$  = Poverty Line (Naira)

$Y_i$  = Per Capital Household Expenditure of the Ginger Women Farmers

$\alpha$  = Non-Negativity Aversion Parameter (0, 1, or 2)

The estimation of poverty status can be decomposed to Prevalence of Poverty ( $P_0$ ), Poverty Depth ( $P_1$ ), and Severity of Poverty ( $P_2$ ). The model is expressed as:

$$P_0 = \frac{q}{N} \text{ (if } \alpha = 0) \dots \dots \dots (3).$$

$$P_1 = \frac{1}{N} \sum_{i=1}^q \left[ \frac{(Z - Y_i)}{Z} \right] \text{ (if } \alpha = 1) \dots \dots \dots (4)$$

$$P_2 = \frac{1}{N} \sum_{i=1}^q \left[ \frac{(Z - Y_i)}{Z} \right]^2 \text{ (if } \alpha = 2) \dots \dots \dots (5)$$

### The Construction of Poverty Line

The poverty line is defined as:

$$PL = \frac{2}{3} \times MPCHE \dots \dots \dots (6)$$

$$MPCHE = \frac{THPHE}{TNR} \dots \dots \dots (7)$$

Where,

$MPCHE$  = Mean Per Capital Household Expenditure (Naira)

$TNR$  = Total Number of Respondents

$THPHE$  = Total Household Per Capital Expenditure (Naira)

$PL$  = Poverty Line

### Tobit Dichotomous Regression Model

The model is explicitly stated as:

$$Y_i = P_i^* = \beta_0 + \beta_1 P_1 + \beta_2 P_2 + \beta_3 P_3 + \beta_4 P_4 + \beta_5 P_5 + \beta_6 P_6 + \mu_i \dots \dots \dots (8)$$

$$Y_i = \begin{cases} P_i^*, & \text{if } P_i^* > 0 \text{ie } (Z > I) \\ 0, & \text{if } P_i^* \leq 0 \text{ie } (Z < I) \end{cases}$$

Where,

$Y_i$  = The Dependent Variable, it is Discrete when the households are not Poor, and Continuous when they are Poor.

$P_i^*$  = Poverty Depth defined as  $\left[ \frac{Z-I}{Z} \right]$

$Z$  = Poverty Line

$I$  = Mean Households Food Expenditure per Adult Equivalent

$\beta_0$  = Constant Term

$\beta_1 - \beta_6$  = Regression Coefficients

$P_1$  = Educational Level (Years)

$P_2$  = Age in Years

$P_3$  = Years of Ginger Farming

$P_4$  = Income from Ginger Farming (Naira)

$P_5$  = Off-Farm Income (Naira)

$P_6$  = Membership of Cooperatives (1, Member; 0, Otherwise)

$\mu_i$  = Noise Term

### PCM (Principal Component Model)

The constraints faced by ginger women producers were submitted to PCM, the model will reduce many interrelated constraints to a few unrelated ones. The principal Component Analysis is stated thus:

$$x = (x_1, x_2, x_3, \dots, x_p) \quad (9)$$

$$\alpha_k = (\alpha_{1k}, \alpha_{2k}, \alpha_{3k}, \dots, \alpha_{pk}) \quad (10)$$

$$\alpha_k^T X = \sum_{j=1}^p \alpha_{kj} X_j \quad (11)$$

$$Var = [\alpha_k^T X] \text{ is Maximum} \quad (12)$$

Subject to:

$$\alpha_k \alpha_k = 1 \quad (13)$$

$$\text{and} \quad cov [\alpha_1^T X - \alpha_2^T X] = 0 \quad (14)$$

The variance of each of the principal components are:

$$Var[\alpha_k^T X] = \lambda_k \quad (15)$$

$$S = \frac{1}{n-1} (X - X)(X - \bar{X})^T \quad (16)$$

$$S = \frac{1}{n-1} \sum_{i=1}^n (X_i - \bar{X}_i)(X_i - \bar{X}_i)^T \quad (17)$$

Where,

$X$  = Vector of  $p$  Random Variables

$\alpha_k$  = Vector  $p$  Components

$\lambda_k$  = Eigen Value

$T$  = Transpose

$S$  = Covariance Matrix

## RESULTS and DISCUSSION

### Summary Information of Factors of Interest

The summary estimates of variables of interest are presented in Table 1. The mean age, years of ginger farming experience, and educational level approximates 48 years, 9 years, and 11 years respectively. Similarly, 98% of respondents are married, 58% are members of cooperative organizations, while 45% have access to credit. In addition, the respondents have an average household

size of 9 persons, and they are smallholder farmers with an average farm size of 0.95ha. This communicates that the ginger women farmers are young, energetic, and resourceful. They are smallholder farmers with less than 5 hectares of ginger farms. About 0.02% of ginger women farmers are not married while 55% of them have no access to credit. In

addition, 42% of ginger women farmers do not belong to any cooperative organizations. This outcome is in accord with the result of Nwaiwu et al. (2022) who documented that the average age of vegetable rural women farmers is 44 years, and 75.56% of them had no access to credit in Imo State, Nigeria.

Table 1. Summary estimates of variables of interest

Variables	Unit of Measurement	$\bar{X}_i$	SD
Age	Years	48	14.56
Household Size	Number	9	2.72
Marital Status	1, Married; 0, Others	0.98	0.73
Cooperative Members	1, Member; 0, Others	0.58	0.47
Years of Farming Experience	Years	9	4.36
Farm Size	Hectares	0.95	0.27
Access to Credit	1, Access; 0, Others	0.45	0.17
Educational Level	Years	11	4.07

Source: Field Survey (2024)

### Poverty Status of Ginger Women Farmers

The poverty status of ginger women farmers is presented in Table 2 and Table 3. The poverty line is estimated from MPCHE. Two-thirds (4, 172.06 Naira) MPCHE is used as the poverty line as documented by Omonona et al. (2007). The poverty incidence ( $P_0$ ), poverty depth ( $P_1$ ), and severity of poverty ( $P_2$ ) were calculated using the Foster, Greer, and Thorbecke (FGT) index approximation ( $0 \leq P \leq 1$ ). The ( $P_0$ ) is estimated as 0.5298, 95% CI[ 0.4575, 0.6021] this represents that 52.98% of ginger women farmers are poor, from the sampling population, approximately 63 ginger women farmers are poor while 57 ginger women farmers were non-poor. The entire population reveals that 4.7 million people were poor, while 4.2 million people were non-poor. The ( $P_1$ ) which estimates the

depth of an average poor ginger women farmer from the poverty line is calculated as 0.2876, 95% [0.2465, 0.3287]. This implies that an average ginger woman farmer would require 28.76% of the poverty line [(28.76% × 4,172.06 Naira)] which was estimated at 1,199.88 Naira to get out of poverty. The ( $P_2$ ) which measures the squares of the poverty gaps relative to the poverty line was calculated at 0.1967, 95% CI[0.1575, 0.2359] this expresses that 19.67% of the ginger women farmers were severely poor. The PCE is calculated at 11, 205.22 Naira (1 USD = 1, 104 Naira) for non-poor, and 1, 884.64 Naira for poor ginger women farmers. This outcome is in consensus with Folorunso et al. (2014) who estimated the poverty line of 7, 599.26 Naira among respondents in Plateau State, Nigeria.

Table 2. Poverty Status of Ginger Women Farmers

Poverty Status	Per Capital Household Expenditure			Total Household Expenditure		
	Non-Poor	Poor	Total	Non-Poor	Poor	Total
Mean	11, 205.22	1, 884.64	6, 258.09	81 440.79	19, 840.71	52, 176.76
Min	4,222,23	195	195	17, 150	1, 950	1 950
Maximum	30, 807.78	3,844	30, 807.78	433, 860	111 860	433, 860
Poverty Line = 4, 172.06 Naira						
FGT			CI Upper Bound	CI Lower Bound		
Poverty Incidence ( $P_0$ ) = 0.5298			0.4575	0.6021		
Poverty Depth ( $P_1$ ) = 0.2876			0.2465	0.3287		
Poverty Severity ( $P_2$ ) = 0.1967			0.1575	0.2359		

Source: Field Survey (2024) 1 USD = 1, 104 Naira, CI = Confidence Interval

Table 3. Distributions of Poverty Indices

Producers	Frequency	Percentage
Poor (Per Capital Income < 4, 172.06 Naira)	63	52
Non-Poor (Per Capital Income ≥ 4, 172.06 Naira)	57	48
Total	120	100.00

Source: Field Survey (2024) 1 USD = 1, 104 Naira

### Factors Influencing Poverty Status among Ginger Women Farmers

The MLEs of the factors influencing poverty status among ginger women producers using the Tobit regression model are presented in Table 4. About five (5) factors were included in the model, and all the factors have negative coefficients and this is in conformity with a priori expectations. The education level and membership of the cooperative are different significantly from zero at  $p=0.000$  probability level, respectively. In addition, the years of ginger farming, income from ginger farming, and off-farm income are different significantly from zero at  $p=0.038$  probability level respectively. The coefficient of income from ginger farming is  $-0.2713$ , 95% CI $[-0.2523, -0.2903]$  and the marginal effect is  $-0.3043$ , this shows that a 1%

increase in income keeping all other factors fixed will give rise to 30.43% decrease in poverty status among ginger women farmers. Similarly, the coefficient of off-farm income is  $-0.1621$ , 95% CI  $[-0.1501, -0.1741]$  and the marginal effect is  $-0.1708$ , this approximates that a 1% increase in off-farm income keeping all other stimuli fixed will give rise to 17.08% decrease in poverty status among ginger women farmers. The Pseudo R square value is 0.8709, this denotes that 87.09% of the poverty status is explained by the stimulus included in the model. The LLF (The Likelihood Function)  $(-142.72)$  is different significantly from zero at the  $p=0.000$  probability level. This is evidence that the data and model are of good fit. This outcome is in accord with Omonona and Agoi (2007) and Igbalajobi et al. (2013).

Table 4. The MLEs (Maximum Likelihood Estimates) of the Factors Influencing Poverty Status among Ginger Women Producers using Tobit Dichotomous Regression Model

Variables	Parameters	Coefficient	Standard Error	t-Value	ME
Constant	$\beta_0$	$-0.7216^{***}$	0.1449	-4.98	-0.0736
Educational Level	$\beta_1$	$-0.3851^{***}$	0.0557	-6.91	-0.2903
Years of Ginger Farming	$\beta_2$	$-0.1472^{**}$	0.0593	-2.48	-0.2412
Income from Ginger Farming	$\beta_3$	$-0.2713^{**}$	0.1076	-2.52	-0.3043
Off Farm Income	$\beta_4$	$-0.1621^{**}$	0.0672	-2.41	-0.1708
Membership of Cooperatives	$\beta_5$	$-0.2101^{***}$	0.0334	-6.28	-0.2704
Diagnostic Statistics	$\beta_5$				
Sigma	0.19743				
$LR_{\chi^2}$ (5)	96.45 <sup>***</sup>				
Pseudo R <sup>2</sup>	0.8709				
LLF (Log Likelihood)	-142.72				
Prob $> \chi^2$	0.00000 <sup>***</sup>				

Source: Field Survey (2024), ME=Marginal Effect

\*Significant at ( $P < 0.10$ )., \*\*Significant at ( $P < 0.05$ ), \*\*\*Significant at ( $P < 0.01$ ).

Table 5. The Challenges Faced by Ginger Women Producers

Constraints	Eigen-Value	Difference	Proportion	Cumulative	Rank
Lack of Credit Facilities	9.4714	5.7570	0.3474	0.3474	1 <sup>st</sup>
Lack of Access to Land	3.7144	0.7871	0.1196	0.4670	2 <sup>nd</sup>
Lack of Farm Inputs	2.9273	0.4121	0.1022	0.5692	3 <sup>rd</sup>
Farmers/ Herders Clash	2.5152	0.1810	0.1005	0.6697	4 <sup>th</sup>
Poor Market Linkages	2.3342	0.2489	0.1002	0.7699	5 <sup>th</sup>
Poor Prices of Commodity	2.0853	1.0981	0.0105	0.7804	6 <sup>th</sup>
Bartlett Test of Sphericity					
$\chi^2$	3579.13 <sup>***</sup>				
KMO	0.839				
Rho	1.00000				

Source: Field Survey (2024), KMO – Kaiser-Meyer-Olken

### The Challenges Encountered by Ginger Women Producers

The challenges faced by ginger women farmers are put through analysis of PCM (Principal Component

Model). The PCM has the capacity to withhold unrelated limitations that have Eigenvalues of more than one, and discard limitations that have Eigenvalues of less than one. Approximate 6 limitations were reserved by the model. The criteria for



selecting those challenges that were retained are explicitly those with Eigenvalue greater than one (1). The first limitation is the lack of credit facilities with an Eigenvalue approximately 9.4714 which explains about 34.74% of all restraints included in the model. The second limitation is the lack of access to farmland with an Eigenvalue of approximately 3.7144 which explained 11.96% of all hindrances included in the model. The third limitation is the lack of farm input with Eigenvalue of approximately 2.9273 which explains about 10.22% of all impediments included in the model. All the limitations withheld by the model explained 78.04% of all the restraints identified by the ginger women farmers. The chi-square value (3579.13) is different significantly from zero at the  $p=0.000$  probability level, this confirmed the use of the PCM for the estimation (Table 5).

### CONCLUSION and RECOMMENDATIONS

The study estimated the poverty status among the ginger women producers in Kaduna State, Nigeria. A multi-stage sampling design was employed. Primary data were utilized based on a well-structured questionnaire. Descriptive and inferential statistics were employed for data analysis. The outcome of the research shows that the average age of ginger women farmers was 48 years. The mean years of farming experience was 9 years. On average, the ginger women farmers had 11 years of school education. Similarly, about 98% of ginger women farmers were married. Approximately 58% of respondents were members of cooperative organizations, while 45% had access to credit. The poverty line is approximately 4, 172.06 Naira (1 USD = 1 104 Naira). The poverty incidence ( $P_0$ ), poverty depth ( $P_1$ ), and severity of poverty ( $P_2$ ) were estimated using Foster, Greer, and Thorbecke (FGT) index approximating ( $0 \leq P \leq 1$ ). The ( $P_0$ ) is approximated at 0.5298, 95% CI [ 0.4575, 0.6021] this corresponds to 52.98% of ginger women farmers are poor, from the sampling populations, this adds up to the fact that 63 ginger women farmers are poor, while 57 ginger women farmers were non-poor. Approximately 4.7 million of the entire population were poor, while 4.2 million were non-poor. The ( $P_1$ ) which explains that the gap between the poor ginger women farmers and the poverty line is 0.2876, 95% CI [0.2465, 0.3287], it will take the poor ginger women farmers [28.76%  $\times$  4, 172.06 Naira) the amount of 1, 199.88 Naira to cover up or make up for the poverty gap. The ( $P_2$ ) which expresses the distance of each poor ginger women farmer to one another was calculated at 0.1967, 95% CI [ 0.1575, 0.2359] this stands for that 19.67% of the ginger women farmers were severely poor. The educational level, years of ginger farming, income from ginger farming, off-farm income, and membership of cooperative were different significantly

from zero in influencing poverty status among ginger women producers. The major limitations faced by ginger women producers were lack of credit facilities (1<sup>st</sup>), lack of access to land (2<sup>nd</sup>), and lack of farm inputs (3<sup>rd</sup>). Established on the outcomes of this research, the following recommendations were made:

- (i) Credit (finance) at single digit interest rate, devoid of cumbersome administrative procedures should be made available to ginger women producers to purchase agrochemicals, fertilizers, and other farm inputs at appropriate times, this will increase productivity and reduce poverty.
- (ii) The government should in terms of policy formulations allow ginger women farmers easy access to farmland, this will increase productivity and reduce the level of poverty.
- (iii) Farm inputs such as improved varieties, fertilizers, and agrochemicals should be provided for ginger women farmers at subsidized prices to increase income and productivity.
- (iv) Ginger women farmers should form themselves into cooperative organizations for easy access to farm inputs, credit, and bulk sales of produce.
- (v) Access to market linkages is necessary for the ginger produce to be sold at appropriate prices.

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## The Dairy Farming Future from Farmers' Perspectives in The Northern Region of Iraq

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### ABSTRACT

The main purpose of this study was to analyze dairy farmers' feelings of safety about the future of dairy farming in the northern region of Iraq and to investigate how environmental factors and demographic characteristics of dairy farms impact their sense of security. Primary data were collected from 280 dairy farmers in four provinces of northern Iraq: Erbil, Sulaymaniyah, Dohuk, and Halabja. Descriptive statistics, the Chi-square test, and binary logistic regression were employed for analysis. The results of binary logistic regression analysis indicated that age, gender, marital status, education, experience, and household size significantly influenced farmers' perceptions of whether dairy farming could secure their future. Moreover, the binary logistic analysis revealed that five elements of dairy farming – technology, dairy farm structure, government policy, marketing, and extension – were significantly associated with farmers' perceptions of future security through dairy farming. However, the study concludes that to promote agricultural development at the macro level, it is essential to prioritize the provision of support services to farmers in policy formulation so that farmers can effectively leverage these services to enhance farm production and income levels.

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## Kuzey Irak'ta Yetiştiricilerin Bakış Açısıyla Süt Sığırcılığı İşletmelerinin Geleceği

### ÖZET

Bu çalışmanın temel amacı, Kuzey Irak'ta süt sığırcılığı işletmelerinin süt hayvancılığının geleceğine ilişkin güvenlik duygularını analiz etmek ve işletmelerin çevresel faktörlerinin ve demografik özelliklerinin güvenlik duygularını nasıl etkilediğini araştırmaktır. Birincil veriler, Irak'ın kuzey bölgesindeki dört ildeki (Erbil, Süleymaniye, Dohuk ve Halepçe) 280 süt çiftçisinden toplanmıştır. Analiz için tanımlayıcı istatistikler, Ki-kare testi ve ikili lojistik regresyon kullanılmıştır. İkili lojistik regresyon analizinin sonuçları, yaş, cinsiyet, medeni durum, eğitim, deneyim ve hane büyüklüğünün çiftçilerin süt hayvancılığının geleceklerini güvence altına alıp alamayacağına ilişkin algılarını önemli ölçüde etkilediğini göstermiştir. Ayrıca, lojistik analizi, süt sığırcılığının beş unsuru olan teknoloji, süt çiftliği yapısı, hükümet politikası, pazarlama ve yayımın çiftçilerin süt hayvancılığı yoluyla gelecekteki güvenlik algılarıyla önemli ölçüde ilişkili olduğunu ortaya koymuştur. Bununla birlikte, çalışma, makro düzeyde tarımsal kalkınmayı teşvik etmek için, politika oluşturma sürecinde çiftçilere destek hizmetlerinin sağlanmasının önceliklendirilmesinin gerekli olduğunu, böylece çiftçilerin bu hizmetlerden etkili bir şekilde yararlanarak çiftlik üretimini ve gelir seviyelerini artırabileceklerini vurgulamaktadır.

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## INTRODUCTION

Governments in developing countries have been progressively engaged in supporting the commercial agricultural crop and livestock sectors recently (Nakane and Tauer, 2009). Many countries have introduced various support programs to increase farm profitability (Smith and Glauber, 2012). Iraq is dependent heavily on milk and other animal products in their regular diet. However, a significant part of the milk and dairy products needed are imported, especially from the Gulf countries and Türkiye (Hakim and Akbay, 2022). Over the past few years, several livestock projects have been implemented in the region for various purposes. For example, the distribution of cultured breed cows has increased significantly in recent years (UNDP, 2019). As a result, the government has recognized the drain of foreign exchange resources flowing out of the country, and, as a relief measure, various policies have been implemented to steer the dairy sector towards development, particularly to increase domestic production (Abid-Al Ammer et al., 2017). Despite this progress, recent policies aimed at addressing related challenges have not been adequately formulated and implemented (Nanakali, 2021).

Due to the current political approach, livestock's share of the country's wealth resources is not properly designed to meet the serious financial needs of producers. On the other hand, inflation and a sharp increase in livestock costs for farmers have resulted in a surge of economic activity in both the national and local economies. In addition, the growing and hidden rent-seeking in the country's layers of management and economy has caused more and more serious challenges for livestock to progress. This is because steps have not been taken to use political and economic management of the land to support livestock and farmers by ignoring irregular livestock imports, supporting importers, and planning to control and regulate imports.

Over the past 30 years, the ability of the Iraqi Ministry of Agriculture and Water Resources and other government institutions to provide agricultural services has deteriorated significantly (Lucani and Saade, 2012; UNDP, 2019). Budget cuts have led to a decline in skilled human resources in agricultural support services. It is necessary to strengthen the capacities of the government and smallholder organizations. Policy gaps must be addressed to help increase the sector's competitiveness and contribute to rural development and poverty alleviation. Furthermore, due to a lack of security and stability, in addition to an uncertain policy environment, the private sector has made no significant investments in agriculture (IFAD, 2017). Security, unclear requirements for business registration and closure,

licensing requirements, limited communication infrastructure and logistics, difficulties in accessing finance, and an uncompetitive business environment with a lack of transparency and clear legal framework for rules-based market competition are all problems in the region. In addition, it is said that obtaining a license is difficult if one does not have the right contacts. Contracts are awarded to farmers who have good relationships with the authorities, who often control important political and governmental positions, and who usually subcontract the work after they have received their share (Jongerden et al., 2018).

To date, there is little academic literature that comprehensively examines the impact of government policies on dairy farms in Iraq's northern region. Bragg and Dalton (2004) investigated factors influencing farmers' decision to exit dairy farming and stated that older producers, those with higher off-farm income, lower returns relative to variable costs, and greater farm income diversification were more likely to abandon dairy farming. Nakane and Tauer (2009) further highlighted that exit decisions are influenced by factors other than milk prices and suggested that national or regional dairy programs should consider strategies other than price support to ensure a stable dairy industry and reduce the rate of dairy farm exit decisions. To optimize the development of dairy farms, the effects of government policy, farmer training, the structure of dairy farms, environmental issues, and milk safety must be investigated. The primary objective of this study is to analyze dairy farmers' feelings of safety concerning the future of dairy farming in the northern region of Iraq. Additionally, the study explores farmers' perceptions of their dairy farms, aiming to identify the reasons behind these perceptions and the environmental factors influencing them. In addition, it investigates the impact of demographic characteristics of dairy farms on farmers' sense of security regarding the future of dairy farming, which has been little studied in previous research. Our hypotheses are:

- ✓ Government policy has a positive and significant influence on the farmers' view of dairy farming's future security.
- ✓ Structural characteristics of a dairy farm have a positive and significant influence on farmers' view of dairy farming's future security.

This research aims to address these gaps by contributing a detailed investigation into the multifaceted challenges faced by dairy farmers. The findings are expected to inform the design of more effective and targeted policy interventions, ensuring sustainable development and stability of the dairy industry in the northern region of Iraq. For the government of the northern region of Iraq, this study is valuable in that it provides general information on



the state of the dairy sector and the financial constraints that influence it in the region. Policymakers will find this study crucial in developing and implementing policies that support and enhance the dairy sector.

### MATERIAL and METOD

A survey was conducted to assess the status of dairy farming in the northern region of Iraq. Four provinces, namely Erbil, Sulaymaniyah, Dohuk, and Halabja were selected for the survey because they encompass the northern region of Iraq, have a high number of dairy animals, and represent a range of geographical and economic conditions. The survey was based on face-to-face interviews with dairy farmers. The sample size was calculated using the following formula (Newbold, 1995):

$$n = \frac{Np(1-p)}{(N-1)\sigma_{p_x}^2 + p(1-p)}$$

where n is the sample size, N is the size of the population (25600), p=0.5 is the probability of examining situations occurring (the absence of preliminary information concerning government policy effect factors), q is the probability that the situation will not occur (q = 1-p), and  $\sigma_{p_x}^2$  is the variance ratio.

The sample size was calculated as 280 to empower the research by using an acceptable marginal error (5.85) and 95% confidence interval. Questionnaires were distributed randomly.

The chi-square test and binary logit regression were used to analyze the effect of dairy farmer characteristics on farmers' confidence about the future of dairy farming. Regression analysis is a multivariate technique that predicts a binary dependent outcome from a set of independent variables to estimate the probability that an event will or will not occur (Draper & Smith, 1981). Logistic regression does not require a linear relationship between the dependent and independent variables. Moreover, the main advantage of the logit model is that it makes no assumptions about the normality, linearity, or homogeneity of variances of the independent variables (Mutura et al., 2015). Logistic regression is widely used in agriculture, biology, medicine, and social sciences (Begizew, 2008). In this study, the logit regression model is used because there are two binary events, farmers consider whether dairy farming secures their future (or not). The binary logistic model used in this study is specified as follows:

$$P = p(Y = 1 / X) = \frac{e^{\beta_0 + \sum_{i=1}^n \beta_i X_i}}{1 + e^{\beta_0 + \sum_{i=1}^n \beta_i X_i}}$$

Where, P is the dairy farming secures their future

(P(Y=1/X),  $X_i$  are the explanatory variables such as age, gender, marital status, education, household size, land size, experience, perceptions, and  $\beta_i$  are coefficients of corresponding variables (Ganiyusufoglu et al., 2022).

$$1 - P = p(Y = 0/X) = \frac{1}{1 + e^{\beta_0 + \sum_{i=1}^n \beta_i X_i}}$$

A transformation of P known as the logit transformation is defined as:

$$\text{Logit } P = \log \left[ \frac{P}{1-P} \right] = \beta_0 + \sum_{i=1}^n \beta_i X_i$$

The Odds ratio (Exp  $\beta$ ) shows how many times (relatively) a unit increase in a single explanatory variable increases the probability of the dependent variable if all other variables are kept constant (Hosmer et al., 2013; Hair et al., 2014).

### RESULTS AND DISCUSSIONS

According to the results of the surveys, 81% of farmers in the research area were male and 65% were married. The average age of the farmers was 42.05 years, with 31% of them aged less than 30 years and 33% aged older than 40 years. The educational level of farmers indicates that 42% were illiterate or had completed elementary school, while 19% had graduated from university. Results indicated that 69% of the farmers had more than four family members, and 76% had experience in the field for more than six years. Social protection programs are absent in the northern region of Iraq, as indicated by the survey results where 76.1% of farmers suggested the necessity to eliminate the deficiency in social protection policies aimed at protecting agriculture. Moreover, 70.4% of the farmers stated that animal husbandry is profitable and that they want to continue dairy farming in the future (Table 1). However, a significant number of farmers stated that it is necessary to be cautious before entering livestock, and they are not sure whether profitability will continue in the future.

The northern region of Iraq faces many challenges that directly impact the country's economic sectors. These challenges include weak institutions, the security situation, and corruption (Lucani and Saade, 2012). According to the results, 72.5% of the farmers stated that corruption in the region affects the economy as well as the dairy sector. Moreover, low productivity and growth rates in the sector are attributed to various problems and government policies. The results reveal that 46.1% of the participants were not satisfied with the government's policies for the expansion of animal husbandry-related service support (Table 1).

Agricultural subsidies are broadly classified into two types: domestic subsidies and export subsidies. They encompass various support mechanisms such as deficiency payments, direct payments, compensatory

payments, crop insurance assistance, livestock assistance, rural development assistance, and environmental set aside (Akbaş et al., 2016). Despite the significant role of subsidies in agriculture (Akbaş and Bilgiç, 2023), the dairy sector has notably received less support from both the government and the public sector. Our analysis of the dairy farms identified both

favorable and negative factors influencing the development of the sector. The findings revealed that approximately 42.5% of farmers initiated dairy farming with financial assistance inherited from relatives, whereas 57.5% started with their resources (Table 2).

Table 1. Farmers' perspectives, needs and satisfaction with dairy farming

*Çizelge 1. Çiftçilerin süt çiftçiliğiyle ilgili bakış açıları, ihtiyaçları ve memnuniyetleri*

Variable	Answer	Number of farms	Percent
Do retired farmers need social protection?	Yes	213	76.1
	No	67	23.9
Will you keep dairy farming?	Yes	197	70.4
	No	83	29.6
Do you think corruption has affected your job?	Yes	203	72.5
	No	77	27.5
Are you satisfied with the level of support you receive from the government?	Satisfied	59	21.1
	A little satisfied	62	22.1
	Not satisfied	129	46.1
	No idea	30	10.7

Table 2. Sources and purposes of loans in dairy farming

*Çizelge 2. Süt sığırcılığı işletmelerinde kullanılan kredilerin kaynakları ve amaçları*

Variables	Number of farms	Percent
<b>Initial funding to set up dairy farming</b>		
From relatives	119	42.5
My own resources	161	57.5
<b>Source of loans</b>		
Friends, relatives, neighbors	238	85.0
Agricultural bank	42	15.0
<b>Purpose of loan</b>		
Land operations	36	12.9
Purchase of in-calf heifers	141	50.4
Purchase of dairy feeds	47	16.7
Others	56	20.0
<b>Reasons for not accessing credit services</b>		
High tax rate	49	17.5
Demand for collateral	79	28.2
Lengthy process	72	25.7
Lack of access	80	28.6
Total	280	100.0

There are no government-owned dairy farms in the northern region of Iraq. Most dairy farms in the northern zone are privately owned. According to the findings, 15% of farmers obtained credit from agricultural banks, while 85% relied on credit from friends, relatives, and neighbors. Credit from these sources was used for various purposes, including production, meeting consumer demands, and fulfilling social obligations. Notably, there are no collateral requirements for credit from these personal sources, and no interest is charged in the area. An oral promise, confidence, trust, and mutuality are frequently sufficient as collateral or security. In contrast,

agricultural bank loans generally entail sensitivity to collateral requirements and are subject to interest charges. Interestingly, this study revealed that many farmers borrowed money from agricultural banks without making projects.

The survey findings underscored the pivotal role of credit in financing essential investments for dairy farming, with expenditures primarily allocated to purchasing in-calf heifers (50.4%), constructing barns or for social purposes (20%), purchasing milk feed (16.7%), and buying land (12.9%). Credit plays a vital role in financing investments and farm processes essential for commercializing dairy farming (Ağır and

Akbay, 2018). Despite its importance, producers in the northern region of Iraq demonstrated limited knowledge of dairy farming credit issues, resulting in low credit utilization rates. Furthermore, it delved into factors influencing credit availability for producers, highlighting key challenges such as lack of access (28.6%), stringent collateral requirements (28.2%), protracted application processes (25.7%), and elevated tax rates (17.5%).

### Impact of Dairy Farmers' Characteristics on the Future Security of Dairy Farming

Table 3 provides detailed descriptions of the explanatory variables used in the Logit model. The Logit model is used in this context because it effectively handles binary outcome variables, like whether farmers are confident or not about the future of dairy farming. At the heart of our inquiry lies a

pivotal question: "Do you feel confident about the future of dairy farming?" This question serves as our dependent variable, delineated as a binary outcome where "satisfied" corresponds to (1) and "dissatisfied" to (0). The findings reveal that 62% of farmers expressed confidence in the future of dairy farming. However, a significant portion, comprising 38% of farmers, were not confident and had a view that working on a dairy farm was the last choice of work. They argue that working on dairy farms requires effort and time, that it is a complicated, dirty, non-potential job that threatens their future, and that it does not pay well. Consequently, they envision alternative pathways for their future endeavors, including aspirations to join the public sector as government employees, army or police officers, among other pursuits.

Table 3. Definition and descriptive statistics of variables used in the model

*Çizelge 3. Modelde kullanılan değişkenlerin tanımı ve tanımlayıcı istatistikleri*

Variables	Definition of variable	Mean	Standard deviation
Dependent variable	Feel confident about the future of dairy farming: 1 for Yes; 0 for No	0.62	0.49
Age1	1 if the household head is aged less than 30; 0 otherwise	0.31	0.47
Age2	1 if the household head is aged between 31 and 40; 0 otherwise	0.36	0.48
Age3	1 if the household head is aged above 40; 0 otherwise	0.33	0.47
Edu1	1 if the household head graduated from elementary school or is illiterate; 0 otherwise	0.42	0.49
Edu2	1 if the household head graduated from high school; 0 otherwise	0.39	0.49
Edu3	1 if the household head graduated from university; 0 otherwise	0.19	0.40
Gender	1 if the household head is male; 0 otherwise	0.81	0.39
Marital status	1 if the household head is married; 0 otherwise	0.64	0.48
Experience	1 if having work experience is above 6 years; 0 otherwise	0.30	0.46
HSize	1 if the household size is $\geq 5$ ; 0 otherwise	0.31	0.47
Land	1 if agricultural land size is $>10$ dunam*; 0 otherwise	0.20	0.40

\*: 1 dunam= 2500 m<sup>2</sup>

The model summary shows that the log-likelihood statistic is 317.17 and the Chi-square test value is 54.31 with a p-value of 0.0001, indicating the joint significance of all coefficient estimates (Table 4). The correctly predicted result indicates that the model predicted 79.1% of the observed ratings correctly, which is considered adequate. Estimated coefficients are tested using standard errors, t-ratios, and p-values. Therefore, all statistically significant coefficients, based on a two-tailed test at  $\alpha = 0.1, 0.05,$  and  $0.01$  levels, were noticeable. In addition, as P-values, statistics obtained for the independent variables indicate that the coefficients for gender, marital status, age and education of farmers, and farm size were statistically significant.

The findings of the binary logit regression reveal a strong relationship between the age of dairy farmers and the future security of dairy farming. Therefore, the age of the farmer is a crucial factor. The results

indicate that farmers aged between 31 and 40 years are 3.19 times more likely to feel that dairy farming secures their future compared with younger farmers. Moreover, for farmers aged over 40 years, this number was 3.18 and positive. The positive sign on the age coefficient indicates that older and middle-aged farmers are 3.2 times more likely to feel that dairy farming secures their future than younger farmers.

Education is thought to play a critical role in farming, especially in the adoption of new technologies, training, and putting into practice the knowledge gained (Lien et al., 2017). According to the statistically significant coefficient, education has a negative effect on dairy farming's future security. The results of the binary logit regression indicate that farmers' education level is strongly related and a critical factor affecting dairy farms. The outcomes indicated that farmers at the high school level were 0.43 times less likely to continue dairy farming to secure their future, and

farmers at the bachelor level were 0.36 times less likely to feel that dairy farming secures their future than farmers who are illiterate or have a primary education degree. The negative impact of education level on the perceived future security of dairy farming might initially appear counterintuitive. However, this result can be attributed to the fact that individuals with higher education often have access to a broader range of career opportunities. These educated individuals may perceive dairy farming as less secure or less appealing compared to other professions that offer better job security, higher income, or improved working conditions. As a result, they are more likely to explore alternative career paths that align with their qualifications and offer greater long-term stability.

The estimation results of a statistically significant coefficient showed that male farmers were 0.47 times less likely to be involved in dairy farms and felt dairy farming secures their future. This is because women are more likely than men to take up dairy farming, given similar opportunities and incentives. However, the country has made only marginal progress in breaking down the cultural barrier that prevented women from owning cattle in their families (Manell and Roberson, 2015). However, the findings for the northern region of Iraq could be explained by the fact that livestock management roles are traditionally performed by women in Kurdish ethnic societies. In the research area, husbands are involved in dairy production in specific roles such as consulting veterinary services, sales, or purchasing animals.

The cultural and social norms related to marital status often emphasize the stability and support system provided by marriage, which is beneficial for farming operations. In many communities, marriage is seen as a partnership where both spouses contribute to the success of the household and its economic activities, including farming. Family members assist each other in making and implementing critical farming decisions (Ramos, 2021). The research indicates a statistical correlation between marital status and future dairy farming security. The model suggests an odds ratio of 2.08 (greater than one), which could mean that married farmers are 2.08 times more likely to continue dairy farming to secure their future, as compared to singles. In addition, some farmers in the village usually prefer to marry at least two wives to have many partners and/or children to help them in their farming or livestock activities.

Another significant socio-demographic characteristic was the household size of dairy farms. Household size is known to be a source of labor for farm and off-farm income-generating activities, sometimes in a village setting (Kuteesa and Waholi, 2018). Large families can provide more labor resources for dairy farming, which is labor-intensive. Family members can assist with various tasks such as feeding, milking, and caring for

the animals, as well as maintaining the farm. This can reduce the need to hire external labor, lowering costs and increasing efficiency. The odds ratio of the coefficient indicates that farmers with large household sizes are 2.6 times more likely than farmers with small household sizes to engage in dairy farm activities.

Considering the statistically significant and positive coefficient of experience, farmers with high farming experience are more likely to continue dairy farming, compared with those with low farming experience. Experience acquired in dairy farming is a de facto reason why experienced farmers are more likely to continue dairy farming than less experienced farmers.

The availability of land is indispensable for dairy farming (Anugrah et al., 2021). Table 4 shows a significant and negative relationship between land size and respondents' satisfaction with dairy farming's future security. This implies that when respondents' landholding size increases, their perceived secure future in dairy farming decreases. The most likely reason for this seems to be that small-scale farms continue to use dairy cattle to survive. On the other hand, on plant production farms, there is a desire to earn more income from dairy farming in the future, where dairy farming is done more intensively in farms with less land. Therefore, enterprises with less land believe that animal husbandry will be more beneficial for their future than enterprises with more land. The most likely reason is that more land allows farmers to increase production, which generates more income that can be used to purchase farm inputs. As a result, farmers with relatively large farm sizes are more likely to use improved technologies. This also implies that respondents with larger farm sizes will seek newer ideas.

Bragg and Dalton (2004) investigated the factors influencing the decision to exit dairy farming. According to their results, the exit decision was significantly influenced by four variables; older producers, those with higher off-farm income, lower returns over variable costs, and greater farm income diversification were more likely to abandon dairy farming. Because exit decisions are influenced by factors other than milk prices, national or regional dairy programs should consider strategies other than price support to secure a stable dairy industry and a lower rate of dairy farm exits.

### **Effect of Perception on Farmers' Feelings of Safety Regarding the Future of Dairy Farming**

The results also support the hypothesis that dairy farming to secure the future of farmers is not related only socioeconomic characteristics of producers but also to the satisfaction of producers with technology, government support, advisory service, veterinary service, marketing, and climate change. A binary logistic model was used to analyze the effect of



dimension factors on the dependent variable. Table 5 gives descriptive statistics of the variables used in the

model. “Do you feel confident about the future of dairy farming.” was used as a dependent variable.

Table 4. Effect of farmers characteristics on farmers' feelings of safety regarding the future of dairy farming  
*Çizelge 4. Çiftçilerin özelliklerinin süt çiftçiliğinin geleceğine ilişkin güvenlik duyguları üzerindeki etkisi*

Variables	Coefficient	Standard error	P-value	Odds ratio
Constant	0.636	0.468	0.174	1.889
Age2	1.155***	0.341	0.001	3.186
Age3	1.159***	0.338	0.001	3.180
Edu2	-0.817**	0.327	0.042	0.432
Edu3	-0.840**	0.412	0.028	0.359
Gender	-0.752*	0.395	0.057	0.472
Marital status	0.778***	0.282	0.006	2.176
Experience	0.769*	0.471	0.100	2.158
HSize	0.974**	0.452	0.031	2.647
Land	-0.711**	0.339	0.036	0.491

-2 Log likelihood: 317.17; Chi-square (P- value): 54.31(0.000); Correctly predicted: 79.1%

Note: \*, \*\* and \*\*\* indicate significance levels at 10%, 5% and 1% respectively

Table 5. Descriptive statistics of the variables on some perceptions of farmers' feeling of safety regarding the future of dairy farming in the model

*Çizelge 5. Modelde süt işletmelerinin geleceğine ilişkin çiftçilerin güvenlik hissine ilişkin bazı algılarla ilgili değişkenlerin tanımlayıcı istatistikleri*

Perceptions	Definition	Definition of variables	Mean	S. D.
Structural characteristics of dairy farm	Relating to herd size, race, lactating, type of cow	1= Dissatisfy.... 5= Satisfy	1.65	0.256
Dairy cattle technology used by farmers	Directly correlated with the technology used by farmers	1= Dissatisfy.... 5= Satisfy	2.23	0.611
Government support and policies	Farmers' opinion about government support	1= Dissatisfy.... 5= Satisfy	1.88	0.256
Extension and advisory services	Information about dairy farming	1= Dissatisfy.... 5= Satisfy	2.29	0.611
Marketing	Selling products through different channels	1= Dissatisfy.... 5= Satisfy	1.89	0.305
Veterinary medicines	Veterinary medicine used by farmers	1= Dissatisfy.... 5=Satisfy	2.22	0.616
Climate change	Perceived effect of climate change on dairy farming	1= Decreasing... 5=Increasing	2.30	0.625

The model summary shows that the log-likelihood statistic is 313.99 and the chi-square test value is 57.49 with a p-value of 0.0001, indicating joint significance of all coefficient estimates. The Correctly predicted result indicates that the model predicted 70% of the observed ratings correctly, which is considered adequate (Table 6). Model results showed that coefficients for dairy farm structure, dairy cattle technology used by farmers, government support and policy, extension and advisory services, and marketing

were significant. The coefficients for veterinary medicine and climate change were found to be insignificant.

The results of the binary logistic regression explained that the structural characteristics of dairy farms had a positive and statistically significant relationship with dairy farming future security, and farmers with a well-structured dairy farm were more likely to be satisfied (P=0.015). There was also a significant relationship between dairy farming future security and farmers

who were satisfied with their use of dairy cattle technology (P=0.001). The results also indicated that dairy farming and government support and policy were statistically significant and negatively correlated (P=0.001). Erdal et al. (2020) stated that the amount of support provided per animal is insufficient and this support item is the most important factor in increasing animal presence in Türkiye. Likewise, there was a negative and statistically significant relationship between extension and advisory services and dairy farming future security (P=0.044).

In our study, we observed significant coefficients reflecting the impact of various factors on dairy farming's future security. Notably, marketing exhibited a positive effect (P=0.046), indicating its role in enhancing the stability and prospects of dairy farming enterprises. Moreover, veterinary medicine

had a negative and insignificant relationship with dairy farming's future security (P=0.381). This result may be since veterinarians are not allowed to access their farms during outbreaks in rural areas. Climate change affects the quantity and quality of feed differently, depending on location, livestock system, and species (Rojas-Downing et al., 2017). Interestingly, a majority of farmers expressed disagreement regarding the perception that their farms have encountered more variable or unusual weather patterns. Additionally, our analysis revealed a non-significant relationship between a farmer's belief in anthropogenic climate change and their observation of more variable weather on their farm (P=0.911). These findings underscore the complexity of climate-related perceptions and their correlation with on-farm experiences.

Table 6. Effect of perceptions on farmers' feelings of safety regarding the future of dairy farming

*Çizelge 6. Alguların çiftçilerin süt işletmeciliğinin geleceğine ilişkin güvenlik duyguları üzerindeki etkisi*

Variables	Coefficient	Standard error	P-value	Odds ratio
Constant	-3.044	1.993	0.127	0.048
Structural characteristics of dairy farm	1.900**	0.783	0.015	6.684
Dairy cattle technology used by farmers	2.878***	0.846	0.001	17.786
Government support and policies	-2.071***	0.599	0.001	0.126
Extension and advisory services	-0.320**	0.163	0.040	0.726
Marketing	0.419**	0.215	0.046	1.521
Veterinary medicines and food security	-0.429	0.490	0.381	0.651
Climate change	-0.094	0.209	0.654	0.911

2 Log likelihood: 313.99; Chi-square (P- value): 57.49 (0.000); Correctly predicted: 70.0%

Note: \*, \*\* and \*\*\* indicate significance levels at 10%, 5% and 1% respectively

## CONCLUSION and RECOMMENDATION

The main objective of this study was to investigate the opinions of producers on the future of dairy farming in northern Iraq. The dairy sector in the region is largely characterized by small-scale, disorganized milk animal holders, as well as scattered, insufficient, and inappropriate animal feeding and health care.

The findings reveal that corruption in the region is a major concern negatively affecting the dairy sector. Farmers' confidence in dairy farming is influenced by age, education, gender, household size, and experience. Older farmers and those with larger households are more optimistic, while female and more educated farmers are less confident. It is recommended that government policies address these gaps, particularly by encouraging female participation and tackling cultural barriers.

Key recommendations include reducing corruption, introducing social protection programs, improving government support, and easing access to credit.

Efforts should also focus on enhancing market competitiveness and addressing production challenges like the high cost of feed. Farmers need better access to training and education on modern dairy practices, which can be achieved through collaboration between public and private institutions.

Improving government support mechanisms and promoting technological adoption are vital for the sector's growth. Large-scale dairy farms should be encouraged for qualified applicants, and ongoing training on livestock management and disease control is essential. To enhance cross-border trade, the government should improve monitoring and control of imported dairy products. Future research should focus on the long-term impacts of advanced technologies, sustainable practices, and the role of government policies in ensuring the sector's sustainability and profitability.

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### Researchers' Contribution Rate Statement

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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## Analysis of Factors Affecting Household Food Consumption Away From Home in Türkiye

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### ABSTRACT

One of the most important changes in consumer food consumption habits in recent years is the trend toward food consumption away from home (FAFH). The effects of socioeconomic and demographic factors on FAFH expenditure and consumption decisions were jointly investigated using the inverse hyperbolic sine double-hurdle (IHS-DH) model, and household survey data consisting of 1655 consumers in 14 cities in Türkiye. According to the results, the consumer being a male, having a high school or university diploma, an extra person generating income outside the family head, having children under the age of 18, and having high income increased the likelihood of consuming FAFH. However, the probability of FAFH consumption spending decreases with the variables of the consumer age, seasonal variables, geographical regions, and family size. On the other hand, consumers' unconditional FAFH consumption expenditure increases when the variables of male, high school or university diploma, internet, children under the age of 18, and visiting shopping centers frequently. However, the presence of regular meals in the family decreases FAFH consumption. This research sheds light on the nuanced interplay of various socioeconomic and demographic factors shaping consumer behaviors regarding FAFH consumption, thereby offering valuable insights for policymakers and stakeholders in the food industry.

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## Türkiye'de Tüketicilerin Ev Dışı Gıda Tüketimi ve Tüketimi Etkileyen Faktörlerin Analizi

### ÖZET

Tüketicilerin gıda tüketim alışkanlıklarında son yıllarda yaşanan en önemli değişikliklerden biri de ev dışında gıda tüketimine (FAFH) yönelik eğilimdir. Sosyoekonomik ve demografik faktörlerin FAFH harcama ve tüketim kararları üzerindeki etkileri, ters hiperbolik sinüs çift engel (IHS-DH) modeli ve Türkiye'deki 14 ildeki 1655 tüketiciden oluşan hane halkı anketi verileri kullanılarak araştırılmıştır. Sonuçlara göre tüketicinin erkek olması, lise veya üniversite diplomasına sahip olması, aile reisi dışında gelir elde eden bir kişi olması, 18 yaş altı çocuk sahibi olması ve gelirin yüksek olması FAFH tüketme olasılığını arttırmaktadır. Ancak FAFH tüketim harcaması olasılığı tüketici yaşı, mevsimsel değişkenler, coğrafi bölgeler ve aile büyüklüğü değişkenleriyle birlikte azalmaktadır. Öte yandan tüketicilerin erkek olması, lise veya üniversite diplomasına sahip olması, internet kullanımı, 18 yaş altı çocuk sahibi olması ve alışveriş merkezlerini sık ziyaret etme değişkenleri göz önüne alındığında tüketicilerin koşulsuz FAFH tüketim harcamaları artmaktadır. Diğer taraftan, ailede öğünlerin düzenli olması FAFH tüketimini azaltmaktadır. Bu araştırma, FAFH tüketimine ilişkin tüketici davranışlarını şekillendiren çeşitli sosyoekonomik ve demografik faktörlerin etkilerine ışık tutmakta ve böylece gıda endüstrisindeki politika yapıcılara ve paydaşlara değerli bilgiler sunmaktadır.

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## INTRODUCTION

Recent economic progression and lifestyle changes in Türkiye have led to an increase in the number of people who consume food outside of the home. In particular, the intense participation of family members in business and the increasing share of working women have changed the lifestyle of individuals as well as their eating habits (Bugra & Cakar, 2010; Wang et al., 2014; Saksena et al., 2018). One of the most important changes in consumer food consumption behavior in recent years is the increasing tendency to consume more food away from home (FAFH). Consumers spend less time preparing food at home (FAH) than in previous years (Smith et al., 2013; Monsivais et al., 2014), leading to an increase in FAFH consumption expenditure. FAFH consumption is primarily related to food and beverage consumption outside the home. However, in the commercial sense, FAFH means that consumers purchase and consume food and beverages produced and served by a commercial enterprise outside the home (Seguin et al., 2016; Saksena et al., 2018). Although this trend is occurring worldwide, there are some structural differences between developed and developing countries. For example, in the United States, households allocate about half of their total food budgets to FAFH consumption expenditures (27% in 1960 compared to 50% in 2010), compared to around 16% in Italy and 25% in Greece, from 25% in 1996 to 30% in 2006 in Spain, from 24% in 2002 to 31% in 2008 in Brazil and from 22% in 1995 to 31% in 2019 in the United Kingdom (UK) (Mihalpoulos & Demoussis, 2001; Montini, 2001; Mutlu & Gracia, 2006; Bezerra et al., 2013; Saksena et al., 2018; Law et al., 2022).

A similar situation has been observed in Türkiye. FAFH consumption increased significantly compared to total food consumption. According to Turkish Statistical Institute (TURKSTAT) data, the share of food expenditure in total expenditure in 1994 was approximately 35%, which decreased to 26% in 2018. On the other hand, the share of FAFH food consumption expenditures in total food expenditure increased from approximately 3% to 15% in the same period (TURKSTAT, 2018). The changes in socio-demographic and economic structure observed in Türkiye in recent years have had an important effect on this change in FAH and the FAFH consumption structure of consumers. Although the share of FAFH consumption expenditure in total food expenditure is not at the level of the United States and other remaining developed countries, the increasing trend observed in recent years is worth emphasizing.

The agricultural sector, which contributes to social

welfare by providing the final food needs of the population, is closely related to such phenomena until the final consumption stage. At this point, consumer decisions guide all sectors. FAH and FAFH consumptions are influenced by both income and price, along with numerous other factors. The change in the demographic structure of the household, the increase in the number of women participating in the business life, more two-earner households (couples), the decreasing household size, the increase in the income level, the increase in the level of education, the use of credit cards, and the rapid increase of different brands of fast-food restaurants and cafeterias and the increased urbanization cause an increase in households' FAFH consumption and expenditure (Byrne et al., 1998; Mihalpoulos & Demoussis, 2001; Montini, 2001; Fanning et al., 2010; Akbay et al., 2007; Angulo et al., 2007; Liu, 2011; Seguin et al., 2016; Saksena et al., 2018).

The rapid increase in the number of domestic franchises and opened fast-food restaurants in recent years, mainly in shopping malls are among the most important inducements for the development of out-of-home food consumption in Türkiye. Especially in metropolitan or big cities, the number of western-origin McDonald's, Pizza Hut, and Burger King restaurants selling pizza and hamburgers has increased significantly. By 2018 among foreign fast-food restaurants in Türkiye, Burger King ranked first with 651 restaurants, followed by Domino's Pizza (381), McDonald's (225), Popeye's (200), Starbucks (161), Sbarro (107), KFC (72), Subway (72), Pizza Hut (45), and Arby's (68 restaurants) (Tavak, 2018). Thus, FAFH consumption not only enriches the food range and development of the Turkish catering industry and other related businesses but also offers great opportunities to attract foreign investors and increase employment in the catering sector in the country.

In addition, many international restaurants operate in Türkiye and pursue expansion policies. Besides these, many local fast-food chains such as Mado (200), Sultanahmet Koftecisi (130), Usta Dönerci (135), Simit Sarayı, and Pizza Pizza (150) operate in Türkiye (Tavak, 2018), most of which are located in large cities and shopping malls. In addition to the fast-food restaurants mentioned above, the number of small and large doner and kebab restaurants in Türkiye is increasing rapidly. It is estimated that approximately half of the total sales consist of doner and kebab sales. For example, meatballs, shish kebabs, lahmacun, pita bread, and doner kebabs offered by peddlers have been indispensable elements of the Turkish food system for many years. In recent years, local hamburger and pizza restaurants and Çiğ Köfte sales places have been

increasing rapidly and they have continued to exist under different names (such as Tatlises Lahmacun, Tadım Pizza, Pizza Hot, Tatlises Çiğ Köfte, Adıyaman Çiğ Köfte<sup>1</sup>).

Although there have been many studies on FAFH in the past (Çabuk and Sengül, 2000; Özçelik and Sürücüoğlu, 1998; Akbay et al., 2007; Tayfun & Tokmak, 2007; Kayisioglu, 2012; Hamsioglu, 2013; Terin, 2019), no national studies have been found that address the socio-demographic, economic, and health perceptions of consumers' FAFH consumption and the food consumption attitudes and behaviors of consumers. In addition, based on the fact that some health problems arise as a result of the increasing tendency of families to consume FAFH, understanding the FAFH consumption and spending behaviors of consumers has become increasingly important for many reasons such as business decisions and health problems (Barrett et al., 2019). Unhealthy eating habits are an important disease problem in individuals of all ages, as they increase with the increase in food consumption outside the home, especially with social and dietary changes (Chen et al., 2021). The current study aims to empirically elicit how consumers' preferences and the factors including dietary behavior and health perceptions that trigger FAFH consumption in Türkiye are determinative on both probability and spending levels, using an inverse hyperbolic sine double-hurdle (IHS-DH) censored model. Unlike previous studies, the current study is the first study to analyze the general structure of household FAFH consumption in Türkiye. Obtaining eclectic empirical results from nationally representative cross-sectional data is crucially important when considering the role of FAFH consumption and providing guidance on healthy FAFH choices in nutritional intervention programs. To create effective marketing channels, it is very important to segregate families into groups according to their role-playing characteristics on FAFH probability and spending levels. The use of household survey data has, therefore, several advantages. First, economic theory can be applied to individual household behaviors by using household survey data. Second, household data are not affected by time-dependent structural changes. Over the past 20 years, Türkiye's demographics have undergone important changes in social and economic structure. Unfortunately, time series analysis is insufficient to elicit changing behavior patterns unique to individuals or families. Therefore, using survey data increases the applicability of demand forecasts with varying household behavioral structures. The results of the current study will also make it easier for businesses to make more informed decisions and will

be a guide for private-sector entrepreneurs. In other words, the investigation of the factors affecting FAFH consumption probabilities and spending levels may have a clear and direct impact on the private sector. However, when the issue is examined extensively, FAFH consumption expenditures can have a direct impact not only on businesses but also on existing health problems, thereby increasing obesity rates. Therefore, understanding the factors that determine consumers' FAFH consumption probability and spending levels is important not only for companies but also for policymakers to make interventionist decisions for effective nutrition programs in the country. Health problems due to increased consumption of FAFH will force the state to have many financial obligations as health expenditures increase.

The structure of the paper is as follows. First, the descriptive analysis of the data and the collection method are presented. This is followed by a brief discussion of the data analysis method. A discussion of the empirical results will be thereafter presented. Finally, we conclude with our main findings and recommendations.

## MATERIAL and METHOD

### Data collection (sampling) method

In this study, supported by the Scientific and Technological Research Council of Türkiye (TÜBİTAK) with project number 113K733, the data were obtained via face-to-face interviews with households determined by an appropriate sampling method. Within the scope of this study, 14 cities in Türkiye were selected to uncover at least one city from 12 NUTS1 regions (Istanbul, Izmir, Bursa, Ankara, Konya, Antalya, Adana, Kayseri, Samsun, Erzurum, Gaziantep, Diyarbakir, Malatya, and Trabzon). The number of households interviewed to represent the study population was determined using the proportional sampling formula (Yamane, 2001):

$$n = \frac{Np(1-p)}{(N-1)\sigma_{px}^2 + p(1-p)} \quad (i)$$

where  $n$  is the sample size,  $N$  is the total number of households in the surveyed provinces (approximately 7.74 million according to the TURKSTAT, 2018), and  $p$  is FAFH consumption share (0.50 will be taken as reaching the maximum sample volume will reduce possible error) and  $\sigma_{px}^2$  is variance ( $\sigma_{px} = 1.96/0.025$ ).

According to the aforementioned formula, the initial sample size was calculated to be 1537, ensuring a 2.5% error margin and a 95% confidence interval, and subsequently increased by 10% to bolster the

pepper, minced meat, tomato paste, onion, parsley, and various spices and consumed without heat treatment (cooking).

<sup>1</sup> Çiğ Köfte (raw meatballs) is a dish belonging to the Southeastern Anatolia (Şanlıurfa and Adıyaman cities) region, prepared by kneading and mixing bulgur, ground hot

statistical power of the study. This enlargement aims to facilitate more dependable and precise estimations of population parameters. After incorporating additional questionnaires, a sample from 1655 households was included in the study.

The descriptive statistics of the factors used in the model according to the information provided by the head of the family are presented in Table 1. The head of the family is 37 years old on average, 56% of them are male, 77% are married, 33 and 37% are high school and university graduates, respectively. Additionally, 15% of respondents reported having health issues, while 24% are currently following a diet program. About 25% pay close attention to their daily food intake, and 15% are classified as obese, with a body

mass index (BMI) exceeding 40 kg/m<sup>2</sup>. Moreover, approximately 21% of respondents drink more than 2.5 liters of water per day. Approximately 15% of individuals visit the shopping center 5 or more times a month. The average family size is 3.5 people, which is in line with the TURKSTAT data. The monthly average family income is around 3500 TL, and we see that about a quarter of this is devoted to food expenditures, which is in line with the TURKSTAT data. The average family spends 139 Turkish Lira (TL) per month with a high standard deviation, and about 87% of families have a dining-out culture in Türkiye. The share of Food-Away-From-Home (FAFH) expenditures accounts for 16.3% of total food expenditures, while its share relative to income is 3.9%.

Table 1. Definition and descriptive statistics of variables  
 Çizelge 1. Değişkenlerin tanımı ve tanımlayıcı istatistikleri

Variable	Definition of variables and measurement units	Mean	Std. Dev.
<b>Dependent Variable</b>			
FAFH expenditure	Household monthly FAFH expenditure	139.467	149.508
DFAFH	1: Consume FAFH; 0 otherwise	0.870	0.336
<b>Independent Variables</b>			
HHAGE	Age of household head (year)	38.707	10.427
HHGENDER	1 if the household head is male; 0 otherwise	0.563	0.496
HHMSTATS	1 if the household head is married; 0 otherwise	0.771	0.420
HGSCHOOL	1 if the household head graduated from high school; 0 otherwise	0.333	0.471
CLSCHOOL	1 if the household head graduated from university; 0 otherwise	0.375	0.484
HHLTHPRB	1 if the head of the family has any health problems; 0 otherwise	0.147	0.354
HHDIYET	1 if the head of the family applies for a daily diet program; 0 otherwise	0.237	0.425
WATCHFUL	1 if the head of the family pays much attention to what he eats daily; 0 otherwise	0.255	0.435
FRQWATER	1 if the head of the family consumes 2.5 lt or more of water daily; 0 otherwise	0.206	0.405
INTERNET	1 if there is internet at home; 0 otherwise	0.730	0.444
HOMECOOK	1 if regular home cooking is cooked at home; 0 otherwise	0.831	0.375
SEASON	1 if the survey was done in summer 1; 0 otherwise	0.476	0.500
MFRTQVST	1 if the individual visits the shopping center 5 or more times a month; 0 otherwise	0.154	0.361
MARMARA	1 if Marmara; 0 otherwise	0.292	0.455
HSIZE	Household size (person)	3.473	1.340
OBESE	Body mass index of household head higher than 40 kg/m <sup>2</sup>	0.153	0.360
INCGRP2	Middle-income household	0.371	0.483
INCGRP3	Higher-income Household	0.310	0.463

\*: In 2016, 1 US Dollar = 3.02 TL

### The Inverse-Hyperbolic Sine Double-hurdle model

The inverse hyperbolic sine double-hurdle model (IHS-

DH), which is one of the generalized censored models, will be used in this study<sup>2</sup>. In presenting the model we

<sup>2</sup> Food away from home consumption is generally based on the utility model introduced by Becker (1965). However,

Becker's utility model failed to attract much attention from many researchers, as it is particularly complex and requires



will uncover its many advantages, but now we will focus on the reasons behind the zero observations in the data. Microdata generally contains zero observations for consumption (quantity) and expenditure variables. These zeros occur for very different reasons. One of the most common reasons for this is either the family's lack of income or the high price of the product at that time. Such reasons are defined as the reason for the emergence of classical censorship. However, although the family may have a sufficient income level and the power to buy the product at a high price, consumers may not be able to consume, for example, out-of-home foods because they find them unhealthy. Or, shift meals of working family members may be uncovered by the company they work for. Or, family members may not consume western origin products by displaying a conservative attitude, or they may not buy them because they are not suitable for their palate. Or they do not need out-of-home meals because the family lives in the heart of the city center. Because of the high cost of time for the family or family members they meet, or with the food they bring from home, they can meet their food needs during work-break hours. As can be seen, there may be too many behavioral restrictions other than income and price in not consuming the product. Methods that ignore such a zero-consumption feature resulting from a consumption action involving a two-stage process jeopardize some basic statistical properties such as unbiasedness, consistency, and efficiency. Censorship and discrete generalized state in the dependent variables usually require the use of limited dependent models such as the DH or the "Sample Selection (SS)" model. The current empirical applications of these models are predominantly based on the bivariate normality distribution for the error terms. However, parameter estimations are inconsistent when models with discrete dependent variables do not fit the assumption of normality and constant variance (29) for cases in which the normal distribution and constant variance assumption do not hold in the well-known censored (Tobit) model of home meals because the family lives in the heart of the city center. Because of the high cost of time for the family or family members they meet, or with the food they bring from home, they can meet their food needs during work-break hours. As can be seen, there may be too many behavioral restrictions other than income and price in not consuming the product. Methods that ignore such a zero-consumption feature resulting from a consumption action involving a two-stage process jeopardize some basic statistical properties such as unbiasedness, consistency, and efficiency. Censorship and discrete generalized state in the dependent

many additional exogenous factors, but few researchers still base food expenditures on the above-mentioned utility model (Yen, 1993; Mutlu & Gracia, 2006; Bai et al., 2016).

variables usually require the use of limited dependent models such as the DH or the "Sample Selection (SS)" model. The current empirical applications of these models are predominantly based on the bivariate normality distribution for the error terms. However, parameter estimations are inconsistent when models with discrete dependent variables do not fit the assumption of normality and constant variance (Arabmazar & Schmidt, 1982) for cases in which the normal distribution and constant variance assumption do not hold in the well-known censored (Tobit) model. The IHS transformation of variable  $y$  (e.g., the dependent variable) with any parameter ( $\theta$ ) is given by the following equation (Reynolds & Shonkwiler, 1991):  $T(y) = \theta^{-1} \sinh(\theta y) = \theta^{-1} \log(\theta y + (\theta^2 y^2 + 1)^{1/2})$  (ii) where  $y$  stands for the dependent variable, e.g., spending levels on FAFH. When transformation  $\theta=0$  or approaching 0, it will resemble the logarithmic transformation ( $\log(2\theta y)$ ) for a substantially linear and large  $y$  value (regardless of the  $y$ -values of the zeros). In addition, the transformation scale is invariant and is suitable for removing the skewness mode of the data by mitigating the effect of outliers above the mean. Therefore, applying the transformation to the dependent variable ( $y$ ) will avoid possible non-normality distribution of error terms and drastically varying variance problems (Reynolds & Shonkwiler, 1991; Su and Yen, 1996; Mihalopoulos & Demoussis, 2001). Let us define the dependent variable  $y_i$  (e.g., the monthly spending levels of food away from home) with the IHS transformation as shown in the equation as  $T(y_i)$ . The IHS-DH model can be expressed as follows:

$$T(y_i) = x_i' \beta + u_{2i} \text{ if } z_i' \alpha + u_{1i} > 0 \text{ and } x_i' \beta + u_{2i} > 0 \\ = 0, \text{ otherwise} \quad (\text{iii})$$

where  $z_i$  and  $x_i$  are explanatory variable vectors,  $\alpha$ , and  $\beta$  are parameters corresponding to these variables and

$[u_{1i}, u_{2i}]'$  are random error terms. The random error terms fit the bivariate normal distribution with an arithmetic mean of zero, variance of  $[1, \sigma^2]'$ , and a correlation coefficient of  $\rho$ :

$$\begin{bmatrix} u_{1i} \\ u_{2i} \end{bmatrix} \sim N \left( \begin{bmatrix} 0 \\ 0 \end{bmatrix}, \begin{bmatrix} 1 & \rho\sigma \\ \rho\sigma & \sigma^2 \end{bmatrix} \right) \quad (\text{iv})$$

where the correlation coefficient,  $\rho$ , measures the linear relationship between the probability of food away from home spending levels and spending levels. Equation 3 assumes that the discrete outputs (zero or positive) occur entirely by a random sampling mechanism. The value of  $x_i' \beta + u_{2i}$  is equivalent to the

Interested reader(s) may refer to this literature on the subject.

latent variable  $y_i^*$ , where if the FAFH spending amount is less than the reservation amount predetermined by their household members or the household, they can consume the meal(s) while dining out. It is worth noting that households will, unfortunately, face income restrictions from the pre-set reservation amount on their out-of-home food consumption, as relatively low-income but large-scale households are expected to be a more disadvantaged group from such income restrictions. In this case, two decision mechanisms must be synchronously processed. In the first decision stage, it should be decided whether to dine out, whereas the second decision stage involves how much they will spend on FAFH, including zero cost. While the dining out decision has a causal relationship with social and behavioral stimuli in the first decision, the spending level decision on FAFH has a causal relationship with economic factors such as budget constraints in the second stage.

The sampling likelihood function for the dependent variable:

$$L = \prod_{y_i=0} \left\{ 1 - \Phi_2 \left( z_i' \alpha, \frac{x_i' \beta}{\sigma_i}, \rho \right) \right\} * \prod_{y_i \neq 0} \left\{ (1 + \theta^2 y_i^2)^{-1/2} \sigma_i^{-1} \phi \left[ \frac{T(y_i) - x_i' \beta}{\sigma_i} \right] \Phi \left[ \frac{z_i' \alpha + (\rho/\sigma_i)(T(y_i) - x_i' \beta)}{(1-\rho^2)^{1/2}} \right] \right\} \quad (v)$$

where  $\Phi_2$  refers to the bivariate standard normal distribution (cdf) and  $\phi$  and  $\Phi$  are the univariate probability density function (pdf) and the cumulative distribution function of the standard normal distribution (cdf), respectively. Zero and positive results in the dependent variable ( $y_i$ ) are managed by a double-hurdle mechanism. The probability of the dependent variable to be  $y_i \neq 0$  is determined by both parameter sets ( $\alpha$  and  $[\beta, \sigma, \theta]$ ) and the parameters are no longer separable with the zero-correlation constraint ( $\rho = 0$ ) in the maximum likelihood function. As with the IHS-DH model, when the variables are  $\theta \rightarrow 0$ ,  $T(y_i) \rightarrow y_i$  and the Jacobian term takes the value 1, the model turns into a conventional error-dependent DH model. When the error terms correlation ( $\rho$ ) is 0, the IHS-DH and DH models will turn into independent IHS-DH and independent DH models, respectively.

$$\text{Prob}(y_i | x_i, z_i, h_i) = \Phi_2 \left( z_i' \alpha, \frac{x_i' \beta}{\sigma_i}, \rho \right)$$

$$E^+ = E[y_i | y_i > 0, x_i, z_i, h_i]$$

$$= \frac{1}{\Phi_2 \left( z_i' \alpha, \frac{x_i' \beta}{\sigma_i}, \rho \right)} \int_0^\infty y_i (1 + \gamma^2 y_i^2) \frac{1}{\sigma_i} \phi \left[ \frac{T(y_i, \gamma) - x_i' \beta}{\sigma_i} \right] \Phi \left[ \frac{z_i' \alpha + \frac{\rho[T(y_i, \gamma) - x_i' \beta]}{\sigma_i}}{\sqrt{1 - \rho^2}} \right] dy_i$$

$$E = E[y_i | x_i, z_i, h_i] = \text{Prob}(y_i | x_i, z_i, h_i) * E[y_i | y_i > 0, x_i, z_i, h_i] \quad (vii)$$

The first equation reflects the associative probability level while the remaining last two equations refer to conditional and unconditional FAFH expenditure levels, respectively. If the derivatives of all equations are taken against an independent variable, the unitary (marginal) effect is reached. The

IHS-DH models used by Yen and Jones (1997) are restricted to error-dependent DH (Blundell & Meghir, 1987), independent IHS-DH (Su & Yen, 1996), and independent DH (Blundell & Meghir, 1987). IHS-DH models can be tested against the internal correlation problem by conventional tests such as the Wald test, LR test, or LM test.

The standard deviation of the dependent variable and error terms was re-parameterized as variable function to avoid problems of varying variance and non-compliance with normal distribution. Heteroscedasticity problems in error terms will make maximum likelihood estimators inconsistent. To correct heteroscedasticity that goes beyond the IHS transformation, the standard deviation " $\sigma$ " of the model corresponding to the FAFH spending level can be parameterized with descriptive variables vector  $w_i$  and  $\gamma$  parameter set:

$$\sigma_i = \exp(w_i' \gamma) \quad (vi)$$

where the first column of  $w_i$  consists of 1's. Similar heteroscedasticity adaptations were applied in some studies (Su & Yen, 1996; Yen & Jones, 1997; Newman et al., 2003; Aristei & Pieroni, 2008). Except for homoscedasticity assumed error terms, nested hypothesis, and the first element of  $\gamma$  is equal to zero, the amounts of all elements can also be estimated with the Wald, LR, or LM tests. To eliminate the problem of identification in the model, different independent variables were used in the equations for FAFH consumption probability and expenditure levels, drawing on the random utility theory (Pudney, 2013). For example, in addition to the common traits in both equations, dummy body mass index variables (30-40 and >40), total food expenditure variable, and only dummy region variables for Istanbul and Ankara are included in the FAFH consumption probability, while the FAFH expenditure equation, body mass index and income are continuously included in the FAFH consumption probability while including some metropolitan indicators as dummy variables in the model in addition to the covariates.

The probability, conditional, and unconditional FAFH expenditure levels are measured as follows:

estimated conditional and unconditional levels of FAFH expenditure were calculated using the numerical method because the function in the integral does not have a closed structure. At the same time, standard deviations of marginal effects were calculated using the delta method. Analyzing the

relationship between consumption probability and expenditures and socioeconomic and demographic factors has an important role in determining and analyzing policies, planning, and developing strategies.

Factors affecting households' spending levels for food away from home (as the explanatory variable in the model) are divided into four main groups. These are demographic factors related to household heads in the household (gender, marital status, education, age, obesity, job status, health insurance), household-related factors (household income, total expenditure, household size, mother's employment status, number of children, and age groups), regional factors (geographical area of residence, distance of shopping centers, availability of fast-food restaurants) and value judgments (prices, health, environment, service, etc.).

## RESULTS and DISCUSSION

Before discussing the model results, we include results of specification tests on the fit of the inverse hyperbolic sine double-hurdle censored model to the data. The error-dependent IHS-DH model has some interesting features unique to it. Therefore, some mandatory tests related to the suitability of the model should be applied. First, if the correlation coefficient  $\rho = 0$  in the model is assumed, the IHS-DH model turns into an error-independent IHS-DH model. On the other hand, assuming the theta parameter ( $\theta$ ) to be 0 in the model, the IHS-DH model turns into a conventional error-dependent double-hurdle censored model (DH). Note that the error-dependent DH model is at the core of the IHS-DH model. Therefore, before obtaining the parameters of the DH model, it is necessary to test whether the IHS-DH model converts to the DH model. If the test result mandates the DH model, the DH model has to be estimated, otherwise, the IHS-DH model must be decided. In addition to the above tests, if both the correlation coefficient and theta parameters in the model are assumed simultaneously  $\rho=\theta=0$ , the IHS-DH model is reduced to the error-independent DH model. All these tests were carried out with the help of conventional Wald and Likelihood tests. Similarly, in the correction of the non-normality and unequal variance status of the model residuals, the variance of the IHS-DH model was assumed as a function of the consumer's age, body mass index, and the number of individuals at home, and income variables. The test of the hypothesis, which suggests that these variables do not affect the variance of the IHS-DH model, was performed with the help of the Wald test. As a result of the tests, the correlation coefficient in the IHS-DH model was found to be different from zero (Wald = 155.37, degrees of freedom (df) = 1, and  $p < 0.01$ ). Therefore, the error-dependent IHS-DH model is not reduced to the error-independent IHS-DH model. In this context, both the probability of FAFH spending and the spending levels of FAFH need to be analyzed

simultaneously with each other. The correlation coefficient ( $\rho$ ) in the IHS-DH model was negative (-0.176) and statistically significant. Therefore, there is an inverse linear relationship between the FAFH expenditure possibility and expenditure levels in the IHS-DH model. This finding indicates that uncontrollable factors in the model increase (or decrease) the probability level of FAFH while they decrease (or increase) the level of FAFH expenditure. Or, as the amount of FAFH expenditure increases (or decreases), the probability of FAFH expenditure decreases (or increases), such that discrete models without correlations compromise some essential properties of the parameters. Also, the zero-hypothesis testing of  $\rho = \theta = 0$  in the IHS-DH model was similarly demonstrated using the Wald test. As a result of the test, the null hypothesis was rejected, so the counter-model-error-free DH model was rejected: both the correlation coefficient and the theta parameters used to correct for outliers in the dependent variable were simultaneously nonzero showing that the dependence of residuals on the likelihood of FAFH and their spending level values and the suitability of inverse hyperbolic sine transformation to ensure normal distribution is appropriate. The zero hypotheses, which suggests that the independent variables used to correct the unequal variance are simultaneously indistinguishable from zero, was rejected (Wald = 1.409, df = 3,  $p < 0.000$ ). The test results show that the independent variables used in correcting the variance were found to be different from zero simultaneously, and the variance in the IHS-DH model changed as the family size and income variables changed, and the variance of the model increased with the increase in these variables. By controlling such a variability, residuals were ensured to have equal variance. Similarly, the standard deviation of the IHS-DH model was estimated as a parameter (0.256) and found statistically significant. AIC and BIC criteria values of the model were found to be 2498.00 and 2734.79, respectively. All independent variables used in the model were found to be different from zero simultaneously, confirming the accuracy of the selection of the variables used in the model.

Most of the maximum likelihood parameters of the IHS-DH model matched our a priori expectations (Tables 2 and 3). However, since the IHS-DH model used is not linear, the maximum likelihood parameters do not reflect marginal effects on both the possibility of FAFH expenditure and FAFH spending levels. Because marginal effects are attributed more than the maximum likelihood parameters estimated in economic science, these effects need to be measured. In the following, we will only present and discuss the marginal effects of the IHS-DH model. The marginal effects of these variables are presented in Table 4, and only the marginal effects of statistically significant

variables will be discussed. FAFH expenditure is inversely related to the age of the household head, and as age increases, the probability of household FAFH expenditure decreases. In general, young people's food tastes and preferences, eating habits, lifestyles, and socialization opportunities greatly differ from those of the elderly, as expected (Heng & Guan, 2007). As the individual ages a year, the probability of household FAFH consumption decreases approximately by 0.1%. Stewart & Yen (2004) also concluded that the aging of the population will reduce fast food consumption approximately by 2%. Such a reduction in FAFH consumption is expected as a result of the decrease in the mobility of the aging population (Mutlu & Garcia, 2006; Angulo et al., 2007; Keelan et al., 2009; Blick et al., 2017).

The probability of FAFH spending is directly related to consumers' gender. The odds of household FAFH expenditure decreases with a household female-

headed and increase when the head of the household is male. As far as the transition between the gender variable is concerned, the probability of family FAFH spending increases approximately by 4.5%. The fact that the consumer is male affects the likelihood of household spending FAFH. This is an expected situation because, in Türkiye, close friendships and relatives among men are high, so it will increase in parallel with the odds of family spending FAFH, as it is likely to spend time eating out collectively. Also, given the fact that women are much more concerned about health and environmental issues than men and their superior ability for home cooking, female-headed households are likely to consume less FAFH. Our finding is in line with the literature (Byrne et al., 1998; Bozoglu et al., 2013; Angulo et al., 2007; Keelan et al., 2009; Fanning et al., 2010; Liu, 2011; Blick et al., 2017; Terin, 2019; Kılıç et al., 2020; Rasool and Akbay, 2021).

Table 2. Maximum likelihood estimates for the DH model  
 Çizelge 2. DH modeli için maksimum olabilirlik tahminleri

Variables	Spending probability		Spending level		Heteroscedasticity	
	Coefficients	t-value	Coefficients	t-value	Coefficients	t-value
Constant	16.069	0.18	0.626	3.02	-	-
HHAGE	0.0349	0.89	-0.001	0.00	0.008***	4.37
HHGENDER	1.144	1.55	0.062	1.14	-	-
HHMSTATS	-3.792	-0.04	0.256***	3.49	-	-
HGSCHOOL	1.094	1.37	0.175***	2.63	-	-
CLSCHOOL	-1.549	-1.64	0.259***	3.23	-	-
HHLTHPRB	0.814	1.05	0.224***	2.75	-	-
SEASON	-0.548	-0.91	-0.005	-0.08	-	-
H SIZE	-1.452***	-3.46	-0.036	-0.57	-0.023**	-2.12
OBESE	-1.760**	-2.14	-0.116*	-1.73	-	-
WATCHFUL	-1.435**	-1.99	0.409***	5.58	-	-
FRQWATER	1.096	1.04	-0.588***	-6.76	-	-
HOMECOOK	2.091*	1.84	0.172***	3.02	-	-
MFRTQVST	-2.369**	-2.46	0.252***	3.37	-	-
MARMARA	-1.154*	-1.70	-0.154**	-2.52	-	-
HHDIYET	0.021	0.03	0.068**	2.42	-	-
INTERNET	-0.673	-1.14	0.198***	2.73	-	-
INCGRP2	-5.421	-1.37	-0.392***	-3.89	-0.755***	-19.66
INCGRP3	-1.803	-0.44	-0.204**	-2.00	-0.526***	-14.35
Sigma	----	----	1.154***	18.50	-----	-----
Rho	----	----	-0.563***	-1,26	-----	-----
Log-Likelihood value	-1860.428					

Note: \*, \*\* and \*\*\* Show statistically important variables at %10, %5 and %1 level.



Table 3. Maximum likelihood estimates of the IHS-DH model  
 Çizelge 3. IHS-DH modelinin maksimum olabilirlik tahminleri

Variable	Spending probability		Spending level		Heteroscedasticity	
	Coefficients	t-value	Coefficients	t-value	Coefficients	t-value
Constant	1.273***	3.89	0.565***	7.23	-	-
HHAGE	-0.007	-1.09	0.0001	0.40	0.006**	2.15
HHGENDER	0.265**	2.45	0.014	0.74	-	-
HHMSTATS	0.231	1.39	0.067***	2.73	-	-
HGSCHOOL	0.427***	3.33	0.028	0.95	-	-
CLSCHOOL	0.825***	4.96	0.049	1.25	-	-
HHLTHPRB	0.238	1.44	0.068**	2.35	-	-
SEASON	0.136	0.96	-0.008	-0.42	-	-
HSIZE	-0.238**	-1.99	-0.006	-0.33	0.009	0.49
OBESE	-0.123	-0.86	-0.041*	-1.94	-	-
WATCHFUL	0.326**	2.54	0.115***	3.83	-	-
FRQWATER	-0.224	-1.06	-0.182***	-5.41	-	-
HOMECOOK	-0.408***	-3.37	0.083***	3.52	-	-
MFRTQVST	0.135	0.73	0.091***	3.56	-	-
MARMARA	-0.130	-1.10	-0.038*	-1.95	-	-
HHDIYET	-0.107**	-2.52	0.028***	3.13	-	-
INTERNET	0.165	1.14	0.047*	1.92	-	-
INCGRP2	-0.003	-0.02	-0.147***	-5.32	-0.363***	-5.84
INCGRP3	0.455***	2.91	-0.084***	-2.87	-0.264***	-4.40
Sigma	-----	-----	0.256 ***	6.10	-----	-----
Rho	-----	-----	-0.176*	-1.93	-----	-----
Gamma	-----	-----	1.409***	5.64	-----	-----
Log-Likelihood value	-1204.004					

Note: \*, \*\* and \*\*\* show statistically important variables at %10, %5 and %1 level.

Table 4. Marginal effects of the IHS-DH model  
 Çizelge 4. IHS-DH modelinin marjinal etkileri

Variables	Spending probability		Unconditional spending level		Conditional spending level	
	Estimate	t-value	Estimate	t-value	Estimate	t-value
HHAGE	-0.0013	-1.36	-0.0042	-1.59	-0.0021	-1.31
HHGENDER	0.0449**	2.53	0.0632*	1.91	0.0157	0.94
HHMSTATS	0.0439*	1.65	0.1447***	3.25	0.0631***	2.88
HGSCHOOL	0.0728***	3.48	0.1121**	2.24	0.0311	1.16
CLSCHOOL	0.1402***	5.15	0.2076***	3.12	0.0549	1.55
HHLTHPRB	0.0451*	1.66	0.1471***	2.88	0.0640**	2.48
HHDIYET	0.0217	0.93	0.0076	0.21	-0.0056	-0.33
WATCHFUL	-0.0397**	-2.03	-0.0471	-1.33	-0.0088	-0.49
FRQWATER	-0.0239	-1.03	-0.0860**	-2.30	-0.0386**	-2.05
INTERNET	0.0637***	3.08	0.2390***	4.89	0.1083***	4.02
HOMECOOK	-0.0528	-1.53	-0.3328***	-5.59	-0.1674***	-5.56
SEASON	-0.0599***	-3.07	0.0741*	1.91	0.0704***	3.33
MFRTQVST	0.0302	0.99	0.1691***	3.48	0.0835***	3.65
MARMARA	-0.0248	-1.28	-0.0829**	-2.39	-0.0363**	-2.06
HSIZE	-0.0156**	-2.30	0.0226	1.13	0.0198*	1.69
OBESE	0.0312	1.32	0.1015**	2.41	0.0441**	2.04
INCGRP2	0.0047	0.22	0.0221	0.33	0.0266	0.68
INCGRP3	0.0808***	3.17	0.1234*	1.80	0.0455	1.17

Note: \*, \*\*, and \*\*\* show statistically important variables at %10, %5, and %1 level. Since the dependent variable is divided into 100 at the model stage, the unitary effects of unconditional and conditional spending levels must be multiplied by 100.

The fact that the household head is a high school and university graduate, including master's and doctoral degrees, increases the likelihood of household spending FAFH whereas having a lower level of education makes households less likely to spend FAFH. Having a certain educational career positively triggers the probability of household spending FAFH by creating a social class in the community and enabling more social and cultural activities outside the home. Compared to secondary education and lower education levels of the household head, high school and university degrees increase the probability of family spending FAFH by approximately 7.3% and 14.0%, respectively. Note that as human capital (education level) increases, the probability of spending FAFH increases gradually. In particular, fast food and other FAFH companies can increase their sales by conducting research on this educational group and making certain promotions for the group. These positive effects of education are consistent with previous findings (Cupak et al., 2016; Piekut, 2016; Traş & Şengül, 2017; Terin, 2019; Ağır and Akbay, 2021).

Having a family head with chronic health problems reduces the odds of household FAFH spending. This is an expected situation: people with chronic illnesses do not have a mobile life and higher health concerns can mitigate the likelihood of household FAFH spending. However the result indicated that households where the head has health problems are 4.51% more likely to spend on FAFH compared to households where the head does not have health problems. The reason for this positive relationship can be explained by the bidirectional relationship. Increased consumption of food-away-from-home (FAFH) could also lead to health problems. FAFH options are often higher in calories, fat, sugar, and sodium than home-cooked meals. Regular consumption of these types of foods can lead to poor nutritional outcomes, including obesity, cardiovascular disease, and other health problems. Moreover, results indicate that households where the head pays close attention to their daily diet are 3.97% less likely to spend on FAFH. This result is statistically significant at the 5% level.

The fact that the family has the odds of spending more on FAFH than those who do not have the internet may be perceived as an opportunity provided by communication in which the internet fulfills the function of information networks such as inviting friends or relatives home or going out to dinner together through social media. It brings the information to be obtained through online research results to a more advantageous position in terms of the likelihood of spending FAFH compared to those who do not have such information. Our result overlaps with the finding of Bozoglu et al. (2013) a positive relationship between having an internet connection at

home and FAFH expenditures.

Meanwhile, seasonal changes shape the probability of household FAFH spending. The fact that the consumer is in winter compared to summer has increased the likelihood of spending FAFH. The rate of increase was approximately 6.0%.

As expected, the probability of spending FAFH decreases with an increasing economic burden as family size increases. The presence of an extra person in the family reduced the likelihood of spending FAFH approximately by 1.6%. Our result overlaps with the literature findings (Heng & Guan, 2007; Akbay et al., 2007; Drescher & Roosen, 2013; Blick et al., 2017). Interestingly, the probability of spending FAFH increases in individuals with a body mass index higher than 40 (Obese). Increased body mass index binds people inside rather than outside the home, and it is likely that while indoor food consumption increases, the probability of out-of-home food consumption decreases, which they may forego because they know they will spend more money while dining out. On the other hand, there is a positive relationship between family income and the odds of family FAFH spending. Although the income variable is not directly included in the equation for the probability of spending FAFH, its effect is indirect. This finding is expected: it triggers more outside activities to increase economic welfare as family income increases. Compared to lower income households, higher income households increase the probability of family spending FAFH by approximately 8.1%. Such income inferences were also consistent with literature findings (Cupak et al., 2016; Piekut, 2016; Blick et al., 2017; Traş & Şengül, 2017; Terin, 2019; Ağır and Akbay, 2021; Rasool and Akbay, 2021).

The marginal effects of the independent variables on the conditional and unconditional food away-from-home spending levels are given in the last four columns of Table 4 with their statistics. In line with our comment, only statistically important variables are emphasized. Similarly, to make a population-oriented inference, the level of FAFH expenditure is emphasized. Looking at the effects of the unitary or marginal impact, men spent more (6.3 TL) on FAFH than women. In line with the Turkish social structure, men spend more FAFH due to reasons such as being more out of the home and being more active than women in terms of socialization. Or, women's health concerns about food consumption outside of the home, increased opportunity costs with the intensity of housework, and more frugal behavior may be the driving forces behind the low level of spending. Angulo et al. (2007) reported that men spend more on FAFH than women in Spain.

Compared to an individual with a secondary or a lower education level, the individual's high school diploma or

university degree has been observed to increase monthly FAFH expenditures (11.2 TL and 20.8 TL, respectively). The increase in FAFH spending is inevitable when the human accumulation gained through education requires more presence outside the home, especially if people with higher education levels look for work fatigue outside the home. Or, the high opportunity cost of higher education levels may push households to consume more out-of-home food. This finding overlaps with international literature findings (Jensen & Yen, 1996; Angulo et al., 2007; Zan & Fan, 2010; Keelan et al., 2009; Liu, 2011). In a study conducted by Akbay et al. (2007), university graduates go to fast food places more frequently. Meanwhile, as expected, a household head who drinks 2.5 lt of water per day decreases home FAFH expenditure. Given such consumer health concerns or diet, it is inevitable to encounter such a finding. Having an internet connection at home increases consumers' FAFH expenditure (23.9 TL more). Considering factors such as the ease of booking reservations in places such as restaurants, the ability to book meals with friends by communicating with social media (e.g., Facebook, Youtube, Instagram, Twitter, Snapchat, etc.), and the quick availability of information about FAFH confirms the existence of such a finding.

A consumer who regularly cooked at home has less FAFH consumption expenditure (33.3 TL) than those who do not have such an advantage. This is an expected situation: the habit of individuals who regularly cook at home with their family members and eat home meals on time mitigates the expenditure on FAFH. A consumer spends more FAFH (7.4 TL) in the summer season than in the winter season. The comfort brought about by the summer season allows the consumer to spend more on FAFH and shows that the amount of FAFH expenditure is affected by the seasonal structure. Firms that offer FAFH spending can take advantage of this summer and increase their profits by adopting a different advertisement and promotion attitude. It has been determined that consumers who visit shopping malls more than 5 times a month spend more on FAFH than expected (16.9 TL more). Families residing in Marmara region spend less on FAFH than their peers residing in other parts of Türkiye (8.3 TL). Thus, we determined that the demographic difference between provinces shapes FAFH consumption. While our findings were consistent with the findings of some previous studies (Keelan et al., 2009; Chang & Yen, 2010; Bozoglu et al., 2013, Cupak et al., 2016; Tıraş & Şengül, 2017), they contradicted some other findings (Heng & Guan, 2007; Akbay et al., 2007; Drescher & Roosen, 2013; Blick et al., 2017).

Interestingly, the amount of FAFH expenditure decreased with increasing body mass index. Households with obese-headed spent more on FAFH

(10.1 TL) than households with non-obese-headed. Therefore, it can be said that as the body mass level increases, the amount of FAFH expenditure also increases. This finding is consistent with those of previous research (De Vogli et al., 2014; Seguin et al., 2016). De Vogli et al. (2014) stated that, as an economic freedom instrumental variable, the relationship between fast food consumption and body mass index is weakened but found to be meaningful. Seguin et al. (2016) found a positive and significant relationship between fast food consumption and body mass index.

As income increases, households consume more FAFH products. This is an expected situation: increased income brings more socialization outside the home, and this attitude is reflected in FAFH expenditure. The results are to be expected because more affluent households tend to spend more on products and services, including entertainment, diversity, and ambiance, and dining facilities such as alcohol service (Bozoglu et al., 2013). Our result is also consistent with findings in the international literature (Jensen and Yen, 1996; Byrne et al., 1998; Montini, 2001; Akbay and Boz, 2005; Akbay et al., 2007; Zan & Fan, 2010; Liu, 2011; Seguin et al., 2016). Montini (2001) emphasized that income has a significant effect on FAFH consumption, and income elasticity is calculated as 1.22. Moreover, Byrne et al. (1998) and Akbay et al. (2007) indicated that income is the most important factor, especially for fast-food restaurant preferences and expenditures.

When looking at the estimated probability of FAFH expenditure in the IHS-DH model, the probability of FAFH expenditure was 0.870, while the model estimated this probability as 0.823. However, the model predicted the unconditional amount of FAFH expenditure at a higher level (176.8 TL)

## RESULTS and RECOMENDATIONS

With economic growth over the last 20 years in Türkiye, there have been important changes in consumer habits. Newly processed foods are placed in the market and have started to take their place in supermarkets, hypermarkets, and other food and beverage offering markets. The wide range of food varieties offered in restaurants and fast-food places and the spread of many new FAFH companies throughout Türkiye offers great opportunities to consumers.

In this study, the factors affecting FAFH expenditure were determined by constructing the IHS-DH model. The model contains two interdependent equations: the first includes FAFH spending probability, and the second includes the level of FAFH spending. The different factors determine each equation. The model structure was tested using various statistical tests. Based on all tests performed, the IHS-DH model was found to be compatible with the data. A negative linear

relationship was found between the probability of spending FAFH and the level of spending. As long as zero spending is observed for factors that are not controlled, the amount of FAFH expenditure decreases when the probability of FAFH expenditure increases. Simultaneously, increasing uncontrolled factors affecting the amount of FAFH will reduce the likelihood of FAFH. The model parameters were obtained using the maximum likelihood function. Most parameters were found to be compatible with economic theory. Because the model is not linear, the marginal effects of regressors on dependent variables are derived and explained in detail in the previous section. When considering the probability of spending FAFH consumption in general, the fact that the household head is male and has a high school or university degree, there are children under the age of 18, and household income increases the likelihood of household FAFH spending. However, the probability of household FAFH spending decreases with the age of the household head and household size.

On the other hand, the overall FAFH consumption of the households will increase if the household head is male, has a high school or university degree, has internet at home, the presence of children under the age of 18, the frequency of shopping mall visits, the season, and an increase in household income. On the other hand, FAFH consumption decreases with a daily water consumption level of 2.5 lt and above and regular cooking in the family. Based on these findings, it will be beneficial for companies in FAFH services to try to deliver their promotions to consumers, especially on the internet. It has been determined that the water consumption level will decrease with the presence of variables such as 2.5 lt and above and the presence of regular meals in the family. Based on these findings, it will be beneficial for companies serving FAFH to try to deliver their promotions to consumers, especially on the Internet. They can also offer meals that are rich in nutrients and a healthy diet, by developing home delivery services to the elderly population and families with female heads. Or, marketing channels can be developed by sharing the steps from the preparation of the dishes to the final presentation with the public through the Metaverse, which will become widespread in the coming years.

Similarly, as the level of education increases, the rate of fast-food consumption also increases. Although this result gives the same result as in the literature, this result should be taken into consideration in terms of human health, especially considering the obesity-fast food relationship. In this study, the most important reason for the positive relationship between education and fast-food consumption is the high level of income among households with high education-headed levels and the fact that the work of family members positively affects their eating out. For these reasons, it will be of

interest to businesses in FAFH services to identify consumers with high school or university degrees and increase their quality of service. Companies serving in the Aegean region are in a more advantageous position, and an increase in the quality of food and services in these areas will lead to an improvement in the company's profits. It will be of interest to companies that provide FAFH services to build playgrounds where they are present and offer their consumers such a service.

The data analysis methods applied in this research are relatively new compared to the method used in the research conducted in the FAFH areas in Türkiye. The econometric method used to explain the social, cultural, and economic dimensions of FAFH consumption adds scientific richness and strength to the existing literature and provides new expansions. The results of the current study are of great importance in terms of raising consumers' awareness of FAFH consumption and evaluating what measures should be taken in terms of food reliability and healthy living during the European Union (EU) negotiation period and membership. According to the results, all organizations in the national and international fast-food chains will satisfy Turkish consumers and enable the development of products that can benefit them. At the same time, the findings obtained from this research increase the added value with the critical importance it carries for policies to protect and satisfy consumers in terms of future health and food safety problems.

The research outputs will serve as a guide for food industry managers and related companies who are wondering what the driving forces behind FAFH spending in Türkiye are. The development of effective marketing programs and policies has always been a major concern in the food distribution and retail industries. The findings provide useful information for managers of food businesses in determining new business opportunities, planning and marketing strategies for better service to consumers, and accurately predicting future consumption patterns.

With rapid urbanization and an increase in women's labor force participation, long working hours, and less physical activity, a diet toward more processed and ready-made foods has started worldwide. Therefore, the increasing effects of supermarkets, hypermarkets, and food and beverage supply chains including catering across the country will ultimately lead to changes in all areas of the food chain. In this context, easily accessible and inexpensive communication systems such as mobile phones, the internet, and even Metaverse can assist consumers in information and market-related services regarding the quality and selectivity of products. Studies should be conducted to raise healthy generations and ensure adequate and balanced nutrition. Understanding the factors



affecting consumers' or households' consumption of FAFH and revealing the profiles of their consuming and not consuming FAFH are not only important for businesses but also for policymakers. Health problems resulting from the increase in FAFH consumption will also increase health expenditures. One of the important contributions of the current study is that it allows us to gain generations with a healthy and balanced diet. In addition, the costs of struggling with fatness and obesity by the Ministry of Health and other public institutions and organizations can be minimized. Moreover, considering the elasticity of fast-food consumption, a tax increase applicable to fast-food consumption will decrease the frequency of visiting a fast-food restaurant and fast-food consumption, or by developing healthier menus, catering services can reduce their tax burden.

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### Researchers' Contribution Rate Statement

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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## Türkiye’de Tarım İşletmelerinin Sosyo-Ekonomik Profili ve Tasarruf Davranışları Arasındaki İlişki: Konya İli Örneği

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### ÖZET

Bu çalışmanın temel amacı kırsal alandaki tarımsal hanehalklarının tasarruf davranışlarına etki eden faktörlerin belirlenmesidir. Bu amaca yönelik olarak Türkiye’de Konya ilinde faaliyet gösteren 268 tarım işletmesiyle anket yapılmıştır. Bu doğrultuda anketler sonucunda tarım işletmelerinin sosyo-ekonomik yapıları ve tasarruf miktarları belirlenmiştir. Türkiye gibi birçok ülkede tarım işletmelerinin sahip oldukları sosyo-ekonomik özellikler göz önünde bulundurulduğunda işletmelerin tamamının tasarruf yapamayacağı ihtimali göz önünde bulundurularak tarım işletmelerinde tasarrufları etkileyen faktörlerin belirlenmesinde tobit model kullanılmıştır. Model sonucunda yaş, aile nüfusu, sermaye, tarımsal gelir, işletme büyüklüğü ve tarım dışı gelir ile işletmelerin tasarruf miktarları üzerinde anlamlı ve pozitif etkisi olduğu belirlenmiştir. Fakat eğitim seviyesi, tecrübe ve sosyal güvence, sağlık durumu, işletme maliyetleri, kredi miktarı ve destekleme miktarının tasarruf miktarı üzerinde anlamlı ama negatif bir etkiye sahip olduğu söylenebilir. İşletmede edilen sonuçlara göre ekonomik büyüme için daha fazla tasarrufu harekete geçirmek için farklı gelir seçenekleri için alternatif kaynakların ihtiyacına işaret edilmektedir. Çünkü kent-kır fark etmeksizin yatırım fonların önemli bir kısmı gayrimenkul veya vasıta fonlarına aktarılmaktadır. Bu sebeple alternatif yatırım araçlarının desteklenerek yaygınlaştırılması gerekmekte olup tasarrufların değerlendirilmesine yönelik politika önerileri hazırlanmıştır.

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Tobit regresyon

## The Relationship Between Socio-Economic Profile and Saving Behavior of Agricultural Enterprises in Turkey: The Case of Konya Province

### ABSTRACT

The primary aim of this study is to identify the factors that impact the saving behavior of agricultural households residing in rural regions. To achieve this, a survey was undertaken with 268 agricultural households located in Konya province, Turkey. Accordingly, the socio-economic structures and amount of savings of agricultural households were determined because of the surveys. Considering the socio-economic characteristics of agricultural businesses in numerous countries, including Turkey, we utilized the tobit model to establish the factors that impact savings in agricultural businesses. The analysis showed that age, household size, capital, income from households, and off-farm business ventures have a significant and positive impact on household savings. Conversely, education level, experience, social security, health status, business expenses, loan amount, and level of subsidies have a significant and negative impact on savings. The research has highlighted the requirement for diverse income options to encourage greater savings, leading to economic growth. Due to the transfer of a substantial amount of investment funds to real estate or vehicle funds without regard to urban-rural differences, the promotion and dissemination of alternative

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investment instruments is essential. Consequently, policy recommendations have been devised for the deployment of savings.

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## INTRODUCTION

Significant advances in agricultural technology since 1950 have transformed production patterns and structures. This has resulted in the replacement of manual labor with machinery, leading to a higher need for working capital due to intensive input use. Additionally, a change in production intensity structures have increased the financial demands of enterprises. The progress in agriculture has stimulated contemplation regarding how and from where households capital necessities will be met. To fulfill these needs in households, internal and external financing sources are employed. Nevertheless, agricultural households are identified by restricted credit access, vulnerability to risk and uncertainty, reliance on climate, and low capital turnover. These economic and structural attributes have decreased households potential revenue, decreasing savings consequently. The poor living conditions and poverty cycle that Agricultural operators face require immediate attention. Therefore, it is crucial to examine savings, which are one of the internal funding sources, to reduce the effects of potential households risks and secure its financial sustainability.

Savings in the agricultural sector refer to the income that is not consumed in the current year and reserved for future investment, consumption, or unforeseen circumstances. Therefore, saving is the ability to control unforeseen risks in the future (Tan ve ark., 2022; Strzelecka ve Zawadzka, 2023; Zeng ve ark., 2023). Therefore, saving is an important economic behavior for households to achieve financial balance. In the world, including Turkey, internal financing programs are needed to increase agricultural production, reduce poverty, prevent income inequality, and provide access to financial services for households (Steinert ve ark., 2018; Abbas, 2022; Karaaslan ve ark., 2022). Savings, the most important internal financing program, can alleviate liquidity constraints in agribusinesses, stabilize consumption, and increase socio-economic welfare. It is emphasized that agribusinesses need to save to invest in health and education, manage household emergencies, and meet other cash needs (Littlefield ve ark., 2003; Yunus, 2006; Morduch, 2009).

Three traditional theories have been widely used in empirical studies on saving formation. These theories are the Life Cycle Hypothesis (LCH) proposed by

Modigliani (1970), the Absolute Income Hypothesis (IHH) proposed by Keynes (1936), and the Permanent Income Hypothesis (PIH) proposed by Friedman (1957). The LIH, it is aimed at maximizing the optimal utility that the individual will obtain throughout life rather than the current period, and it is known that they will consume and save according to their past and future income. Keynesian theory has identified absolute disposable income as an important determinant of saving. It defines saving as the amount remaining after subtracting the cost of consumption expenditures from the disposable income earned in each period. Friedman states that capital has a significant effect on consumption according to the SGH theory and that saving rates may vary as income varies from year to year. The implication of these theories is that income and expenditures have an effect on savings over time, the marginal benefit of saving is higher when income is low, and the marginal benefit of saving is lower when income is high. These theories have been the subject of many empirical studies and studies on saving have been conducted so far. Empirical studies have examined the behavior of entrepreneurs towards savings (Léon ve Rainelli, 1976; Spence ve Mapp, 1976; Hamaker ve Patrick, 1996; Mapesa, 2015), the relationship between income and capital accumulation and consumption and savings (Jensen ve Pope, 2004), the savings instruments used by the rural population (Horioka ve Wan, 2007; Subhashree ve ark., 2016) and the factors affecting savings in households (Gedela, 2012; Alessie ve ark., 2013; Chamon ve ark., 2013; Fletschner ve Kenney, 2014; Karlan ve ark., 2014; Komal ve Abbas, 2015; Zhou ve ark., 2019). Studies have established that saving behaviors are prevalent in agriculture in developed countries. Climatic conditions, natural disasters, and uncertainty in agricultural markets force agricultural operators to save more than operators in other sectors; there is a significant relationship between savings in the agricultural sector and income; high marginal consumption and low marginal saving tendencies prevail in low-income agriculture. In addition, studies have also found that income growth supports the life cycle hypothesis (Horioka ve Wan, 2007) and that savings rates depend on seasonal income rather than regular income (Kulikov ve ark., 2007). Studies on savings in Turkey have mainly focused on household savings, which are considered the most significant aspect of domestic

savings. In these studies, which were conducted without sectoral distinction, the reasons why household savings are low and the factors affecting it were determined (Bozkuş ve Üçdoğruk, 2007; Erdem, 2017; Zengin ve ark., 2018). In these studies, in which the main factor affecting savings is income, saving tendencies differ according to household income levels. This situation has been the subject of many studies and studies measuring saving behavior according to different household incomes have been conducted (Çiçek, 2000; Çelik, 2009; Çolak ve Öztürkler, 2012). While Cefri (2009) found a high correlation between poverty and savings in Adana province in Turkey, other studies have revealed that the most important factors affecting savings other than income are age, education level, and social opportunities (Karataş ve Gavcar, 2001; Bozkuş ve Üçdoğruk, 2007; Çelik, 2009; Aktas ve ark., 2010). Accordingly, in parallel with the increase in the education level of households, there is an increase in their savings rates (Hayta, 2008; Kanık ve Dinç, 2017). In addition, there are studies that conclude that savings will increase with an increase in labor force participation (Ceritoğlu ve Eren, 2013). As can be seen, household saving surveys conducted in Turkey have been analyzed with macro data without considering sectoral breakdown (Çiçek, 2000; Bozkuş ve Üçdoğruk, 2007; Hayta, 2008; Çelik, 2009; Aktas ve ark., 2010; Çolak ve Öztürkler, 2012; Ceritoğlu ve Eren, 2013; Erdem, 2017; Zengin ve ark., 2018) and there is limited sectoral and area-based information. As a matter of fact, saving behavior differs even among national, regional, and local households. In macro analyses, factors such as social, economic, demographic, geographical, etc. among households are analyzed assuming that these data are constant. In addition, due to the structural and economic differences of households by sectors, in empirical studies to be conducted with savings theories, households in each sector should be analyzed with their dynamics (Kutlu, 2016; Akın, 2018). For this reason, the factors affecting the savings in the

agricultural sector with their dynamics were determined in this study.

## MATERIAL and METHOD

### Sampling Method

The Konya province, selected as the research area, shows the highest level of capital movements in land, livestock, and equipment machinery. A stratified random sampling method was employed to select a sample of 106,833 farmers from the total population of the region, with 268 farms to be surveyed (Table 1). According to the stratified random sampling method, the number of samples to be studied was calculated using the formula below. In determining the number of samples drawn from the main mass, 5% error and 99% confidence limits were used (Yamane, 1967).

$$n = \frac{(\sum N_h \cdot S_h)^2}{N^2 \cdot D^2 + \sum (N_h \cdot S_h^2)} \quad D^2 = d^2 / z^2$$

n= Number of samples

N<sub>h</sub>= h. Number of units in the layer

S<sub>h</sub>= h. Standard deviation in the stratum

N= Total number of observations

D=d/z

d= Deviation from the mean at a certain rate

Z=If the number of units is above 30, z value is used in the t distribution.

After determining the number of samples, the Neyman method was used to determine a single sample volume for all strata by considering the weights of the mean and variance of each stratum, and this method aimed to increase the efficiency of sampling. The formula used to distribute the sample volume determined according to the Neyman method according to the strata is shown below.

$$n = \frac{N_h S_h * n}{\sum N_h S_h}$$

Table 1. Number of Sample Enterprises according to Enterprise Size Groups (number)

Çizelge 1. İşletme Büyüklük Gruplarına Göre Örnek İşletme Sayısı (adet)

Enterprises Size (ha)	N <sub>h</sub>	S <sub>h</sub>	Mean	CV	N <sub>h</sub> *S <sub>h</sub>	N <sub>h</sub> *(S <sub>h</sub> ) <sup>2</sup>	n
0-5	18.888,00	9.94	3.2	31.05	187.654,98	1.864.379	18
5.1-15	28.873,00	28.47	9.15	31.09	821.892,08	23.395.788	78
15.1-50	18.900,00	81.42	24.70	32.95	1.538.797,84	125.285.651	145
50.1-+	1.477,00	195.27	60.55	32.25	288.409,85	56.317.021	27
Total	68.138,00				2.836.754,75	206.862.839,00	268

### Statistical Analysis

The tobit model is widely used when there are unobservable values in the dependent variable. As a matter of fact, in recent years, it has been stated in the international literature that the model that should be used in determining the factors affecting the savings of households is tobit (Obayelu, 2013; Mapesa, 2015;

Sallawu ve ark., 2016; Lidi ve ark., 2017). The tobit regression model, developed by Tobin (1958), was originally used to investigate the connection between income levels and household spending. In cases where households do not exceed a particular income threshold, their expenses were assumed to be zero. In the study, unobservable variables were either excluded

or assigned a zero value. The present inquiry aims to employ the tobit model in elucidating the factors that affect farm savings. Certain agricultural practices may not generate savings due to insufficient income. Therefore, the study used the left-censored regression (Tobit) approach to account for situations where dependent variables are not observable and take a value of zero while corresponding independent variables are observable. The study identified that 94 farmers were unable to generate savings, resulting in the savings data being incorporated into the model as "censored data."

Restricted dependent variables are estimated using both the tobit and probit models. Nonetheless, the parameters derived from the tobit model are more efficient than those from the probit model (Üçdoğruk ve ark., 2001). For this reason, the tobit model was used in this study and the general representation of this model is as follows (Greene, 2003).

$$Y_i^* = X_i\beta + \mu_i, \quad i = 1, 2, \dots, n$$

$$Y_i = Y_i^* \quad \text{if } Y_i^* > 0$$

$$Y_i = 0 \quad \text{if } Y_i^* \leq 0$$

$Y_i$ : the observed amount of household savings

$Y_i^*$  is the latent variable which is not observed

$\beta$  is the Vector of unknown parameters

$X_i$  is the vector of independent variables affecting household savings.

In the equation,  $Y^*$  is the unobservable (latent) variable,  $\beta$  is the  $(k \times k)$  dimensional parameter vector,  $X$  is the  $(k \times k)$  dimensional vector of independent variables,  $\mu$  is the error term and  $Y$  is the observable variables (Akgüngör ve ark., 1999). In the equation to be prepared within the scope of the Tobit model, the amount of savings is taken as the dependent variable and the independent variables are age, education, experience, social security, household size, health status, capital, agricultural income, operating costs, land size, non-agricultural income, loan amount, subsidy amount and household consumption. In this study, which examines how the saving behavior of the operator changes according to demographic, financial, and environmental factors, in particular, the effects of these resources on savings were investigated since there are studies in the literature (Mishra ve Chang, 2009) that the amount of support provided not only reduces their profitability by reducing their savings opportunities but also reduces the level of social welfare. Among the demographic factors included in the model, variables such as age, education, gender, marital status, social security status, and health status will be obtained through surveys, and the capital structure of household, the value of movable

and immovable assets, annual operating results, land size, subsidy amount, loan utilization amount and household consumption expenditures are included in the model.

The Tobit model is formulated in a manner analogous to that of a standard regression model. However, in this case, the observability of the dependent variable is controlled by a limiting mechanism. In the Tobit model, the limiting mechanism causes the observed dependent variable to remain below or above a certain threshold. This necessitates the utilization of specific methodologies for the estimation and analysis of the model. Censoring is typically classified into two principal categories: left censoring and right censoring. Left censoring occurs when the dependent variable is situated below a predefined threshold value, whereas right censoring occurs when the dependent variable is positioned above the specified threshold value. In this context, the Tobit model with left censoring is employed and expressed as follows:

If the observed  $y_i$ :

$$\begin{cases} Y_i^* & \text{if } L < y_i^* < U \\ L & \text{if } y_i^* \geq L \\ U & \text{if } y_i^* \leq U \end{cases}$$

In this context,  $L$  represents the lower bound and  $U$  the upper bound. Once the bounds have been established, the tobit model is estimated using the maximum likelihood method. This method is designed to identify the values of the model parameters that maximize the probability density function. The probability density function (PDF) and log-likelihood function of the Tobit model are defined as follows.

$$L(\beta, \sigma | y) = \prod_{i=1}^n \left[ \Phi \left( \frac{\beta' x_i}{\sigma} \right) \right]^{1-d_i} \left[ \frac{1}{\sigma} \phi \left( \frac{y_i - \beta' x_i}{\sigma} \right) \right]^{d_i}$$

In this context,  $\Phi$  represents the cumulative distribution function of the normal distribution, whereas  $\phi$  denotes the density function of the normal distribution.  $d_i$  serves as an indicator of whether the observations are restricted. The maximum likelihood method estimates the parameters by maximizing this log-likelihood function. This method is employed to estimate the parameters of the model with greater accuracy and to consider the effects of the restricted data. The log-likelihood value, AIC, and BIC criteria, and p-values are utilized to ascertain the performance of the model.

## RESULTS and DISCUSSION

The development and continuity of agriculture rely on the creation of internal resources. Since the employment of external resources is limited and such resources are not adequately developed for high-yield investments, the importance of internal financing

resources increases. In addition, the shallow financial system in the agricultural sector, coupled with low savings levels, further emphasizes the significance of creating internal resources in the agricultural sector of Turkey. The analysis of the elements influencing savings rates and net profit margins in agriculture

bestows significant revelations facilitating the formulation of strategies that can augment the sector's potential to create internal wealth. The amelioration of domestic savings can remarkably advance investments, thereby fostering economic growth.

Table 2. Variables in the Prediction Model Used to Determine the Factors Affecting Amount of Savings.

*Çizelge 2. Tasarruf Miktarını Etkileyen Faktörleri Belirlemek için Kullanılan Tahmin Modelindeki Değişkenler.*

Variable Name	Description	Expected
Age	Year	+/-
Education	(1: Primary School, 2: Middle School, 3: High School, 4: University)	+
Experience	Year	+/-
Social Security	(0: None, 1: SSI, 2: Insuranced Self-Employed Institution, 3: Retirement Fund, 4: Green Card)	+/-
Household Size	Number	+/-
Health Status	(1: Very Bad, 2: Bad, 3: Fair, 4: Good, 5: Very Good)	-
Capital	\$	+
Agricultural Income	\$	+
Operating Cost	\$	-
Land Size	ha	+
Non-Agricultural Income	\$	+/-
Loan Amount	\$	+
Subsidy Amount	\$	+
Household Consumption	\$	-

Therefore, to identify appropriate policies that can improve resource availability for development, it is essential to comprehend the objective factors that drive household savings. Thus, the study examined the determinants of savings in the agricultural industry.

Within the study, the analysis of factors that affect savings rates in households is divided into two groups: endogenous and exogenous. The former includes the personal characteristics of operators and economic structures in households, which are examined using micro-level data. The latter, however, is categorized at a macro level and consequently determined to have side effects on operators' savings. The analysis focuses on the personal factors that pertain to the household, particularly the characteristics of the household head. In rural regions, the head of the household holds significant influence in family decision-making and affects a range of familial behaviors, such as saving. The household head's age, gender, education, expectations, socio-cultural background, health status, and psychological state can result in distinct approaches. Empirical studies reveal that savings of household differ depending on these factors (Bozkuş ve Üçdoğruk, 2007; Mishkin, 2007; Özcan ve Günay, 2012; Temel Nalın, 2013; Şengür ve Taban, 2016). The characteristics, desires, and aspirations of households in households are of great importance for expenditure and savings. However, in addition to these, some factors (economic and environmental) have an impact

on savings. In this case, determining which other factors besides demographic factors affect savings behavior and formulating policies in this direction are among the first things to be done.

Farm savings are affected by internal factors, including farm income, farm size, working capital, and household average ratios, as well as external financial factors, such as inflation, exchange rates, loan interest rates and utilization, and public savings. Environmental factors such as climate change, technological advancements, risks and uncertainties, population pressure, and market instability can restrict the economic mobility of agricultural practices, resulting in fluctuations in savings and expenditure rates. The macro-level analysis is commonly used to evaluate savings and expenditures, while the influence of financial factors on savings is measured through time-series analysis in micro-level studies. Therefore, this study has identified endogenous factors that impact savings formation and expenditures, playing a crucial role in achieving capital accumulation within the agricultural sector. Recommendations have been prepared for policymakers based on these findings.

The savings behavior of agricultural households is influenced by the demographic, economic and social characteristics of the operator, alongside macroeconomic indicators and environmental factors. To examine the factors that impact savings, we created a Tobit regression model that incorporates these



variables. Descriptive statistics of the variables used for the analysis are given in Table 3. The results of the analysis showed the necessity of using a one-sided Tobit model with left-hand and right-hand censoring.

We have identified twelve farms classified as censored, which report no savings (i.e. savings  $\leq 0$ ). Consequently, the model is one-sided.

Table 3. Descriptive Statistics on Factors Affecting Agricultural Savings

*Çizelge 3. Tarımsal Tasarrufları Etkileyen Faktörlere İlişkin Tanımlayıcı İstatistikler*

Variables	Mean	Mod	Standard Deviation	Minimum	Maximum
<b>Savings Amount</b>	70582.03	-63554.62	647878.56	-63554.62	4460050.78
<b>Age</b>	51.82	65.00	13.40	18.00	77.00
<b>Education</b>	1.76	1.00	0.96	0.00	4.00
<b>Experience</b>	28.76	30.00	13.40	3.00	60.00
<b>Social Security</b>	1.36	1.00	0.55	0.00	3.00
<b>Household Size</b>	3.74	2.00	1.86	1.00	10.00
<b>Health Status</b>	3.46	5.00	1.44	1.00	5.00
<b>Capital</b>	3525309.46	1106000.00	3511417.79	307000.00	35533500.00
<b>Agricultural Income</b>	520084.39	23623.73 <sup>a</sup>	695543.76	23623.73	4684962.57
<b>Operating Costs</b>	315454.79	21184.50 <sup>a</sup>	296021.52	21184.50	2485979.42
<b>Farming Size</b>	281.64	60.00 <sup>a</sup>	354.02	0.00	4200.00
<b>Non-Agricultural Income</b>	24707.99	0.00	47063.23	0.00	521000.00
<b>Loan Amount</b>	172492.54	0.00	322847.38	0.00	3000000.00
<b>Support Amount</b>	35998.93	0.00	129101.32	0.00	1405500.00
<b>Household Consumption</b>	96978.75	42960.00	58453.85	10656.00	272412.00

The operator's age constitutes the initial personal factor in the model, interacting with other factors such as experience, financial status, and goals. Technical term abbreviations are elucidated upon first use. The results of our modeling indicate that age has a notable positive effect on savings, with a statistical significance of  $p < 0.15$  (for details, please refer to Table 4). In fact, those anticipating a decrease in their future income tend to save enough to cover their consumption expenses. Conversely, if the expectations for the future are optimistic, they tend to save less. This can account for why the population who are not in employment and especially the elderly who have retired save comparatively less than those who are employed. Although individuals may encounter limitations when saving during their youth and old age, they typically have a higher tendency to save during their working years (Modigliani, 1970). It is therefore anticipated that savings will rise in line with an increase in the proportion of the population engaged in productive work. In other words, savings are likely to grow in tandem with an expansion in the employment rate of the active population. (Lahiri, 1989; Edwards, 1996; Thimann ve Dayal-Gulati, 1997; Loayza ve ark., 2000).

The second most important personal factor affecting savings is "education" (Table 4). In the Tobit model, education has a positive and significant effect on the amount of savings ( $p < 0.10$ ). While individuals with higher levels of education are known to save more, savings show a reverse trend as the level of education decreases (Bernheim ve ark., 2001; Qi ve ark., 2004; Cilasun ve Kirdar, 2009; Aktas ve ark., 2010; Özcan ve

Günay, 2012; Temel Nalın, 2013; Şengür ve Taban, 2016). In some empirical findings, a negative relationship between savings and the education of the head of the household has been determined and it has been determined that savings decrease as the level of education increases due to higher wages spent on education (Burney ve Khan, 1992; Abid ve Afridi, 2010). Individuals with higher levels of education possess greater financial assets than their less-educated counterparts. As a result, raising the country's overall education level would promote an increase in national savings rates. In essence, the so-called "Theory of Bounded Rationality" and related mindset positing that emotions and thoughts prevent individuals from acting rationally, is not particularly fitting for agricultural operators. It has been revealed that with an increase in the education level of operators, their income tends to increase, leading them to save for specific purposes. Furthermore, higher education is associated with higher income, and the low investment in education by low-income farmers may contribute to transferring income inequality across generations. Therefore, it is necessary to enhance the level of education to develop human capital and increase savings, contributing to the economic growth of the country, region, and agriculture. Additionally, "experience" (Table 4) is another endogenous factor that affects savings in households, referring to the accumulation of experience by agricultural operators, promoting specialization and sustainable production in the agricultural industry. Therefore, experience enhances the potential to produce under market conditions and is a crucial acquisition for building strategies against

hazards and uncertainties. Households with extensive knowledge and proficiency not only in cultivation but also in the market, marketing, economy, consumer behavior, and various other aspects can utilize their

production resources judiciously and shape their socio-cultural existence accordingly. It was discovered in this investigation that expertise enhances the sum of savings ( $p < 0.15$ ).

Table 4. Factors Affecting Agricultural Savings in Farm  
*Çizelge 4. Çiftlikte Tarımsal Tasarrufları Etkileyen Faktörler*

Tobit regression		Total Number of Observations	=	<b>268</b>	
Log likelihood function= <b>-3715.202</b>		Maximum Likelihood Ratio Significance Level > Chi-squared	=	<b>184.54</b>	
		Coefficient of Determination	=	<b>0.0000</b>	
				<b>0.6593</b>	
	Coefficient	Standard Error	t	P> t	[95% Confidence Interval]
Age	783.6879	3383.719	0.23	0.117****	-5880.032 7447.407
Education	59890.82	34967.64	1.71	0.088***	128754.3 8972.625
Experience	4552.032	3235.412	1.41	0.111****	10923.68 1819.619
Social Security	3963.889	54741.87	0.07	0.042**	-103841.9 111769.7
Household Size	-5734.708	16877.16	-0.34	0.034**	-38971.69 27502.28
Health Status	-17688.11	20588.6	-0.86	0.091***	-58234.22 22858.01
Capital	.0098176	.0099717	0.98	0.126****	-.00982 .0294553
Agricultural Income	.4690053	.1104686	4.25	0.000*	.2514543 .6865563
Operating Costs	-.399559	.3350225	-1.19	0.034**	-1.059335 .2602168
Farming Size	731.3031	121.5947	6.01	0.000*	491.8409 970.7652
Non-Agricultural Income	.2595576	.6825522	0.38	0.104****	-1.084625 1.60374
Loan Amount	-.8002965	.1077735	1.11	0.067***	-.2922731 .1322138
Support Amount.	-.1836924	.2442531	-0.75	0.053***	-.6647116 .2973269
Household Consumption.	-.2831779	.5060649	-0.56	0.076***	-1.279796 .7134398
Constant	264194.6	218420.2	1.21	0.028**	-165950.7 694339.9
	468066	20748.64			427204.7 508927.3

p-value: \*.000, \*\*.005, \*\*\*.010, \*\*\*\*.015

12 Number of left-censored observations

256 Number of uncensored observations

0 Number of right-censored observations

"Social security", classified as an endogenous factor impacting savings in agriculture, offers the state guarantee for operators to sustain their livelihoods, primarily in terms of health and income (Table 4). Alongside the growth of insurance frameworks, private insurance businesses furnish substantial future coverage. These insurances are sometimes denominated as precautionary savings, and operators can gain from these insurance processes against possible risks. Although a positive correlation exists between the insurance system and savings in developed countries, a comparable effect was also observed in Turkish households ( $p < 0.05$ ).

Households have embraced the agricultural sector as a way of life and continue its activities as part of a socio-cultural lifestyle. This is particularly evident in small-scale households, where low incomes and lack of socio-cultural infrastructure impact savings. In numerous household situations, socio-cultural needs increase

with household size. Moreover, resources are transferred out of agriculture, which is expected to have a negative impact on agricultural savings. The empirical analysis reveals that agricultural savings are negatively impacted by "Household size" ( $p < 0.05$ ). This outcome concurs with the conclusions drawn from empirical studies (Browning ve Lusardi, 1996; Loayza ve ark., 2000; Abid ve Afridi, 2010; Obayelu, 2013). There is a significant correlation between the "health status" and agricultural savings, with the knowledge that greater savings are made in the case of unexpected future health-related situations ( $p < 0.10$ ). Further analysis has confirmed this and revealed that the health status of the operator indeed has an adverse impact on savings (Table 4).

In the Turkish economy, there is a need for macro policies that will increase income, increase production, and reduce economic vulnerabilities. This is also true for savings, and the second of the endogenous factors

affecting agricultural savings is "economic factors". While economic indicators in the world and in Turkey have a rapid change trend, it is important for savers to adapt to this trend. Increasing "business income", which is the most important among economic factors, affects the financial situation of the household and increases savings. Especially in developing countries, income plays an important role in determining household savings. Indeed, the willingness and ability to save depends on having more resources than those allocated to basic needs.

An increased income results in higher cash inflows for a business, thereby impacting its ability to save and boost its financial stability. Moreover, business owners have more opportunities for savings. By utilizing the additional income, businesses can invest, establish emergency funds, or make long-term savings, ultimately enhancing their financial security and facilitating their expansion and growth prospects. When analyzing the relationship between business income and savings using the Tobit model, it is shown that agricultural income has a positive impact on savings ( $p < 0.01$ ). Additionally, it is estimated that increasing the income will also increase the amount of savings, as shown in Table 4.

Working capital is another significant economic factor in the model, with intensive capital being necessary to meet population pressure in agricultural production. Implementing technology requires capital, making it an asset. Productivity can be best achieved through the rational use of capital. The excessive use of fixed capital leads to increased interest and depreciation costs, resulting in higher operating costs. It is crucial to maintain a balance between capital items for the sustainability of the household. Thus, savings are necessary for rationalizing working capital. Within the context of this study, Table 4 shows that a capital increase has a positive effect ( $p < 0.15$ ) on savings in the household industry. It is anticipated that a further increase in capital will lead to further savings.

Another factor that affects internal savings in households is the cost of operating. This cost encompasses both fixed and variable expenses resulting from production activities in the household and is projected to negatively impact savings. The study found a negative effect of operating costs on savings ( $p < 0.05$ ). Hence, it is anticipated that decreasing operating costs will lead to increased profits and, in turn, result in higher savings (refer to Table 4).

The size of farms is an additional economic factor influencing savings. In fact, 99% of agricultural operations in Turkey are considered small and below the optimum scale, resulting in decreased savings. This study produced a similar outcome, identifying that the size of a farm has a positive effect ( $p < 0.01$ ) on the amount of savings (Table 4). Policies that promote household expansion can aid in savings and capital

accumulation. However, the household industry faces challenges in auditing, informality, and under-reporting activities, impeding the ability to increase savings. Ultimately, the impact of financial variables on savings hinges on sectoral development.

Within the scope of the study, the impact of exogenous factors on savings is analyzed with the support of the literature by utilizing macro indicators as well as micro indicators. Accordingly, it is observed in the literature that the real exchange rate has a negative effect on savings (Montiel ve Servén, 2008; Hassan ve ark., 2011; Torun ve Karanfil, 2016). Especially in an import-oriented economy, the appreciation of the exchange rate against the value of the national currency causes production to become more expensive, which in turn leads to a decrease in production and loss of income. Therefore, monetary and exchange rate policies are needed to control overvaluation. In addition, policies and measures aimed at limiting rapid credit growth that encourages consumption growth will increase savings.

Apart from income and exchange rates, the most important factors affecting savings are "interest rates and inflation". An increase in interest rates increases savings. Empirical studies have shown that interest rates increase saving tendencies in developed countries (Koskela ve Virén, 1982; Balassa, 1992). This is the case in the Turkish economy and especially the decline in interest rates has caused savings to move downwards. When the changes in interest rates between 1998-2018 are analyzed, it is seen that the decline in interest rates and inflation has led to a downward trend in household savings. In countries with high interest rates and inflation, savers prefer different investment channels (Schaefer, 1993). Therefore, following economic trends is important for the evaluation of savings.

In recent years, fluctuations in agricultural incomes have resulted from changes in supply and demand structure, consumption habits, and increased production costs. To offset income shortfalls, farmers often rely on alternative sources of income. Pension, rental income, salary, and profit partnerships are common examples of additional income sources within the agricultural industry. In this study, we incorporated income from non-agricultural sources into the model under the assumption of a positive impact on savings. The results of our analysis, as presented in Table 4, indicate a statistically significant positive effect at the 0.15 level.

The global and Turkish markets have seen a surge in demand for agricultural products, the principal source of essential nutrients, prompting increased production to meet this demand. However, in Turkey, as well as globally, arable land has reached its maximum capacity. The only way to increase agricultural production is by improving productivity per unit area

or animal. Achieving this higher productivity is directly linked to the intensive use of capital. Thus, farmers must rely on their savings to meet this challenge. When farmers utilize external resources for intensive capital utilization, they may require more external resources if the harvest income falls below expectations. This can lead to decreased sustainability of the household. The research conducted revealed that increased credit usage has a negative effect ( $p < 0.10$ ) on savings (refer to Table 4). Consequently, we can conclude that greater credit usage reduces the tendency to save in households.

Other factors that facilitate the shift from agriculture to other industries include the significant reliance on weather patterns in agricultural production, the prevalence of risks and uncertainties, and the instability of prevalent market conditions. These obstacles create disparities in earnings among farmers. As a result, the agricultural sector is sustained to minimize these inequalities and guarantee the acquisition of capital. In Turkey, around 25 billion TL is allocated to the agricultural sector via public subsidies (Kıymaz, 2023). However, there are empirical studies indicating that the amount of support provided to households not only reduces their profitability by reducing their savings opportunities but also reduces the level of social welfare (Mishra ve Chang, 2009). The Tobit model was employed to examine the impact of public subsidies on the savings rates of households. The analysis revealed a negative effect of subsidies on savings ( $p < 0.10$ ) (Table 4), indicating that agricultural support measures in Turkey decrease savings. Savings ought to be deemed a risk management instrument, as it stabilizes operators' income, enkindles deposit-taking during times of excess income, and furnishes cash flow during low-income periods. Thus, agricultural households can meet their subsistence and operating expenses by using their savings and can secure their income without relying on state intervention through the creation of a precautionary fund for household managers and their families.

"Consumption expenditures" refers to necessary expenses such as food, healthcare, transportation, communication, social and cultural activities, and so forth, within a year - another factor impacting savings in households. Humans are social beings who aim to satisfy other needs after working for a certain time. Therefore, consumption expenditures are made to enhance individuals' productivity and maintain their livelihood. Despite the known issue of the impact of consumption expenditures on savings, they have been incorporated into the model to assess their strength of influence. Analysis suggests a significant and strong positive impact of consumption expenditures on savings, along with a negative and statistically significant effect ( $p < 0.05$ ) (refer to Table 4).

Environmental factors that affect savings in agricultural households were not considered in the model due to the necessity for macro data, and the relevant data set will be treated as a time series. However, climate conditions, soil fertility, water resources, market conditions, and risks and uncertainties do impact the profitability of agricultural household and its ability to save. Developing risk management strategies and making long-term plans are crucial for efficient households. However, in macro analyses, particularly in relation to climatic factors, it is essential to follow pertinent statistics over time. Therefore, evaluating the impact of environmental factors using cross-sectional data is not considered appropriate.

### CONCLUSION and RECOMMENDATIONS

An understanding of the factors influencing savings formation in the agricultural sector is of great value in the design of policies that enhance the sector's capacity to generate internal resources. It is anticipated that the findings will provide valuable information that can be utilized in the formulation of effective policies that favor the household community. The objective of this study is to examine the relationship between individual and economic variables and the level of agricultural savings. The Tobit regression analysis indicates that age, household size, capital, agricultural income, farm size, and non-agricultural income exert a significant and positive influence on farm savings. It can therefore be posited that as the age, household size, capital, agricultural income, farm size, and non-farm income of operators increase, savings are expected to increase. Conversely, a decline in educational attainment, coupled with inadequate social security, deteriorating health, rising operating costs, loan values, and subsidies, have a significant and detrimental impact on savings levels. It is noteworthy that lower levels of education are associated with lower levels of savings, and business expenditures lead to an increase in total expenditures, reducing disposable income and causing a decline in total savings.

It is therefore recommended that alternative sources of income be investigated with a view to stimulating economic growth by mobilizing greater savings. As the data analysis reveals, a significant proportion of investment capital in Turkey is allocated to real estate or vehicle funds, irrespective of location. It is therefore imperative to provide support and encouragement for alternative investment instruments, rather than relying on property ownership as the sole mechanism for investment. Firstly, it is of great importance to enhance existing incentives, such as those pertaining to housing account contributions, the KKDF, and the BİTT, for first-time buyers. Another issue that requires attention is the way tax arrangements, such



as those pertaining to value increase and property tax, are structured. This is with a view to preventing the acceptance of real estate and vehicles as investment instruments if a second real estate asset is owned. Furthermore, it is recommended that practices for opening savings accounts by expenditure items be implemented in agricultural enterprises, considering the amount and the duration of the amount in the account. It would be beneficial for the government to provide state support for the establishment of a small number of aggregated savings accounts, which should then be made widespread. Savings accounts should be established for expenditures on education, health, holidays, culture, etc., especially for consumption goods, and savings accounts should be established with the help of tax deductions, incentives, or other monetary mechanisms.

Upon analysis of the data in Turkey, it becomes evident that a considerable proportion of investment funds are directed towards real estate or vehicle funds, irrespective of whether the location in question is urban or rural. It is therefore necessary to provide support and promote alternative investment instruments, rather than housing ownership as an investment vehicle. One approach to achieving this would be to guarantee existing investment funds. It is also important to ensure the reliability of mutual funds to attract the savings of enterprises into the system. Finally, it would be beneficial to provide guarantees for the funds of agricultural enterprises, which could be secured by supporting appropriate sectoral and non-sectoral investment funds.

It is anticipated that as the level of education within households increases, so too will awareness of the importance of savings and the role of savings institutions. It is, therefore, crucial to prioritize the raising of awareness and the provision of support to households through the implementation of targeted training and awareness programs, which are designed to address the specific needs of agribusinesses. In this context, the dissemination of information using brochures, booklets, educational visual aids, seminars, and training activities to different segments of the population is of paramount importance. The objective is to enhance comprehension of the principles of saving, investment, budgeting, and financial planning. It is essential to promote a range of financial products and services and to facilitate an understanding of the risks associated with financial markets. This will enable individuals to make informed decisions and to apply for assistance when necessary. It is of the utmost importance to implement initiatives that will enhance financial awareness within the community. Seminars, conferences, and TV radio programs can serve as effective platforms for disseminating knowledge on financial literacy. Training modules on fundamental financial concepts, budget planning, credit and debt

management, and saving methods should be developed for agricultural enterprises and farmer organizations. The involvement of all relevant stakeholders, particularly universities, is crucial in the preparation of these training modules.

It is recommended that policy interventions in Turkey prioritize enhancing the accessibility and availability of financial institutions in rural areas, with the objective of fostering household savings. It is important to acknowledge that financial services such as loans and subsidies have a detrimental and pronounced effect on savings. It is, therefore, imperative that loans, grants, incentives, and subsidies be restructured to guarantee the economic sustainability of agriculture and promote diversification. It is recommended that state-supported financial systems be established with the objective of increasing the proportion of savings that are converted into investments. In this context, the promotion of mobile applications and web platforms that facilitate communication between agricultural operators and financial institutions should be encouraged. Such systems must be based on an accurate assessment of credit risks, which can be achieved through the analysis of agribusiness data and the utilization of artificial intelligence-supported credit evaluation systems. Furthermore, efforts should be made to specialize in agricultural loans by establishing local public and private banks and diversifying collateral in financial services, as well as accepting alternative collateral sources. In particular, the bureaucratic obstacles between financial institutions and agricultural operators should be removed and the legislation on agricultural loans should be simplified. Furthermore, the establishment of funds that provide credit guarantees specific to the sector should be supported. Another action to be taken in this area is to reinforce the financial structures of cooperatives and unions. At the level of cooperatives and unions, collective credit should be made available with the objective of reducing the costs associated with buying and selling transactions, as well as the costs borne by members. Professional consultancy services should be made mandatory, and the financial management skills of these organizations should be enhanced. It would be beneficial for these organizations to establish funds in which members can make collective savings, and to encourage the utilization of these savings for joint investments. Another significant issue is the inverse correlation between public subsidies and savings. It is therefore necessary to combine appropriate items by providing subsidies on an enterprise basis and to ensure the traceability of these transfers by providing them to micro-enterprises.

In conclusion, it can be argued that an increase in the financial savings of agricultural operators will support

the sustainability of agricultural production and ensure their economic security. In order to achieve this, a number of strategies should be adopted, including training programs, government support, digitalization, the strengthening of cooperatives, and the improvement of banking services. By implementing these measures, it is possible to increase the level of financial awareness of farmers, thereby enabling them to save and become economically stronger.

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## Summary of Researchers' Contribution Rate Declaration

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

## Conflict of Interest Statement

We declare that there is no conflict of interest between us as the article authors.

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## Anatomic and Phytochemical Investigation of Herbal Tea Bags Sold as Lemon Balm (*Melissa officinalis* L.) in the Market

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### ABSTRACT

*Melissa officinalis* L., an ethnobotanically valuable plant, has been used for the treatment of several diseases since ancient times. However, different plants with the same name are sold instead of lemon balm in markets that sell herbal products in Türkiye. For this purpose, 15 different brands of tea bags in crushed form, sold as lemon balm (*Melissa officinalis* L.) in markets, were analyzed. The total phenolic, flavonoid, and antioxidant capacities of the hydroalcoholic extracts of these tea samples along with the stomatal structure were investigated. Additionally, chemical compositions and rosmarinic acid contents were determined by the high-performance thin-layer chromatography method. Among the samples examined, S1, S2, S9, and S12 tea samples were found to meet the eligibility criteria. The leaves of these samples had diacytic stomata and the rosmarinic acid ratio in their phytochemical composition was over 2%. Furthermore, caffeic acid was detected in these samples.

### Food Science

### Research Article

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## Piyasada Tıbbi Melisa (*Melissa officinalis* L.) Olarak Satılan Bitkisel Poşet Çayların Anatomik ve Fitokimyasal Olarak İncelenmesi

### ÖZET

Etnobotanik olarak değerli bir bitki olan melisa eski çağlardan beri birçok hastalığın tedavisinde kullanılmaktadır. Buna karşın, ülkemizde aynı isme sahip farklı bitkiler piyasada tıbbi melisa (*Melissa officinalis* L.) yerine satılmaktadır. Bu amaçla, piyasada *M. officinalis* olarak satılan öğütülmüş formda 15 farklı markalı poşet çay analiz edildi. Bu örneklerin hidroalkolik ekstratlarının toplam fenol, flavonoid ve antioksidan kapasiteleri ile birlikte stoma yapıları incelendi. Buna ek olarak, kimyasal birleşimleri ve yapılarında bulunan rosmarinik asit miktarları Yüksek Performanslı İnce Tabaka Yöntemi ile belirlendi. İncelenen örnekler arasında, S1, S2, S9 ve S12 kodlu örneklerin uygunluk kriterlerini karşıladığı bulundu. Buna göre bu örneklerin yaprakları diasitik stoma yapısına sahip olduğu ve kimyasal yapılarında rosmarinik asit oranlarının %2'nin üzerinde olduğu belirlendi. Ayrıca bu örneklerde kafeik asidin de olduğu tespit edildi.

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### INTRODUCTION

Lemon balm (*Melissa officinalis* L.), known as "Melisa, oğulotu" in Türkiye, is a perennial herb belonging to the Lamiaceae family. It typically grows to a length of about 30-120 cm, and its stem and leaf parts are covered with hairs. *Melissa officinalis*, which grows naturally in Southern Europe, the Caucasus, Northern

Iran, Northern Iraq, and Türkiye, is extensively cultivated worldwide (Mill, 1982; Güner et al., 2012; Shakeri et al., 2016).

The medicinal use of the plant dates back centuries. Tea prepared with the leaf part of *M. officinalis* is especially beneficial for sleep and gastrointestinal problems (Carnat et al., 1998). In addition, clinical

studies have shown that it possesses antioxidant, antiviral, antifungal, antibacterial, antitumor, antianxiety, and antihyperlipidemic properties (Mimica-Dukic et al., 2004; Ferreira et al., 2006; Nolkemper et al., 2006; Cases et al., 2011). *M. officinalis* contains various phytochemicals in its structure. Aerial parts contain flavones such as luteolin, apigenin, flavanol (catechin, epicatechin), flavanone (naringenin, hesperidin), and essential oil compounds like citral. It also contains triterpenes (ursolic and oleanolic acid) and intense phenolic acids (rosmarinic acid, caffeic acid, chlorogenic acid) (Patora & Klimek, 2002; Dastmalchi et al., 2008; Pereira et al., 2014; Miraj et al., 2017). In particular, its pharmacological activity is thought to be due to rosmarinic acid, and its standardization in leaves and extracts is based on this phenolic compound according to the European Pharmacopoeia (Ramanauskiene et al., 2016; Anonymous, 2023; Ulgen et al., 2023).

There are different plants with the name "Melisa" in Türkiye. The leaves of beebush (*Aloysia* L. sp.), which have a similar odor, are sold in many markets instead of *M. officinalis* (Saskara et al., 2010). However, no studies have been conducted on grounded-type teas so far. This study aimed to compare *M. officinalis* tea bags purchased from 15 different markets as anatomical and phytochemically. For this purpose, the stomata type of the samples was examined. Additionally, total phenolic, and flavonoid contents as well as antioxidant activities were determined. Furthermore, rosmarinic acid content in the structure of the samples was quantitatively determined by the high-performance thin layer chromatography (HPTLC) method.

## MATERIALS and METHODS

### Plant Materials and Chemicals

15 different brands of tea bags (coded S1-S15) sold as "*M. officinalis* leaves" in the markets were purchased and stored at room temperature throughout the experiment (Figure 1). *Melissa officinalis* standardized extract was obtained from Martin Bauer (Germany) for comparison with other samples in a thin-layer chromatography study. Other chemicals used in experiments were purchased from Sigma.

### Preparation of Hydroalcoholic Extract

250±1 mg of herbal tea was extracted with 25 ml of 75% ethanol in an ultrasonic bath for 20 minutes. It was filtered with a 0.45 µm syringe filter and the liquid portion was evaporated using a rotary evaporator under 45°C and 50 mbar pressure conditions. After calculating the extract yields, stock solutions of 5 mg ml<sup>-1</sup> were prepared for each sample to be used in the experiments (Table 1). These prepared stock solutions were stored in the refrigerator at +4°C throughout the study.



Figure 1. The general appearance of the herbal material used in the experiments. (Sample 3)

Şekil 1. Çalışmalarda kullanılan bitkisel materyalin genel görünümü. (Örnek 3)

### Anatomical Analysis

The type of stomata, stomatal length, width, and outline dimensions (Figure 2) of the samples were observed and photographed at 40x magnifications using the Zeiss (Oberkochen, Germany), Axio Lab A1 microscope with 50% chloral hydrate solution according to the microscopic examination of the *Melissae folium* section in the European Pharmacopoeia (Anonymous, 2023).

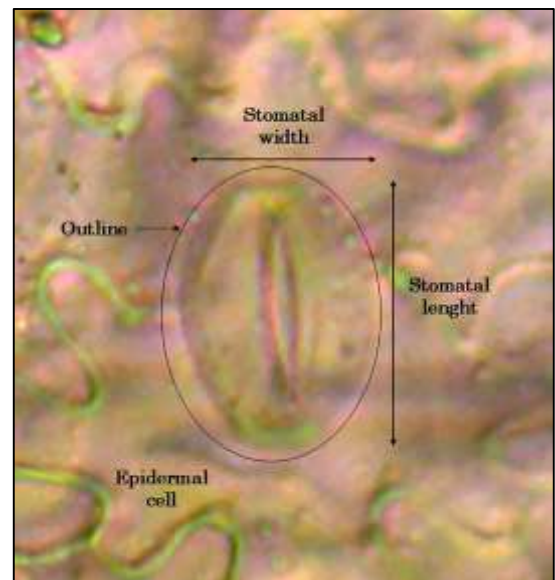


Figure 2. The stomatal sections were examined at 40x magnification using a 50% chloral hydrate solution during anatomical analysis

Şekil 2. Anatomik analizde %50 kloral hidrat solüsyonu ile 40x büyütmede incelenen stoma bölümleri.

Table 1 Extract Yields and Dilution Volumes  
*Çizelge 1. Ekstre Verimleri ve Seyreltme Oranları*

Samples	Sample weights (mg)	Extract (mg)	Yield (%)	Volume used to prepare 5 mg ml <sup>-1</sup> stock solution (ml)
S1	250.4	53.2	21.2	10.64
S2	250.0	50.1	20.0	10.02
S3	250.5	56.8	22.7	11.36
S4	250.0	63.1	25.2	12.62
S5	250.8	38.9	15.5	7.78
S6	250.9	41.6	16.6	8.32
S7	250.6	49.5	19.8	9.9
S8	250.9	49.3	19.7	9.86
S9	250.1	36.5	14.6	7.3
S10	250.6	78	31.2	15.6
S11	250.5	55.8	22.3	11.16
S12	249.7	34.1	13.6	6.82
S13	250.0	57.5	23.0	11.5
S14	249.8	66.8	26.7	13.36
S15	250.8	59.2	23.6	11.84

### Total Phenolic Content

25 µl of 0.5 mg ml<sup>-1</sup> sample or blank (water), 25 µl of distilled water, 125 µl of 10% Folin Ciocalteu reagent, and 100 µl of 7.5% sodium carbonate were added directly into the 96-well plate and incubated at 37°C for 30 minutes (Singleton & Rossi, 1965). After incubation, the absorbance of the solutions was measured with a Thermo Scientific (Massachusetts, ABD) Varioskan Lux microplate reader at a wavelength of 760 nm. The total phenolic content of tea samples was expressed as mg gallic acid (4-125 µg ml<sup>-1</sup>;  $y = 66.656x - 0.0303$ ,  $R^2 = 0.9974$ ) equivalent per g of extract.

### Total Flavonoid Content

#### AlCl<sub>3</sub> Colorimetric assay in the absence of NaNO<sub>2</sub>

30 µl of 5 mg ml<sup>-1</sup> sample or blank (ethanol), 60 µl of 70% ethanol, 30 µl of 10% AlCl<sub>3</sub>, 30 µl of 1 M sodium acetate, and 150 µl of methanol were added into the wells. After the 15-minute incubation period, the absorbance of the reactions was measured at a wavelength of 415 nm (Degirmencioglu et al., 2019). Results were shown as mg quercetin (4-250 µg ml<sup>-1</sup>;  $37.126x + 0.0069$ ,  $R^2 = 0.9999$ ) equivalent to total flavonoid content per g Of extract.

#### AlCl<sub>3</sub> Colorimetric assay in the presence of NaNO<sub>2</sub>

200 µl of 0.5 mg ml<sup>-1</sup> sample, 200 µl of water, and 30 µl of 5% sodium nitrite were mixed and kept for 5 minutes. Afterward, 30 µl of 10% AlCl<sub>3</sub> was added, mixed by a vortex again, and left at room temperature for 6 minutes. Lastly, 200 µl of 1 M NaOH and 340 µl water were added to the solution, mixed, and kept for 15 minutes (Zhishen et al., 1999). Absorbances were defined at a wavelength of 510 nm and results were specified as mg catechin (16-500 µg ml<sup>-1</sup>;  $y = 15.33x -$

0.0118,  $R^2 = 0.9976$ ) equivalent per g of extract.

### Total Anthocyanin Content

40 µl of 5 mg ml<sup>-1</sup> sample and 160 µl of pH1 and Ph4.5 buffer solutions (pH1 buffer was prepared with 25 mM of potassium chloride and pH4.5 buffer was prepared with 400 mM of sodium acetate, pH of both buffer solutions was adjusted using diluted hydrochloric acid) were mixed separately. 15 minutes later absorbances were observed at 520 and 700 nm wavelengths (Lee et al., 2005). The absorbance of anthocyanin was determined according to Equation 1. The total anthocyanin content of the examined tea sample was given as mg cyanidin 3-glucoside (4-125 µg ml<sup>-1</sup>;  $y = 29.296x + 0.0067$ ,  $R^2 = 0.9999$ ) equivalent per g of extract.

$$\text{Anthocyanin absorbance} = \frac{(A_{520} - A_{700})_{\text{pH1}} - (A_{520} - A_{700})_{\text{pH4.5}}}{\text{(Eq. 1)}}$$

### Total Antioxidant Contents

#### DPPH

280 µl of 0.1 mM ethanolic DPPH solution (2,2-Diphenyl-1-picrylhydrazyl, absorbance ~0.7) was added to 20 µl of 0.5 mg ml<sup>-1</sup> sample or blank (ethanol) and left in the dark environment for half an hour at room temperature. The absorbance of the formed color changes was measured at a wavelength of 520 nm (Blois, 1958). The results were expressed as mg Trolox (8-125 µg ml<sup>-1</sup>;  $y = 79.756x - 0.0047$ ,  $R^2 = 0.9998$ ) equivalent to total antioxidant content per g of extract.

#### ABTS Radical scavenging assay

20 µl sample or blank (methanol) and 280 µl ABTS reagent ( $7 \times 10^{-3}$  M of ABTS and  $2.45 \times 10^{-3}$  M of potassium persulfate) were mixed in equal volumes and kept for 12-16 hours at room temperature in a dark



environment, then the solution was diluted at a ratio of 1:10 with methanol and adjusted to an absorbance of approximately 0.7) were added into the wells and incubated for 6 minutes at room temperature. The absorbance of solutions was read at 734 nm wavelength (Re et al., 1999). Results were defined as mg Trolox ( $8-125 \mu\text{g ml}^{-1}$ ;  $y=84.011x-0.0047$ ,  $R^2 = 0.9931$ ) equivalent to total antioxidant content per g of extract.

#### Ferric-reducing antioxidant power assay (FRAP)

20  $\mu\text{l}$  of sample or blank (water) and 280  $\mu\text{l}$  of freshly prepared FRAP reagent ( $2 \times 10^{-2}$  M  $\text{FeCl}_3$ ,  $1 \times 10^{-2}$  M TPTZ, and pH 3.6 sodium acetate buffer) were mixed in a ratio of 1:1:10, respectively) were mixed and kept for 6 minutes at room temperature. Then, absorbances of the reaction were determined at a wavelength of 595 nm (Benzie & Strain, 1996). Results were stated as mg Trolox ( $8-250 \mu\text{g ml}^{-1}$ ;  $y=102.5x+0.061$ ,  $R^2 = 0.9992$ ) equivalent to total antioxidant content per g of extract.

#### CUPRAC

The Cupric Reducing Antioxidant Capacity assay was determined according to the method of Apak et al. (2004) with minor modifications. Initially, 20  $\mu\text{l}$  sample or blank (water) solutions were added well plate, then 280  $\mu\text{l}$  of reagent consisting of  $1 \times 10^{-2}$  M copper (II) chloride,  $7.5 \times 10^{-3}$  M neocuproine and 1 M ammonium acetate (pH 7) was added onto solution and kept in the dark at room temperature for half an hour. The absorbance of the yellow color formed at the end of the reaction was measured at a wavelength of 450 nm. Results of the tea samples were given as mg Trolox ( $16-250 \mu\text{g ml}^{-1}$ ;  $y=67.53x-0.0029$ ,  $R^2=0.9998$ ) equivalent to the total antioxidant content per g of extract.

#### Qualitative and Quantitative Analysis by High-Performance Thin Layer Chromatography

The chromatographic separations of rosmarinic acid, caffeic acid, and verbascoside compounds in the tea extracts were visually determined by the HPTLC method, qualitatively. References including 200  $\mu\text{g ml}^{-1}$  of standardized *M. officinalis* extract, rosmarinic acid, and caffeic acid with 5  $\text{mg ml}^{-1}$  of samples were implemented 5  $\mu\text{l}$  on a glass-backed plate coated with silica gel 60 F<sub>254</sub> as an 8 mm wide band by an automatic Camag Limonat V sample spotter (Muttentz, Switzerland). For the development process, two mobile phase systems were utilized. For detection of rosmarinic acid and caffeic acid; chloroform: ethyl acetate: formic acid (5:4:1, v:v:v), for the verbascoside; ethyl acetate: water: formic acid: acetic acid (15:2:1:1, v:v:v:v) systems were used. After development, the plates were heated at 105 °C for 3 min and then derivatized with NP and PEG reagents respectively. Derivatized plate image was captured at 254 and 366 nm wavelengths by HPTLC imaging device.

The quantitative determination of rosmarinic acid content in the tea extracts was performed using the HPTLC method. A solution containing 0.2 to 1  $\mu\text{g}$  of rosmarinic acid and 0.5  $\text{mg ml}^{-1}$  of sample extracts was applied on the silica plate in triplicate by a sample applicator equipped with a 100  $\mu\text{l}$  syringe (Hamilton, Bonaduz, Switzerland) connected to a nitrogen tank. The twin trough chamber was pre-conditioned with the vapor of the developing solvent system of chloroform: ethyl acetate: formic acid (5:4:1, v:v:v) for 20 minutes. Subsequently, it was developed up to 7 cm in the Camag Automatic Developing Chamber, with relative humidity fixed at 33% using magnesium chloride hexahydrate solution. After the development process, plates were automatically dried for 5 minutes and scanned in absorption/reflectance mode at 330 nm wavelength by Camag TLC Scanner 3. Rosmarinic acid contents were established through peak area via polynomial regression ( $y=378.304+202.602x-0.007x^2$ ,  $R^2=0.99910$ ).

#### Statistical Analysis

Statistical differences among the samples were analyzed using Minitab 17 software. The statistical significance of the results was determined through a one-way analysis of variance (ANOVA), and the significance levels were indicated using the Tukey post hoc test with a significance level of  $p \leq 0.05$ . The experiments were conducted in triplicate, and the results are presented as means  $\pm$  standard error.

## RESULTS and DISCUSSION

#### Anatomical Analysis

The stomatal structures of the samples were examined using chloral hydrate solution under a light microscope at 40x magnification (Figure 3). Among the samples, diacritic stomata surrounded by wavy epidermal cells were observed in six samples (S1, S2, S9, S12, S14, and S15), while anomocytic stomata with striated epidermal cells were present in seven samples (S3, S4, S6, S7, and S8). Additionally, paracytic stomata were identified in two other samples (S11 and S5). Furthermore, a few diacytic stomata were observed alongside anomocytic stomata in the S10 and S13 samples.

Among the observed stomatal types, diacritic stomata were found to have a width ranging from 15.17 to 17.54  $\mu\text{m}$ , a height between 20.10 and 24.63  $\mu\text{m}$ , and an outline area of 256.64 to 357.02  $\mu\text{m}^2$ . Additionally, paracytic stomata exhibited an average width of 20.40 to 21.95  $\mu\text{m}$ , a height of 23.80 to 25.49  $\mu\text{m}$ , and an outline area of 396.58 to 442.81  $\mu\text{m}^2$ . Anomocytic stomata, on the other hand, displayed an average width of 19.77 to 22.74  $\mu\text{m}$ , a height ranging from 26.13 to 32.73  $\mu\text{m}$ , and an outline area of 433.30 to 534.60  $\mu\text{m}^2$  (Table 2).



Table 2. The results regarding stomatal width, height, and outline in the tea samples. (\*: Multiple stoma types were identified in samples S10 and S13, and only the measurements of the anomocytic stomata type were utilized)

*Çizelge 2. Çay örneklerinde stoma genişliği, yüksekliği ve ana hatlarına ilişkin sonuçlar. (\*: S10 ve S13 örneklerinde birden fazla stoma tipi belirlenmiş olup sadece anomositik stoma tipinin ölçüleri kullanıldı)*

	Type of stomata	Epidermal cells	Stomatal width (µm)	Stomatal height (µm)	Outline (µm <sup>2</sup> )
S1	Diacritic	Sinuuous	15.60±0.06	22.72±0.82	270.64±22.78
S2	Diacytic	Sinuuous	15.68±0.77	20.10±1.32	256.64±32.25
S3	Anomocytic	Irregular and striated	19.77±0.29	28.66±0.81	433.30±4.69
S4	Anomocytic	Irregular and striated	22.74±0.14	26.13±1.65	456.19±12.93
S5	Paracytic	Polygonal	21.90±1.01	25.49±0.12	442.81±6.82
S6	Anomocytic	Irregular and striated	21.78±0.18	32.73±1.11	532.84±11.22
S7	Anomocytic	Irregular and striated	21.99±0.63	31.28±0.84	534.60±2.28
S8	Anomocytic	Irregular and striated	21.53±1.94	30.55±0.71	468.66±46.20
S9	Diacytic	Sinuuous	17.08±0.14	23.88±2.14	315.67±7.43
S10*	Anomocytic	Irregular and striated	21.60±1.07	32.37±1.39	529.12±36.91
S11	Paracytic	Polygonal	20.40±2.02	23.80±1.39	396.58±55.25
S12	Diacytic	Sinuuous	17.54±0.35	24.25±1.56	335.09±27.59
S13*	Anomocytic	Irregular and striated	22.19±0.94	26.86±1.02	467.34±8.38
S14	Diacytic	Sinuuous	16.83±1.93	24.63±0.90	357.02±28.75
S15	Diacytic	Sinuuous	15.17±1.04	24.08±1.47	280.03±10.98

### Total Phenolic, Flavonoid, Anthocyanin and Antioxidant Contents

S1 and S2 samples showed the highest results in all analyses except for the total flavonoid experiment conducted without NaNO<sub>2</sub> in the medium, while S5 and S11 samples yielded the lowest results.

The total phenolic content of tea extracts varied between 305.08-117.80 mg gallic acid equivalent per gr extract. The S2 sample possessed the highest content with 305.08±2.32 mg gr<sup>-1</sup> of hydroalcoholic extract (Table 3).

In the total flavonoid experiment, the two widely applied methods in other studies were used. In the absence of sodium nitrite, aluminum chloride, and sodium acetate reagent, the reaction occurs only with flavonols and luteolin in the flavonoid subclasses. However, in the presence of sodium nitrite, the reaction is more specific for rutin, luteolin, and catechins (Pekal & Pyszynska, 2014; Shraim et al., 2021). The results showed that the S3 sample had the highest total flavonoid amount with 48.83±0.89 mg gr<sup>-1</sup> of extract in the reaction without NaNO<sub>2</sub>. When NaNO<sub>2</sub> was added to the reaction, the S2 sample exhibited the highest results with 260.17±2.96 mg gr<sup>-1</sup> compared to other extracts (Table 3).

According to the thin-layer chromatography test, anthocyanin subclass compounds were identified on the plate in S15 (Figure 3). In the total monomeric anthocyanin content experiment; 2.03±0.21 mg cyanidin 3-glucoside equivalent total anthocyanin per g of extract was detected in the structure of tea extract

coded as S15.

In the antioxidant experiments, it was defined that the S1 and S2 samples owned a higher antioxidant content than the other extracts. In the FRAP, ABTS, DPPH, and CUPRAC assays, the S1 sample yielded the following results; 410.91±9.99, 356.37±5.61, 300.96±5.70, 534.28±11.99 mg total antioxidant content per gram of extract, respectively. The S2 sample possessed 460.15±13.41 mg, 375.81±5.07 mg, 309.60±4.76 mg, and 643.05±11.58 mg of total antioxidant content per gram of extract in the same assays (Table 3).

### High-Performance Thin Layer Chromatography

Rosmarinic acid and caffeic acid, which are found in high amounts in *M. officinalis* along with verbascoside, the main compound of lemon verbena, were visually examined (Figure 4). Rosmarinic acid was detected in 10 samples (S2, S3, S4, S7, S9, S10, S12, S13, S14, and S15), verbascoside in 7 samples (S3, S4, S6, S7, S8, S10, and S13), and caffeic acid in 4 samples (S1, S2, S9, and S12) based on chromatograms.

Anthocyanins are substances that can be observed in white light without any derivatization on a chromatogram plate. They exhibit maximum absorbance between wavelengths of 500-550 nm (Saha et al., 2020). Accordingly, the red substances separated in white light in S15 were identified as anthocyanin (Figure 5). Furthermore, in the chromatographic bands of S5 and S11 samples, completely different substances were observed.

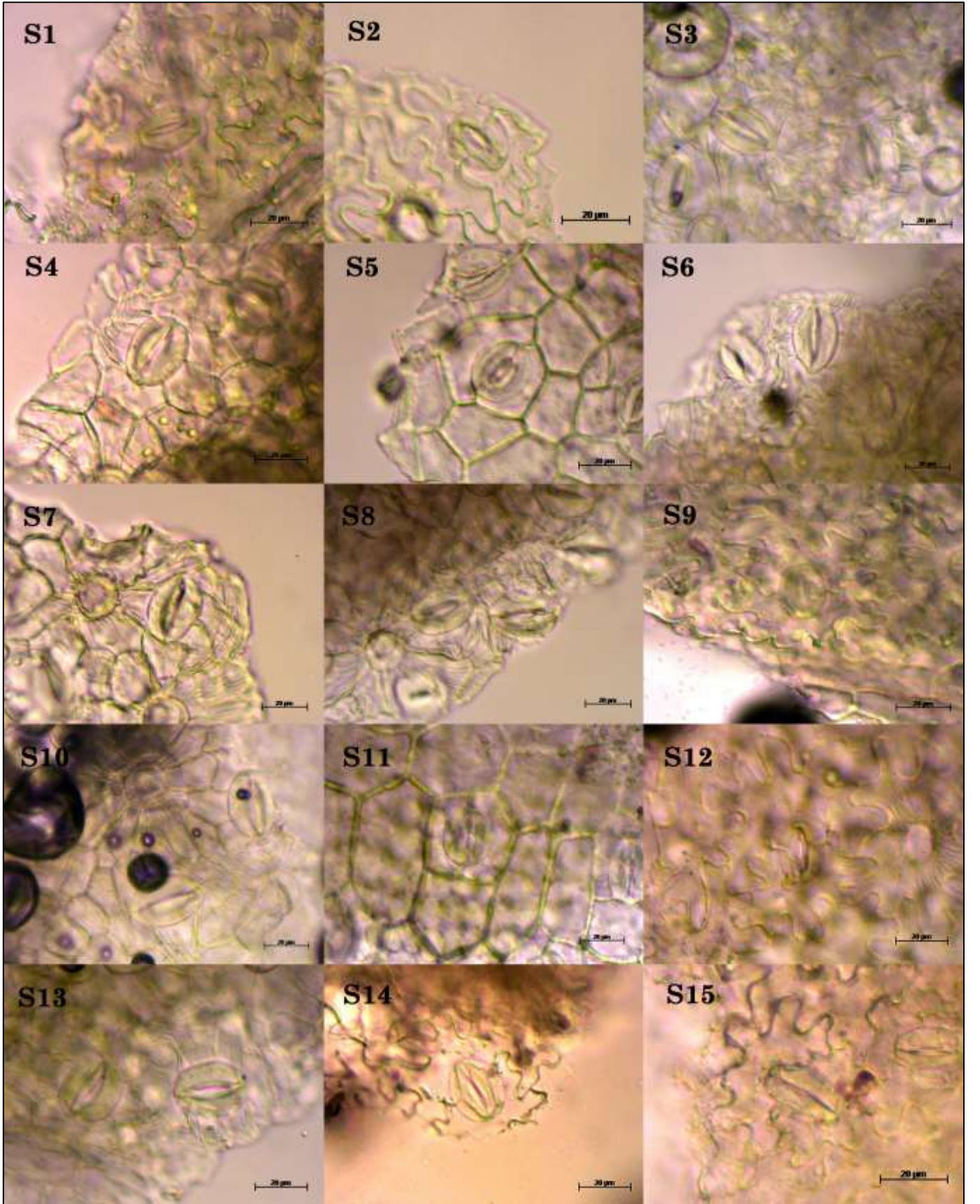


Figure 3. Major stomata types of tea samples with 40x magnification  
*Şekil 3. 40x büyütmede çay örneklerinin ana stomaları*

Table 3. Total phenolic, flavonoid, antioxidant, and rosmarinic acid contents in *Melissa officinalis* L. tea extracts<sup>6</sup>. (N.D.: not detected)  
 Çizelge 3. *Melissa officinalis* L. çay örneklerindeki toplam fenolik, flavonoid, antioksidan ve rosmarinik asit miktarları (N.D.: tespit edilmedi)

	Total phenolic content <sup>1</sup>	Total flavonoid content <sup>2, 3</sup>			Total antioxidant content <sup>4</sup>			Rosmarinic acid content <sup>5</sup>
		AlCl <sub>3</sub>	NaNO <sub>2</sub>	FRAP	ABTS	DPPH	CUPRAC	
<b>S1</b>	268.53±5.45 <sup>B</sup>	27.86±0.33 <sup>EF</sup>	228.75±0.14 <sup>B</sup>	410.91±9.99 <sup>B</sup>	356.37±5.61 <sup>A</sup>	300.96±5.70 <sup>A</sup>	534.28±11.99 <sup>B</sup>	60.08±1.87 <sup>A</sup>
<b>S2</b>	305.08±2.32 <sup>A</sup>	32.49±1.02 <sup>CD</sup>	260.17±2.96 <sup>A</sup>	460.15±13.41 <sup>A</sup>	375.81±5.07 <sup>A</sup>	309.60±4.76 <sup>A</sup>	643.05±11.58 <sup>A</sup>	52.45±0.61 <sup>B</sup>
<b>S3</b>	148.94±1.13 <sup>E</sup>	48.83±0.89 <sup>A</sup>	135.60±4.68 <sup>E</sup>	164.34±1.75 <sup>F</sup>	188.86±3.00 <sup>F</sup>	190.35±6.39 <sup>DEF</sup>	261.45±2.93 <sup>E</sup>	1.10±0.11 <sup>FG</sup>
<b>S4</b>	121.43±3.76 <sup>F</sup>	27.81±1.41 <sup>EF</sup>	117.29±1.56 <sup>FG</sup>	125.11±2.68 <sup>HI</sup>	176.11±3.50 <sup>FG</sup>	158.83±4.30 <sup>GH</sup>	196.14±6.76 <sup>G</sup>	N.D.
<b>S5</b>	104.64±1.60 <sup>F</sup>	44.42±0.82 <sup>B</sup>	82.78±1.22 <sup>H</sup>	82.05±0.57 <sup>J</sup>	158.62±1.36 <sup>G</sup>	135.27±2.46 <sup>H</sup>	169.04±4.89 <sup>G</sup>	N.D.
<b>S6</b>	182.95±2.22 <sup>D</sup>	41.46±0.47 <sup>B</sup>	154.56±5.25 <sup>D</sup>	203±7.70 <sup>E</sup>	236.86±7.87 <sup>C</sup>	228.95±8.90 <sup>C</sup>	318.64±6.10 <sup>D</sup>	N.D.
<b>S7</b>	147.89±7.74 <sup>E</sup>	34.16±0.73 <sup>CD</sup>	134.68±5.66 <sup>E</sup>	156.92±2.22 <sup>FGH</sup>	204.18±2.99 <sup>DEF</sup>	197.88±6.10 <sup>DE</sup>	242.56±8.78 <sup>EF</sup>	1.80±0.18 <sup>FG</sup>
<b>S8</b>	158.36±2.58 <sup>E</sup>	34.50±0.53 <sup>CD</sup>	140.33±1.62 <sup>E</sup>	150.90±2.75 <sup>FGH</sup>	202±6.29 <sup>DEF</sup>	190.37±2.25 <sup>DEF</sup>	243.18±2.25 <sup>EF</sup>	N.D.
<b>S9</b>	215.46±1.27 <sup>C</sup>	30.54±0.53 <sup>DEF</sup>	156.25±1.29 <sup>D</sup>	277.12±3.00 <sup>D</sup>	305.77±6.78 <sup>B</sup>	259.44±2.16 <sup>B</sup>	420.84±10.91 <sup>C</sup>	35.73±1.46 <sup>C</sup>
<b>S10</b>	150.96±2.65 <sup>E</sup>	31.67±0.47 <sup>CDEF</sup>	127.46±0.11 <sup>EF</sup>	163.06±2.63 <sup>F</sup>	199.39±2.52 <sup>DEF</sup>	188.84±0.87 <sup>DEF</sup>	261.35±4.07 <sup>E</sup>	3.65±0.34 <sup>F</sup>
<b>S11</b>	118.42±3.88 <sup>F</sup>	34.78±0.82 <sup>C</sup>	45.34±0.33 <sup>J</sup>	98.47±2.19 <sup>IJ</sup>	227.16±1.59 <sup>CD</sup>	170.71±4.70 <sup>FG</sup>	173.06±12.33 <sup>G</sup>	N.D.
<b>S12</b>	253.02±2.40 <sup>B</sup>	27.71±0.34 <sup>F</sup>	194.17±1.61 <sup>C</sup>	333.28±13.77 <sup>C</sup>	312.35±13.34 <sup>B</sup>	273.90±5.06 <sup>B</sup>	528.58±10.84 <sup>B</sup>	57.68±0.72 <sup>A</sup>
<b>S13</b>	143.95±1.22 <sup>E</sup>	31.91±0.45 <sup>CDE</sup>	109.76±2.01 <sup>G</sup>	145.19±5.75 <sup>FGH</sup>	183.54±2.01 <sup>FG</sup>	177.54±2.96 <sup>EFG</sup>	240.34±6.99 <sup>EF</sup>	3.63±0.18 <sup>F</sup>
<b>S14</b>	117.80±0.76 <sup>F</sup>	22.27±1.50 <sup>G</sup>	62.06±1.09 <sup>I</sup>	128.56±3.73 <sup>GHI</sup>	197.90±2.65 <sup>EF</sup>	166.81±1.65 <sup>FG</sup>	205.15±0.51 <sup>FG</sup>	7.25±0.17 <sup>E</sup>
<b>S15</b>	159.25±2.19 <sup>E</sup>	35.37±0.54 <sup>C</sup>	113.44±2.05 <sup>FG</sup>	162.49±5.62 <sup>FG</sup>	220.63±4.20 <sup>CDE</sup>	212.02±9.15 <sup>CD</sup>	271.23±11.41 <sup>E</sup>	13.41±0.43 <sup>D</sup>

<sup>1</sup> mg gallic acid equivalent total phenolic content in gr extract

<sup>2</sup> AlCl<sub>3</sub> procedure, mg quercetin equivalent total flavonoid content in gr extract

<sup>3</sup> NaNO<sub>2</sub> procedure, mg catechin equivalent total flavonoid content in gr extract

<sup>4</sup> mg Trolox equivalent total antioxidant content in gr extract

<sup>5</sup> mg rosmarinic acid per gr extract

<sup>6</sup> Different letters in the same column indicate significantly different values at P ≤ 0.05



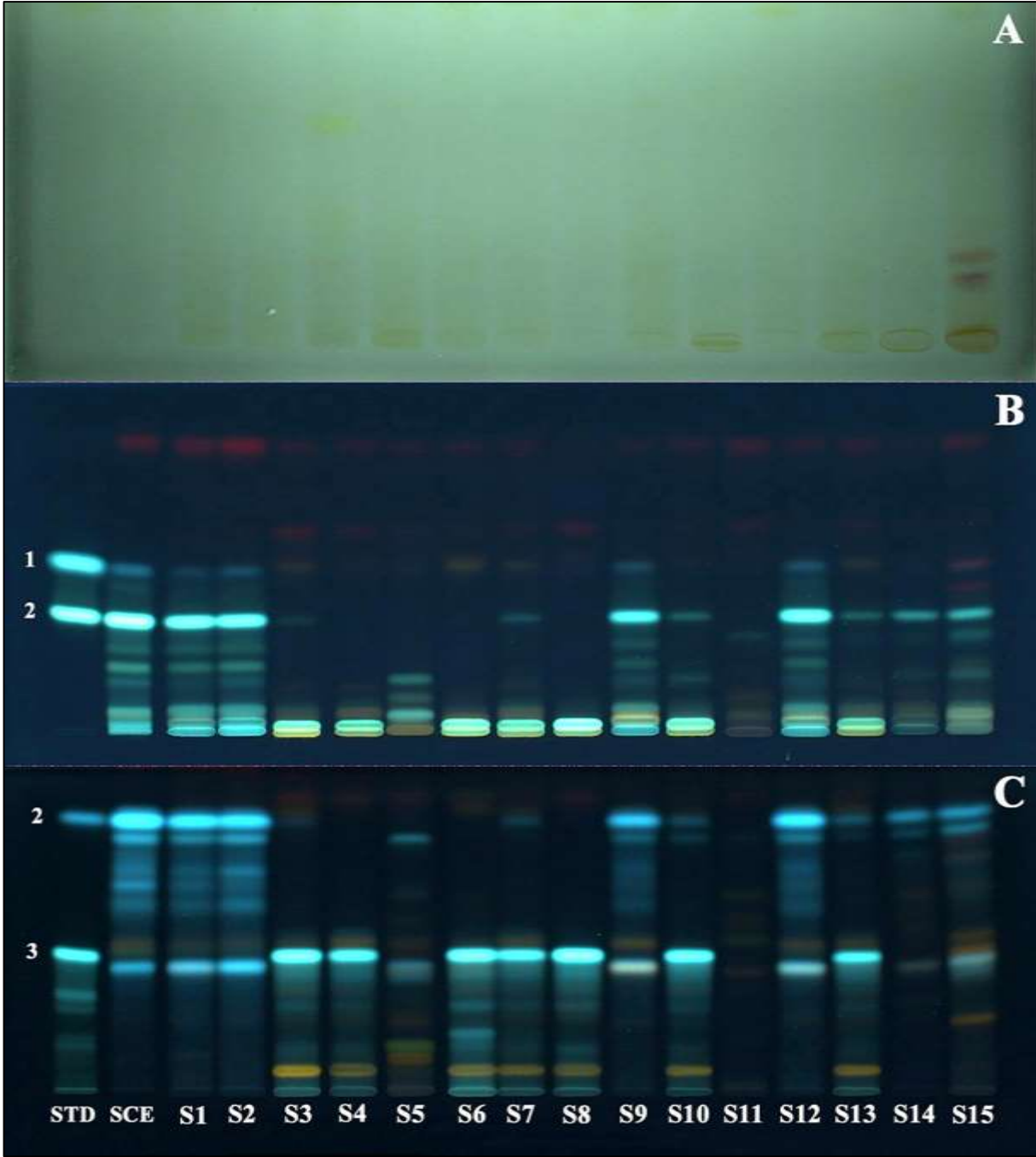


Figure 4. HPTLC chromatograms of tea extracts. STD: standards. 1: caffeic acid, 2: rosmarinic acid and 3: verbascoside. SCE: standardized commercial *M. officinalis* extract. (A) at white light, mobile phase: ethyl acetate–formic acid–acetic acid–water, (100:11:11:26, v:v:v:v), (B) at 366 nm and derivatized with NP/PEG, mobile phase: chloroform–ethyl acetate–formic acid (5:4:1, v:v:v:v), (C) at 366 nm and derivatized with NP/PEG, mobile phase: ethyl acetate–water–formic acid–acetic acid (15:2:1:1, v:v:v:v).

Şekil 4. Çay örneklerinin YPİTK kromatogramları. STD: referanslar. 1: kafeik asit, 2: rosmarinik asit ve 3: verbaskozit. SCE: standardize ticari *M. officinalis* ekstresi. (A) beyaz ışıkta, hareketli faz: etil asetat-formik asit-asetik asit-su, (100:11:11:26, v:v:v:v), (B) 366 nm'de ve NP/PEG ile türevlendirilmiş, hareketli faz: kloroform-etil asetat-formik asit (5:4:1, v:v:v), (C) 366 nm'de ve NP/PEG ile türevlendirilmiş, hareketli faz: etil asetat-su-formik asit-asetik asit (15:2:1:1, v:v:v:v).



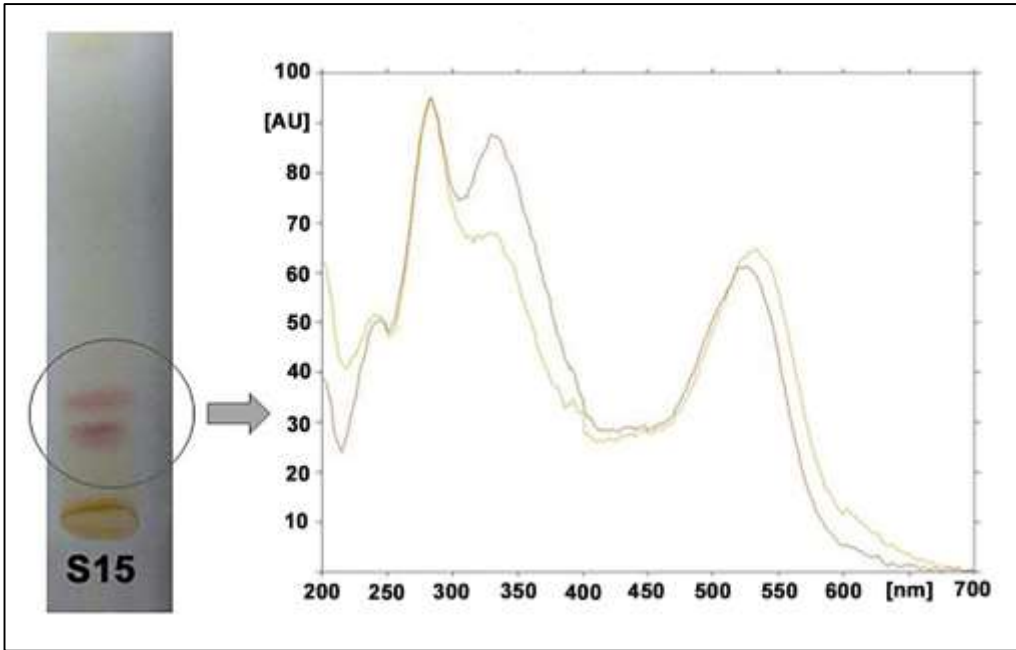


Figure 5. Overlaid spectra of the red-colored spots in S15 between 200 and 700 nm wavelength  
 Şekil 5. S15'teki 200 ve 700 nm dalga boyu arasındaki kırmızı renkli noktaların üst üste bindirilmiş spektrumları

**Quantitative Rosmarinic Acid Content by HPTLC**

According to the European Pharmacopeia, *M. officinalis* leaf extract must contain at least 2% rosmarinic acid (Anonymous, 2023). In the analysis, rosmarinic acid was found in 11 of the 15 samples and defined only 4 tea extracts (S1: 6%, S2: 5.2%, S9: 3.6%,

and S12: 5.8%) possessed rosmarinic acid in their chemical structure above 2% (Table 2). While the S1 sample was the extract with the highest content of rosmarinic acid with  $60.08 \pm 1.87 \text{ mg gr}^{-1}$  extract in its chemical composition (Figure 6), it was observed there was no rosmarinic acid in the structure of the 5 tea extracts (S4, S5, S6, S8, and S11).

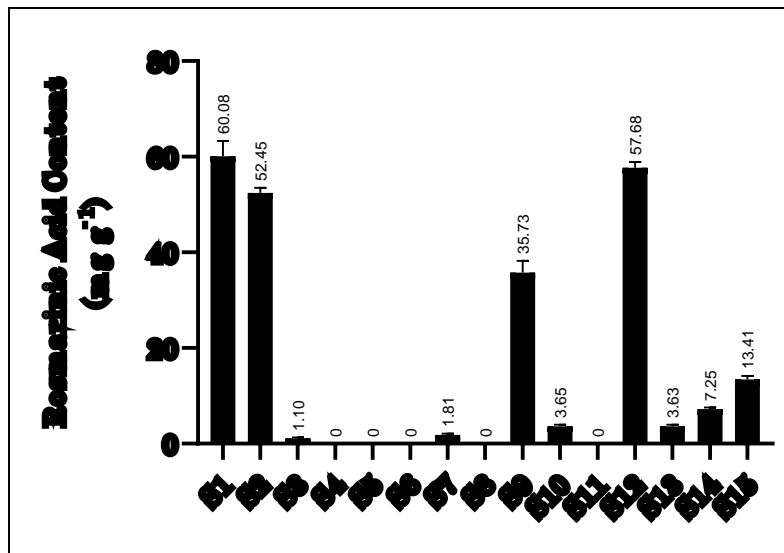


Figure 6. Rosmarinic acid content in samples  
 Şekil 6. Örneklerdeki rosmarinik asit miktarları

From an anatomical perspective, three types of stomata were identified in the examined samples: diacritic, anomocytic, and paracytic. The stomatal structure of the *M. officinalis* leaf, which belongs to the Lamiaceae family, is diacritic (Anonymous, 2023), and this stomatal structure was observed in six samples

(S1, S2, S9, S12, S14 and S15). Additionally, it was found that the sizes of these three stoma types varied, with diacritic stomata being smaller than the others in width, height, and outline (Table 2).

The most abundant substance in the chemical composition of *M. officinalis* is rosmarinic acid

(Shakeri et al., 2016), and over 2% of rosmarinic acid was detected in the structure of 4 samples (S1, S2, S9, and S12) of these 6 samples. In some studies on lemon balm, the ratio of rosmarinic acid in the chemical composition of the hydroalcoholic extracts of the lemon balm leaf varies between 3-8% (Dastmalchi et al., 2008; Kim et al., 2010; Shekarchi et al., 2012; Calleja et al., 2017; Kittler et al., 2018; Kittler et al., 2018).

In addition to chlorogenic acid, caffeic acid, another major substance found in the structure of *M. officinalis*, was also detected in the chromatogram of S1, S2, S9, and S12 samples. Hydroxycinnamic acids are substances with antioxidant properties (Ockun et al., 2022), and Papoti et al. (2019) stated that a higher hydroxycinnamic acid content increased the total phenol and antioxidant content of the *M. officinalis* extract obtained using different extraction methods. Therefore, these four examples have higher total phenol and antioxidant content compared to other samples. However, these samples demonstrated low results in the absence of NaNO<sub>2</sub> in terms of total flavonoid content due to the lower amount of flavonols in their structures compared to other samples. Due to the high content of phenolic acid in the structure of *M. officinalis*, these samples reached the highest total flavonoid content in the presence of NaNO<sub>2</sub>. Although the presence of NaNO<sub>2</sub> increases the selectivity in flavonoids, phenolic acids also exhibit the same absorbance with flavonoids at 510 nm wavelength (Pełal & Pyrzyńska, 2014).

Anomocytic stomata were observed in 7 samples (S3, S4, S6, S7, S8, S10, and S13), and verbascoside (acetone), the main compound found in *Aloysia* (Bahramsoltani et al., 2018; Costa de Melo et al., 2019; Tammar et al., 2021), was found in the thin layer chromatography chromatogram of these samples. However, in the phytochemical studies, verbascoside was not identified in the chemical composition of *M. officinalis* (Shakeri et al., 2016). Interestingly, diabetic stomata, resembling the epidermal surface of *M. officinalis*, were also found in 2 of these 7 samples (S10 and S13). This suggests that these two specimens may have been adulterated and sold as *M. officinalis*.

S5 and S11 samples were considered to be completely different plants from *M. officinalis* and *Aloysia* species. They exhibited paracytic stomata type, and the substances separated in their chromatograms were distinct from those in other samples. Additionally, their phenolic and antioxidant contents were lower compared to the other samples.

The low rosmarinic acid, total phenolic, and antioxidant content in the S14 sample may be attributed to unfavorable storage conditions for this product.

Despite the showing of 1.34% rosmarinic acid in the phytochemical composition of the S15 sample and its

anatomical diacytic stomata structure, the presence of anthocyanin in its structure is an indication that this sample was likely adulterated.

## CONCLUSION

As a result of this study, appropriate stomatal structure, rosmarinic acid ratio above 2%, and presence of caffeic acid in its composition were determined in only 4 samples (S1, S2, S9, and S12). While substituting *Aloysia* for *M. officinalis* as a herb in the markets poses a problem, much more significant issues were observed in crushed-type herbal teas. These issues include selling different plants instead of lemon balm, adulteration, and low rosmarinic acid content. For these reasons, it is recommended to frequently conduct quality controls, especially in markets selling medicinal herbs and teas.

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## Geometrik Morfometri Analiz Yöntemi Kullanılarak Genç ve Erişkin Gökkuşığı Alabalıklarında Cinsiyet Tayini

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### ÖZET

Gökkuşığı alabalıklarında cinsiyet ayrımındaki genel yaklaşım, eşeysel olgunluğa erişmiş bireylerde vücut şekli ve renginin öznel değerlendirmesidir. Bu çalışmanın amacı, genç ve erişkin gökkuşığı alabalıkları arasındaki vücut geometrisine dayalı cinsiyet farklılıklarını geometrik morfometri ile değerlendirmektir. Çalışma 40 genç (20 dişi, 20 erkek) ve 40 erişkin (20 dişi, 20 erkek) olmak üzere toplam 80 adet gökkuşığı alabalığı (*Oncorhynchus mykiss*) üzerinde gerçekleştirildi. Tüm alabalıkların sol lateral yönlü görüntüleri üzerinde 16 adet homolog landmark kullanılmıştır. Cinsiyetler arasında ayrımın yapılabilmesi için genç ve erişkin balıklara ayrı ayrı geometrik morfometrik prosedürler uygulanmıştır. Yapılan analizler sonucunda, genç erkek alabalık örneklerinde dişilere göre, pelvik yüzgecin orijin noktasının posteroventral yönlü olduğu ve burnun uç noktasının ise anteriodorsal yönelim gösterdiği belirlendi. Erişkin erkek alabalıkların dişilerle karşılaştırıldığında, burnun uç noktasının anteriodorsal yönlü olduğu, dorsal yüzgecin anteriodorsal köşe noktasının ise dorsal yönlü olduğu tespit edildi. PCA (Principal Component) skorları, PCA 1-2'nin cinsiyet grupları arasındaki toplam varyansın gençlerde %61.49'unu erişkinlerde %43.48'ini açıkladığını göstermektedir. Geometrik morfometri ile özellikle genç, kısmen de erişkin gökkuşığı alabalıklarında eşeysel farklılıklar kolaylıkla tespit edildi. Sonuç olarak ucuz, invaziv olmayan ve herkesin erişebileceği pratik bir yöntem olan geometrik morfometrinin cinsiyetin tanımlamasına yardımcı olarak, alabalık yetiştiriciliği yapan işletmelerin kısa sürede kolay, sürdürülebilir, ekonomik ve katma değeri yüksek balık yetiştiriciliği yapabilmelerine katkı sağlayabileceğini düşünüyoruz.

### Su Ürünleri

### Araştırma Makalesi

### Makale Tarihçesi

Geliş Tarihi : 18.07.2024

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### Anahtar Kelimeler

Alabalık

Morfometri

Eşeysel dimorfizm

## Sex Determination in Young and Adult Rainbow Trout Using Geometric Morphometrics Analysis

### ABSTRACT

Sex determination in rainbow trout generally involves the subjective evaluation of body shape and color in sexually mature individuals. The present study aimed to evaluate sex differences in body geometry in both young and adult individuals of rainbow trout using geometric morphometry. A total of 80 rainbow trout (*Oncorhynchus mykiss*) individuals were studied, including 40 young individuals (20 females and 20 males) and 40 adult individuals (20 females and 20 males). Sixteen homologous landmarks were evaluated in the left lateral images of the studied trout. Geometric morphometrics procedures were applied separately to young and adult fish to determine the sex differences. The results revealed that in young male trout individuals, the origin point of the pelvic fin was posteroventral, and the anterior tip of the nose was anterodorsal, compared to females. When adult male trout fish were compared to their female counterparts, the anterior tip of the nose was observed to be anterodorsal, and the anterodorsal corner of the dorsal fin was dorsal. In the Principal Component Analysis (PCA), PCA 1-2 explained 61.49% of the total variance between the sexes in young trout and 43.48% of the total variation in adult trout. The use of geometric

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morphometry enabled easy determination of sex differences, particularly in young rainbow trout and partly in adult rainbow trout. Therefore, geometric morphometry could serve as a cost-effective, non-invasive, and feasible approach for the sex determination of fish in trout farms to achieve simple, sustainable, economical, and high-value-added fish farming within a short duration.

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## INTRODUCTION

Rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) is a member species of the Salmonidae family. This Pacific salmonid species is carnivorous and has a characteristic adipose fin (Scott & Crossman, 1973). The species is particularly preferred in aquaculture due to its adaptability to the existing environmental conditions, short incubation period, ease of feeding, resistance to diseases, and high level of nutritional value (Bristow, 1992; Çelikkale, 1994; Baki et al., 2021; Özyılmaz et al., 2023). The males and females of this species reach sexual maturity in 2 years and 3 years, respectively. Adult rainbow trout fish are sexually dimorphic, although the evaluation of body structure and color changes in these fish for sex determination is conducted subjectively (Ekingen, 1975; Bristow, 1992). In the case of young trout, the general morphological differences that would facilitate sex determination have not been established to date.

The study of fish morphology generally involves evaluating the length, depth, and width of the body and the shape, color, and location of the other body structures of the fish (Strauss & Bond, 1990; Oliveira & Almada, 1995; Berns, 2013; Gurkan & Innal, 2021). External appearance does not always facilitate the determination of sex differences in fish as the fish may not exhibit evident sexual characteristics, which depend on the larval stage and the completion of morphological development of juvenile fish, as well as on the genetic and environmental factors of the fish (Hanson et al., 2008; Wearmouth & Sims, 2008; Tenugu & Senthilkumaran, 2022). Experts may be able to distinguish sexes in fish by examining the morphological features, although such morphological differences may not be distinctly visible or could be absent entirely. Surgical methods and ultrasonography may, therefore, be used for sex determination in fish. Surgical procedures are, however, not suitable for all species as these may cause stress to the fish. Ultrasonography, on the other hand, is an expensive procedure requiring specific equipment (Baroiller et al., 1999; Guiguen et al., 1999; Baroiller & D'Cotta, 2001; Sarıeyüpoğlu et al., 2003).

Morphometry is the approach of conducting multiple variance analyses using quantitative variables such as

length, height, width, etc. Followed by a mathematical shape analysis to reveal shape variations (Bookstein, 1991; Dryden, 2014). The greatest progress in determining shape differences in biological structures has been achieved by using the landmark-based Geometric Morphometric (GM) method (Rohlf & Marcus, 1993; Adams et al., 2004). This method enables an inexpensive and feasible determination of the relationship between anatomical points through the statistical analysis of the data obtained from reproducible and reliable anatomical points (Zelditch et al., 2012). According to Takács et al. (2016), the GM method is effective due to the similarity between the measurers and the reduced effect of the measures, which reduces or eliminates the data-related errors. GM is used widely to determine the morphological differences between individuals in various fields of science, such as biology, anatomy, forensic medicine, and anthropology. Sex or shape differences have been determined using the GM method in various fish species, such as *Cyphotilapia frontosa* (Altun et al., 2015), *Hysteroecarpus traskii* (Parvis, 2016), *Barbus balcanicus* (Radojković et al., 2019), *Danio rerio* (Duff et al., 2019), *Salvelinus confluentus* (Nitychoruk et al., 2013), *Salmo trutta* (Monet et al., 2006; Závorka et al., 2020; Špelić et al., 2021; Salehi et al., 2022), *Oncorhynchus kisutch* (Hard et al., 2000), *Oncorhynchus tshawytscha* (Wessel et al., 2006), and *Oncorhynchus mykiss* (Salinas et al., 2022; Sevastei et al., 2024). However, sex differences in rainbow trout (*O. mykiss*), one of the most preferred species in aquaculture, have not been determined using morphometry to date. Therefore, the present study aimed to determine whether geometric differences at the morphometric level exist between sexes in young and adult rainbow trout. The effect of sex on body shape was also investigated using homologous landmarks. Sex determination in fish is important for both aquaculture production and maintaining fish sex ratios in the wild.

## MATERIALS and METHODS

### Selection and Description of Subjects

The present study was conducted with 1–2-year-old young (20 females and 20 males) and 2–4-year-old

adult (20 females and 20 males) rainbow trout (*O. mykiss*) individuals sampled from a trout production facility in Konya/Sarayönü region. All procedures were conducted after receiving approval from the Ethics Committee (2015/90) of the Experimental Animal Production and the Research Center at the Faculty of Veterinary Medicine, Selcuk University. The young trout individuals were photographed on the same day they were obtained from the production facility, following which the fish were examined for their gonads through necropsy and then placed in containers filled with formaldehyde. Since two fish in the young female trout fish group were determined to be male during the autopsy, these individuals were removed from the group, and in their place, two female samples were added to the group. The adult rainbow trout individuals were photographed immediately after milking under anesthesia [25 mg/L MS-222 (tricaine methanesulfonate)] (Topic Popovic et al., 2012). All rainbow trout individuals were first compared in terms of sex at the macro-anatomical level, following which geometric morphometric analyses were performed.

### Geometric Morphometrics

Young and adult trout individuals were placed on a flat surface and then photographed from a distance of 35 cm in the left lateral direction using a tripod (Sony Alpha DSLR-A330 digital camera). All obtained images of trout fish in JPEG format were then transferred to the TpsUtil v.1.69 software program on a personal computer. A total of 16 homologous landmarks were determined in all images of trout fish using the TpsDig2 (v.2.26) software (Figure 1) (Rohlf, 2016).

The identified homologous landmarks were subjected to a validation analysis using the TpsSmall (v.1.33) software (Rohlf, 2015). TpsRelw (v.1.62) and Past (v.3.12) were employed to perform translation, rotation, and scaling for all samples (Hammer et al., 2016; Rohlf, 2016). The distance values of the landmark points to each other were stabilized using the GPA (Generalized Procrustes Analysis)-superimposition method, and an average consensus image was generated. This enables the elimination of the differences in shape caused by any reason other than the original image. TpsRelw and Past software were then employed to determine the displacement, direction, slope, and deformation of the homologous landmark points identified in the trout images, and the groups were accordingly compared in terms of sex. A Principal Component Analysis (PCA-Principal Component & RWA-Relative Warp) was performed for each image, in which evaluations were presented on the X-Y coordinate axis, and PCA percentages of the components were determined. The GM procedures were applied separately to young and adult trout individuals.

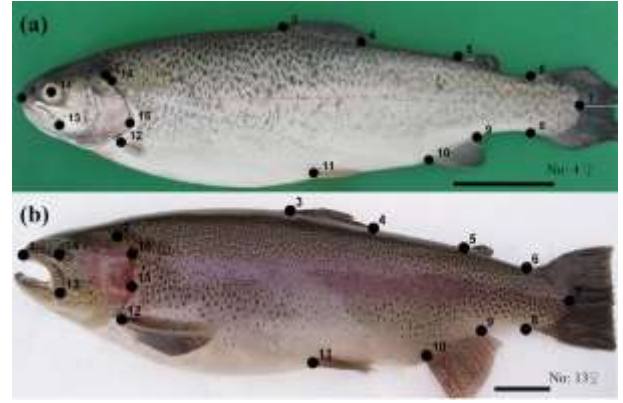


Figure 1. A total of 16 homolog landmarks. (a) Young female rainbow trout. (b) Adult female rainbow trout. 1, anterior tip of nose; 2, most dorsal point of the operculum; 3, anterior and 4, posterior of base of the dorsal fin; 5, anterior of base of adipose fin; 6, anteriodorsal point of the caudal fin; 7, posterior end point of the lateral line; 8, anteroventral point of the caudal fin; 9, posterior and 10, anterior of base of anal fin; 11, origin of the pelvic fin; 12, origin of the pectoral fin; 13, the posterior point of maxilla; 14, midpoint of the eye; 15, most posterior point of the operculum; 16, anterior endpoint of the lateral line. Scale bar=50mm.

Şekil 1. Toplam 16 homolog landmark. (a) Genç dişi gökkuşağı alabalığı. (b) Erişkin dişi gökkuşağı alabalığı. 1, burnun anterior uç noktası; 2, operkulum'un dorsal orijin noktası; 3, dorsal (sırt) yüzgecinin antero-dorsal köşe noktası; 4, dorsal yüzgecinin postero-dorsal köşe noktası; 5, yağ yüzgecinin antero-dorsal köşe noktası; 6, caudal (kuyruk) yüzgecinin antero-dorsal köşe noktası; 7, lateral (yanal) çizginin caudal sonlanma noktası; 8, caudal yüzgecinin antero-ventral köşe noktası; 9, anal yüzgecin postero-ventral köşe noktası; 10, anal yüzgecin antero-ventral köşe noktası; 11, pelvic yüzgecin orijin noktası; 12, pektoral yüzgecin orijin noktası; 13, maksilla'nın posterior köşe noktası; 14, gözün orta noktası; 15, operkulum'un posterior köşesi; 16, lateral çizginin cranial orijin noktası.

In the present study, the anatomical structures of the fish were named according to The Laboratory Fish (Ostrand et al., 2000) and Nomina Anatomica Veterinaria (NAV, 2017). GM analyses were performed using the SB Morphometrics software programs (<https://www.sbmorphometrics.org>). The results of the morphometric analysis were visualized using Adobe Photoshop CC 2015.5 (Version: 25.5.0, Adobe system, San Jose, CA, USA).

### RESULTS and DISCUSSION

The general morphological examination of rainbow trout fish individuals revealed certain non-specific color and structure differences among the trout samples. In particular, certain young male trout individuals exhibited a slightly brighter stripe along the lateral line than that observed in their female



counterparts. The lower jaw was elongated in certain, although not all, adult male rainbow trout individuals, and the tip of the lower jaw in these fish was curved backward and shaped like a hook.

In living organisms, sex-related differences in the form of different colors have been reported in several species of birds, fish, mammals, etc. (Oliveira & Almada, 1995; Berns, 2013). Willson (1997) reported that the male members of migratory salmon exhibit brighter colors. Arslan et al. (2010) reported that male trout fish exhibit color darkening close to the breeding season. In the present study, six male and ten female adult trout individuals were darker in color compared to the other trout fish, as reported by Arslan et al. (2010).

In the male members of salmon, the anterior part of the nose is hook-shaped, and a similar condition is observed in certain female counterparts. In *Salmo* and most members of *Salvelinus* and *Oncorhynchus*, the development of a hooked lower jaw in breeding individuals is reported as a sex differentiator. The development of this hook structure is greater in stream-dwelling and anadromous forms compared to the lake-spawning or freshwater forms. While this structure is further developed in males, it has also been observed in the females of certain species (Willson, 1997). In adult trout (Brook trout) individuals sampled from the tributaries in the Savage River basin in western Maryland, males ( $n = 36$ ) reportedly had a blunt snout and a rounded head, while females ( $n = 29$ ) had an angular head, a pointed snout, and a well-developed lower jaw (Holloway, 2012). The body of the adult female rainbow trout is swollen, while the body of the adult male fish is flat with a hook-shaped lower jaw (Ekingen, 1975; Bristow, 1992; Aydın, 2009; Altun et al., 2015). Ouillet et al. (2004) reported that the secondary sex characteristics of adult rainbow trout may remain hidden in the case of intermediate sexuality. The authors examined the gonads of young (6–13-month-old,  $n = 288$ ) and adult (2–4-year-old,  $n = 203$ ) rainbow trout fish, identifying 50 inter-sex gonads in the young trout and 23 inter-sex gonads in the adult fish in both gonads. In the present study, similar to the studies reported in the literature (Ekingen, 1975; Bristow, 1992; Aydın, 2009; Altun et al., 2015), no differences were observed in the head, nose, and body structure of adult fish at the macro-anatomical level, although certain adult male individuals had an extended-forward and hook-shaped lower jaw as reported in the literature (Willson, 1997; Holloway, 2012).

In certain kinds of Pacific salmon (*Oncorhynchus spp.*), males have a larger body structure than females, while in certain kinds of coho salmon (*O. kisutch*), females have a larger body structure than males (Tamate, 2004). In 34 species of the fish genus *Sebastes*, males are shorter than the females (Lenarz & Echeverria, 1991). Holloway (2012) reported that Pacific salmon

males exhibit larger values of the length and height of adipose fin than females. In pink salmon males, the head size and dorsal hump are larger than those in the females (Cadrin, 2000). Sockeye salmon (*O. nerka*) males have larger dorsal humps, and longer jaws compared to females (Quinn & Foote, 1994). In the morphological evaluations conducted in the present study, hump structures were observed neither in adult trout nor in young trout. Moreover, no differences in the body and fin structure were observed in adult trout, while young male fish had larger body structures than their female counterparts, as reported in previous studies for Pacific salmon (Tamate, 2004). It is generally accepted that male rainbow trout reach sexual maturity earlier than their female counterparts. Dimensional differences have also been reported as the fish approach sexual maturity. These findings might explain the differences in the size between the sexes in these fish (Ekingen, 1975; Bristow, 1992).

The GM analyses conducted in the present study revealed sexual differences, especially in young rainbow trout and partly in adult rainbow trout (*O. mykiss*). The TpsSmall analysis, which was the first of the geometric morphometric analyses conducted in the present study, revealed the slope and correlation values of 0.999728 and 1.000000, respectively, for the landmark points determined for the young trout individuals. The slope and correlation values determined for the adult trout individuals were 0.999868 and 1.000000, respectively. These values confirmed that the landmark points used were correctly placed.

In the GPA and TPS (Thin-Plate Spline) analyses, the young rainbow trout fish that differed from the consensus image were distinctly visible in the graphics and vector figures. The young male rainbow trout fish, unlike their female counterparts, had anterodorsal landmarks 1, 5, 6, and 7 and anteroventral landmark 12. In addition, landmark points 9, 10, and 11 were posteroventral, while landmark points 3 and 4 were posterodorsal in male fish compared to female fish. Partial morphometric differences were observed in the remaining landmark points. These results were consistent with the TPS link graph (Figures 2 and 3). Morphometric differences between the sexes were also distinctly observed in the RWA-PCA analysis graphs of young trout fish. Polarization between the sexes was distinctly visible in the Cartesian coordinate system. While the male trout fish clustered on the upper left side of the graph, female trout fish clustered on the lower right side of the graph. According to the PCA scores, the first two PCA components (PCA-1 = 45.98% and PCA-2 = 15.51%) together explained 61.49% of the total variance between the groups. In addition, PCA-3 (7.91%) and PCA-4 (6.09%) together explained 14% of the total variance between the groups (Figure 3).



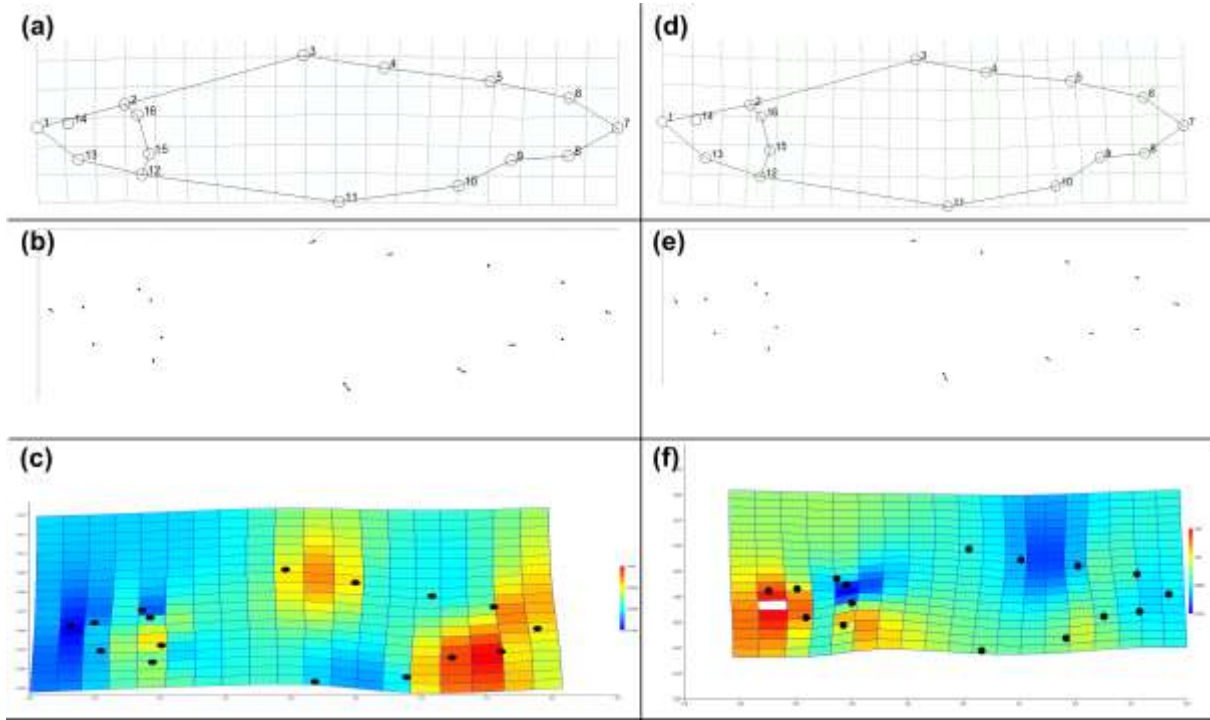


Figure 2. Thin-Plate Spline (TPS) and vectorial deformation analysis graphics. (a, b, c) Young female rainbow trout. (d, e, f) Young male rainbow trout.

Şekil 2. TPS ve vektörel deformasyon analiz grafiği. (a, b, c) Genç dişi gökkuşuğu alabalığı. (d, e, f) Genç erkek gökkuşuğu alabalığı.

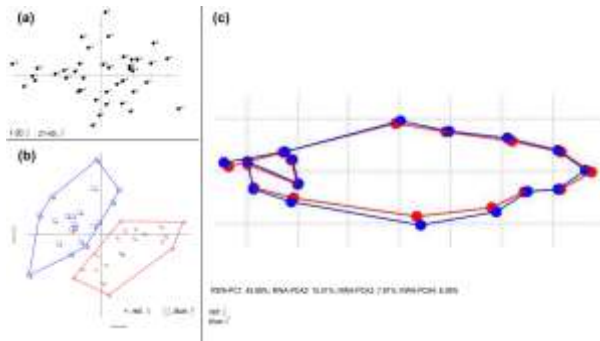


Figure 3. Analyses in young rainbow trout. (a) Relative Warp Analysis (RWA). (b) Principal Component Analysis (PCA). (c) Thin-Plate Spline (TPS) link graph.

Şekil 3. Genç gökkuşuğu alabalığında analizler. (a) Relative Warp Analysis (RWA). (b) Principal Component Analysis (PCA). (c) Thin-Plate Spline (TPS) link grafiği.

Further, according to the results of the GPA and TPS (Thin-Plate Spline) analyses, the adult rainbow trout fish individuals that differed from the consensus image were partially observed in the graphics and vector figures. The adult male fish, unlike their female counterparts, had anterodorsal landmark point 1, dorsal landmark point 3, posterior landmark point 15, posteroventral landmark point 12, and posterior landmark point 16. No morphometric deformations in

any of the remaining landmark points were observed. These results obtained were corroborated in the TPS link graph (Figures 4 and 5). Sex differences were partially observed in adult trout individuals when RWA and PCA analyses were conducted. The adult female fish were clustered to the upper left side of the Cartesian coordinate axis, while the adult males were clustered on the lower right side. According to the PCA scores, the first two PCA components (PCA-1 = 29.79% and PCA-2 = 13.69%) together explained 43.48% of the total variance between the groups. In addition, PCA-3 (12.28%) and PCA-4 (9.74%) together explained 22.02% of the total variance between the groups (Figure 5).

Morphometric differences between males and females based on the results of GM analyses have been reported in different fish species. According to Parvis (2016), the most significant difference between the sexes in Tule perch (*H. traski*) was that the anterior endpoint of the anal fin was more posterior in females than in males. In another study, it was indicated that the caudal peduncle is shorter anteriorly in the females of *C. frontosa* than their male counterparts and that the main difference is in the head region (Altun et al., 2015). In *B. balcanicus*, the dorsal fin was reported to be positioned further back, and the caudal and pectoral fins were observed to be positioned slightly more anteriorly than those in the consensus image

(Radojković et al., 2019). In zebrafish (Duff et al., 2019), the eyes are reportedly positioned slightly more ventrally in males than in females. In the present study, the anterior endpoint of the anal fin in young trout fish was observed to be more anterodorsal in females than in males, and as reported by Parvis (2016), it was slightly more posterior in adult females

than in adult males. No differences were observed between the sexes of adult fish in terms of the anterodorsal and anteroventral points of the caudal fin, while in young female rainbow trout, these points were more posterior than in males, which is different from the findings reported in the literature (Altun et al., 2015; Duff et al., 2019; Radojković et al., 2019).

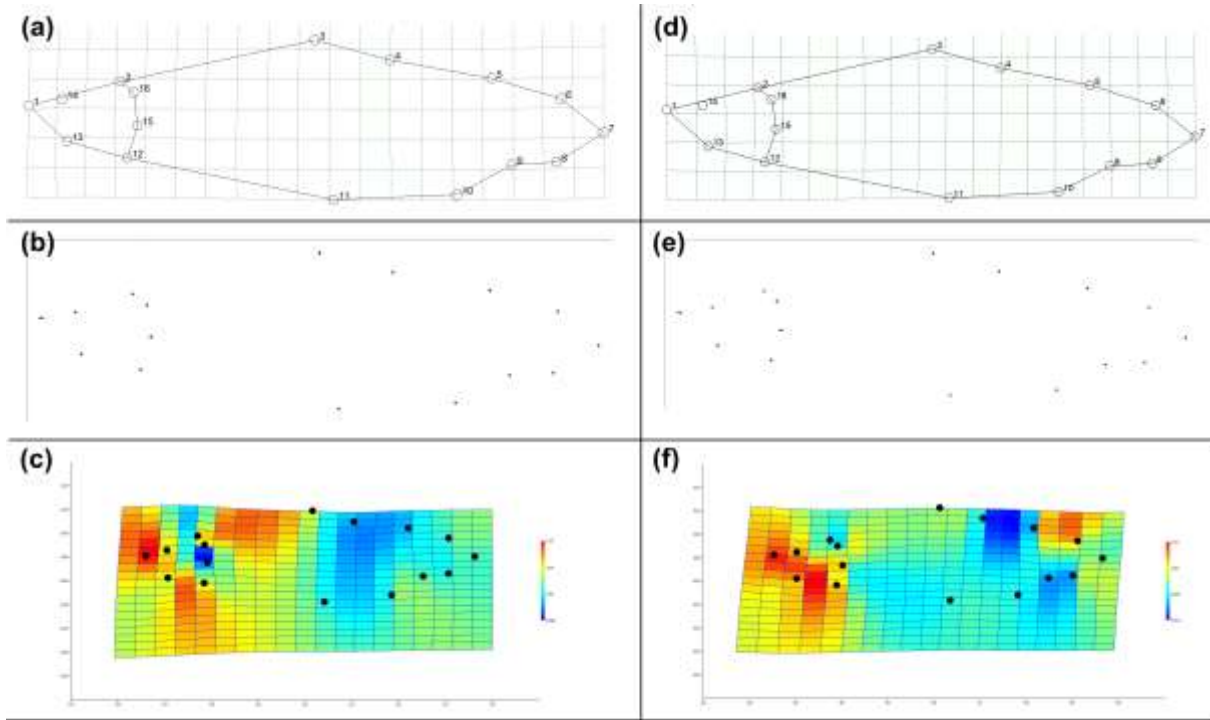


Figure 4. Thin-Plate Spline (TPS) and vectorial deformation analysis graphics. (a, b, c) Adult female rainbow trout. (d, e, f) Adult male rainbow trout.

Şekil 4. TPS ve vektörel deformasyon analiz grafiği. (a, b, c) Erişkin dişi gökkuşuğu alabalığı. (d, e, f) Erişkin erkek gökkuşuğu alabalığı.

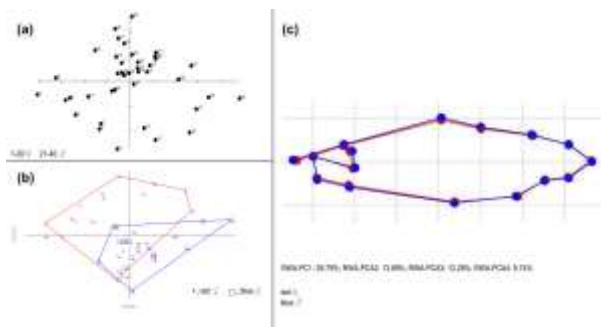


Figure 5. Analyses in adult rainbow trout. (a) Relative Warp Analysis (RWA). (b) Principal Component Analysis (PCA). (c) Thin-Plate Spline (TPS) link graph.

Şekil 5. Erişkin gökkuşuğu alabalığında analizler. (a) Relative Warp Analysis (RWA). (b) Principal Component Analysis (PCA). (c) Thin-Plate Spline (TPS) link grafiği.

Hard et al. (2000) reported that the pectoral and pelvic fins of adult farm-raised coho salmon males were more posterior than those of their female counterparts. The morphometric analyses conducted with brown trout by Monet et al. (2006) revealed that the dorsal end point of the head, the tip of the nose, and the anterior and posterior end points of the dorsal fin were in an anterior position in males compared to females. In addition, the localization of the eyes differed between the sexes. Špelić et al. (2021) reported that in brown trout of Danubian, Atlantic, and Hybrid lineages collected from ten different streams, the Danubian and Hybrid individuals had more streamlined bodies and longer heads with larger eyes compared to the Atlantic lineage. In addition, the individuals of Danubian lineages had longer heads than those of the hybrid individuals. Another study conducted with the same fish species examined the body shape of five different populations of this species in the Caspian Sea, Namak, and Urmia basins and reported that the Jajrud River population had a greater body depth, a shallower head,

and a lower anterior pectoral fin origin compared to the other populations (Salehi et al., 2022). A study investigating the effect of competition on head shape in allopatric (brown trout only) and a sympatric (brown and brook trout co-occurring) trout revealed that sympatric trout had smaller eyes, shorter lower jaws, and a more terminal mouth than their allopatric counterparts (Závorka et al., 2020). In the rainbow trout populations of Valcheta (n = 140) and Guillelmo (n = 128) streams in Patagonia, body shape GM data could be distinguished among different populations as well as among Parr, smolt, and adult individuals (Sevastei et al. 2024). In a study investigating the effects of GM on body shape in the neomales and males of *O. mykiss* parr reared at the temperatures of 8 °C and 16 °C, dimorphism was observed in the dorsal and caudal fin regions of males for which the traditional morphometrics data could not be determined (Salinas et al., 2022). In the present study, no significant sex differences were noted in the pelvic and pectoral fins in adult fish; the origin point of the pelvic fin was posteroventral in young male fish compared to females, as also reported for coho salmon (Hard et al., 2000) and brown trout (Jajrud River population) in previous studies (Salehi et al., 2022). The origin points of the pectoral fin, on the other hand, was anteroventral. In addition, the tip of the nose in fish was observed to be anterodorsal in young male rainbow trout and anterior in adult male rainbow trout compared to their respective female counterparts, as also reported for brown trout in a previous study (Monet et al., 2006). In the present study, the dorsal fin was revealed to exhibit dimorphism between young and adult fish, while the caudal fin exhibited dimorphism among the young fish, as reported for the males of *O. mykiss* parr (Salinas et al., 2022). Further, different from the findings reported for brown trout (Monet et al., 2006), the anterior and posterior points of the dorsal fin were posterior in the young males and dorsal in the adult males of rainbow trout compared to their female counterparts. Finally, while no sex difference was noted in the localization of the midpoint of the eye in adult trout fish, this point was slightly more dorsal in young male fish compared to young female fish.

## CONCLUSION

Sex determination is important when fish populations must be compared, and the behavior and ecological life of a species must be studied (Mank et al., 2006). Appropriate sex ratios must be maintained during individual selection and broodstock management. In aquaculture, populations comprising entirely male or female fish individuals are generally preferred. In tilapia, the stocks comprising only male individuals are preferred, while in trout, stocks comprising only female individuals are preferred (Altunok et al., 2008).

Further, with the increasing demand for food worldwide, the preference for healthy and natural animal products is also increasing. The limited existing resources, therefore, must be sustainable.

In the above context, the use of a limited number of animals from a limited region and the lack of geometric morphometrics data of related species may be considered limitations of the present study. Nonetheless, the study pioneers in providing detailed data on the sex differences, particularly in young rainbow trout and partly in adult rainbow trout. These data contribute to the morphological information on rainbow trout and would be beneficial for the aquaculture sector. In addition, these data may be used in various future studies and compared or evaluated using different methods for further validation.

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## Author's Contributions

Nimet Turgut, Saadettin Tıprıdamaz, and Hakan Yalçın designed the study and collected by Nimet Turgut the data. Nimet Turgut and Hakan Yalçın executed the analysis executed the analysis. Nimet TURGUT wrote the article and critically reviewed it by Saadettin Tıprıdamaz and Hakan Yalçın.

## Conflicts of Interest

The authors have declared no conflict of interest

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## Sığınmacı İşgücünün Hayvancılık Sektöründeki İstihdama Etkilerinin Yerel Aktör Perspektifinden Değerlendirilmesi: Konya İli Örneği

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### ÖZET

Kırsal alanlardaki yerleşik nüfusun kentsel alanlara/yerleşimlere hareketliliği çalışan sayısında azalmayı ve hayvancılık sektöründe istihdam sorununu beraberinde getirmiştir. Bu sorunun aşılması için sığınmacı işçi gücünün bu alanlarda istihdamı yaygın hale gelmiştir. Çalışma sığınmacıların Türkiye işgücü piyasasındaki istihdam alanlarından birisi olan hayvancılık sektöründeki etkilerini ele almıştır. Bu kapsamda sektörün lokomotif illerinden biri olan Konya özelinde değerlendirmeler yapılmış, sığınmacıların hayvancılık sektöründeki istihdamı, katkıları hem işverenler hem de çalışanlar için mevcut sorunlar ve çözüm önerileri tartışılmıştır. Nitekim Türkiye’de sığınmacıların istihdamı ile ilgili mevcut çalışmaların büyük bir kısmı kentsel alanlara ve özellikle de sanayi sektörüne odaklanırken, sığınmacıların kırsal alanlarda özellikle hayvancılık sektöründeki konumları yeterince ele alınmamıştır. Bu bağlamda araştırma, alanda bu eksikliği gidermeyi, literatüre özgün katkılar sağlamayı hedeflemektedir. Çalışmada nitel yöntem ve yarı yapılandırılmış mülakat tekniği tercih edilerek Konya’da hayvancılık sektörünün kurumsal temsilcileri, yerel yöneticiler ile üreticilerle görüşmeler gerçekleştirilmiş ve elde edilen veriler temel sorun alanlarına göre tasnif edilmiştir. Araştırma sonuçları, hayvancılık sektöründe işgücü eksikliği yaşandığını ve bu nedenle sığınmacıların istihdamının kaçınılmaz olduğunu göstermekte ancak mevcut politikaların yetersiz olduğu ve istihdamın düzenlenmesi ile ilgili sorunların çözülmesi gerektiği vurgulanmaktadır.

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Sığınmacı istihdamı  
Konya

## Evaluation of the Effects of Refugee Labor Force on Employment in the Livestock Sector from the Perspective of Local Actors: The Case of Konya Province

### ABSTRACT

The mobility of the resident population from rural areas to urban areas/settlements has decreased the number of employees and employment problems in the livestock sector. To overcome this problem, the employment of the asylum-seeker labour force in these areas has become widespread. The study examined the effects of asylum seekers in the livestock sector, which is one of the employment areas in the Turkish labour market. In this context, evaluations were made in Konya, one of the locomotive provinces of the sector, and the employment of asylum seekers in the livestock sector, their contributions, existing problems and solution suggestions for both employers and employees were discussed. While most of the existing studies on the employment of asylum seekers in Turkey focus on urban areas and especially on the industrial sector, the position of asylum seekers in rural areas, especially in the livestock sector, has not been sufficiently addressed. In this context, the research aims to fill this gap in the field and make original contributions to the literature. In the study, qualitative method and semi-structured interview technique were preferred and interviews were conducted with institutional representatives of the livestock sector, local administrators and producers in Konya. The data obtained were classified according to

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the main problem areas. The results of the research show that there is a labour shortage in the livestock sector and therefore the employment of asylum seekers is inevitable. Still, it is emphasized that the current policies are insufficient and the problems related to the regulation of employment need to be solved.

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## GİRİŞ

Hayvancılık sektörü, nüfusun besin ihtiyacının karşılanması, et, süt, deri, tekstil, kozmetik ve ilaç sanayine hammadde sağlanması, bölgeler ve sektörler arası dengeli kalkınma hedefine ulaşılması, kırsal refahın gelişmesi, önemli bir istihdam alanı olması sebebiyle yıllara göre önemini yitirmeyen bir sektör olarak karşılık bulmaktadır. Sektör; yem, et ve mamulleri, süt ve mamulleri, dericilik ve tekstil, veteriner ilaçları ve hayvancılık ekipman vb. bileşenleri için yeni istihdam alanları oluşturmakta ve hayvansal ürünlerin işlenmesi ile katma değer üretmektedir (TİGEM, 2019). Yanı sıra ülkelerin gelişmişlik düzeylerinin belirlenmesinde kullanılan önemli kriterlerden birisi de kişi başına tüketilen hayvansal besin miktarıdır ve bu miktar gelişmişlik düzeylerinin bir göstergesi olarak kabul edilmektedir (Bakır & Kibar, 2019). Bu bağlamda hayvansal protein tüketimi ile kalkınmışlık arasında sebep sonuç ilişkisi kurulduğu görülmektedir (TİGEM, 2019). Ekonomide sağladığı gelir ve istihdam potansiyeli açısından önemli bir paydada yer alan hayvancılık sektörü

dünyada olduğu gibi Türkiye’de de ülke ekonomisi ve ülke ekonomisinin karşı karşıya olduğu sorunlara çözüm getirme potansiyeline sahiptir. Çünkü hayvancılıkta sağlanacak verimlilik artışı, hayvansal üretim dışında diğer üretim dallarını da olumlu yönde etkilemektedir. Dahası ve önemlisi, hayvancılık, toplumların yeterli ve dengeli beslenme ihtiyaçlarını karşılamada kritik bir rol oynamaktadır (Akbaş & Bilgiç, 2023). Özellikle son yıllarda dünya genelinde yaşanan gıda krizleri dikkate alındığında gıda güvencesi ve güvenliği açısından sektörün stratejik bir öneme sahip olduğu ve konunun sadece kâr bağlamında ele alınamayacağı da ortadadır.

Konuya bu açıdan bakıldığında hayvancılık sektörünün Türkiye için ne denli önemli olduğu bir kez daha ortaya çıkmaktadır. Nitekim Avrupa İstatistik Ofisi (Eurostat, 2023) ve Türkiye İstatistik Kurumu (TÜİK, 2023) verileri karşılaştırıldığında 2020 yılından itibaren küçükbaş ve büyükbaş hayvan sayısında Türkiye’nin AB ülkelerini geride bıraktığı ve lider konuma yükseldiği görülmektedir.

**Çizelge 1.** 2023 Yılı Avrupa Birliği Ülkeleri ve Türkiye'nin Büyükbaş, Koyun ve Keçi Varlığı Karşılaştırması  
**Table 1.** 2023 Comparison of Bovine Cattle, Sheep and Goat Assets of European Union Countries and Türkiye

Ülke	Büyükbaş Hayvan Sayısı (Milyon)	Ülke	Koyun Sayısı (Milyon)	Ülke	Keçi Sayısı (Milyon)
Fransa	16.807	Türkiye	42.060	Türkiye	10.303
Türkiye	16.583	İspanya	13.596	Yunanistan	2.877
Almanya	10.836	Romanya	10.192	İspanya	2.293
İrlanda	6.525	Yunanistan	7.251	Rpmanya	1.292
Polonya	6.435	Fransa	6.576	Fransa	1.275
İspanya	6.295	İtalya	6497	İtalya	0.980
İtalya	5.999	İrlanda	3.995	Hollanda	0.561
Hollanda	3.723	Portekiz	2.082		

Kaynak: Eurostat 2023; TÜİK 2023

Çizelgeden de görüleceği üzere Türkiye, büyükbaş hayvan varlığında Fransa dışındaki bütün AB ülkelerini geçerek ikinci sıraya yerleşmiştir. 2023 yılı itibarıyla Türkiye, küçükbaş hayvan sayısında Avrupa Birliği ülkelerine göre önemli bir üstünlük sağlamıştır. Koyun varlığında Türkiye, Avrupa'daki en yakın rakibi olan İspanya'yı 3'e katlamış, keçi varlığı açısından ise Avrupa Birliği ülkelerinin tamamını geride bırakmıştır. En yakın rakibi Yunanistan'ın keçi

sayısı 2 milyon 877 bin olup, Türkiye bu sayının 3 katını aşmıştır. Görüldüğü üzere bu veriler, Türkiye'nin küçükbaş hayvan yetiştiriciliğinde Avrupa'nın en önde gelen ülkesi olduğunu ve diğer ülkelerle karşılaştırıldığında önemli bir üstünlük sağladığını göstermektedir. Söz konusu veriler hayvancılık sektörünün Türkiye için önemini ve sektörde Türkiye'nin rolünü açıkça ortaya koymaktadır.

**Çizelge 2.** Türkiye'de Kırmızı Et Üretiminin Yıllara Göre Dağılımı (Ton)  
**Table 2.** Distribution of Red Meat Production in Türkiye by Years (Tons)

	2005	2010	2015	2020	2023
Kırmızı Et Üretimi (Ton)	737 220	879 819	1 187 018	1 785 952	2 384 047
Kırmızı Et Üretim Oranları	0.2	3.9	5.7	2.6	8.8

Kaynak: TÜİK Kırmızı Et Üretim İstatistikleri, 2023

Türkiye'nin kırmızı et üretiminin yıllara göre dağılımı incelendiğinde, üretim miktarlarında belirgin bir artış gözlemlenmektedir. Bu artış eğilimi, hem Türkiye'nin hayvancılık sektöründe üretim kapasitesinin arttığını hem de kırmızı ete olan talebin sürdürülebilir bir

şekilde yükseldiğini göstermektedir. Veriler yıllara göre analiz edildiğinde, kırmızı et üretiminde her dönem belirli oranlarda bir artış yaşandığı görülmektedir.

**Çizelge 3.** Türkiye Küçükbaş Kırmızı Et Arz ve Kullanımı (Ton)  
**Table 3.** Supply and Utilization of Ovine Red Meat in Türkiye (Tons)

	2018	2019	2020	2021	2022	2023
<b>Arz</b>						
Üretim	374.017	403.296	436.296	480.488	468.476	491.900
İthalat	400	364	153	203	342	318
Toplam Arz	374.417	403.660	436.449	480.691	468.818	492.218
<b>Kullanım</b>						
Tüketim	373.910	402.880	436.073	479.878	463.840	491.942
İhracat	507	780	376	813	4.978	276
Toplam Kullanım	374.417	403.660	436.449	480.691	468.818	492.218
Kişi Başına Tük. (kg.)	4.38	4.72	5.11	5.63	5.5	5.8

Kaynak: TEPGE 2023

Çizelge 3 de görüldüğü üzere Türkiye'nin küçükbaş kırmızı et üretiminde hem iç tüketimi karşılayabilecek hem de zaman zaman ihracat potansiyelini artırabilecek bir yapıya sahip olduğunu göstermektedir. Söz konusu veriler, hayvancılık sektörünün Türkiye için ne denli önem arz ettiği ve Türkiye'nin sektördeki konumunu ortaya koması

bakımından manidardır. Bununla birlikte Türkiye'de hayvancılık sektörü birtakım sorunlarla karşı karşıyadır. Yem bitkileri üretiminde tohum, ilaç, gübre, mazot ve bu gibi girdilerde maliyetlerin yüksek olması, yem katkı maddelerinin üretilmemesi, mera alanlarının ıslah edilememesi, alternatif yem kaynaklarının devreye sokulamaması, her bölge için



en uygun hayvan ırkının belirlenememesi ve ırk ıslahı, hastalıklarla etkin mücadele, hayvansal ürünlerde verim, kalite ve ürün çeşitliliğinin artırılmaması, artan vergiler, üretici ile sanayici entegrasyonun sağlanamaması, canlı hayvan ve et ürün borsalarının geliştirilememesi, miras hukuku, kayıt dışı istihdamın artması sebebiyle oluşan haksız rekabet ve adaletsizlik vb. pek çok yapısal sorun bulunmaktadır. Özellikle son yıllarda kırsal alanlardan kente göçün artması tarım ve hayvancılık sektöründe istihdam sorununu öncelikli sorun alanlarından biri haline getirmiş ve üreticiler sorunu aşabilmek için sığınmacı istihdamına yönelmiştir. Kısaca ifade edildiğinde işsizliğin azaltılması, kırsaldan kente göçün önüne geçilmesi gibi birçok fonksiyonları üstlenen tarım ve hayvancılık aracılığıyla gerçekleştirilecek olan ekonomik kalkınma sayesinde ülke ekonomisinin gelişim kaydetmesi beklenen bir durumdur. Bu durum çalışmanın konusu olan istihdam problemini de yeniden gündeme getirmiştir. Bu çalışma da Konya özelinde gerçekleştirilen saha çalışması verilerinden hareketle kırsal alanlarda, özellikle hayvancılık sektöründe sığınmacı işgücünün istihdamına ilişkin sorunları, beklentileri ve çözüm önerilerini incelemeyi amaçlamaktadır. Sığınmacıların istihdamı ile ilgili mevcut çalışmaların büyük bir kısmı kentsel alanlara ve özellikle de sanayi sektörüne odaklanırken (Özer & Beyazıt; Koç, Görücü & Akbıyık; Dikkaya & Dinçer), sığınmacıların kırsal alanlardaki özellikle de hayvancılık sektöründeki konumları, sorunları, beklentiler ve çözüm önerileri yeterince ele alınmamıştır. Kırsal alanlardaki Suriyeliler ile ilgili Kalkınma Atölyesi'nin mevsimlik işçi göçü bağlamında gerçekleştirdiği "Türkiye'de Mevsimlik Tarımsal Üretimde Yabancı Göçmen İşçiler" (Kalkınma Atölyesi, 2016a) ve "Bereketli Topraklar, Zehir Gibi Yaşamlar" (Kalkınma Atölyesi, 2016b) başlıklı raporlar dışında bir çalışma mevcut değildir. Mevcut konu ile ilgili çalışmalar daha çok sığınmacıların sosyal ve kültürel uyumları (Millet, 2021; Kara & Kaya Tilbe, 2023) çerçevesinde değerlendirilmektedir. Bu bağlamda çalışma bir ilk olup, alandaki bu eksikliği gidermeyi, sığınmacı işgücünün hayvancılık sektörünün sürdürülebilirliği ve gelişimi için nasıl daha etkili kullanılabileceğine dair kapsamlı analizler ile literatüre özgün katkılar sağlamayı hedeflemektedir.

## KONYA'DA HAYVANCILIK SEKTÖRÜ

Tarım ve hayvancılık söz konusu olduğunda Türkiye'de ilk akla gelen illerden biri de Konya'dır. Konya, hem Türkiye'nin hem de İç Anadolu Bölgesinin tarım, ticaret, sanayi ve turizm alanında cazibe merkezlerinden biridir. Bölgedeki diğer illere lokomotiflik görevi yapan, ülke ekonomisine ciddi katkıları bulunan bir üretim üssüdür. Konya, 41 bin km<sup>2</sup>'lik alanı ile Türkiye'nin en büyük ilidir. En fazla

ilçe sayısı ile de ikinci sırada yer alırken yüzölçümü olarak Dünya'daki yaklaşık 50 ülkeden daha büyüktür (Konya Valiliği, 2024).

Konya, sahip olduğu tarım alanları ve barındırdığı hayvan sayıları ile tarım ve hayvancılık alanında üretim, ticaret ve istihdam açısından merkezi bir konumda yer almaktadır (Üstün, Çelik vd. 2020). 18,6 milyon dekarlık tarım alanı ile ülkenin toplam tarım alanının %8'ini bünyesinde barındıran Konya, Türkiye'nin en büyük tarım alanına sahip ilidir. Konya ili arazilerinin %55.1'ini tarım, %18.7'sini çayır-mera, %13.2'sini orman-fundalık ve %13'ünü de tarım dışı araziler oluşturmaktadır (MEVKA, 2012). Zihinlerde sürekli tahıl ambarı olarak iz bıraksa da Konya sadece tarımda değil hayvancılık alanında da önemli bir konuma sahiptir. 2023 yılı itibarıyla 930 bin büyükbaş ile Türkiye'de birinci, 3 milyon 46 bin küçükbaş ile ikinci ve 9 milyon kanatlı hayvan ile üçüncü sıradadır. Son 10 yılda Konya ilinde, büyükbaş hayvan sayısı %114 ve süt üretimi %151 artış göstermiştir. Konya'daki hayvansal üretim göstergeleri, bölgenin tarım ve hayvancılık sektöründeki önemli rolünü vurgulamaktadır. 2024 yılı verilerine göre, Konya'da hayvansal üretimin yaygın olduğu ve çiftliklerin çeşitli ölçeklerde faaliyet gösterdiğini görülmektedir. Bölgedeki büyükbaş işletme sayısı 48 bin, küçükbaş işletme sayısı ise 18 bin olarak kaydedilmiştir. Büyükbaş ve küçükbaş işletmelerin ortalama büyüklükleri sırasıyla 20 ve 176 olarak belirlenmiştir ki bu durum Konya'daki işletmelerin genellikle orta ölçekli olduğunu göstermektedir. Ayrıca, ari ve onaylı işletme sayısı 25 olarak rapor edilmiştir, bu da bölgede yetiştiricilerin genellikle kaliteli ve izlenebilir hayvanların üretimine odaklandığını yansıtmaktadır. (Konya İl Tarım ve Orman Müdürlüğü, 2023a). 2019 itibarıyla de yukarı yönlü bir artış dikkati çekmektedir. İnek sütü üretiminde 1,3 milyon ton ile Türkiye birincisi, koyun sütü üretiminde ise 88 bin ton ile Türkiye üçüncüsüdür. 12 bin ton keçi sütü, 479 ton manda sütü üretiminin yanı sıra kanatlı hayvancılık sektöründe de önemli bir konuma sahiptir. Nitekim yumurta tavuğu üretiminde yaklaşık 13 milyon ile Konya, Afyonkarahisar'dan sonra Türkiye'de ikinci sıradadır (Konya İl Tarım ve Orman Müdürlüğü, 2024).

Hayvansal ürünler değerlerine bakıldığında da Konya yaklaşık 4 milyar lira ile Türkiye birincisidir. Türkiye'deki hayvansal ürünler değerinin %3,52'sine sahiptir. Dahası ve önemlisi Konya'daki hayvansal ürünler değer olarak son on yıldır artış yaşamaktadır. Konya, canlı hayvan varlığı değerlerinde de liderliği elinde bulundurmaktadır. Buna göre Konya yaklaşık 9 milyar lira ile Türkiye birincisi olup Türkiye'deki canlı hayvan değerinin %5,4'üne sahiptir. Hayvansal ürünler değerlerinde olduğu gibi canlı hayvan değeri de beş yıldır düzenli olarak artış göstermektedir (Yetkin, 2021). 2020 verilerine göre 2,8 milyon hayvan

ile Türkiye'deki küçükbaş hayvanların %5'ini barındıran Konya, beş yıl içerisinde hayvan varlığını yaklaşık 800 bin artırmıştır. Küçükbaş hayvan sayısına göre Konya'yı 2,5 milyon ile Şanlıurfa, 2,1 milyon ile Diyarbakır ve 2 milyon ile Ankara takip etmektedir. 2021 yılı itibariyle büyükbaş sayısı yaklaşık 958 bin küçükbaş sayısı ise 3 milyon 59 bine çıkmıştır. Kanatlı hayvan varlığı yaklaşık 10 milyon, arı kovana ise 117 binin üzerindedir. 2020 itibariyle su ürünleri üretim miktarı ise 1.418 tondur. Süt ve süt ürünleri ile ilgili işletme sayısı 134, et ve et ürünleri 81, su ürünleri işleme 6, yumurta paketleme 91, yem ile ilgili işletme sayısı ise 134'tür (Yetkin, 2021). Uygun mera ve yem bitkisi üretim alanlarının mevcut olması, büyükbaş ve küçükbaş hayvancılık için uygun büyüklükte mera alanlarına ve yem bitkisi üretimi için geniş arazilere sahip olması, doğal ve kültür bitkisi bakımından arıcılığa hitap eden geniş bir bitki deseni, coğrafi konumu, hava, kara ve demiryolu ulaşımı imkanı ile Türkiye'nin lojistik üssü olmaya aday potansiyeli, işleme ve pazarlama sıkıntısının olmaması, sektör için avantaj sağlamaktadır. İlde nüfusun fazla olması, batı ve güney bölgelere ulaşımın kolay olması ve yoğun olarak tarımsal ürün yetiştiriciliğinin yapılması sektörü cazip hale getirmektedir.

Bu bağlamda tarım dışı alanların hayvancılık için kullanıldığı düşünüldüğünde Konya'nın hayvancılıkta önde gelen potansiyele sahip olması için yeterli alana sahip olduğunu söylemek mümkündür. Ancak hayvancılık konusunda yeterli alanlara sahip olan Konya'nın bu sektörde istikrarlı bir işgücü ihtiyacı bulunmaktadır. Nitekim hayvancılık sektörü yerli nüfusun en az tercih ettiği iş kollarından birisidir ve hem diğer sektörlerin sürekliliği hem de ülke ekonomisinin geleceği için işgücü ihtiyacının zorunlu olarak karşılanması gereken bir alan olarak karşımıza çıkmaktadır. Kırsal alanlarda yaşanan istihdam sorunu Türkiye'ye göç eden yabancıların işgücüne dahil edilmesi ile çözülmeye çalışılmaktadır. Bilindiği üzere ulusötesi göç hareketliliği konusunda 2000'li yıllara kadar transit ülke konumunda bulunan Türkiye, çeşitli nedenlere bağlı olarak ülkelerini terk eden yabancı uyruklular için hedef ülke konuma gelmiştir. Türkiye'de kalma biçimlerine göre ikincil koruma ya da geçici koruma statüsünde yer alan bireyler temel ihtiyaçlarını karşılamak amacıyla daha çok yerli nüfusun tercih etmediği alanlarda istihdama dahil olmaktadır. Özellikle hayvancılık sektörü ele alındığında başta Afganistan uyruklular olmak üzere, Suriye, Türkmenistan gibi farklı uyruklardan yabancıların sektörde aktif faaliyet gösterdiği görülmektedir. Yukarıda zikredilen verilerden de anlaşılacağı üzere Konya, hayvancılık sektöründe Türkiye'nin önemli merkezlerinden biri olup söz konusu istihdam sorununu derinden hisseden illerin başında gelmektedir. Bu durum, Konya'da özellikle

kırsal alanlarda özelde ise hayvancılık sektöründe yabancı istihdamını adeta bir zorunluluk haline getirmiştir.

## İSTİHDAMDA SIĞINMACI İŞGÜCÜ

Türkiye'de 1950'lerde başlayan ve halihazırda devam eden kırdan kente göç, tarımdan tarım dışına işgücü göçü ve tarım dışında yüksek miktarda istihdam yaratılması gerekliliği hem kırsal alanlarda hem de kentsel alanlarda farklı etkiler yaratmıştır (Tatlidil & Xanthacou, 2002). Kırsal alanlar özelinde tarım ve hayvancılık sektöründe işgücü arzını mevcut nüfus üzerinden kullanamayan hayvancılık sektörü için dışarıdan alınan göçler istihdam alanında yaşanan sorunlara çözüm getirme potansiyeline sahiptir. Gelişmiş ülkelerde kitlesel üretim sağlayan işletmelerin teknolojik gelişmeler paralelinde zamanla emek yoğun üretimi terk ederek sermaye/teknoloji yoğun üretime geçiş yapmaları bu ülkelerde ikincil sektörün yavaş yavaş ortadan kalkmasına sebep olmaktadır. Bu durum ikincil sektör işçilerine dolayısıyla göçmen işçilere olan ihtiyacın gelişmiş ülkelerde azalacağını bir göstergesi olarak düşünülebilir. Bununla birlikte sanayi sektörü dışında, tarım, hayvancılık ve hizmet sektörlerinin ikincil sektör olarak faaliyetlerini devam ettirdiği dikkate alındığında bu sektörlerde göçmen işçilere ihtiyacın devam ettiği görülmektedir (Toksöz, 2006). Bu noktada emek yoğun işlerin genel olarak gelişmekte olan ülkelere kaydığı ve buna bağlı olarak göçmen işçilerin gelişmekte olan ülkelere yöneleceği söylenebilir. Türkiye'ye 2000 sonrası yönelen uluslararası göçler de -gönüllü bir göç sürecinin sonucu olmasa da- uygun politikalar ile desteklendiği takdirde bu durumun Türkiye özelinde bir ihtiyaca karşılık verebilecek nitelikte olduğunu söylemek mümkündür. Nitekim bugün kırsal alanlara bakıldığında başta Afganistan ve Suriye olmak üzere yabancı uyruklular sığınmacı işgücünün sektördeki istihdam açığını kapattığı görülmektedir.

"Asya'nın kalbi" olarak nitelendirilen, yıllarca savaş, işgal, siyasi belirsizlikler, iç çatışmalar ve terör saldırılarıyla dünya gündeminden düşmeyen, tarihi boyunca sığınmacı üreten ülkelere biri olan Afganistan ile 2011 yılı itibariyle Suriye'de yaşanan savaş ve iç çatışmalar sonucu Türkiye'ye yönelen göçler Türkiye'nin göç serüveni açısından bir kırılmaya sebep olmuştur. Afganistan'dan Türkiye'ye yönelen göçlerin tarihi oldukça uzun bir geçmişe dayansa da Afganistan'ın Sovyet Rusya tarafından işgal edilmesi süreci hızlandıran faktörlerden biri olmuştur. 1990'ların başında Taliban'ın yönetimi ele geçirmesi ve ardından çıkan iç çatışmalar, 2001 yılındaki ABD işgali ve son olarak 2021'de ABD'nin ülkeden çekilme kararı ülkede siyasi kargaşa ve iç çatışmaları artırmış birçok Afganistan vatandaşı ülkeyi terk etmiş ya da terk etmek zorunda

birakılmıştır. Göçün başladığı ilk yıllarda Afganistan vatandaşları için Avrupa yolunda transit bir ülke olan Türkiye, özellikle 2000 sonrası Türk Kızılayı Göç ve Mülteci Hizmetleri Müdürlüğü'nün Göç İstatistik Raporu'na (2017) da yansıdığı üzere hedef ülke konumuna gelmiştir. Süreç içerisinde Afgan uyruklular genel olarak Türkiye'de turizm, eğlence, perakende, satış, inşaat gibi sektörlerde işçi olarak çalışmışlar, dahası son yıllarda emek piyasası içerisinde birtakım sorunlar yaşamaları Afganlı göçmenleri girişimci bir özneye dönüştürmüş (Arıcıoğlu & Afkari, 2023) olsa da Afgan uyrukluların hali hazırda en yoğun faaliyet gösterdikleri sektör hayvancılık sektörüdür. Ticaret Bakanı Ömer Bolat'ın 07 Temmuz 2024'de verdiği bir röportajda “*25 bin Afgan çoban gitse tarım, hayvancılık kalmaz*” ifadeleri mevcut durumu net olarak ortaya koymaktadır (Altınok, 2024).

Suriye'de yaşanan iç savaş neticesinde de ilk etapta 1 milyondan fazla Suriyeli Türkiye'ye sığınmış ve 2022 yılı itibarıyla bu sayı yaklaşık dört katına ulaşmıştır. Suriye krizi sonrasında Türk hükümeti bu sürecin kısa sürede sonlanacağı ve geri dönüşlerin gerçekleşeceği düşüncesiyle ülkeye sığınan Suriyelilere “Geçici Koruma” statüsü verilmiş ancak aradan 13 yıl geçmesine rağmen Suriye'de savaş son bulmamıştır. Türkiye'nin hazırlıksız yakalandığı bu göç akını sığınmacıların Türkiye'nin neredeyse tüm kentlerine dağılmasıyla sonuçlanmıştır. Böylece sığınmacıların entegrasyonu, istihdamı, barınma, yaşadıkları sorunlar, talep ve beklentileri, ulusal ve yerel aktörlerin süreçteki konumu, halkın izlenen politika ve sığınmacılara ilişkin görüşleri ve toplumsal kabul düzeyi gibi birçok tartışmayı da beraberinde getirmiştir. Halihazırda yaklaşık 5 milyon ile dünyada en fazla sığınmacıya ev sahipliği yapan ülke konumunda olan Türkiye'de gerek medya da gerekse yerel halk arasında tartışmalar içinde öncelikli sorun alanlarının başında konunun ekonomik boyutu gelmektedir. Tartışmalar daha ziyade göçle gelen külfet özellikle de sığınmacı istihdamı üzerine sürdürülmektedir. Henüz gelişmekte olan ve ekonomik göstergeler açısından kırılğan bir yapıya sahip Türk ekonomisinin bu kadar fazla sığınmacıya ev sahipliği yapmasının ekonomiye büyük bir yük getireceği ve özellikle de sığınmacı istihdamı ile Türk vatandaşlarının işten çıkarıldığı, iş bulamadığı ya da hak ettikleri ücretleri alamadıkları en sık dillendirilen konuların başında gelmektedir. Elbette göç söz konusu olduğunda istihdam, işten çıkarma, ucuz emek, çocuk işçiliği, işsizlik, yoksulluk, kamu yardımları gibi konular ilk elden gündeme gelmekte ve daha ziyade olumsuz etkiler vurgulanmaktadır. Başta medya olmak üzere yerel halk arasında sıklıkla dile getirilen bu hususların toplumsal gerçekliğin sadece bir yanını yansıttığı da unutulmamalıdır. Ancak konuyu sadece külfet ya da olumsuzluklar üzerinden ele almak her

şeyden önce göçün karakteri ile örtüşmemektedir. Nitekim her göç kendi ekonomisini de beraberinde getirmektedir. Bu noktada üzerinde durulması gereken önemli bir husus da göç edilen kentin başta ekonomik imkanları olmak üzere sosyal, kültürel ve siyasi potansiyeli ile ilgilidir. Ekonomisi gelişmiş kentler de özellikle mevsimlik sektörlerde ve imalat sanayinde nitelikli ara eleman ve vasıfsız eleman ihtiyacı ile tarımsal alanlardaki işgücü açığının sığınmacılar aracılığı ile giderilmeye çalışıldığı dikkati çekmektedir (Koyuncu, 2016). Nitekim TÜİK'in İl Bazlı İşsizlik Oranlarına bakıldığında tarım ve sanayisi gelişmiş şehirlerde Suriyelilerin göçü ile artması beklenen işsizlik oranlarının düştüğü görülmektedir (Koyuncu, 2016). Bu şartlara hem ailesinin geçimini sağlamak ve kente tutunmak adına zorlukları ve sıkıntıları göz ardı eden sığınmacılar hem de vasıfsız eleman bulmakta güçlük çeken sektör temsilcileri için bir imkana dönüşmüştür. Türkiye'de tarımda çalışan yabancı işgücü üzerine akademik çalışmalar her ne kadar sınırlı olsa da mevcut araştırmalar sığınmacıların mevcut tarımsal işgücü boşluğunu doldurduğunu göstermektedir (Ulukan & Çiğerci Ulukan, 2009; Akay Ertürk, 2016; Millet, 2021). Türkiye'nin tahıl ambarı olarak bilinen ve daha ziyade tarımsal faaliyetleri ile ön plana çıksa da Konya hayvancılık sektöründe de Türkiye'nin en önemli illerinden biri olup işgücü ve istihdam sorunu sektör temsilcilerinin öncelikli sorunları arasında yer almaktadır. Bu sebeple sektör temsilcileri söz konusu sorunun giderilmesinde sığınmacı istihdamını kimi zaman bir fırsat kimi zaman bir zorunluluk olarak nitelendirmektedir.

## MATERYAL ve METOD

Araştırmada, kırsal alanlarda özellikle de hayvancılık sektöründe sığınmacı istihdamına ilişkin sorun alanlarının, beklenti ve çözüm önerilerinin ortaya konulabilmesi amaçlanmıştır. Olguların bağlamlar içerisinde incelenmesi amacıyla nitel bir araştırma yaklaşımı benimsenmiştir. Derinlemesine ve detay bilgilere ulaşmak amacıyla yöntemine uygun olarak yarı yapılandırılmış mülakat tekniği ile katılımcılarla yüz yüze görüşülerek veriler toplanmıştır. Katılımcılara ulaşmak için maksimum çeşitlilik örneklem seçim tekniği tercih edilmiştir. Bilindiği üzere maksimum örnekleme, araştırılan konuya taraf olabilecek bireylerin çeşitliliğini maksimum derecede yansıtmaya amaçındadır (Christensen, Johnson & Turner, 2015) ve nitel araştırmalarda en yaygın kullanılan örnekleme tekniklerinden biridir. Temel amaç, katılımcıların konuya ilişkin kanaatlerini, farklı ve özgün değerlendirmelerini ortaya çıkarmak ve bu yolla karmaşık bir olgunun farklı boyutlarına dair bilgi elde edebilmektedir (Creswell & Clark, 2014). Bu kapsamda araştırmanın örnekleme oluşturulurken Konya'da hayvancılık sektöründe faaliyet gösteren



çeşitli paydaşları temsil kabiliyeti dikkate alınmış ve hem kurumsal temsilciler hem de yerel yöneticiler ile görüşmeler gerçekleştirilmiştir. Bu bağlamda kurumsal olarak Konya Kırmızı Et Üreticileri Birliği, Konya Damızlık ve Sığır Yetiştiricileri Birliği, Konya Damızlık Koyun Keçi Yetiştiricileri Birliği, Kadınhanı Ziraat Odası, Konya Koyun Keçi Yetiştiricileri Birliği temsilcileri ile bunun dışında hayvancılık faaliyetlerinin yoğun olarak yapıldığı ve bu süreçte sığınmacı istihdam eden Kaşınhanı, Çarıklar, Kuyulusebil, İhsaniye ve Çeşmelisebil'de yer alan 19 işletme sahibi olmak üzere toplam 28 kişi ile mülakatlar yapılmıştır. Böylece sektördeki farklı bakış açıları ve deneyimler yansıtılmak istenmiştir. Katılımcıların izni dahilinde ses kaydı alınmış ve izni olmayan katılımcıların verileri anlık olarak not alma şeklinde kaydedilmiştir. Ses kaydına müsaade etmeyen katılımcıların verileri ise görüşme sonrasında hemen düzenlenmiş ve mevcut verilerin kaybına izin verilmemiştir. Araştırmada, elde edilen verilerin sınıflandırılması, yorumlanması ve yapılandırılması için söylem analizi kullanılmıştır. Söylem analizi, araştırmanın sonuçlarını daha kapsamlı bir şekilde anlamak (Baş & Akturan 2008) ve sığınmacı iş gücünün hayvancılık sektöründeki etkilerini detaylı bir biçimde değerlendirmek amacıyla tercih edilmiştir. Nitekim söylem analizi bütüncül bir teori, metot ve uygulamadan ziyade farklı disiplinlerin teorik bakış açılarından beslenen ve yine bu disiplinlerin teorik görüşleri ile korunan, farklı araştırma geleneklerinin bir araya geldiği nitel bir araştırma yöntemidir (Tonkiss, 2006). Bu anlamda, söylem analizi, en basit anlatımı ile dilin incelenmesidir. Ancak bu inceleme sadece dilsel öğelerin basit bir incelenmesi olmayıp ifadelerin altında yatan anlamı ve içeriği incelemeyi gerektirmektedir (Çelik ve Ekşi, 2008, s. 105). Van Dijk'in de (1997) ifade ettiği gibi söylem analizi, söylem ya da dil kullanımının sadece biçimsel yönü ile sınırlı değildir. Analiz, ilgi odağını sosyal ve kültürel bağlam içinde iletişim kuran dil kullanıcılarının oluşturduğu sosyal olaylara çevirir (Barker ve Galasinski, 2001). Bulgular sorun alanlarına göre tasnif edilmiş ve "Bir Fırsat Olarak Sığınmacı İşgücü", "Sığınmacı İstihdamında Sorun Alanları", "Sığınmacı İş Gücünün Potansiyel Riskleri" ve "Sığınmacı İş Gücünde Çözüm Yolları" başlıkları altında analitik bir çözümlenmeye tabi tutulmuştur.

## BULGULAR

### Niçin Sığınmacı İstihdamı? Ya da Bir Zorunluluk Olarak Sığınmacı İstihdamı

Türkiye'de işgücü piyasasındaki sığınmacılar çalışma izni olanlar ve çalışma izni olmadan çalışanlar olarak iki grupta yer almaktadır. Çalışma izni almadan çalışan sığınmacılar, genellikle tarım, hayvancılık, turizm, eğlence ve ev içi hizmetleri gibi sektörlerde güvencesiz bir şekilde çalışmaktadır (Akbaş Demirel,

2015). Yerli işgücünün temin edilemediği hayvancılık sektöründe ise sığınmacı işgücünün önemli bir boşluğu doldurduğu görülmektedir. Sektöre ilişkin pek çok yapısal problem olduğu görülmektedir. Konya'da tarım arazilerinin yaklaşık %70'inde kuru tarım yapılması, sulama suyunun yetersizliği ve maliyet yüksekliği, meraların zayıf karakterde olması, hayvancılıkta kaliteli kaba yem yetersizliği, yüksek enerji, akaryakıt ve gübre giderleri, teknoloji kullanımı, küçük işletmelerin sayıca fazla olması, işletmelerin profesyonel olarak yönetilememesi, tarımsal örgütlenmenin yetersiz oluşu gibi başlıklar temel sorun alanları arasında yer almaktadır. Bununla birlikte söz konusu sorun alanları arasında istihdam öncelikli konu başlığıdır. Araştırma sürecine dahil olan katılımcıların istisnasız tamamının hemfikir olduğu ve yabancı istihdamını bir zorunluluk haline getiren temel sorun alanları ise şunlardır:

- Hızlı kentleşmeye bağlı olarak köyden kente göçün artması,
- Hizmetler ve sanayinin sektörel payında meydana gelen artış,
- Birçok ebeveynin çocuklarını tarım ve hayvancılık dışı sektörlerle yönlendirmesi,
- Genç nüfusun tarım ve hayvancılık dışındaki alanlardaki iş imkanlarına yönelmesi,
- 12 yıllık zorunlu eğitim ve eğitim amaçlı kente göç,
- Gençlerin, özellikle evlilik çağındaki genç kadınların eş seçiminde kentte yaşamayı önlemesi,
- Hayvancılık sektörlerindeki iş kalemlerinin emek yoğun olması,
- Hayvancılık sektörünün mevsimlik değil yıl boyu süren emek gücü talep etmesi dolayısıyla mevsimlik işçilerin soruna çözüm olamaması,
- Türkiye Cumhuriyeti vatandaşlarının ücretleri yetersiz bulması,
- Sektörün mesai ve hafta sonu tatili gibi mefhumlardan azade olması, kırsal alanlarda özellikle hayvancılık sektöründe istihdam sorununun ortaya çıkmasına zemin hazırlamıştır.

Nitekim, araştırmaya dahil olan katılımcıların hemfikir olduğu bu temel sorunlar, Türkiye'nin kentsel dönüşüm sürecindeki karmaşık dinamikleri ve ekonomik yapıdaki değişimleri yansıtmaktadır. Hızlı kentleşme ve kırsal göçün artması tarım ve hayvancılık gibi geleneksel sektörlerden uzaklaşmayı ve kentsel yaşamı tercih etmeyi teşvik etmektedir. Ayrıca, hizmetler ve sanayi sektörlerindeki büyüme, iş olanaklarının kırsal kesimde azalmasına ve gençlerin tarım ve hayvancılık dışındaki alanlara yönelmesine neden olmaktadır. Tüm bu faktörler, kırsal kesimdeki ekonomik ve sosyal yapıyı etkileyen karmaşık bir dizi



sorun alanını ortaya koymaktadır. Bu sorunların çözümü, hem kırsal kesimdeki iş gücünün sürdürülebilirliğini sağlamak hem de kentlerdeki nüfus artışının yönetilmesi açısından önemlidir.

*“Çalışmaya gidiyorlar, okumaya gidiyorlar, evlenip gidiyorlar ama yine etlerini, sütlerini, yoğurtlarını biz gönderiyoruz. Bize dönüp de iş var mı yardıma gelelim mi diyen yok ama şehirde yaşasalar da bizim elimize muhtaçlar. Yardıma bari gelin deyince gelen yok”* (Konya Kırmızı Et Üreticileri Birliği Başkanı).

*“Okumak için çıkıyor evden bir daha ara ki bulasın. Köyde iş fazla bu işi yapacak çoluğun çocuğun olacak ki sana kalan iyi kalsın. Tarla var ekilecek, çalışacak adam yok, Hayvan var güdülecek, güdecek çoban yok. Giden çoluk çocuk geri geldiğinde tavuğa yem atmasını bile bilmiyor. Bu yaştan sonra dönse ne olur dönmese ne olur”.* (S.H., Çarıklar, Erkek, 68 Yaşında).

Köylerde iş gücü eksikliği gençlerin köyde kalmak istememesinin temel nedenlerinden biridir. Köyde iş olanaklarının sınırlı olması gençleri tarım ve hayvancılık gibi sektörlerde istihdam bulma konusunda tereddüt yaşamalarına neden olmaktadır. Arazilerin çoğunlukla miras sebebiyle parçalı hale gelmesi, sulama imkanlarının yetersizliği, iklim ve coğrafi koşullardan kaynaklı ürün çeşitliliğinin ve verimin düşük olması özellikle gençlerin tarım ve hayvancılık dışı alanlarda istihdama yönelmelerine zemin hazırlamaktadır. Bu sebeple kırsalda yaşayan gençler eğitim alma ve kariyer yapma hedefleri ile kente yönelmektedir. Köydeki iş fırsatlarının ve gelir düzeyinin sınırlı olduğunu düşünen gençler için bir diğer temel sorun da köydeki özellikle de hayvancılık sektöründeki iş ve emek yoğunluğudur. Bu sebeple masa başı olarak tarif edilen ve köye nispetle konfor alanı çok daha yüksek sektörlerde iş bulma ümidi gençlerin kentlere yönelmesine ve kentlerde daha çeşitli iş imkanları aramasına yol açmaktadır. Ayrıca, köyde yaşlı nüfusun gençlere yardım çağrılarında rağmen gençlerin dönüp köyde iş aramaması, göçün birkaç boyutunu yansıtmaktadır.

*“Allah bereket versin. Kazancımız iyi. Bu parayı şehirde kazanabilir misin? Kazanamazsın. Ama burası zor. Daha güneş doğmadan başlıyoruz çalışmaya. Hayvanların sütünü sağ, silajını hazırla, yemini ver, ahırını temizle, gübreyi taşı, malı yaylıma çıkar... Bahçeye bak, ekine git, çapa yap, tarlayı sür. Sulaması, hasadı derken iş bitmez burda. Mesai diye birşey yok yani. Bir bakarsın gece hayvan hastalanır. Doğum yapar. Hadi bakalım*

*gece vakti veteriner bul. Gecesi gündüzü yok bu işin. Şehir rahat tabi. İşin belli. Mesain belli. Hafta sonu var, bayram tatili var. Gez, toz, eğlen. Kim istemez.”* (M.E., Çarıklar, Erkek, 18 Yaşında).

Nüfus yoğunluğunun kentsel bölgelerde artması bu bölgelerin kırsaldaki insanları kendisine doğru çekmesine neden olmuştur. Öyle ki kırsaldaki birçok kişi için yakın akrabalarının kentlerde yaşıyor olması bile başlı başına kentin çekici gücünü oluşturmaktadır (Güreşçi & Yurttaş, 2008). Gençlerin kente göç etmelerinde sadece ekonomik nedenlerin değil, aynı zamanda kentin sosyal, kültürel imkanları, yaşam kalitesi ve konfor alanları gibi unsurlarının öncelikler arasında yer aldığı görülmektedir (Beauchemin & Schoumaker, 2005). Bu noktada araştırma verileri arasında yer alan dikkat çekici bir başka diğer sonuçta gençlerin statü arayışına ilişkindir. Bilindiği üzere son yıllarda kırsal alanlarda da imkanların ciddi anlamda arttığı görülmektedir. Günümüzde özellikle ikamet edilen evlerde kent imkanlarını aratmayan donatılar mevcuttur. Arık evler kaloriferli, bütün beyaz eşyalar eksiksiz mevcuttur. Özellikle orta ve büyük ölçekli işletmeler de bahsi geçen yorucu ve zorlu işlerin tamamı çoğu sığınmacı olmak üzere işçiler tarafından yapılmaktadır. Buna karşın başta genç kadınlar olmak üzere gençlerin temel sorunu köylü statüsünden kurtulmaktır. Bu durum sadece hayvancılık sektörünün gerektirdiği zorlu, emek yoğun süreçle ilgili değildir. Daha ziyade eğitimsiz, kaba, adab-ı muâşeret kurallarından habersiz vb. olumsuz yüklemeler içeren köylülük tanımlamasından duyulan rahatsızlık ve statüden kurtulma çabasının kente göçe zemin hazırladığı dikkati çekmektedir. Genç erkekler için bu sorun genç kadınlara göre öncelik sıralamasının üst sıralarında yer almamaktadır. Bu durum, köylerde yaşam standardının ve sosyal olanakların gençler için cazip olmadığını göstermektedir. Nitekim, hızlı kentleşme, köyden kente göçü arttırarak tarım ve hayvancılık sektöründeki işgücü kaybını tetiklemiş, genç nüfus kentlerde iş arayışına yönelmiş ve bu durum köylerde işgücü eksikliği yaratmıştır. Sanayi ve hizmet sektörünün gelişme kaydetmesi ise tarım ve hayvancılığın önemini azaltmada önemli bir pay elde etmiş böylelikle gençlerin bu sektörlere yönelimi büyük oranda düşüş yaşamıştır. Evlilik ve yaşam tercihleri de işaret edilen bir diğer sorun alanlarıdır. Gençlerin evlilik ve yaşam tercihlerinde kentin avantajlarını öncelemesi, kırsal alanlarda tarım ve hayvancılık sektörüne olan ilgi azalmıştır.

*“Ne kızımız ne oğlumuz köyde kalıyor. Hepsi okul için, iş için, evlilik için köyden gitmek istiyor. Köyden birisine köydeki kızını veremezsin, köyde kalacaksak varmam der. Oğlu da aynı kız da aynı artık kimse durmuyor”* (Kaşınhanı

Muhtarı).

*“Evlenemiyorum. Çünkü kimse köyde yaşamı kabul edip gelmek istemeye gönüllü olmuyor. Şehirde iş bulamadım köye dönüş yaptım. Görüştüğüm, konuştuğum hiç bir kız uzun vadede bu konuşmayı sürdürmedi köylü olduğum için”* (S. H. Kuyulusebil, 34 Yaşında, Erkek).

Sektör temsilcilerinin ve üreticilerin ifadelerine de yansıdığı üzere sorunun giderilmesi noktasında sığınmacılar önemli bir rol üstlenmiş olup, halihazırda ticari amaçlı faaliyet gösteren işletmelerin tamamına yakını sığınmacı çalışmaktadır. 2017 yılında dönemin Gıda, Tarım ve Hayvancılık Bakanı Ahmet Eşref Fakıbaba *“üreten insanlar bunu hobi haline getirmişler, biz bu insanlara biraz destek verirsek Türkiye'nin sulanabilir arazileri ile her şeyi yapabiliriz. Ben bunu yüzde yüz ile de çalıştırabilirsem benim işsizlik oranım bitecek. Şu an 3,5 milyon bizim Suriye'den gelen misafirlerimiz var, kendileri gitse biz onları göndermeyeceğiz, bizim ihtiyacımız var”* (CNN TÜRK, 2017) sözleri ile sığınmacı iş gücüne duyulan ihtiyacı dile getirmiştir. Bu noktada genelde Türkiye ekonomisinde özelde ise Konya'nın tarım ve hayvancılık sektöründe sığınmacı işgücüne ihtiyacı bulunmaktadır. Uygulanan doğru ve dinamik göç politikaları ile sığınmacıların hangi sektörlerde yer alacağı, hangi sektörlerde sığınmacılara ihtiyaç duyulduğu, bu sektörlerdeki işgücünün niteliği, teknoloji kullanımı, ücret, fırsat ve riskler bağlamında değerlendirilmesi gerekmektedir.

### **Bir Fırsat Olarak Sığınmacı İşgücü**

Emek yoğun faaliyet gösteren hayvancılık sektöründe çalışan işçinin süreklilik sağlaması hem üretim sürecinde hem kendi kendine yeterlilikte hem de ihracat noktasında oldukça önemlidir. Bu bağlamda hayvancılık, daha çok kırsal alanda yaşayan bireylerin hane üyeleri ile sürdürdükleri iş kollarından birisidir. Tüm aile üyeleri üretim sürecine dahil olmakta ve kendi kendine yetebilen bir potansiyel oluşturmaktadırlar. Yıllara göre değişen imkanlar, iş sahasının tarım ve hayvancılık alanı dışına yönelmesi, eğitim, evlilik ya da kentte yaşam arzusu gibi faktörler, hane üyelerini kente yönlendirmiş ve kırsalda yaşamaya devam eden bireyler istihdamda yaşadıkları sorunları kalan bireyler ile çözmeye çalışmışlardır. Süreç içerisinde kalan bireylerin kente yönelmesi ya da genel olarak sektörün ağır iş yükü ya da ücretlerin yetersiz bulunması gibi farklı nedenlerle hayvancılıkta en büyük problemlerden biri sektörde istihdam edilecek işçinin bulunmamasıdır. Bu sebeple, ihtiyaç duyulan iş gücü sığınmacılar ile sağlanmış ve bu anlamda sığınmacı işgücü bir fırsat olarak değerlendirilmiştir.

*“Tarım ve hayvancılıkta insan gücüne*

*ihtiyaç yoğun ve bu yoğunluğun karşılanabilmesi anlamında ciddi problemler yaşıyoruz. Eğitim sistemindeki değişiklikler, tarım ve hayvancılıkta maliyetlerden dolayı iş gücüne ihtiyacımız var. Sığınmacılar da bu ihtiyacın karşılanması için bir fırsat oldu. İşgücü büyük ölçüde onlarla karşılanıyor”* (Konya Damızlık ve Sığır Yetiştiricileri Birliği Başkan Vekili).

*“İhtiyaç çok var hem de. Ben çok sıkışıyorum. Türkler çok para istiyor, sigorta istiyor ama Suriyeli hemen geliyor. Ama onlarda da işte Türkler gibi çalışmıyorlar. Ama yine yani ben şimdi 10 yılı geçti bu işi yapalı Suriyeliler geldiğinden beri adama sıkışmadım. Kurtardı yani beni iyi kötü bir şekilde. Keşke daha çok olsa. Ama yine yetersiz yani Suriyeli daha çok olsa çalışır yani ihtiyaç var”* (S. L. Kuyulusebil, 56 Yaşında, Erkek).

Kırsal alanlardan kente göçün derinden etkilediği iş gücü açığı tarımsal alanlarda günlük yevmiyeli işçiler ve özellikle çapa ve hasat zamanında mevsimlik işçiler aracılığıyla, hayvancılık alanında ise daha ziyade Afganistan uyrukluların ağırlıkta olduğu yabancı uyruklular ve yerel halktan işçiler aracılığıyla çözüme kavuşturulmaya çalışılmıştır. Afganların çoğunluğu oluşturduğu hayvancılık sektöründe yanı sıra Suriyelileri de görmek mümkündür. Suriyelilerin Türkiye'ye göçünün ardından kentsel alanlarda tutunamayan Suriyeliler kırsal alanlara yönelmişler ve birçok sektörde olduğu gibi kırsal alanlarda da Suriyelilerin istihdamına kapı aralanmıştır. Bu durum, yerel halkın tarım ve hayvancılık sektöründe nitelik ve nicelik olarak ihtiyaç duyduğu insan kaynağı noktasında sığınmacılardan beklentileri, iş yapabilmeye kapasiteleri ve iş ahlakı, yerel halkın kültürel farklılıklara bakışı, birlikte yaşama tecrübesi vb. hususlar temel sorun alanları haline gelmiştir.

*“Bizim oğlumuzdan gelinimizden çalıştıracak insan bulma ihtimalimiz sıfır. Şimdiye kadar hep yerli insanlar vardı ama bu insanların aileleri gittikten sonra yerli çobanlar da işi boşladılar. Bu işi yapacak yerli çoban yok. Yeni nesilden çobanlık yapacak kişi yok. Madem (sığınmacılar) geldiler biz de tarım ve hayvancılıkta değerlendirelim”* (Konya Damızlık Koyun Keçi Yetiştiricileri Birliği Başkanı).

*“Eskiden çoban bulmak kolaydı. Şimdi bu işi yapacak kimse yok. Mecbur dışardan geleni çalıştırmak zorundayız yoksa mümkün değil. Çalışacak adam bulmak çok zor artık”* (M. N. Çeşmelisebil, 62

Yaşında, Erkek).

*“Artık eskisi gibi değil. Şimdi köydeki gençler okul diyor şehre gidiyor. Onlar da haklı. Köyde sosyal hayat yok niye dursunlar. Şahsen ben kızlarım dursun istemem, diğerleri de istemiyor. Bir de çiftçilik ağır geliyor buradakilere”* (R. K. İhsaniye, 38 Yaşında, Erkek).

Çalışmaya kaynaklık eden görüşmecilerin dikkat çektiği nokta ilk olarak kendi aile üyelerinden hayvancılığı sürdürecektir kimsenin olmaması üzerinedir. Genç nüfusun kırsal yaşamdan ziyade kenti öncelendiği, kırsalda kalan ve hayvancılık ile uğraşanların daha büyük yaş grubu olduğunu söylemek mümkündür.

*“Tüm tarımsal üretimde olduğu gibi kırmızı et üretiminde de yabancı işçilerden yararlanma mecburiyeti var. Vasıfsız işçi bulmakta sıkıntı çekiyoruz. Bütün sosyal ihtiyaçlarında yardımcı olup çalıştırıyoruz”* (Konya Kırmızı Et Üreticileri Birliği Başkanı).

Sığınmacıların emek piyasalarında özellikle Türklerin çok da tercih etmedikleri sektörlerde kalifiye ara eleman ve özellikle de vasıfsız eleman açığını kapattıkları, bu sektörlerin sürdürülebilirliğine ve kapasite artışına büyük katkı sağladıkları, sığınmacı istihdamına son verilmesi halinde birçok sektörün “kapağa kilit vuracağı” bizzat sektör temsilcileri tarafından ifade edilmiştir. Bu nedenle sığınmacı iş gücünden yararlanılması sadece işçi ve işveren arasında değil aynı zamanda devletin ekonomik kalkınmasında olumlu çıktılara sahip olması anlamına gelmektedir. Benzer bir tecrübeyi geçmişte deneyimleyen Avrupa, II. Dünya savaşında kaybettiği nüfusun yerine göçmen nüfusu kullanarak sanayi sektöründe kalkınma sağlamıştır. Günümüz koşullarında Türkiye'nin de hayvancılık sektöründe çalışacak nüfusa ulaşamaması geçmişin hafızasına sığınması gerektiğini hissettirmektedir. Bu noktada yerli nüfusun hayvancılık sektöründe işgücü açığının karşılamaması sebebiyle bu açığın sığınmacılar tarafından doldurulacak olması bir fırsat olarak görülmektedir.

*“Devletten destekle hayvan alıp köye yerleştirdim. Hayvanlarda çoğaldı, borcumu kapattım ama gel gelelim hayvanları kıra götürecek çoban meselesinin bir mesele haline geldiğini bilemedim. Çocuklar var yetişmiş ama kentte doğdular yetiştirilmedi. Yardım ederler sağ olsunlar ama çobanlık yapamazlar. Tetikteyiz sürekli çoban arıyoruz. Bulduğumuz zaman da en iyi imkanlarla kalması için elimizden geleni yapıyoruz”* (L. H. Kuyulusebil, 62 Yaşında, Erkek).

*“Bu Afganlar olmazsa ben bu sabah koyuncululuğu bırakırım. Bir tane yerli çoban bulma şansım yok. Mecliste karar mı çıkacak ne yapılacaksa bir şey yapılması lazım”* (Kadınhanı Ziraat Odası Başkanı).

Kadınhanı Ziraat Odası Başkanı sığınmacıların istihdamı konusunda işgücü piyasası için uygun politikaların üretilmesi gerektiğine işaret etmektedir. Zira sığınmacıların hayvancılık sektöründen çekilmesi ivedi bir şekilde işverenin de bu sektörden ayrılacağı anlamına gelmektedir. Çünkü işveren hayvancılık sektöründe sığınmacıların dışında Türk vatandaşların istihdamı konusunda sıkıntı yaşayacağını bilincindedir. Katılımcının dikkat çektiği bir diğer nokta ise “Afganlar” vurgusudur. Sığınmacıların kendi aralarında belirli iş kolları üzerine yoğunlaştığı ve hayvancılık sektörü için daha çok Afganlıların tercih edildiği ya da Afganlıların hayvancılık sektörlerini tercih ettiği görülmektedir.

*“Afganlar daha çok çalışıyor. Hayvancılıkta mesai yok. Biz mecburen Afganlı çalıştırmak zorundayız. Onlar da tamamen kaçak yollardan giriyor ve hepsi kayıtsız. Bizim Afganların çoğunluğu kayıt olamıyor. Bunun için ne gerekiyorsa kanun çıkacaksa bunu yapalım. Biz bunu yapmazsak tarım ve hayvancılık stop edecek”* (Kaşınhanı Muhtarı).

Afganlıların hayvancılık sektörü içerisinde diğer sığınmacılara kıyasla daha çok tercih edilir olması çalışma pratikleri ile ilişkilendirilmektedir. Afganistan'da edindikleri beceri ve deneyimlerini Türkiye'de kullanabilme fırsatları, sığınmacıların uyum süreçlerinde etkili olan temel unsurlardan biridir. Bu bulgu, istekler ve yetkinlikler yaklaşımının, göçmenlerin göç öncesinde kazandıkları beceri ve yetkinliklerin, göç sonrası başarılarıyla ilişkili olduğu savını desteklemektedir. Buna göre, bireylerin sahip oldukları yetenek ve becerileri göç sonrası süreçte etkin şekilde kullanabilmeleri, yüksek düzeyde öznel iyi oluşa katkıda bulunmaktadır (Kara & Kaya Tilbe, 2023).

Sığınmacılar, işverenler açısından genel olarak tembel ya da çalışkan olarak kodlanmakta ve belirli iş kolları üzerinde bu etiketler aracılığı ile istihdam edilmektedir. Bu algının arkasında yatan temel sebeplerden biri Afganistan uyruklularının memleketlerinde de benzer iş kolunda faaliyet göstermeleri sebebi ile sektörün gereklilikleri (özellikle hayvan sağlığı ve bakımı) konusunda tecrübeli ve birikimli olmalarından kaynaklıdır. Afganistan uyruklularına yönelik olumlu vurgunun bir diğer sebebi de bu kesimlerin büyük çoğunluğunun bekar olması, geldiği ülkenin koşullarına nispetle beklentilerinin düşük olması ve mesai kavramı gözetmeksizin çalışmalarınıdır. Elbette bu durum,



maliyet-fayda dengesinde işverenin lehine sonuçlanmaktadır. Çünkü canlı varlıkların beslenmesi, tedavisi, üreme koşulları zamanın bağlayıcı sınırları dışında işlemektedir. Bu anlamda hayvancılık sektöründe çalışma şartlarına uyum sağlayan bireylerin saat gözetmeksizin çalışmaya hazır olması gerekmektedir.

*“Suriyelilerin mesai kavramı var. Fakat hayvancılıkta 7/24 bir çalışma lazım bu yüzden Afganlar bu işe daha uygunlar. Ama onlar da artık pazarlık etmeye başladılar. Neden? Bizim gençlerimizden hayır olmadığını gördüler. Eskiden ne versek tamam diyorlardı”* (Konya Koyun Keçi Yetiştiricileri Birliği Başkanı).

Katılımcı tarafından işaret edilen “Ama onlar da artık pazarlık etmeye başladılar” söylemi dikkat çekicidir. Bu söylem esas olarak hem işveren hem de istihdamda kullanılan nüfus için süreç içerisinde tecrübe edilen değişime işaret etmektedir. Nitekim bahsi geçen işgücü belirli maddi ve manevi yetersizlikler amacıyla daha iyi imkanları elde etmek adına göç eden işgücüdür. Bu işgücü istihdam da kendisine pay edinmek için ilk olarak işverenin tüm şartlarını kabul eden ancak daha sonra işverenin de esasında kendisine muhtaç olduğunu öğrendiği zaman işverenin şartları karşısında duran bir konuma yükselmiştir. Katılımcının tabiri ile eskiden ne verilirse tamam diyen işgücü artık hayır demesini öğrenmiş ve kendi şartları ile istihdamda var olmuştur. Başka bir ifadeyle sektördeki işgücü açığının bir mecburiyet ilişkisine evrildiği ve bu zorunluluğun Afgan uyruklu sığınmacıların talep ve beklentilerine de hatta kimi zaman tehditlerine yansıdığı dikkatlerden kaçmamaktadır.

*“Bugün senle pazarlık eder anlarsın yarın bir başkası daha çok verir ertesi gün seni ortada bırakır gider haber bile vermez”* (Konya Koyun Keçi Yetiştiricileri Birliği Başkanı).

*“İş beğenmeme, iş seçme var onlarda da var artık. Afgan çok değerli herkes ona muhtaç bu yüzden Afgan’ı elinde tutmak için verebildiğin kadar çok şey veriyorsun”* (Konya Damızlık ve Sığır Yetiştiricileri Birliği Başkan Vekili).

*“Bekar Afganlar elimiz kolumuz oldular ama bekar olmalarından kaynaklı bir sıkıntımız var. Bugün burada ailesi olmadan malımızı emanet ettiğimiz Afgan, yarın daha iyi ücreti bulduğunda seni bir anda ortada bırakır. Çok geldi başımıza. Bu yüzden gelen Afganların aileleri ile gelmesi bir anda seni terk etmemesini sağlar. Evini bırakıp bir gecede başka bir köye çoban olarak*

*gitmez. O zaman da bizim mağduriyetimiz ortadan kalkar.”* (A. K. Kuyulusebil, 63 Yaşında, Erkek).

Afgan uyruklu sığınmacıların istihdam sürecinde tercih edilir olması beraberinde başka problematik sorunların yaşanmasına da neden olmaktadır. Zira resmi olmayan yollarla Türkiye’ye giriş yapan Afgan uyrukluların doğal olarak çalışma izinlerinin olmaması hem işverenleri hem de sığınmacıları sıkıntılı bir duruma sokmaktadır. Bu sebeple özellikle Afgan uyruklulara hayvancılık sektöründe duyulan ihtiyaç ve bu ihtiyacın giderilmesi noktasında çalışma izinlerinin ve kayıtlarının olmaması bir diğer gerilim kaynağıdır. İşverenin ihtiyaç duyduğu işgücünün çalışma izni olmayan bireylerden oluşması ve bu işgücünün hiçbir prosedüre tabi olmaksızın istihdamdan çekilme tehlikesi hayvancılık sektörü için bir tehdit olarak değerlendirilmektedir.

### Sığınmacı İstihdamında Sorun Alanları

Sığınmacı istihdamındaki temel sorun alanı kayıt dışı istihdamdır. Genel olarak istihdam sürecinde olan bireylerin hizmetlerinin gün veya ücret bilgilerinin ilgili kurum ve kuruluşlara eksik bildirilmesi ya da hiç bildirilmemesi (SGK, 2022) olarak tanımlanan kayıt dışı istihdam, yapısı itibariyle kısa sürede olumlu etkiler ortaya çıkarsa da uzun dönemde birtakım problemleri açığa çıkarmaktadır.

*“Kayıtsız çalışma en büyük sorun. Çalışma izninden muaf çalıştırılırsa ne olur. Mevzuat bilgisi yok, ne işverende ne de sığınmacılarda. Bu yüzden tedirginler. Çalıştıkları zaman sosyal haklarına dair bilgileri yok sığınmacıların. Özellikle kayıtsızlığı düzeltmemiz gerekiyor. Bu artık bir gerçek ve orta ve uzun vadede bizim bu iş gücüne ihtiyacımız var”* (Konya Damızlık ve Sığır Yetiştiricileri Birliği Başkan Vekili).

Katılımcılarının tümünün kanaatlerini özetleyen bu alıntı sahadaki durumu özetler niteliktedir. Gelişmiş, gelişmekte olan ve az gelişmiş ülkelerin ekonomik seviyelerine göre yoğunlukları değişen kayıt dışı istihdam sorunu işsizlik, enflasyon, kırdan kente göç ya da kitlesel göç gibi nedenlere bağlı olarak görünürlük elde etmektedir. Bu nedenlerin doğru bir şekilde analiz edilmesi kayıt dışı istihdamı önlemede uygulanacak doğru politikaların belirlenmesi için önemlidir. Zira kayıt dışı istihdam hem çalışanlar hem işverenler hem de devlet açısından birtakım sorunları barındırmaktadır. Sığınmacıların hukuki statüleri ve vatandaşlık alma ihtimallerinin zayıf olması sebebiyle kayıt altına alınsalar bile emekli olamamaları, yatırılan sigorta primlerinin doğrudan kendilerine verilmesi talebini beraberinde getirmektedir. Ayrıca cüzi bile olsa daha fazla ücret teklif edildiğinde hiçbir hukuki yaptırım olmaksızın iş bırakma lüksünü



kaybetmek istememektedirler. Ancak kayıt dışı istihdamda bulunan ve kayıt dışı olduğu için sigortasız çalışmak zorunda kalan bireyler, çalışanların sahip olduğu birçok yasal haklardan yararlanamazlar. Çalışma günleri emeklilikleri için geçerli sayılmaz ve herhangi bir hastalık sonucunda malulen emekli aylığından yararlanamazlar. Ölüm sonrası aile üyeleri ölüm aylığı hakkından yararlanamaz ve genel sağlık sigortalarını kendileri ödemek zorundadırlar. Herhangi bir iş kazası sonrası yasal olarak verilecek iş göremezlik gelirlerinden yararlanamazlar ve işsizlik sonrası işsizlik sigortası alamazlar. İşten ayrılma sonrası kıdem tazminatı alamadıkları gibi yıllık, haftalık gibi izin haklarından yararlanamazlar. İşyerinde kayıt dışı işçi çalıştıran işveren ise idari ve adli yaptırımlarla karşılaşabilir, mali teşvik, hibe, kredi ve vergi avantajlarından faydalanamazlar (SGK 2022). Devlet açısından ise kayıt dışı istihdam prim kaybı ve sosyal güvenlik açıklarının artması ile sonuçlanmaktadır bu durum da gelir dağılımındaki adaletsizliklerin ve ahlaki dejenerasyonun artışına sebep olmaktadır.

*“İnternet veriyoruz, ücreti yüksek tutuyoruz, yemek bizden içmek bizden ev bizden her şey bizden. Facebookta çeşitli gruplar üzerinden ilan vermeye başladık işte internet, konaklama, yemek şu kadar ücrete şeklinde. Her imkanı sağlıyoruz ama bir kuruş fazla veren olduğu zaman seni anında yarı yolda bırakıyor”* (M. H. Çeşmelisebil, 66 Yaşında, Erkek).

*“Kolay değil iş bilmeyen adama iş öğretmek. Çoban deyip geçme onun da bilmesi gereken şeyler var. Kırdan hayvanın başına bir şey gelse yatırıp kesmesini de bilmeli telef etmemeli. İlaç yapmalı bizle birlikte. Öğretiyoruz, öğreniyor da her şeyi ama çok kolay gidiyor. Bu gün var yarın yok gibi”*. (H. K. Kuyulusebil, 64 Yaşında, Erkek).

*“Hayvancılığı sevmeleri lazım bu adamlar sadece işi olmadıkları için bu işi yapıyorlar. Mesela bana Afgan işçim dedi ki ‘ne öğreteceksin sen bana tezek çekmek öğrenilir mi’ dedi? Halbuki hayvancılık doktorluk kadar hassastır, bebek gibi... Bilgin olmazsa hayvan ölüp gider. Su kalmamış adam doldurmuyor. Diyorum ki amcam şu hayvanların olduğu doldurulacak altı çekilecek iş bu”* (S.H., Çarıklar, Erkek, 68 Yaşında).

Konut ve istihdam gibi entegrasyonun farklı boyutlarının sağlanmasına (Wang & Ning 2016) karşın katılımcıların ifadelerinden de anlaşılacağı üzere sığınmacı işgücünde karşılaşılan bir diğer sorun alanı taraflar arasında iş akdinin olmamasıdır. İş akdinin olmaması konumlanma sürecini zedelemekte

ve sığınmacıların işverene hiçbir bilgi vermeden işi bırakmasına imkan sağlamaktadır. Özellikle işverenin uzun süre bilgi ve tecrübelerini aktararak yetiştirdiği sığınmacı işgücünün çok cüzi ücret artışları için iş bırakması iş ahlakını ve işletmelerin sürdürülebilirliğini zedelemektedir. Sığınmacıların iş mekanlarına uyumu da bir başka sorun alanıdır. Sığınmacıların iş mekanlarına uyumu sağlamaları, işin gerekliliklerini öğrenmeleri süreç isteyen bir konudur. Tarım sektöründe kullanılan ekim, sulama gibi konuların öğrenilmesi daha önce işi bilmeyen Sığınmacılar için zaman alıcı bir süreç olduğu görülmektedir. Ancak işverenin işçinin eğitimi için harcadığı bu emek karşısında işçinin bir gün gidecek olması endişe yaratmaktadır. Bu nedenle işveren, işçisini elinde tutmak için iş yapılmayan dönemlerde dahi ücret ödemediği görülmektedir. Bu bağlamda sığınmacıların iş mekanlarına ve iş ortamına uyumlarının ücretlerini aldığı sürece olduğu söylenebilir.

Sağlıklı bir iletişimin temel şartı olan dil bariyeri halen geçerliliğini korumaya devam etmektedir. İstihdam edilen sığınmacıların iş kolunun gerekliliklerini anlama, yapılacak işin ne olduğunu ve nasıl yapılması gerektiğini kavrama konusunda ciddi sorunlar yaşanmaktadır.

*“Vaktimiz ilk zamanlarda birbirimizi anlamakla geçiyor. Onlar Türkçe bilmiyor ben başka bir dil bilmiyorum ama zamanla anlaşıyoruz ama zaman alıyor”* (Kaşınhanı Muhtarı).

*“Bir derdi var söyleyemiyor, sen de nasıl soracağını bilmiyorsun. Tariflerle anlaşıyoruz tarif ederek anlatıyoruz. Traktörü kullan dedim traktörü bozmuş, hayvana gerektiğinde iğne yapması lazım onu anlatamıyorsun bazen ciddi zararımız oluyor”* (Konya Koyun Keçi Yetiştiricileri Birliği Başkanı).

Bu durum kimi zaman yüksek maliyetli hataların ortaya çıkmasına sebep olmakta ve işverene ekonomik külfet oluşturmaktadır. Aynı zamanda, çalışma mekanları, çalışma ortamı ve iş arkadaşlarına uyum sorunlarını da beraberinde getirmektedir. Son olarak görüşmelerde sığınmacıların siyasetin bir malzemesi haline getirilmesi ve oy devşirmek adına sığınmacılar üzerinden üretilen dışlayıcı politik retoriğin yerel halkta öfke ve nefret söylemine evrilmesinin olumsuz sonuçları vurgulanmıştır.

*“Biz Afgan, Suriyeli istemiyoruz diyorlar! Eee sen gel çalış o zaman adamların sana ne zararı var senin dönüp beğenmediğin işleri yapıyor bu insanlar”* (Konya Koyun Keçi Yetiştiricileri Birliği Başkanı).

*“Mülteci istemiyorum, sığınmacı istemiyorum diyenlerin hiçbiri onlar”*

*giderse nasıl sorunlar yaşarız bilmiyorlar. Durduğu yerden konuşması kolay ama gerçek öyle değil bugün bu adamlar gönderilse ya da gitse biz yarın iflasın eşliğine geliriz”* (Konya Damızlık ve Sığır Yetiştiricileri Birliği Başkan Vekili).

*“Afganlar, Suriyeliler geldi biz işsiz kaldık diyorlar. Gel çalış desen gelip çalışmaz. Bu adamlar işleri senin elinden almadı sen çalışmadığın için bu adamları ben çalıştırıyorum. Yarın bu insanlar gönderilse sen gelip çalışmazsın, oğlum kızım bile olsa gelip çalışmaz ama anlamıyorlar”* (Kaşınhamı Muhtarı).

Bu ifadelerde Afganistan ve Suriyelilerin siyasi amaçlar için kullanılması ve dışlayıcı politik retorik oluşturmak için araç olarak ele alınması vurgulanmaktadır. Siyasi figürlerin veya kuruluşların bu insanları hedef alarak popülist bir dil kullanarak kitleleri etkilediği belirtilmektedir. İfadelerde, Afgan ve Suriyelilere karşı olumsuz tutumların ve tepkilerin yerel halk içinde nasıl evrildiği ve nasıl güçlendiği açıklanmaktadır. İşsizlik gibi yerel sorunlarla ilişkilendirilerek, yerel halkın bu konudaki endişelerinin siyasi manipülasyonlarla nasıl şekillendiği anlatılmaktadır. Katılımcılar, sığınmacıların yerel ekonomiye katkıları ve iş gücü açısından nasıl değerlendirilmesi gerektiği konusunda bir perspektif sunulmaktadır. Sığınmacıların işgücü piyasasına olumlu katkılar sağladığı ve bu bakımdan olumsuz algıların gözden geçirilmesi gerektiği vurgulanmaktadır. Siyasi figürlerin sığınmacılara yönelik olumsuz retoriği ve bu retoriğin toplumda nasıl öfke ve nefret söylemine dönüştüğünü ele almaktadır. Bu durum sadece sığınmacı çalışanları değil aynı zamanda sektörde çalışacak personel bulamayan işverenleri de kaygılanmaktadır. Son dönemde başta sosyal medya üzere yazılı ve görsel medyada sıklıkla dile getirilen “Afgan/Suriyeli istemiyoruz” ile başlayan hakaret, nefret ve tehdit diline evrilen paylaşımların gerçeği yansıtmadığı, kırsal alanlardan elde edilen ürünlerde sığınmacıların rolü ve emeğinin yok sayıldığı belirtilmiştir. Dolayısıyla sığınmacıların bir külfet değil nimet, bir lütuf olduğu, aksi halde tahıl, meyve, sebze ve tüm hayvansal ürünlerin dışa bağımlı bir biçimde (ithal edilmek suretiyle) karşılanacağı ve bunun maliyetinin yine Türk halkına çıkacağı ifade edilmiştir.

### **Sığınmacı İş Gücünün Potansiyel Riskleri**

Söz konusu yabancı uyrukluların başta hukuki statülerinden kaynaklanan belirsizlik, vatandaşlık alınamadığı için emekli olamama, mevcut mevzuatın bilinmemesinin yanı sıra mevcut mevzuatın sorunları giderememesi, yardım bağımlılığı, iş ahlakı, meslek etiği ve işletmelerin sürdürülebilirliği, güven esası çalışma ortamının tesis edilememesi gibi hayvancılık

sektöründe sığınmacı iş gücünün potansiyel riskleri değerlendirildiğinde hem işverenler hem de işçiler açısından birtakım sorunların olduğu görülmektedir.

*“İş kazası oluyor hastaneye götüremiyoruz polis ne der diye. Biz bu insanları çalıştırmaya devam edeceğiz”* (Konya Koyun Keçi Yetiştiricileri Birliği Başkanı).

İşçi gücünün potansiyel riskleri işçiler açısından değerlendirildiğinde ilk olarak ortaya çıkan sorun sağlığa ilişkin yaşanacak herhangi bir problemde tedavi sürecinin iş verende bir gerilime neden olduğudur. Sığınmacılar yaşam koşulları nedeni ile sağlık açısından diğer gruplara göre daha fazla risk altındadırlar (Küçükkendirci ve Batı, 2020). Zira yasadışı yollarla Türkiye’de bulunan kayıt dışı istihdamın çalıştırılması yasal olarak mümkün değildir. Sağlık alanında resmi otorite ile karşılaşacak olan işgücü ve işveren tedavi sonrasında yüklü miktarda cezalarla karşılaşacaktır. Bunun bilincinde olan sağlık söz konusu olduğunda ilk olarak hastayı düşünmek yerine karşılaşacağı cezayı düşünmektedir. Bu durum hastanın erken tedavi edilmemesi gibi birtakım sorunlara yol açması muhtemel görülmektedir.

*“Bir sıkıntımız var, tanımadığımız bilmediğimiz insanları çalıştırdığımız için sıkıntı yaşıyoruz. Ülkeye girerken arka planları araştırılıp suça yönelik olup olmadıklarının araştırılmasını talep ediyoruz. Suça karışmamış sicili temiz insanların yönlendirilmesiyle daha rahat edilecek. İnsanların burada kalmasında sıkıntımız yok”* (Konya Kırmızı Et Üreticileri Birliği).

Bir diğer risk potansiyeli ise işçinin kim olduğuna ilişkin bilgilerin edilememesinden kaynaklanmaktadır. Yasa dışı yollarla Türkiye’ye göç eden sığınmacıların geldikleri ülkelerde herhangi bir suç kayıtlarının olup olmadığının bilinmemesi işverenleri potansiyel suç mağduru olarak konumlanmasına neden olmaktadır. Ancak işveren işçiye duyduğu zorunlu ihtiyaç neticesinde kim olduğunu bilmediği sığınmacıyı çalıştırmak zorundadır.

Bireylerin uygun şartlar altında harcadığı emek ile orantılı bir şekilde ücret alması sosyal devlet anlayışının görevleri arasındadır. Kayıt dışı ekonomide yer alan bireylerin ise haklarının belirli yasalar etrafında korunması mümkün değildir. Bu durum beraberinde sigorta şartlarının sağlanmaması, emeklilik haklarının elde edilememesi, sağlık kuruluşlarından cüzi miktarda yararlanılmaması gibi sonuçlara neden olmaktadır. Hayvancılık sektöründe sürekliliğin sağlanabilmesi için ilk olarak sığınmacıların şartlarının iyileştirilmesi

gerekmektedir. Bu şartlar ücret dahilinden ziyade kayıt dışı istihdamdan kaynaklanan sorunların önüne geçilmesi ile çözüme kavuşturulacaktır. Zira işverenler hayvancılık sektöründe Afganistanlılar dışında çalıştıracak işçi bulamadıklarını ifade etmektedirler. Bu işçilerin süreklilik dahilinde kalması için ise asgari ücret düzeyinin üstünde maaş verdiklerini belirtmektedirler. Afganistanlıların gitmesine karşı duyulan kaygı neticesinde yüksek olarak ücretlendirdikleri düşünülen katılımcılar için uygun politikaların geliştirilmesi ile yasal bir zemine oturtulan çalışma hakkının işverenleri rahatlatacağı düşünülmektedir.

### Sığınmacı İş Gücünde Çözüm Yolları

Tarım ve hayvancılık sektöründe hem bir ihtiyaca cevap verilmesi hem de kırsal ekonomide artışın yaşanması için sığınmacı işgücüne zorunlu olarak ihtiyaç duyulmaktadır. Bu çerçevede mevcut göç politikaların yeniden değerlendirilmesi gerekmektedir. Sektörde yaşanan istihdam sorunu dikkate alınarak Türkiye'ye göç etmek isteyen yabancı işgücünün engellenmemesi, sınırları kapatmak, duvarlar örmek, gözetleme kuleleri inşa etmek yerine tüm sektörler için bir ihtiyaç analizi yapılarak hazırlanacak stratejik planlar doğrultusunda yetenek ve niteliklerine göre yabancıların istihdama kazandırılmasının önü açılmalıdır. Bu noktada işçinin işin niteliğine göre seçilerek ülkeye getirilmesi de teklifler arasındadır.

*“Mülteciler ülkelerinde çalıştıkları işlerden farklı işlerde çalışıyorlar, ülkelerindeki yaptıkları işlere göre sınıflandırmaları gerekiyor. Özellikle ülkemize girişlerinden sonra mesleki alışkanlık ve yeterliliklerine göre bir sınıflandırma yapılması gerekiyor daha rahat istihdam edilebilmeleri için. Mesleki eğitim çalışmalarında Halk Eğitim Merkezlerinin biraz daha gayretli olup çalışması gerekiyor. Bunların hepsi yapılırsa amaçladığımız iş gücünü daha verimli kullanmış oluruz”* (Konya Damızlık ve Sığır Yetiştiricileri Birliği Başkan Vekili).

Nitekim tarım ve hayvancılık sektöründe sığınmacı işgücüne zorunlu olarak ihtiyaç vardır. Bu ihtiyacın karşılanması için gerekirse farklı ülkelerden işgücü talep edilmesi bir zorunluluk olarak değerlendirilmektedir. Bu bağlamda her bir sektör ve iş kolu için ihtiyaç analizlerinin yapılması ve analizler doğrultusunda işgücü talebinin ortaya konulması son derece önemlidir. Kayıt dışı istihdam sorununun giderilmesi için 2000 yılına kadar Suudi Arabistan'da yürürlükte olan kefalet ya da kefillik sisteminin hayata geçirilmesi teklifler arasındadır. Keffilik sistemi, yabancı bir işçinin çalışabilmesi için belli bir

ücret karşılığında kendisine kefalet verecek Suudi Arabistan vatandaşı bulması esasına dayanmaktadır. Kefili olmayan kişi ülkede çalışma izni alamamaktadır. Bu sistem bir taraftan sığınmacı işgücünün kayıt altına alınması diğer taraftan da sürdürülebilir ve güven esaslı çalışma koşullarının tesisi açısından bir alternatif olarak sunulmuştur. Elbette bu sistemde özellikle sığınmacı işgücünün olası mağduriyetleri karşısında devletin denetim mekanizmalarını sağlıklı bir biçimde işletmesi oldukça önemlidir. Tarım ve hayvancılık sektöründe kayıt dışı istihdam konusunda en temel sorun alanlarından biri de sektörü domine eden Afganistan kökenli yabancıların istihdamda yer alabilecekleri bir statüye kavuşturulmalarıdır. Bu konuda acilen bir yasal düzenlemeye gidilmesi ya da tıpkı Suriyeliler gibi geçici koruma statüsüne geçirilmeleri öneriler arasında yer almaktadır.

### SONUÇ ve ÖNERİLER

Konya'da tarım ve hayvancılık sektöründe çalışacak işçi gücünün olmaması ve var olan işçi gücünün çalıştırılması yönünde mevcut politikaların yetersiz kalması ön plana çıkmaktadır. Özellikle Türkiye yüzdesinde önemli bir pay elde eden Konya için durum değerlendirildiğinde bu sektöre acil olarak uygun politikaların geliştirilmesi ve desteklenmesi gerektiği görülmektedir. Doğru politikaların uygulanması halinde Türkiye'nin son on yıllarda deneyimlediği göçlerin istihdama katkı sağlaması düşünülmektedir. Konya için sığınmacıların Konya'ya işgücü hareketliliği tarım ve hayvancılık sektörü için önem arz etmektedir. Hayvanların yayılımı, bakımı, beslenmesi ya da sağımı; tarımsal ürünlerin ekimi, ilaçlanması, gübrelenmesi, sulanması ya da tarımsal araçların kullanımı bu sektörlerde talep edilen işgücünün niteliğini ortaya koymaktadır. Ancak potansiyelde bu niteliklere sahip işçilerin azlığı ya da var olan işçinin kente göç etmesi bu sektörde yer alacak olan sığınmacıların niteliğinin sorgulamaksızın işe alımını zorunlu hale getirmiştir. Böylece hem işveren işçinin yetişmesi için daha fazla emek harcamakta hem de gelecekte olup olmayacağı belli olmayan işçi için daha çok kaygılanmaktadır. İşverenin bu kaygılarla baş etmesi için işçinin işyerine bağlı kalması hususunda politikaların geliştirilmesi gerekmektedir.

Hali hazırda sektörlerin en temel problemi kayıt dışılık ve buna bağlı kayıt dışı istihdamdır. Bu bağlamda başta Afganistan kökenli yabancılar olmak üzere sığınmacıların tarım ve hayvancılık sektöründe kayıtlı işgücü olarak çalışmaları için yasal mevzuatın yeniden gözden geçirilmesi ve göç politikaları belirlenirken bu hususun göz ardı edilmemesi elzemdir. Tarım ve hayvancılık sektöründe üretimin sürekliliği ve gelişimi için sığınmacı işgücüne zorunlu olarak ihtiyaç duyulmaktadır. Bu iş gücünün kayıt dışı şekilde



gerçekleşmesi ise sadece işçi ve işveren arasında bir sorun olmadığı, daha büyük çerçevede ileride yaşanması muhtemel sorunları gün yüzüne çıkaracağı düşünülmektedir. Kayıt dışı istihdamın önüne geçilmesi işveren içinde önemli bir konudur. İşverenin kaygısı işçinin daha cazip ücret karşılığında kendisini yarıda bırakmasıdır. Bu kaygı işveren için önemli bir sorundur zira giden işçinin arda bıraktığı istihdam boşluğunu kırsal alanda doldurmak kolay değildir. İşçilerin kayıt altına alınması ise işverenin böyle kaygılar taşımamasına neden olmayacaktır.

Son olarak sığınmacıların toplumda yarattığı algının, özellikle siyaset malzemesi olarak kullanılmasının önlenmesi, toplumsal barış ve uyum açısından kritik bir öneme sahiptir. Bu bağlamda, yabancı düşmanlığı, nefret söylemi ve ırkçılığa karşı yasal düzenlemelerin yapılması gereklidir. Medyada sıklıkla karşılaşılan “rızkımızı/işimizi elimizden alıyorlar” şeklindeki söylemler, toplumda olumsuz bir izlenim yaratmakta ve bu söylemler gerçeği yansıtmamaktadır. Araştırmalar göstermektedir ki sığınmacılar genellikle yerel halkın tercih etmediği iş kollarında istihdam edilmektedirler. Dolayısıyla, sığınmacıların istihdamının sona ermesi, belirli sektörlerde ciddi bir daralma yaratacak ve bu durumun ekonomik maliyeti yerel halkın üzerine yüklenecektir. Bu nedenle, sığınmacıların yerel ekonomik yapıya katkılarının topluma anlatılması önemlidir. Sektör temsilcileri, somut verilerle bu durumu açıklamalı ve sığınmacıların çalıştığı sektörlerdeki durumun ekonomik sonuçlarını vurgulamalıdır. İmkanlar dâhilinde, kamu spotları ve bilgilendirme kampanyaları aracılığıyla yerel halk ile bu bilgilerin paylaşılması, algının değiştirilmesi adına önemli bir adım olacaktır. Politika düzeyinde öneriler geliştirmek için sığınmacıların istihdamında yerel halkın ihtiyaçlarını göz önünde bulunduran ve istihdam politikalarını destekleyen bir yaklaşım benimsenmelidir. Ayrıca, sığınmacılara yönelik entegrasyon programları geliştirilmeli, bu programlar aracılığıyla yerel halk ile etkileşim ve işbirliği fırsatları artırılmalıdır. Bu tür önlemler, hem toplumsal uyumun sağlanmasına hem de ekonomik sürdürülebilirliğin desteklenmesine katkıda bulunacaktır.

### 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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## Şavak Akkaraman Koyunlarının Süt Verimi, Meme Özellikleri ve Meme Ölçüm Değerleri Arası İlişkiler

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### ÖZET

Bu araştırmada, ekstansif şartlarda yetiştirilen Şavak Akkaraman koyunlarında süt verimi özellikleri ile meme özellikleri ve bunlar arasındaki ilişkiler incelenmiştir. Araştırmanın hayvan materyalini, Erzincan ili Şavak Akkaraman koyunu Halk Elinde Islahı Projesi kapsamında üç farklı işletmede yetiştirilen koyunlar (n=172) oluşturmuştur. Ortalama laktasyon süresi, sağımda kalınan süre ve emiştirme süresi sırasıyla 205.8±2.0, 144 ve 61.8 gün; Trapez II (ICAR: International Committee for Animal Recording) ile hesaplanan laktasyon (sağılan) süt verimi ve günlük ortalama süt verimi sırasıyla; 88.8±3.5 kg, 616.5±2.5 g olarak belirlenmiştir. Ortalama laktasyon süresi ve emiştirme süresine, yaş ( $p<.05$ ), doğum tipi ve işletmenin ( $p<.01$ ), laktasyon süt verimi ve ortalama günlük süt verimine ise doğum tipi ve işletmenin ( $p<.01$ ) etkileri önemli bulunmuştur. Sağım döneminin 3 farklı zamanında, alınan meme ölçülerinden; meme derinliği (MD) 15.8±0.2, meme genişliği (MG) 13.3±0.2, meme çevresi (MÇ) 40.3±0.5, sağ/sol meme başı uzunluğu Sağ/SolMBU) 2.9±0.6, 2.8±0.1, sağ/sol meme çapı (Sağ/SolMBC) 2.1±0.1, 2.0±0.0, meme başları arası mesafe (MBAM) 15.7±0.2, meme başı yüksekliği (MBY) 28.0±0.4, meme alt yüksekliği (MAY) 27.9±0.4 cm'dir. Meme ölçülerinin laktasyonun farklı dönemlerinde önemli ( $p<.05$ ;  $p<.01$ ) derecede değiştiği görülmüştür. Meme ölçüleri arası ve bunların günlük süt verimi (GSV) ile ilişkilerinin tamamı önemli ( $p<.01$ ) ve korelasyon katsayıları genel olarak yüksek bulunmuştur. Bu durum ve ölçüm zamanı ile olan korelasyonlar; meme yapısının simetrik olduğunun, süt veriminin yüksekliği ile orantılı şekilde büyüdüğünün ve yere yaklaştığının, süt verimi azaldıkça yine orantılı olarak küçüldüğünün işareti sayılabilir. Sonuç olarak, elde edilen bulgulara göre; Şavak Akkaraman koyunlarından sağılan süt miktarının tatmin edici olduğu ve tespit edilen varyasyonun seleksiyon için iyi bir potansiyel barındırdığı söylenebilir.

### Zootekni

### Araştırma Makalesi

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### Anahtar Kelimeler

Şavak Akkaraman

Süt verimi

Meme özellikleri

## Relationships Between Milk Yield, Udder Characteristics and Udder Measurement Values of Şavak Akkaraman Sheep

### ABSTRACT

In this research, milk yield characteristics and udder characteristics and the relationships between them were examined in Şavak Akkaraman ewes raised under extensive conditions. The animal material of the research consisted of ewes (n=172) raised in three different farms within the scope of the Şavak Akkaraman sheep Public Breeding Project in Erzincan province. The average lactation duration, milking duration, and suckling duration were 205.8±2.0, 144, and 61.8 days, respectively. Lactation (milked) milk yield and daily average milk yield calculated by Trapez II (ICAR: International Committee for Animal Recording) were respectively; It was determined as 88.8±3.5 kg, 616.5±2.5 g. The effects of age ( $p<.05$ ), type of birth, and farm ( $p<.01$ ) on average lactation duration and suckling time, and effects of type of birth and farm ( $p<.01$ ) on lactation milk

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yield and average daily milk yield were found to be significant. Udder measurements taken at 3 different times of the milking period were measured as udder depth (UD)  $15.8 \pm 0.2$ , udder width (UW)  $13.3 \pm 0.2$ , udder circumference (UC)  $40.3 \pm 0.5$ , right/left udder length R/LUL)  $2.9 \pm 0.6$ ,  $2.8 \pm 0.1$ , right/left udder diameter ( R/LUD)  $2.1 \pm 0.1$ ,  $2.0 \pm 0.0$ , teat distance (TD)  $15.7 \pm 0.2$ , teat height (TH)  $28.0 \pm 0.4$ , udder bottom height (UBH)  $27.9 \pm 0.4$  cm. It was observed that udder measurements changed significantly ( $p < .05$ ;  $p < .01$ ) in different periods of lactation. All of the relationships between udder measurements and their Daily Milk Yield (DMY) were found to be significant ( $p < .01$ ) and their correlation coefficients were high. This situation and correlations with measurement time; It can be considered a sign that the udder structure is symmetrical, that it grows proportionally and moves closer to the ground when milk yield is high, and that it shrinks proportionally as milk yield decreases. As a result, according to the findings; it can be said that the amount of milk produced from Şavak Akkaraman ewes is satisfactory, and the detected variation has a good potential for selection.

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## GİRİŞ

Son yıllarda koyun sütünün önemi gittikçe artmakta ve kaliteli peynir, yoğurt çeşitleri yüksek fiyatlara satılabilmektedir. Türkiye’de hayvan yetiştiriciliğinde koyunculüğün geçmişten günümüze ayrı bir önemi vardır (Kaymakçı, 2016). TÜİK, (2024) verilerine göre; 2023 yılı Türkiye koyun varlığı 42 milyonun üzerinde olup koyun sütü üretimi 933.579 ton olarak belirlenmiş, koyun sütünün toplam çiğ süt üretimi (21.481.567 ton) içerisindeki payı %4.34 olarak gerçekleşmiştir. Son yıllardaki anaç koyun varlığının azalmasına bağlı olarak koyun sütü üretiminin son üç yılda %15.2 oranında azaldığı gözlemlenmiştir.

Türkiye, dünyada Çin’den sonra en fazla koyun sütü üreten ülke konumundadır. Türkiye koyun sayısının son yıllarda azalış göstermesine rağmen mevcut hayvanlarını azami şekilde üretimde kullanmasının avantajı ile dünya koyun sütü üretiminin yaklaşık %11’ini üretmektedir. Avrupa Birliğinde ise et veya yapağı koyunculugu yaygın olarak yapılmaktadır. Türkiye, Avrupa Birliği ülkelerinin ürettiği koyun sütünün %42 kadarını tek başına üretebilmektedir (FAO, 2024). Verilen bu özet değerler; koyun yetiştiriciliğinin hem Türkiye’de, hem bulunduğu ve girmeye aday olduğu bölgede, hem de dünyada önemli bir üretici konumunda olduğunu ve bunun Türkiye açısından önemini ortaya koymaktadır.

Türkiye’de yetiştirilen koyun ırklarından en fazla olan (%38) Akkaraman koyun ırkının birçok tipi bulunmaktadır. Bunlar; daha çok Sivas ve Malatya ili çevresinde yetiştirilen “Kangal”, Diyarbakır, Van yöresinde yetiştirilen “Karakas”, Mersin yöresinde yetiştirilen “Güney Karaman” ve Diyarbakır yöresinde yetiştirilen “Zom Koyunu” olarak bilinen koyun

tipleridir. Bunlardan biri de son yıllarda önemi fark edilen ve özellikle süt üretimi için Erzincan, Elazığ ve Tunceli yörelerinde yetiştirilen ve yöreye ait tulum peyniri üretiminde önemli bir yere sahip olan “Şavak koyunu” olarak adlandırılan koyun tipidir (Yağcı, 2017).

Şavak Akkaraman koyunu, adını, bölgede yaşayan bir aşiretten almaktadır. Şavak koyunlarını, yetiştirildiği coğrafya ve iklim şartlarına uygun ve daha fazla süt üretebilecek seviyeye ulaştırabilmek için geleneksel yöntemlerle, yıllardır süregelen çalışmalarda bulunulmuştur. Öncelikle sürülerde en iyi damızlıklar seçilmeye çalışılmış ve yakın çevrelerden damızlık koçlar getirilerek sürüler kullanmıştır. Bölge yetiştiricisinin günümüze kadar gelen bu süreç zarfında uyguladığı yetiştiricilik sistemi sayesinde coğrafyaya uyum sağlamış ve süt verimi yönünden de azımsanmayacak düzeyde olan bir koyun popülasyonu ortaya çıkmıştır (Yağcı, 2017).

Şavak Akkaraman koyunu yetiştiricileri süt üretimini öncelikli amaç olarak gördüklerinden, koyunlarını daha uzun süre sağmaya gayret etmektedirler. Bu nedenle Şavak koyunu süt veriminin Türkiye’nin diğer bölgelerindeki Akkaraman sürülerinden genellikle yüksek olduğu bilinmektedir. Sağılan sütün büyük bir kısmı “Şavak Tulum Peyniri” olarak bilinen peynir yapımında kullanılmakta ve üretilen peynirden oldukça iyi düzeyde gelir elde edilmektedir. Şavak Tulum Peyniri üretimi bölgenin tulum peynirinin merkezi olma konumunda büyük önem arz etmektedir (Yağcı, 2017).

Koyunculüğün gelişmesi ve istenilen hedeflere ulaşabilmesi için mevcut durumun bilinmesi gerekmektedir. Bu konuda yapılacak her bilimsel



çalışma bu nedenle önem taşımaktadır. (Köseman ve ark., 2022). Bu gerçekten hareketle bu çalışma, Türkiye’de yoğun olarak koyun süt sağımının yapıldığı Erzincan ve civarı bölgede yetiştirilen Şavak Akkaraman koyunlarının morfolojik ve fizyolojik özelliklerini belirleme kapsamında süt verimi ve meme özellikleri bakımından tanıtıcı bilgiler elde etmek, diğer Akkaraman koyunları içindeki yerini ve benzerlik veya farklılıklarını ortaya koymak amacıyla yapılmıştır.

## MATERIAL ve METOD

Araştırmanın materyalini; süt verimi özellikleri için 172, meme özellikleri için 171 baş koyundan alınan veriler oluşturmuştur. Koyunlar genel olarak merada, kış mevsiminde ise ağılda kuru ot samanı ve arpa kırması ile beslenmiştir. Araştırmanın yapıldığı işletmelerde koçlar yaklaşık 60-70 gün süre ile serbest aşım için Ekim ayında sürüye katılmıştır. Kuzular süttten kesime kadar (yaklaşık 2 ay) ana sütü ile beslenmiştir. Kuzular doğum sonrası 2-3 gün süreyle analarıyla birlikte tutulmuş ve daha sonra analarından ayrılmış, sabah ve akşam olmak üzere günde 2 kez emzirilmiştir.

Koyunlar doğumların ardından hemen sağılmamış, kuzular belirli bir süre analarıyla birlikte bırakılarak analarını serbestçe emmeleri sağlanmış ve sonra sürü bazında süttten kesim uygulaması yapılarak kuzular analarından ayrılmıştır. Ancak, süttten kesim uygulaması yapılmadan kısa süre önce kuzular bir öğün emiştirilerek alıştırmış, diğer öğünde ise koyunlar sağılmıştır. Süttten kesim sonrası sürüler peyderpey yaylaya çıkarılmıştır. Kontrol sağımları uygulanacak sürülerde sağımlar 20 Nisan tarihinde başlatılmış ve ilk kontrol tam bir ay sonra 20 Mayıs’ta gerçekleştirilmiştir. İlk üç kontrol ayda bir, son iki kontrol ise 21 gün arayla toplam 5 kontrol yapılmıştır. 1 Eylül’de yapılan son kontrolden 10 gün sonra koyunların bir kısmı kuruya çıkarılmış ve sağıma devam edilen az sayıda hayvanın süt verimleri 100 ml’nin altında olduğundan değerlendirmeye alınmamış ve son kontrolden 10 gün sonra laktasyon sonlandırılmıştır. Sağım kontrolleri, sabah ve akşam elle sağım yöntemiyle yapılmıştır. Süt miktarı küçük plastik kaplara sağılıp terazide tartılarak g cinsinden belirlenmiştir. Elde edilen verilerden her koyunun günlük süt verimi, laktasyon süt verimi, laktasyon süresi, süt verimi ve kuzu emiştirme süresi hesaplanmıştır.

Koyunların doğum yaptıkları tarih ile kuzuların süttten kesildiği tarih arası emiştirme süresi, doğumdan son kontrolün 10 gün sonrasına kadar olan süre ise laktasyon süresi olarak kabul edilmiştir. İlk

sağımın başladığı tarih ile sonlandırıldığı tarih arasındaki süre ise koyunun sağımda kaldığı süre olup, söz konusu sağım süresi olarak hesaplanmıştır.

Laktasyon (sağılan) süt veriminin tahmininde Trapez I, Trapez II (ICAR), Hollanda, Vogel ve İsveç Metodu olarak bilinen 5 yöntem kullanılmıştır. Sağım kontrollerinde sağılan süt miktarı kontrol günü süt verimi olarak kabul edilmiştir. Günlük Ortalama Süt Verimi (GOSV) iki farklı şekilde hesaplanmış olup; ilki, kontrol sağımlarında ölçülen süt miktarları toplanarak toplam kontrol sayısına bölünmesiyle hesaplanmış (GOSV1), ikincisi ise; laktasyon süt verimini hesaplamada kullanılan yöntemlerinden Trapez II ile hesaplanan toplam süt miktarının sağım süresine bölünmesiyle elde edilmiştir (GOSV2).

Meme ölçümlerine doğum sonrası ilk ay içerisinde başlanmış olup yaklaşık 45 günlük ara ile toplamda 3 kez ölçüm yapılmıştır. İncelenen sürülerde meme ölçümleri; doğumdan hemen sonra (20 Şubat), kuzuların süttten kesim esnasında yaylaya çıkmadan önce (18 Nisan) ve laktasyonun ortasında yaylada (06 Temmuz) gerçekleştirilmiştir. Bu ölçümlerle eş zamanlı olarak, meme ölçüleri ile süt verimi arasındaki ilişkileri belirlemek amacıyla günlük süt verimi kontrolleri yapılmıştır. Ölçümler sağımdan hemen önce yapılmıştır. Meme ölçüleri; meme derinliği (MD), meme çevresi (MÇ), meme genişliği (MG), sağ ve sol meme başı uzunlukları (SağMBU, SolMBU), sağ ve sol meme başı çapları (SağMBÇ, SolMBÇ), meme başları arası mesafe (MBAM), meme başı yüksekliği (MBY) ve meme alt yüksekliği (MAY) olmak üzere 10 farklı ölçüden oluşmuştur (Şekil 1). Ölçümlerde kumpas aleti, şerit metre ve mezura kullanılmıştır.

Şavak Akkaraman koyunlarda meme ve süt verim özelliklerinin istatistiki analizinde etkili olduğu varsayılan faktörlere göre her bir özellik için bir istatistik model oluşturulmuş ve veriler faktöriyel deneme düzeninde varyans analizine tabi tutulmuştur. Varyans analizinde GLM (Generalized Linear Models) kullanılarak LSM (Least Squares Means) hesaplanmış ve alt grup ortalamalarının karşılaştırılmasında ise Duncan çoklu karşılaştırma testi kullanılmıştır.

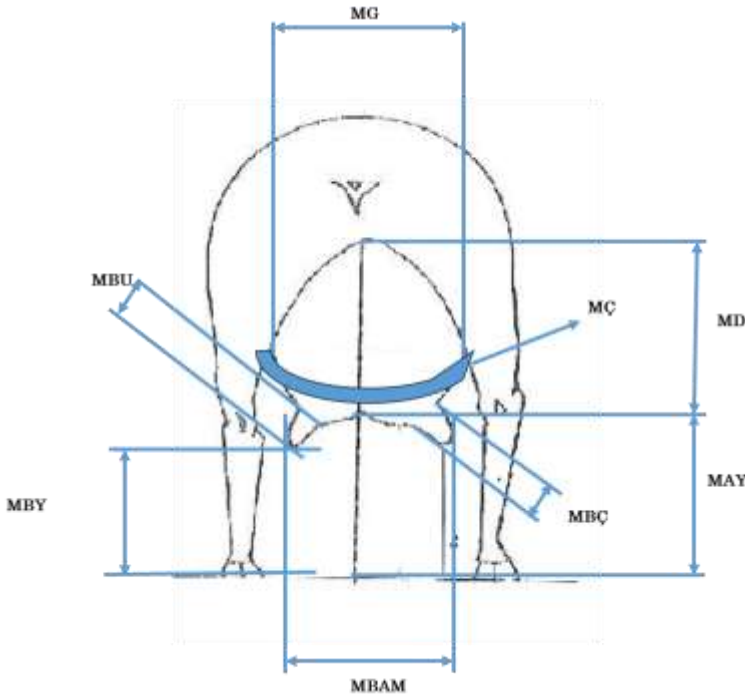
Genel olarak  $a_i$  = yaş,  $b_j$  = işletme,  $c_k$  = cinsiyet ve  $d_l$  = doğum tipi olmak üzere analizde benimsenen istatistiki modeller;

Süt verimi özellikleri için;

$$Y_{ijls} = \mu + a_i + b_j + d_l + e_{ijls}$$

Meme özelliklerinin analizinde yukarıdaki modele ölçüm zamanı ( $f_n$ ) faktörü eklenmiştir.

İstatistik analizlerde SPSS 22.0 paket programı kullanılmıştır (SPSS, 2013).



Şekil 1. Meme ölçüleri  
Figure 1. Udder measurements

## BULGULAR

### Laktasyon ve emiştirme süresi

Şavak Akkaraman koyunlarının laktasyon ve emiştirme sürelerine ait en küçük kareler ortalamaları, standart hataları, önemlilik ve çoklu karşılaştırma testi sonuçları Çizelge 1'de sunulmuştur.

Şavak Akkaraman koyunlarının laktasyon süresi  $205.8 \pm 2.0$  gün, emiştirme süresi ise  $61.8 \pm 2.0$  gün olarak hesaplanmıştır. En az ve en çok olarak tespit edilen laktasyon süreleri 165-229 gün, emiştirme süreleri ise 21-85 gündür. Laktasyon ve emiştirme sürelerine etkileri incelenen faktörlerden koyunun yaşı önemli ( $p=0.037$ ), doğum şekli ve işletme faktörleri ise çok önemli ( $p<.001$ ) düzeyde etkiye sahiptir. Laktasyon süreleri yaşa bağlı olarak 200.2 gün ile 213.6 gün arasında değişim göstermektedir ( $P<0.05$ ). En düşük ve en yüksek değerleri veren sırasıyla 7 ve 9 yaş gruplarının dışındaki diğer yaş grupları arasında da farklı gruplanmalar söz konusudur. Laktasyon süreleri bakımından 2, 3, 4 ve 6 yaşlı koyunlar aynı grupta, 5 ve 7 yaşlı koyunlar da farklı bir grupta yer almıştır. Aynı şekilde emiştirme süreleri bakımından da paralel bir seyir gözlenmiştir. En yüksek ve en düşük değerler yine 7 ve 9 yaşlı koyunlarda sırasıyla 69.6 ve 56.2 gün olarak gerçekleşmiştir ve yaş grupları arasında farklılık önemli bulunmuştur ( $P<0.05$ ). Emiştirme süresi en uzun 5 ve 7 yaşlı koyunlarda görülürken, en kısa 2, 4, 6 ve 9 yaşlı koyunlarda gözlenmiştir. En yüksek ve en düşük emiştirme süresine sahip yaş grupları kendi içinde benzer iken, gruplar birbirinden farklılık göstermiştir ( $p<.05$ ).

Meme derinliği (MD) (UD)  
Meme genişliği (MG) (UW)  
Meme çevresi (MÇ) (UC)  
Meme başı uzunluğu (MBU) (TL)  
Meme başı çapı (MBÇ) (TD)  
Meme başları arası mesafe (MBAM) (DBT)  
Meme başı yüksekliği (MBY) (TH)  
Meme alt yüksekliği (MAY) (UBH)  
UD: Udder depth  
UW: Udder width  
UC: Udder circumference  
TL: Teat length  
TD: Teat diameter  
DBT: Distance between teat  
TH: Teat height  
UBH: Udder bottom height

Koyunun doğurduğu kuzunun tekiz veya ikiz oluşu, laktasyon süresinin çok önemli ( $p<.004$ ) ölçüde değişmesine sebep olmaktadır. İkiz doğuran koyunların laktasyon sürelerinin 10 gün daha kısa olduğu hesaplanmıştır. Aynı şekilde, ikiz doğuran koyunların emiştirme süreleri de 10 gün daha kısa olarak belirlenmiştir. Laktasyon ve emiştirme süreleri bakımından işletmeler arasında varyasyon söz konusudur ( $p<.001$ ). En yüksek ve en düşük süreler bakımından işletme ortalamaları arasında 17.3 günlük bir farklılık söz konusu olup istatistik olarak önemlidir ( $p<.05$ ). Ancak, 1 ve 3 nolu ile kodlanan işletmeler arasında da benzerlik söz konusudur. İşletmelerde en az ve en çok olarak belirlenen laktasyon süreleri işletme sırasıyla 199-222 gün, 170-222 gün, 165-229 gün, emiştirme süreleri ise aynı sırayla 55-78 gün, 26-78 gün, 21-85 gündür.

### Laktasyon süt verimi

Şavak Akkaraman koyunlarında Trapez I, Trapez II (ICAR), Hollanda, İsveç ve Vogel yöntemleriyle hesaplanan toplam süt verimi (kuzunun emdiği süt miktarı hariç) ortalamaları sırasıyla  $86.3 \pm 3.5$ ,  $88.8 \pm 3.5$ ,  $91.4 \pm 3.6$ ,  $91.6 \pm 3.6$  ve  $79.7 \pm 3.1$  kg olarak hesaplanmıştır (Çizelge 2). Trapez II (ICAR), Hollanda ve İsveç metodu ile hesaplanan toplam süt verimlerinin birbirlerine oldukça yakın olduğu gözlenmiştir. Trapez II yöntemiyle düzeltilmiş süt verimi en düşük 55 kg, en yüksek ise 168.6 kg olarak hesaplanmıştır.

Çizelge 1. Şavak Akkaraman koyunlarının laktasyon ve emiştirme sürelerine ait ortalama değerler, standart hataları, önemlilik ve çoklu karşılaştırma testi sonuçları.

Table 1. Mean values, standard errors, significance and multiple comparison test results of lactation and suckling periods of Şavak Akkaraman ewes.

	n	Laktasyon Süresi (gün)	Emiştirme Süresi (gün)
		<i>Lactation periods (days)</i>	<i>Suckling periods (days)</i>
		$\bar{X} \pm S_{\bar{x}}$	$\bar{X} \pm S_{\bar{x}}$
<b>Ortalama (Means)</b>	172	205.8 ± 2.0	61.8 ± 2.03
<b>Yaş (Ages)</b>		(F=2.302; p=0.037)	(F=2.302; p=0.037)
2	25	203.1 ± 2.6 <sup>cd</sup>	59.1 ± 2.6 <sup>cd</sup>
3	29	207.4 ± 2.6 <sup>bc</sup>	63.4 ± 2.6 <sup>bc</sup>
4	40	203.3 ± 2.6 <sup>cd</sup>	59.3 ± 2.5 <sup>cd</sup>
5	49	211.7 ± 2.2 <sup>ab</sup>	67.7 ± 2.2 <sup>ab</sup>
6	4	201.1 ± 5.5 <sup>cd</sup>	57.1 ± 5.5 <sup>d</sup>
7	4	213.6 ± 5.5 <sup>a</sup>	69.6 ± 5.5 <sup>a</sup>
9	21	200.2 ± 3.3 <sup>d</sup>	56.2 ± 3.3 <sup>d</sup>
<b>Doğum Şekli (Birth types)</b>		(F=8.502; p=0.004)	(F=8.502; p=0.004)
Tek	162	210.8 ± 1.2 <sup>a</sup>	66.8 ± 1.2 <sup>b</sup>
İkiz	10	200.8 ± 3.6 <sup>b</sup>	56.8 ± 3.6 <sup>a</sup>
<b>İşletme (Farms)</b>		(F=22.064; p<.001)	(F=22.064; p<.001)
1	57	212.4 ± 2.7 <sup>a</sup>	68.4 ± 2.7 <sup>a</sup>
2	60	195.1 ± 2.8 <sup>b</sup>	51.1 ± 2.8 <sup>b</sup>
3	55	209.9 ± 2.1 <sup>a</sup>	65.9 ± 2.1 <sup>a</sup>
<b>R<sup>2</sup></b>		0.295	0.295

a,b,c,d: Aynı harfi taşıyan alt grup ortalamaları arasındaki farklılık önemsiz, farklı harf taşıyanlar önemlidir (p<.05).

a,b,c,d: The difference between subgroup averages with the same letter is insignificant, those with different letters are important (p<.05).

Toplam süt verimi üzerine koyunun yaşının etkisi tüm yöntemlerde önemsiz çıkmıştır. İkiz ya da tekiz doğumun etkisi ve verilerin elde edildiği işletme etkisi önemli (p<.001) düzeyde varyasyon meydana getirmektedir. Toplam süt veriminin yaşa göre değişimi izlendiğinde, farklılıklar önemli olmamakla birlikte, 2 yaşından itibaren artış, 3 ve 4 yaşlarında üst seviyede kısmen sabitleme, 4 yaşından itibaren azalma eğilimi (6 yaşına kadar) gözlenmektedir. İkiz doğuran koyunların toplam süt verimleri beklendiği gibi tek doğuranlardan daha yüksektir. İkiz doğuran koyunlar lehine olan üstünlük Trapez I, Trapez II (ICAR), Hollanda, İsveç ve Vogel yöntemlerinde sırasıyla; 21.8, 22.2, 21.5, 22.6 ve 18.7 kg olarak hesaplanmıştır. Ortaya çıkan farklılıklar önemlidir (p<.05).

İncelenen işletmeler arasında en yüksek toplam süt verimi bütün yöntemlere göre 3 nolu işletmede gerçekleşmiştir. Bu işletmenin toplam süt verimi, 2 nolu işletmede Trapez I, Trapez II ve İsveç yöntemlerine göre hesaplanan ortalamalar ile benzerlik gösterirken diğer yöntemlerde farklılıklar (p<.05) tespit edilmiştir. 1 nolu işletmeye ait toplam süt verimi ise bütün yöntemlere göre diğer işletmelerden önemli seviyede (p<.05) düşük hesaplanmıştır. En yüksek ve en düşük ortalamaya sahip işletmeler arasındaki farklılıklar hesaplama yöntemlerinde yukarıdaki sıraya göre; 19.7, 20.4, 21.7, 21.3 ve 19.0 kg olarak hesaplanmıştır. İşletmelerde

Trapez II yöntemiyle hesaplanan en düşük ve en yüksek düzeltilmiş süt verimleri işletme sırasıyla 56.7-120 kg, 55-168.6 kg, 63.3-116.6 kg olarak belirlenmiştir. En düşük-en yüksek değer farkı olarak işletme 2'de gözlenen değer (113.6 kg), işletme 1 ve 3'de belirlenen değerlerin (sırasıyla 63 ve 53.3 kg) neredeyse iki katı kadar olması üzerinde durulması gereken önemli bir husustur. İşletmeler arasında bakım-besleme-idare farklılıklarından kaynaklanan varyasyon beklenen bir durumdur. Ancak, burada işletmelerin kendi içinde, özellikle işletme 2'de hayvanlar arasında geniş bir varyasyon gösterdiği görülmektedir.

#### Kontrol günü süt verimi

İncelenen sürülerde tespit edilen kontrol günü günlük süt miktarlarının en küçük kareler ortalamaları, standart hataları, önemlilik ve çoklu karşılaştırma testi sonuçları Çizelge 3'de sunulmuştur.

Şavak Akkaraman koyunlarının ilk sağım kontrolünde tespit edilen günlük süt miktarı ortalamaları 813.0±38.4 g iken 2., 3., 4. ve 5. kontrollerde giderek azalmış ve sırasıyla; 753.2±36.0, 536.6±23.7, 476.1±15.0 ve 250.1±16.6 g olarak ölçülmüştür (Çizelge 3). Kontrol günlerinde tespit edilen en düşük ve en yüksek değerler sırasıyla 230-1640 g, 235-1548 g, 230-896 g, 230-740 g ve 90-540 g olarak belirlenmiştir. Kontrol günü verimlerinde yaşların oluşturduğu varyasyon bütün dönemlerde önemsiz bulunmuştur.

Koyunun doğurduğu kuzunun tek veya ikiz oluşu ise 1. ve 2. kontrollerde süt veriminde çok önemli ( $p=.001$ ) farklılığa yol açarken, 3. ve 4. kontrollerde farklılıklar

azalarak önemli ( $p=.0086$  ve  $p=.010$ ) düzeyine çekilmiş, 5. kontrolde ise doğum tipinden kaynaklanan farklılık kaybolmuştur ( $p=.0114$ ).

Çizelge 2. Şavak Akkaraman koyunlarında farklı süt verimi hesaplama yöntemleri ile hesaplanan süt verimine ait ortalamalar, standart hataları, önemlilik ve çoklu karşılaştırma testi sonuçları.

Table 2. Means, standard errors, significance and multiple comparison test results of milk yield calculated with different milk yield calculation methods in Şavak Akkaraman ewes.

		Laktasyon (Sağılan) Süt Verimi (kg) (Milking Milk Yield)				
		Trapez I	Trapez II	Hollanda	İsveç	Vogel
		$\bar{X} \pm S_{\bar{x}}$	$\bar{X} \pm S_{\bar{x}}$	$\bar{X} \pm S_{\bar{x}}$	$\bar{X} \pm S_{\bar{x}}$	$\bar{X} \pm S_{\bar{x}}$
<b>Ortalama (Means)</b>	172	86.3 ± 3.5	88.8 ± 3.5	91.4 ± 3.6	91.6 ± 3.6	79.7 ± 3.1
<b>Yaş (Ages)</b>		(F=1.039; $p=.0402$ )	(F=0.993; $p=.0432$ )	(F=1.029; $p=.0409$ )	(F=1.006; $p=.0424$ )	(F=1.027; $p=.0410$ )
2	25	81.8 ± 4.4	84.5 ± 4.5	87.4 ± 4.5	87.4 ± 4.6	76.2 ± 3.9
3	29	90.3 ± 4.4	93.0 ± 4.5	96.3 ± 4.6	96.1 ± 4.6	84.0 ± 4.0
4	40	90.6 ± 4.3	93.2 ± 4.4	95.7 ± 4.5	96.1 ± 4.5	83.4 ± 3.9
5	49	84.5 ± 3.8	87.2 ± 3.8	90.0 ± 3.9	90.0 ± 3.9	78.5 ± 3.4
6	4	80.8 ± 9.3	82.9 ± 9.5	84.9 ± 9.6	85.4 ± 9.8	74.0 ± 8.3
7	4	90.0 ± 9.3	92.3 ± 9.5	94.3 ± 9.6	95.1 ± 9.8	82.3 ± 8.3
9	21	85.9 ± 5.6	88.3 ± 5.7	91.1 ± 5.7	91.3 ± 5.8	79.4 ± 5.0
<b>Doğum Şekli (Birth types)</b>		(F=13.983; $p<.001$ )	(F=13.951; $p<.001$ )	(F=12.881; $p<.001$ )	(F=13.713; $p<.001$ )	(F=12.884; $p<.001$ )
Tek (Single)	162	75.4 ± 2.1 <sup>b</sup>	77.7 ± 2.1 <sup>b</sup>	80.6 ± 2.1	80.3 ± 2.2 <sup>b</sup>	70.3 ± 1.8 <sup>b</sup>
İkiz (Twin)	10	97.2 ± 6.1 <sup>a</sup>	99.9 ± 6.2 <sup>a</sup>	102.1 ± 6.2	102.9 ± 6.3 <sup>a</sup>	89.0 ± 5.4 <sup>a</sup>
<b>İşletme (Farms)</b>		(F=10.240; $p<.001$ )	(F=10.446; $p<.001$ )	(F=12.029; $p<.001$ )	(F=10.955; $p<.001$ )	(F=12.159; $p<.001$ )
1	57	74.5 ± 4.6 <sup>b</sup>	76.6 ± 4.7 <sup>b</sup>	79.3 ± 4.7 <sup>c</sup>	79.2 ± 4.8 <sup>b</sup>	69.2 ± 4.1 <sup>c</sup>
2	60	90.1 ± 4.8 <sup>a</sup>	92.7 ± 4.9 <sup>a</sup>	93.7 ± 4.9 <sup>b</sup>	95.1 ± 5.0 <sup>a</sup>	81.7 ± 4.3 <sup>b</sup>
3	55	94.2 ± 3.6 <sup>a</sup>	97.0 ± 3.7 <sup>a</sup>	101.0 ± 3.7 <sup>a</sup>	100.5 ± 3.8 <sup>a</sup>	88.2 ± 3.3 <sup>a</sup>
<b>R<sup>2</sup></b>		0.303	0.306	0.312	0.313	0.307

a,b,c: Aynı harfi taşıyan alt grup ortalamaları arasındaki farklılık önemsiz, farklı harf taşıyanlar önemlidir ( $p<.05$ ).

a,b,c: The difference between subgroup averages with the same letter is insignificant, those with different letters are important ( $p<.05$ ).

Kontrol sağlımlarında en yüksek günlük süt miktarına 2., 3. ve 4. kontrollerde 3 yaş grubu koyunlar sahip olurken, bu yaş grubu 5. kontrolde 2 yaş gruplarının hemen ardından yer almıştır. 1. kontrolde ise 7 yaş grubu en yüksek verime sahip olmuştur. En düşük günlük süt verimini ise 1. ve 2. kontrollerde 2 yaş grubunda, diğer kontrollerde ise 6 yaş grubunda gerçekleşmiştir. Kontrol günü verimleri bakımından işletmeler arası varyasyon 5. kontrolde önemli ( $p=.011$ ), diğer kontrollerde ise çok önemlidir ( $p=.01$ ). Tespit edilen en düşük ve en yüksek süt verimleri arası farklılık işletme 1'de kontrol günü sırasına göre 695, 695, 522, 284, 230 g; işletme 2'de 1220, 1204, 616, 510, 420 g; işletme 3'te 714, 485, 661, 309, 440 g olarak hesaplanmıştır. Belirlenen bu değerlerin işletmelere göre ortalamaları olan 485, 794 ve 522 g değerleri bakım, besleme ve idare farklılıklarını yansıtan işletmeler arası farklılıkları gösterdiği kadar özellikle işletme 2'nin kendi içinde işletmeler arası farklılıktan daha büyük bir varyasyonu barındırdığına işaret etmektedir.

### Günlük ortalama süt verimi

Şavak Akkaraman koyunlarının her iki hesaplama yoluyla elde edilen GOSV'lerine ait en küçük kareler ortalamaları, standart hataları, önemlilik ve çoklu karşılaştırma testi sonuçları Çizelge 4'de sunulmuştur.

İncelenen sürülerde Şavak Akkaraman koyunlarında iki farklı şekilde hesaplanan Günlük Ortalama Süt Verimi (GOSV) sırasıyla 565.8±1.5 ve 616.5±24.5 g olarak belirlenmiştir. En düşük ve en yüksek değerler aynı sıraya göre 258-1028 g ve 263-1153 g'dır. GOSV2'nin daha yüksek olarak hesaplanmış olması tüm sağım döneminin ve özellikle süt veriminin daha yüksek olduğu ilk kontrol öncesi dönemin dikkate alınmış olmasından kaynaklanmaktadır. GOSV'nin varyasyonunda koyunun yaşı önemli bir etkiye sahip değilken doğum tipi ve işletme faktörleri çok önemli ( $p<.001$ ) düzeyde varyasyona sebep olmuştur.



Çizelge 3. Şavak Akkaraman koyunlarının kontrol sağımalarında elde edilen süt miktarlarına ait ortalamalar, standart hatalar, önemlilik ve çoklu karşılaştırma testi sonuçları.

Table 3. Means, standard errors, significance and multiple comparison test results of the milk amounts obtained during control milking of Şavak Akkaraman ewes.

Kontrol Günü Süt Verimleri (KGSV) (g) (Control Days Milk Yields) (CDMY)						
	N	KG 1 $\bar{X} \pm S_{\bar{x}}$	KG 2 $\bar{X} \pm S_{\bar{x}}$	KG 3 $\bar{X} \pm S_{\bar{x}}$	KG 4 $\bar{X} \pm S_{\bar{x}}$	KG 5 $\bar{X} \pm S_{\bar{x}}$
<b>Ortalama (Means)</b>	172	813.0 ± 38.4	753.2 ± 36.0	536.6 ± 23.7	476.1 ± 15.0	250.1 ± 16.6
<b>Yaş (Ages)</b>		(F=0.0945; <i>p</i> =0.464)	(F=1.043; <i>p</i> =0.399)	(F=1.303; <i>p</i> =0.259)	(F=1.205; <i>p</i> =0.306)	(F=0.645; <i>p</i> =0.694)
<b>2</b>	25	758.0 ± 48.5	695.4 ± 45.5	529.2 ± 30.0	460.4 ± 19.0	275.7 ± 20.9
<b>3</b>	29	823.1 ± 49.3	795.0 ± 46.2	588.6 ± 30.4	510.5 ± 19.3	270.6 ± 21.2
<b>4</b>	40	864.8 ± 48.1	792.9 ± 45.1	558.7 ± 29.7	483.0 ± 18.8	254.7 ± 20.7
<b>5</b>	49	783.4 ± 41.9	743.3 ± 39.3	525.2 ± 25.9	478.4 ± 16.4	264.8 ± 18.1
<b>6</b>	4	776.1 ± 103.6	720.3 ± 97.1	467.3 ± 64.0	443.6 ± 40.5	215.2 ± 44.6
<b>7</b>	4	877.1 ± 103.6	786.3 ± 97.1	533.3 ± 64.0	483.9 ± 40.5	227.4 ± 44.6
<b>9</b>	21	808.1 ± 61.7	739.4 ± 57.8	554.1 ± 38.1	472.5 ± 24.1	242.0 ± 26.6
<b>Doğum Şekli (Birth type)</b>		(F=16.574; <i>p</i> <.001)	(F=11.127; <i>p</i> =0.001)	(F=2.983; <i>p</i> =0.086)	(F=6.742; <i>p</i> =0.010)	(F=2.527; <i>p</i> =0.114)
<b>Tek (Single)</b>	162	681.0 ± 22.8 <sup>b</sup>	651.9 ± 21.4 <sup>b</sup>	502.0 ± 14.1 <sup>b</sup>	443.1 ± 8.9 <sup>b</sup>	227.9 ± 9.8
<b>İkiz (Twin)</b>	10	944.9 ± 67.3 <sup>a</sup>	854.5 ± 63.1 <sup>a</sup>	571.2 ± 41.6 <sup>a</sup>	509.0 ± 26.8 <sup>a</sup>	272.3 ± 29.0
<b>İşletme (Farms)</b>		(F=8.083; <i>p</i> <.001)	(F=6.528; <i>p</i> =0.002)	(F=35.891; <i>p</i> <.001)	(F=41.175; <i>p</i> <.001)	(F=4.649; <i>p</i> =0.011)
<b>1</b>	57	682.7 ± 50.8 <sup>c</sup>	643.0 ± 47.6 <sup>b</sup>	485.6 ± 31.4 <sup>b</sup>	436.1 ± 19.9 <sup>b</sup>	216.2 ± 21.9 <sup>b</sup>
<b>2</b>	60	913.9 ± 53.2 <sup>a</sup>	819.7 ± 49.8 <sup>a</sup>	463.1 ± 32.8 <sup>b</sup>	431.0 ± 20.8 <sup>b</sup>	255.4 ± 22.9 <sup>a</sup>
<b>3</b>	55	842.3 ± 40.4 <sup>b</sup>	797.0 ± 37.9 <sup>a</sup>	661.2 ± 25.0 <sup>a</sup>	561.1 ± 15.8 <sup>a</sup>	278.6 ± 17.4 <sup>a</sup>
<b>R<sup>2</sup></b>		0.292	0.271	0.419	0.465	0.158

a,b,c: Aynı harfi taşıyan alt grup ortalamaları arasındaki farklılık önemsiz, farklı harf taşıyanlar önemlidir (*p*<.05).

a,b,c: The difference between subgroup averages with the same letter is insignificant, those with different letters are important (*p*<.05).

Her iki GOSV değerinin yaşlara göre seyri, paralel şekilde, 3 yaşına kadar artış, 3 ve 4 yaşlarında kısmen sabitleme 5 ve 6 yaşlarında azalma şeklinde beklentilere kısmen uygun bir seyir izlemiştir. İkiz doğuran koyunların GOSV'leri tekiz doğuranlardan daha yüksektir (*p*<.05). Bu üstünlük GOSV1'de 129 g, GOSV2'de ise 155 g olarak tespit edilmiştir.

İşletmeler arasında GOSV bakımından önemli farklılıklar görülmüştür. GOSV bakımından en yüksek ve en düşük değere sahip işletmeler arasındaki farklılık her iki hesaplama şekline göre sırasıyla 136 ve 141 g olarak gerçekleşmiştir (*p*<.05). İşletme 1, 2 ve 3'te en düşük ve en yüksek değerler GOSV1 için sırasıyla 257.8-645.4 g, 319.2-1027.6 g ve 416-768 g; GOSV2 için ise sırasıyla 262.9-702.3 g, 348.4-1150.2 g ve 410.5-819.7 g olarak belirlenmiştir. GOSV1 ve GOSV2 bakımından işletme 2'nin en düşük-en yüksek farklılığının (sırasıyla 708.4 ve 801.8 g) diğer iki işletmedeki farklılığın (sırasıyla işletme 1 için 387.6 ve 352 g; işletme 3 için 439.4 ve 409.2 g) neredeyse iki katı olması işletmeler arası varyasyondan ziyade işletme içi varyasyonun ön plana çıktığını göstermektedir.

### Meme özellikleri

Şavak Akkaraman koyunlarının ölçümleri yapılan meme özelliklerine etkili faktörlerin alt seviyelerine ait en küçük kareler ortalamaları ve standart hataları Çizelge 5'te verilmiştir. Çizelgelerde ayrıca varyans analizi ve önemlilik testi sonuçları da özetlenmiştir.

İncelenen sürülerde meme özelliklerini belirlemek amacıyla 3 kez ölçüm ve bu ölçümlerle eş zamanlı olarak, meme ölçümleri ile süt verimi arasındaki ilişkileri belirlemek amacıyla günlük süt verimi kontrolleri yapılmıştır. İlk ölçüm esnasında, yetiştiriciler koyunları sağmayıp kuzuları emiştirmeye bıraktığından, kontrol sağımı yapılmamıştır. Dolayısıyla ilk ölçüm tarihinde Günlük Süt Verimi (GSV) tespiti yapılmamıştır. Bundan sonraki 2 ölçüm tarihinde yapılan süt denetimlerinden; yavla dönemine denk gelen kontrol sağımında, sütten kesim dönemine denk gelen kontrol sağımına göre yaklaşık 80 g daha yüksek günlük süt verimi tespit edilmiştir. Buna göre; 3. ölçümün yapıldığı esnada koyunların laktasyonun pik döneminde olduğu ifade edilebilir. Bununla birlikte,

yaylaların besleme kapasitesi ve süt verimine olumlu etkisi de gözden uzak tutulmamalıdır. Nitekim, Şavak koyunlarının yaz döneminde götürüldüğü yaylalar arasında süt ve dolayısıyla tulum peyniri üretim

kapasiteleri bakımından farklılıklar olduğuna ilişkin gözlemler bulunmaktadır. Belirli yaylaların olduğu bölgelerden daha çok ve daha lezzetli tulum peynirleri geldiğine dair yaygın bir inanış vardır.

Çizelge 4. Şavak Akkaraman koyunlarının Günlük Ortalama Süt Verimlerine ait ortalama değerler, standart hataları, önemlilik ve çoklu karşılaştırma testi sonuçları.

Table 4. Mean values, standard errors, significance and multiple comparison test results of Daily Average Milk Yield of Şavak Akkaraman ewes.

Günlük Ortalama Süt Verimi (GOSV) (g) (Daily Average Milk Yield (DAM)) (g)			
	n	GOSV 1 (DAM 1) $\bar{X} \pm S_{\bar{x}}$	GOSV 2 (DAM2) $\bar{X} \pm S_{\bar{x}}$
<b>Ortalama (Means)</b>	172	565.8 ± 1.5	616.5 ± 24.5
<b>Yaş (Ages)</b>		(F=0.999; p=0.428)	(F=0.993; p=0.432)
2	25	543.7 ± 27.1	586.9 ± 31.0
3	29	587.5 ± 27.5	645.6 ± 31.4
4	40	590.8 ± 26.9	646.9 ± 30.7
5	49	559.0 ± 23.4	605.4 ± 26.7
6	4	524.5 ± 57.9	575.9 ± 66.1
7	4	581.6 ± 57.9	641.1 ± 66.1
9	21	563.2 ± 34.5	613.4 ± 39.4
<b>Doğum Şekli (Birth types)</b>		(F=12.726; p<.001)	(F=13.951; p<.001)
Tek (Single)	162	501.2 ± 12.7 <sup>b</sup>	539.2 ± 14.6 <sup>b</sup>
İkiz (Twin)	10	630.4 ± 37.6 <sup>a</sup>	693.7 ± 43.0 <sup>a</sup>
<b>İşletme (Farms)</b>		(F=13.070; p<.001)	(F=10.446; p<.001)
1	57	492.4 ± 28.4 <sup>c</sup>	532.2 ± 32.4 <sup>b</sup>
2	60	576.6 ± 29.7 <sup>b</sup>	643.6 ± 33.9 <sup>a</sup>
3	55	628.0 ± 22.6 <sup>a</sup>	673.5 ± 25.8 <sup>a</sup>
<b>R<sup>2</sup></b>		0.321	0.306

a,b,c: Aynı harfi taşıyan alt grup ortalamaları arasındaki farklılık önemsiz, farklı harf taşıyanlar önemlidir (p<.05).

a,b,c: The difference between subgroup averages with the same letter is insignificant, those with different letters are important (p<.05).

Bu anlamda, Şavak Akkaraman koyunlarında meme ölçümlerinde tespit edilen meme özelliklerine ait en küçük kareler ortalama değerleri; MD için 15.8±0.2, MG için 13.3±0.2, MÇ için 40.3±0.5, SağMBU için 2.9±0.6, SolMBU için 2.8±0.1, SağMBC için 2.1±0.0, SolMBC için 2.0±0.0, MBAM için 15.7±0.2, MBY için 28.0±0.4, MAY için 27.9±0.4 cm ve GSV ise 0.829±0.037 kg olarak tespit edilmiştir.

Çizelgede görüldüğü üzere, meme özelliklerine etkili olduğu varsayılan faktörlerden koyunun yaşı SolMBU ve SağMBC ölçüleri üzerine etkisi önemsiz, MÇ ve SağMBU ölçüleri üzerine etkisi önemli (p=0.038 ve 0.040) bulunurken, diğer bütün özelliklerde çok önemli (p=0.008) seviyede etkilidir. Doğum tipi ise SolMBC (p=0.018), SağMBU, SolMBU ve SağMBC (p=0.006) dışındaki ölçümlere etkisi önemsiz bulunmuştur. SolMBU (p=0.034) ve SolMBC (p=0.023) özellikleri dışında bütün özelliklerde işletmeler arası farklılığın istatistik anlamda çok önemli (p=0.001) düzeyde varyasyona sebep olduğu anlaşılmaktadır. Bir varyasyon kaynağı olarak işletmeler karşılaştırıldığında işletmelerin birbirinden farklı (p<.05) 2 veya 3 grup oluşturdukları görülmektedir. Özelliklerin çoğunda 2 nolu işletme en yüksek

değerlere sahip olmuştur.

Meme özelliklerinin laktasyon boyunca değişimini izlemek üzere farklı zamanlarda yapılan ölçümlerde, MBAM (p=0.441) ve MBY (p=0.907) özellikleri hariç, diğer tüm özelliklerin önemli (p=0.018-0.050) veya çok önemli düzeyde (p=0.001) değiştiği gözlenmiştir.

#### Meme özellikleri arası ilişkiler

Meme özellikleriyle ilgili olarak laktasyonun 3 farklı döneminde gerçekleştirilen 10 farklı meme özelliğine ait ölçüm değerlerinin hem birbirleriyle hem de Günlük Süt Verimi (GSV) ile ilişkilerini incelemek üzere korelasyon analizi yapılmıştır. Bu analizde ayrıca meme ölçümlerinin birbirleriyle ve GSV ile ilişkileri yanında ölçüm zamanlarıyla olan korelasyonları da incelemek amaçlanmıştır. Meme ölçüleri arası ilişkilere ilişkin korelasyon analizi sonucunda hesaplanan Pearson Korelasyon katsayıları ve önemlilik seviyeleri Çizelge 6'de verilmiştir.

Çizelge 5. Şavak Akkaraman koyunlarında laktasyonun farklı dönemlerinde ölçülen meme özelliklerine (cm) ve günlük süt verimine (kg) etkili faktörlerin alt gruplarına ait en küçük kareler ortalamaları, standart hataları, varyans analizi ve çoklu karşılaştırma testi sonuçları

Table 5. Least squares means, standard errors, analysis of variance and multiple comparison test results of the subgroups of factors affecting daily milk yield (kg) and the udder characteristics (cm) measured in different periods of lactation in Şavak Akkaraman ewes

	n	MD (UD)	MG (UW)	MÇ (UC)	Sağ MBU (RTL)	Sol MBU (LTL)	Sağ MBC (RTD)	Sol MBC (LTD)	MBAM (DBT)	MBY (TH)	MAY (UBH)	n	Günlük Süt Verimi (DMY)
		$\bar{x} \pm S_{\bar{x}}$	$\bar{x} \pm S_{\bar{x}}$	$\bar{x} \pm S_{\bar{x}}$	$\bar{x} \pm S_{\bar{x}}$	$\bar{x} \pm S_{\bar{x}}$	$\bar{x} \pm S_{\bar{x}}$	$\bar{x} \pm S_{\bar{x}}$	$\bar{x} \pm S_{\bar{x}}$	$\bar{x} \pm S_{\bar{x}}$	$\bar{x} \pm S_{\bar{x}}$		$\bar{x} \pm S_{\bar{x}}$
<b>Ortalama (Means)</b>	513	15.8±0.2	13.3±0.2	40.3±0.5	2.9±0.6	2.8±0.1	2.1±0.1	2.04±0.04	15.7±0.2	28.0±0.4	27.9±0.4		0.829±0.037
<b>Ölçüm (Measure)</b>		(F=3.582; p=0.029)	(F=6.980; p=0.001)	(F=55.966; p<.001)	(F=7.019; p=0.001)	(F=8.115; p<.001)	(F=3.551; p=0.029)	(F=3.953; p=0.020)	(F=0.819; p=0.441)	(F=0.098; p=0.907)	(F=5.331; p=0.05)		(F=5.717; p=0.018)
<b>1</b>	171	15.5±0.3 <sup>b</sup>	13.5±0.2 <sup>a</sup>	42.7±0.6 <sup>a</sup>	2.9±0.1 <sup>a</sup>	2.8±0.1 <sup>a</sup>	2.07±0.04 <sup>b</sup>	2.02±0.04 <sup>b</sup>	15.9±0.2	27.9±0.4	27.2±0.4 <sup>b</sup>	-	-
<b>2</b>	171	15.8±0.3 <sup>ab</sup>	13.4±0.2 <sup>a</sup>	40.7±0.6 <sup>b</sup>	2.7±0.1 <sup>b</sup>	2.7±0.1 <sup>b</sup>	2.05±0.04 <sup>b</sup>	2.00±0.04 <sup>b</sup>	15.7±0.2	28.0±0.5	28.2±0.5 <sup>a</sup>	<b>171</b>	0.790±0.035 <sup>b</sup>
<b>3</b>	171	16.1±0.3 <sup>a</sup>	12.9±0.2 <sup>b</sup>	37.5±0.6 <sup>c</sup>	2.9±0.1 <sup>a</sup>	2.9±0.1 <sup>a</sup>	2.14±0.04 <sup>a</sup>	2.10±0.04 <sup>a</sup>	15.7±0.2	28.1±0.5	28.4±0.5 <sup>a</sup>	<b>64</b>	0.868±0.045 <sup>a</sup>
<b>Yaş (Ages)</b>		(F=12.281; p<.001)	(F=2.925; p=0.008)	(F=2.248; p=0.038)	(F=2.223; p=0.040)	(F=1.946; p=0.072)	(F=1.903; p=0.079)	(F=4.110; p<.001)	(F=7.634; p<.001)	(F=3.154; p=0.005)	(F=5.560; p<.001)		(F=2.929; p=0.009)
<b>2</b>	61	14.6±0.4 <sup>d</sup>	12.6±0.3 <sup>c</sup>	38.8±0.8 <sup>d</sup>	2.8±0.1 <sup>ab</sup>	2.8±0.1	2.05±0.05	1.96±0.06 <sup>b</sup>	15.1±0.3 <sup>c</sup>	28.7±0.6 <sup>a</sup>	29.1±0.6 <sup>a</sup>	<b>21</b>	0.686±0.054 <sup>c</sup>
<b>3</b>	76	15.1±0.3 <sup>cd</sup>	13.2±0.2 <sup>b</sup>	40.6±0.7 <sup>abc</sup>	2.7±0.1 <sup>b</sup>	2.6±0.1	2.03±0.05	1.96±0.05 <sup>b</sup>	15.9±0.2 <sup>b</sup>	28.4±0.5 <sup>ab</sup>	28.4±0.5 <sup>ab</sup>	<b>33</b>	0.908±0.049 <sup>a</sup>
<b>4</b>	113	15.5±0.3 <sup>bc</sup>	13.5±0.2 <sup>ab</sup>	41.2±0.7 <sup>ab</sup>	2.9±0.1 <sup>ab</sup>	2.8±0.1	2.10±2.04	2.07±0.05 <sup>ab</sup>	15.9±0.2 <sup>b</sup>	29.0±0.5 <sup>a</sup>	29.1±0.5 <sup>a</sup>	<b>72</b>	0.831±0.045 <sup>b</sup>
<b>5</b>	90	16.2±0.3 <sup>b</sup>	13.2±0.2 <sup>ab</sup>	39.8±0.7 <sup>bcd</sup>	2.8±0.1 <sup>b</sup>	2.7±0.1	2.04±0.05	1.99±0.05 <sup>b</sup>	15.0±0.2 <sup>c</sup>	27.9±0.5 <sup>abc</sup>	27.6±0.5 <sup>bcd</sup>	<b>37</b>	0.835±0.048 <sup>b</sup>
<b>6</b>	39	16.8±0.4 <sup>ab</sup>	13.7±0.3 <sup>a</sup>	41.7±0.9 <sup>a</sup>	2.9±0.1 <sup>b</sup>	2.8±0.1	2.08±0.07	2.03±0.07 <sup>b</sup>	16.7±0.3 <sup>a</sup>	27.3±0.7 <sup>bc</sup>	26.6±0.7 <sup>d</sup>	<b>15</b>	0.861±0.063 <sup>ab</sup>
<b>7</b>	20	15.2±0.5 <sup>cd</sup>	13.1±0.4 <sup>bc</sup>	39.5±1.2 <sup>cd</sup>	3.0±0.1 <sup>a</sup>	2.9±0.2	2.13±0.08	2.09±0.09 <sup>ab</sup>	15.6±0.4 <sup>b</sup>	27.9±0.9 <sup>abc</sup>	28.0±0.9 <sup>abc</sup>	<b>8</b>	0.821±0.078 <sup>b</sup>
<b>8</b>	114	17.3±0.3 <sup>a</sup>	13.4±0.2 <sup>ab</sup>	40.5±0.6 <sup>abcd</sup>	3.0±0.1 <sup>a</sup>	2.9±0.1	2.18±0.05	2.18±0.05 <sup>a</sup>	15.9±0.2 <sup>b</sup>	26.9±0.5 <sup>c</sup>	26.7±0.5 <sup>cd</sup>	<b>49</b>	0.857±0.042 <sup>ab</sup>
<b>Doğum Şekli (BT)</b>		(F=0.581; p=0.446)	(F=2.397; p=0.122)	(F=0.397; p=0.529)	(F=9.124; p=0.003)	(F=7.491; p=0.006)	(F=8.844; p=0.003)	(F=5.591; p=0.018)	(F=2.639; p=0.124)	(F=0.016; p=0.899)	(F=0.397; p=0.529)		(F=1.222; p=0.291)
<b>Tek (S)</b>	489	15.6±0.1	13.0±0.1	40.0±0.3	2.7±0.0 <sup>b</sup>	2.6±0.0 <sup>b</sup>	1.98±0.02 <sup>b</sup>	1.95±0.02 <sup>b</sup>	15.5±0.1	28.0±0.2	27.7±0.2	<b>224</b>	0.796±0.022
<b>İkiz (T)</b>	24	16.0±0.5	13.5±0.3	40.6±1.0	3.1±0.1 <sup>a</sup>	3.0±0.1 <sup>a</sup>	2.19±0.07 <sup>a</sup>	2.12±0.07 <sup>a</sup>	16.0±0.3	28.0±0.7	28.2±0.7	<b>11</b>	0.861±0.064
<b>İşletme (Farms)</b>		(F=16.616; p<.001)	(F=5.521; p=0.001)	(F=12.649; p<.001)	(F=7.681; p<.001)	(F=2.905; p=0.034)	(F=7.825; p<.001)	(F=3.191; p=0.023)	(F=17.192; p<.001)	(F=15.488; p<.001)	(F=13.476; p<.001)		(F=10.873; p<.001)
<b>1</b>	162	15.3±0.3 <sup>b</sup>	13.4±0.2 <sup>a</sup>	43.0±0.7 <sup>a</sup>	2.9±0.9 <sup>b</sup>	2.8±0.1 <sup>ab</sup>	2.13±0.05 <sup>a</sup>	2.04±0.05 <sup>a</sup>	16.5±0.2 <sup>a</sup>	26.7±0.5 <sup>c</sup>	26.3±0.5 <sup>c</sup>	<b>59</b>	0.779±0.048 <sup>c</sup>
<b>2</b>	45	17.5±0.4 <sup>a</sup>	13.8±0.3 <sup>a</sup>	41.4±0.8 <sup>a</sup>	3.1±0.1 <sup>a</sup>	2.9±0.1 <sup>a</sup>	2.16±0.06 <sup>a</sup>	2.10±0.06 <sup>a</sup>	16.1±0.3 <sup>b</sup>	27.2±0.6 <sup>c</sup>	27.6±0.6 <sup>c</sup>	<b>15</b>	1.005±0.062 <sup>a</sup>
<b>3</b>	120	14.7±0.3 <sup>c</sup>	12.9±0.2 <sup>b</sup>	38.7±0.7 <sup>b</sup>	2.6±0.1 <sup>c</sup>	2.7±0.1 <sup>b</sup>	1.93±0.05 <sup>b</sup>	1.94±0.05 <sup>b</sup>	15.1±0.2 <sup>c</sup>	30.0±0.5 <sup>a</sup>	29.6±0.5 <sup>a</sup>	<b>40</b>	0.678±0.047 <sup>d</sup>
<b>4</b>	186	15.8±0.3 <sup>b</sup>	13.0±0.2 <sup>b</sup>	38.7±0.6 <sup>b</sup>	2.9±0.1 <sup>b</sup>	2.9±0.1 <sup>a</sup>	2.12±0.05 <sup>a</sup>	2.08±0.05 <sup>a</sup>	15.3±0.2 <sup>c</sup>	28.1±0.5 <sup>b</sup>	28.2±0.5 <sup>b</sup>	<b>121</b>	0.852±0.037 <sup>b</sup>
<b>R<sup>2</sup></b>		0.207	0.089	0.251	0.140	0.108	0.143	0.128	0.168	0.183	0.221		0.266

a,b,c,d: Aynı harfi taşıyan alt grup ortalamaları arasındaki farklılık önemsiz, farklı harf taşıyanlar önemlidir (p<.05). MD: Meme Derinliği, MG: Meme Genişliği, MÇ: Meme Çapı, MBU: Meme Baş Uzunluğu, MBC: Meme Baş Çapı, MBAM: Meme Başları Mesafe, MBY: Meme Baş Yüksekliği, MAY: Meme Alt Yüksekliği, a,b,c,d: The difference between subgroup averages with the same letter is insignificant, those with different letters are important (p<.05). UD: Udder Depth, UW: Udder Width, UC: Udder Circumference, RTL: Right Teat Length, LTL: Left Teat Length, RTD: Right Teat Diameter, LTD: Left Teat Diameter, DBT: Distance Between Teats, TH: Teat Height, UBH: Udder Bottom Height, DMY: Daily Milk Yield

Çizelge 6. Şavak Akkaraman koyunlarının bazı meme ölçüleri, ölçüm zamanı ve günlük süt verimleri arası korelasyon katsayıları ve önem seviyeleri  
 Table 6. Correlation coefficients and significance levels between some udder measurements, measurement time and daily milk yield of Şavak Akkaraman sheep

		MD (UD)	MÇ (UC)	MG (UW)	SağMBU (RTL)	Sol MBU (LTL)	Sağ MBÇ (RTD)	Sol MBÇ (LTD)	MBAM (DBT)	MBY (TH)	MAY (UBH)	GSV (DMY)
MÇ (UC)	Katsayı	.373**										
	Önem	<0.001										
MG (UW)	Katsayı	.384**	.675**									
	Önem	<0.001	<0.001									
Sağ MBU (RTL)	Katsayı	.260**	.265**	.258**								
	Önem	<0.001	<0.001	<0.001								
Sol MBU (LTL)	Katsayı	.245**	.221**	.274**	.867**							
	Önem	<0.001	<0.001	<0.001	<0.001							
Sağ MBÇ (RTD)	Katsayı	.367**	.285**	.304**	.796**	.765**						
	Önem	<0.001	<0.001	<0.001	<0.001	<0.001						
Sol MBÇ (LTD)	Katsayı	.394**	.283**	.297**	.702**	.795**	.852**					
	Önem	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001					
MBAM (DBT)	Katsayı	.468**	.618**	.577**	.420**	.410**	.459**	.441**				
	Önem	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001				
MBY (TH)	Katsayı	-.578**	-.344**	-.340**	-.294**	-.260**	-.398**	-.372**	-.327**			
	Önem	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001			
MAY (UBH)	Katsayı	-.540**	-.333**	-.281**	-.137**	-.104*	-.256**	-.211**	-.315**	.828**		
	Önem	<0.001	<0.001	<0.001	.002	.019	<0.001	<0.001	<0.001	<0.001	<0.001	
GSV (DMY)	Katsayı	.365**	.404**	.461**	.353**	.360**	.416**	.363**	.480**	-.214**	-.178**	
	Önem	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	.001	.006	
Ölçümler (1,2,3) (M)	Katsayı	.106*	-.406**	-.144**	-.018	.046	.084	.081	-.049	.016	.121**	.274**
	Önem	.016	<0.001	.001	.689	.293	.058	.067	.263	.724	.006	<0.001

İncelenen meme özelliklerinden MD, MÇ, MG, SağMBU, SolMBU, SağMBÇ, SolMBÇ, MBAM, MBY ve MAY ölçüleri arası korelasyonların tamamı çok önemli ( $p<.001$ ) bulunmuştur. Bu korelasyonlardan sadece MAY-SolMBU arası korelasyon önemlidir ( $p<.019$ ). Bu korelasyon katsayılarından MBY ve MAY ile MD, MÇ, MG, SağMBU, SolMBU, SağMBÇ, SolMBÇ ve MBAM arasındaki korelasyonlar negatif, diğer meme ölçüleri arası korelasyonlar pozitifdir. Sağ ve SolMBU ve MBÇ arasında belirlenen yüksek pozitif korelasyon katsayıları (0.702 ile 0.867 arası) beklentiler yönündedir ve meme lobları arasındaki simetriyi göstermesi bakımından önemlidir. Aynı şekilde MG ile MÇ arasındaki yüksek korelasyon katsayısı (0.675) da beklentilere uygundur. Benzer şekilde, MBAM ile MD, Sağ ve Sol MBU ve MBÇ arasındaki korelasyon katsayıları da kısmen yüksek ve pozitifdir (0.410 ile 0.468 arasında). MBAM ile en yüksek korelasyon MÇ ve MG arasında tespit edilmiştir.

Meme derinliği, çevresi, genişliği ve meme başlarına ilişkin ölçülerin artması meme büyüklüğünün arttığının göstergesidir. Bu korelasyon katsayılarından MD ile MBY ve MAY arasındakiler (sırasıyla -0.578 ve -0.540) negatif ve yüksek olması ile diğerlerinden belirgin biçimde ayrılmaktadır. MBY ile MAY arasındaki korelasyon katsayısının oldukça yüksek ve pozitif çıkması (0.828) memenin orantılı biçimde büyüdüğü ve yere yaklaştığı görüşünü desteklemektedir.

Meme ölçülerinin GSV ile olan ilişkileri incelendiğinde; genel olarak orta seviyede olduğu söylenebilir. İlişkiler çok önemli ( $p=0.006$ ) bulunmuş olmakla birlikte, ilişki katsayıları 0.5 seviyesinin altında hesaplanmıştır. GSV ile MBAM ve MG arasında hesaplanan korelasyon katsayıları (sırasıyla 0.480 ve 0.461) diğerlerinden daha yüksek ve bir ölçüde daha anlamlıdır. Zira, meme dokusunda süt salgısı arttıkça memelerin daha dolgun ve daha geniş olması ve buna bağlı olarak meme başları arası mesafenin açılması beklenebilir. Benzer şekilde, katsayılar kısmen küçük ve negatif olmakla birlikte GSV ile MBY ve MAY arası ilişkiler (sırasıyla -0.214 ve -0.178) günlük süt verimi yüksek hayvanların diğerlerine göre memelerinin büyük olmaları sebebiyle yere daha yakın olduğu tezini desteklemektedir.

Meme ölçülerinin laktasyonun farklı zamanlarındaki değişimlerini belirlemek üzere, ölçüm zamanı ile meme ölçüleri arasında hesaplanan korelasyon katsayılarının düşük ve önemsiz olduğu tespit edilmiştir. Ölçüm zamanının Sağ ve Sol MBU ve MBÇ, MBAM ve MBY ile olan ilişkileri önemsiz çıkmıştır. Ölçüm zamanı ile MÇ, MG, MAY ve GSV arasındaki ilişkiler çok önemli ( $p=0.006$ ), MD arasında olan ilişki ise önemlidir ( $p=0.016$ ). Bu korelasyon katsayılarından ölçüm zamanı- MÇ arasında olan hariç (-0.406) diğerleri oldukça küçüktür.



## TARTIŞMA

### Süt Verim Özellikleri

Bu çalışmada, Şavak Akkaraman koyunlarında kontrol sağımlarından Trapez II (ICAR) yöntemiyle hesaplanan kuzunun emdiği süt miktarı hariç toplam süt verimi ( $88.8 \pm 3.5$  kg); Akçapınar ve ark., (1982), Odabaşoğlu (1983), Küçük (1995), Yardımcı (2001), Altın (2001), Denk (2003) ve Mundan (2003)'ün Akkaraman koyunu üzerine yaptıkları çalışmalarda bildirdikleri değerlerden (sırasıyla 50.5 kg, 73.6 kg, 57 kg, 51.8 kg, 62,3 kg, 39.73 kg ve 43.1 kg), Yüceer ve ark., (2015)'nin Sakız x Akkaraman G1 koyunu (82.28 kg ve 68.79 kg), Koyuncu & Altınçekiç (2011)'in Tahirova (51.91 kg), Kıvırcık (65.57 kg) ve Karacabey Merinosu koyunu (39.83 kg) üzerindeki çalışmalarda bildirdikleri değerlerden yüksek bulunmuştur.

Karakaş koyunu üzerinde yapılan çalışmalarda Gökdal ve ark., (2002)'nin bildirdiği (54.8 kg) değerler yüksek, Karaca ve ark., (2003)'nin bildirdiği değerlerden (103.1 kg) ise düşük bulunmuştur.

Türkiye'de yetiştirilen diğer koyun ırklarından Morkaraman koyunu üzerine yapılan çalışmalardan, Akçapınar ve ark., (1982), Odabaşoğlu (1983), Macit (1994), Özbeyaz & Akcan (2000) ve Kırmızıbayrak ve ark., (2005) tarafından bildirilen değerler (77.6 ile 96.4 kg aralığında) bu çalışmada elde edilen laktasyon süt verimi değerlerine benzerlik göstermektedir.

Aynı zamanda, bu çalışmada elde edilen laktasyon süt verimi, Özbaşer (2010)'ün Acıpayam koyunu (170.5 kg), Bingöl ve ark., (2008)'nin Norduz koyunu (132.8 kg), Özbeyaz & Akcan (2000), Mckusick ve ark., (1999) ve Reiad ve ark., (2010) ve Kaygısız ve Dağ (2017)'in İvesi koyunu üzerinde yaptıkları çalışmalarda bildirdikleri (109.9 kg ile 248.3 kg arasında) değerlerden düşük belirlenmiştir.

Bu çalışmada Şavak Akkaraman koyunlarının laktasyon süresi  $205.8 \pm 2.0$  gün değeri; diğer Akkaraman koyunlarında yapılan çalışmalardan Aktaş (1970), Odabaşoğlu (1983), Yardımcı (2001), Mundan (2003), Küçük (1995), Akçapınar ve ark., (1982), Denk (2003) ve Altın (2001) bildirdikleri değerlerden (sırasıyla 144.8, 146.9, 148, 155.8, 155.8, 130.3, 122 ve 173 gün) yüksek bulunmuştur..

Çalışmada bulunan laktasyon süresi Kangal Akkaramanın koyunlarında yapılan çalışmada bildirilenden (166.9 gün) yüksek bulunmuştur (Altıoğlu, 2007). Van yöresinde yetiştirilen Karakaşlarda ise laktasyon süresi de (177-197.8 gün) düşük bulunmuştur. (Gökdal ve ark., 2002; Karaca ve ark., 2003).

Morkaraman koyunlarında tespit edilen laktasyon süresi (137-188.8 gün) bu çalışmada tespit edilenden düşük olarak belirlenmiştir (Akçapınar ve ark., 1982, 1984; Odabaşoğlu 1983, 1985; Macit, 1994; Özbeyaz ve Akcan 2000; Kırmızıbayrak ve ark., 2005).

Laktasyon süresi, İvesilerde 169.4 gün (Özbeyaz &

Akcan, 2000) ve 173.8 gün (Kaygısız ve Dağ, 2017) olarak verilmiştir. Bu araştırma bulgusu, Norduz koyunlarında 183.4 gün (Bingöl ve ark., 2008), Kıvırcık koyunlarında ise 65.6 gün (Koyuncu & Altınçekiç, 2011) bildirilenlerden yüksek, 207.3 gün (Yılmaz & Altın, 2004) olarak bildirilenden ise düşük belirlenmiştir.

Bu çalışmada tespit edilen Şavak Akkaraman koyununun günlük ortalama süt verimi Trapez II ile hesaplanan toplam süt miktarının sağım süresine bölünmesiyle elde edilen değerdir (GOSV2). Buna göre günlük ortalama süt verimi  $616.5 \pm 24.5$  g olarak belirlenmiştir.

Söz konusu bu değer; Akkaraman koyunlarında günlük ortalama süt verimi olarak 282 g (Mundan, 2003) 334 g (Denk, 2003), 353 g (Yardımcı, 2001), 360 g (Küçük, 1995; Altın, 2001), 388 g (Akçapınar ve ark., 1982), 501 g (Odabaşoğlu, 1983), 567 g (Dağ, 2000) dan yüksek, 1090 g (Aktaş, 1970) değerinden düşük olarak tespit edilmiştir.

Günlük ortalama süt verimi Akkaraman koyun ırkının varyetelerinden Karakaş koyunlarında 277 g (Gökdal ve ark, 2002) olarak tespit edilenden yüksek, Kangal Akkaraman koyunlarında tespit edilen 906 g (Altıoğlu, 2007) değerinden düşük bulunmuştur.

Süt verim özellikleri üzerine etkileri araştırılan çevre faktörlerinden özellikle koyunun yaşının genel olarak önemsiz çıkması işletmeler arası ve işletme içi varyasyonun yaş grupları arası varyasyondan çok daha fazla olması ve yaşlar arası farklılığı gölgelemesi ile açıklanabilir. Nitekim, Trapez II ile hesaplanan süt veriminde yaş grupları arası farklılık 11 kg iken, işletmeler arası farklılık 20 kg'a; GOSV1 için bu farklılık sırasıyla 66 ve 136 g'a; GOSV2 için sırasıyla 71 ve 142 g'a; kontrol grubu süt verimleri için ise sırasıyla 119 ve 231 g, 98 ve 176 g, 121 ve 198 g, 67 ve 130 g, 60 ve 62 g'a ulaşmaktadır. Bu alt grup ortalamalarını ortaya koyan alt grup içi değerler incelendiğinde grup içi farklılıkların da işletmelerde yaş gruplarından daha fazla olduğu görülmektedir. Doğum tipinin süt verimi özellikleri üzerine etkilerinin beklenen yönde gerçekleştiği söylenebilir. İkiz doğuran anaların laktasyon ve emiştirme sürelerinin 10 gün daha kısa olması, yavrularını daha iyi besleyebilmek için ilk dönemlerde daha fazla süt vermeleriyle ilişkili olabilir. Nitekim, ikiz doğuran analar lehine olan üstünlüğün Trapez II toplam süt veriminde 22 kg, GOSV1 ve 2'de sırasıyla 129 ve 154 g kadar olduğu görülmektedir. Doğum tipinden kaynaklanan farklılığın zaman içinde ortadan kalktığı bilgisini destekleyen bulgu ise kontrol günü süt verimlerinde izlenmektedir ve tek-ikiz farkı kontrol günü sırasıyla 264, 203, 69, 66 ve 44 g olarak tespit edilmiştir. Benzer bulgular başka araştırmalarda da ortaya konmuştur (Yüceer ve ark., 2015; Kaygısız ve Dağ, 2017).

### Meme Özellikleri

Türkiye'nin farklı yerlerinde meme özelliklerine ilişkin yapılan çalışmalardan; laktasyonun 60., 90. ve 120. günlerindeki ölçümlerde meme derinliği ölçüsü olarak Akkaraman koyunlarında Odabaşoğlu ve ark. (1985) 14, 11.7 ve 11.8 cm; Küçük (1995) 13.2, 12.9 ve 11.6 cm; Yardımcı (2001) 12.8, 12.3 ve 11.8 cm; Mundan (2003) 13.2, 12.6 ve 11.1 cm; Acıpayam koyunlarında Özbaşer (2010) 13.1, 9.8 ve 8.1 cm değerlerini vermişlerdir. Mundan ve Özbeyaz (2004) 60., 90. ve 150. günlerde yaptıkları ölçümlerde bu değerleri Akkaramanlarda 13.2, 12.3 ve 10.3 cm, Kıvırcık x Akkaraman melezlerinde 12.8, 11.9 ve 9.4 cm, Sakız x Akkaraman melezlerinde 15.2, 13.6 ve 11.4 cm olarak belirlemişlerdir. Ünal ve ark. (2008) Bafra koyunlarında 42., 70. ve 98. günlerde farklı yaş gruplarında yaptıkları ölçümlerden 17.1-17.9, 15.6-16.6 ve 13.6-14.2 cm değerlerini tespit etmişlerdir. Koyuncu ve Altınçekiç (2011) ise laktasyonun başında ve sonunda yaptıkları ölçümlerden oldukça farklı sonuçlar elde etmişler ve bu değerleri Tahirova için 9.0-5.5 cm, Kıvırcık için 9.4-5.3 cm ve Konya Merinosu için 9.6-5.1 cm olarak bulmuşlardır. Bu değer Karya koyunlarında 15.8 cm (Karaca ve ark., 2009), Morkaraman, Tuj ve İvesi koyunlarında 12.8, 12.9 ve 14.1 cm (Özyürek ve ark., 2015) olarak tespit edilmiştir.

Bu anlamda, yapılan araştırmada ortalama meme derinliği (MD) ölçüsü, Odabaşoğlu (1983), Küçük (1995), Yardımcı (2001), Mundan (2003), Mundan & Özbeyaz (2004), Karaca ve ark., (2009), Özbaşer (2010), Koyuncu & Altınçekiç (2011), ve Özyürek ve ark., (2015)'nin bulunduğu değerlerden yüksek, Ünal ve ark., (2008)'nin bulunduğu değerlerden düşük bulunmuştur.

Laktasyonun 60., 90. ve 120. günlerindeki ölçümlerde meme genişliği ölçüsü olarak Akkaraman koyunlarında Odabaşoğlu ve ark. (1985) 13.4, 11.6 ve 11.6 cm; Küçük (1995) 13.7, 13.2 ve 11.9 cm; Yardımcı (2001) 13.4, 13.2 ve 12.4 cm; Mundan (2003) 14.2, 13.5 ve 11.8 cm; Acıpayam koyunlarında Özbaşer (2010) 14.4, 11.4 ve 10.4 cm; Sakız x Akkaraman G1 koyunlarının iki farklı yaş grubunda Yüceer ve ark. (2015) 13.4-13.7, 14.6-15.4 ve 11.6-12.5 cm değerlerini vermişlerdir. Mundan & Özbeyaz (2004) 60., 90. ve 150. günlerde yaptıkları ölçümlerde bu değerleri Akkaramanlarda 14.0, 13.5 ve 11.2 cm, Kıvırcık x Akkaraman melezlerinde 13.5, 12.9 ve 11.3 cm, Sakız x Akkaraman melezlerinde 15.1, 14.3 ve 12.7 cm olarak belirlemişlerdir. Ünal ve ark. (2008) Bafra koyunlarında 42., 70. ve 98. günlerde farklı yaş gruplarında yaptıkları ölçümlerden 15.2-15.6, 14.1-14.5 ve 10.4-10.6 cm değerlerini tespit etmişlerdir. Koyuncu & Altınçekiç (2011) ise laktasyonun başında ve sonunda yaptıkları ölçümlerden oldukça farklı sonuçlar elde etmişler ve bu değerleri Tahirova için 17.3-9.3 cm, Kıvırcık için 16.3-8.5 cm ve Konya

Merinosu için 14.9-9.7 cm olarak bulmuşlardır. Kıvırcık koyunlarından laktasyon boyunca dokuz dönemde alınan ölçülerde başlangıçta 21 cm civarında olan meme genişliğinin 17 cm'ye kadar düştüğü belirlenmiştir (Akgün ve Koyuncu, 2021). Bu değer Karya koyunlarında 12.6 cm (Karaca ve ark., 2009), Morkaraman, Tuj ve İvesi koyunlarında 13.4, 12.4 ve 13.1 cm (Özyürek ve ark., 2015) olarak tespit edilmiştir.

Çalışmada ortalama meme genişliği (MG) ölçüsü, Karaca ve ark., (2009), Özbaşer (2010), Özyürek ve ark., (2015), Yüceer ve ark., (2015)'nin bulunduğu değerlerden yüksek, Mundan ve Özbeyaz (2004), Ünal ve ark. (2008)'nin bulunduğu değerlerden düşük, Odabaşoğlu (1983), Küçük (1995), Yardımcı (2001), Mundan (2003)'nin bulunduğu değerlere benzerlik göstermektedir.

Laktasyonun 60., 90. ve 120. günlerindeki ölçümlerde meme çevresi ölçüsü olarak Akkaraman koyunlarında Odabaşoğlu ve ark. (1985) 45.8, 40.2 ve 42.4 cm; Küçük (1995) 42.1, 41.0 ve 36.8 cm; Yardımcı (2001) 47.8, 49.1 ve 45.1 cm; Mundan (2003) 44.3, 44.5 ve 40.6 cm; Acıpayam koyunlarında Özbaşer (2010) 45.4, 40.6 ve 34.4 cm; Sakız x Akkaraman G1 koyunlarının iki farklı yaş grubunda Yüceer ve ark. (2015) 43.7-39.8, 46.3-42.1 ve 40.7-39.6 cm değerlerini vermişlerdir. Mundan ve Özbeyaz (2004) 60., 90. ve 150. günlerde yaptıkları ölçümlerde bu değerleri Akkaramanlarda 43.8, 44.4 ve 39.0 cm, Kıvırcık x Akkaraman melezlerinde 44.6, 44.9 ve 40.0 cm, Sakız x Akkaraman melezlerinde 51.0, 50.2 ve 45.1 cm olarak belirlemişlerdir. Ünal ve ark. (2008) Bafra koyunlarında 42., 70. ve 98. günlerde farklı yaş gruplarında yaptıkları ölçümlerden 47.7-48.7, 43.7-44.3 ve 34.7-36.5 cm değerlerini tespit etmişlerdir. Koyuncu & Altınçekiç (2011) ise laktasyonun başında ve sonunda yaptıkları ölçümlerden oldukça farklı sonuçlar elde etmişler ve bu değerleri Tahirova için 45.1-26.6 cm, Kıvırcık için 45.1-25.1 cm ve Konya Merinosu için 42.8-26.6 cm olarak bulmuşlardır. Kıvırcık koyunlarından laktasyon boyunca dokuz dönemde alınan ölçülerde başlangıçta 41 cm civarında olan meme çevresinin 33 cm'ye kadar düştüğü belirlenmiştir (Akgün ve Koyuncu, 2021). Bu değer Karya koyunlarında 40.0 cm (Karaca ve ark., 2009), Anadolu Merinosu için 46.0 cm (Doğan ve ark., 2013), Morkaraman, Tuj ve İvesi koyunlarında 36.3, 36.8 ve 36.3 cm (Özyürek ve ark., 2015) olarak tespit edilmiştir.

Bu çalışmada ortalama meme çevresi (MÇ) ölçüsü, Karaca ve ark., (2009) ve Özyürek ve ark., (2015)'nin bulunduğu değerlerden yüksek, Odabaşoğlu (1983), Küçük (1995), Yardımcı (2001), Mundan (2003), Mundan ve Özbeyaz (2004), Kırmızıbayrak ve ark., (2005), Ünal ve ark., (2008), Koyuncu ve ark., (2011) ve Doğan ve ark., (2013)'nin bulunduğu değerlerden düşük bulunmuştur.

Meme çevresine ait değerler laktasyonun ilk

döneminde artmış ve sonuna doğru azalmıştır. Meme çevresi ile süt verimi arasında pozitif bir ilişki mevcuttur. Bundan dolayı süt verimindeki azalışa bağlı olarak meme çevresi değerlerinde azalma meydana gelmiştir.

Laktasyonun 60., 90. ve 120. günlerindeki ölçümlerde meme başları arası mesafe ölçüsü olarak Akkaraman koyunlarında Odabaşoğlu ve ark. (1985) 16.0, 14.0 ve 14.5 cm; Küçük (1995) 16.6, 16.1 ve 14.3 cm; Yardımcı (2001) 16.6, 16.2 ve 15.7 cm; Mundan (2003) 17.6, 16.2 ve 14.8 cm; Acıpayam koyunlarında Özbaşer (2010) 19.4, 18.4 ve 16.3 cm; Sakız x Akkaraman G1 koyunlarının iki farklı yaş grubunda Yüceer ve ark. (2015) 15.4-16.2, 17.0-17.4 ve 14.9-15.1 cm değerlerini vermişlerdir. Mundan & Özbeyaz (2004) 60., 90. ve 150. günlerde yaptıkları ölçümlerde bu değerleri Akkaramanlarda 17.6, 15.9 ve 13.8 cm, Kıvrıkcık x Akkaraman melezlerinde 14.6, 13.5 ve 10.7 cm, Sakız x Akkaraman melezlerinde 16.5, 15.4 ve 12.8 cm olarak belirlemişlerdir. Ünal ve ark. (2008) Bafra koyunlarında 42., 70. ve 98. günlerde farklı yaş gruplarında yaptıkları ölçümlerden 12.6-14.0, 11.7-12.8 ve 9.2-9.8 cm değerlerini tespit etmişlerdir. Bu değer Anadolu Merinosu için 16.1 cm (Doğan ve ark., 2013), Morkaraman, Tuj ve İvesi koyunlarında 14.3, 14.4 ve 14.6 cm (Özyürek ve ark., 2015) olarak tespit edilmiştir. Kıvrıkcık koyunlarından laktasyon boyunca dokuz dönemde alınan ölçülerde meme başı uzunluğunun 2.-3.0 cm aralığında değiştiği belirlenmiştir (Akgün ve Koyuncu, 2021).

Araştırmada tespit edilen ortalama meme başları arası uzaklık (MBAM) ölçüsü, Ünal ve ark. (2008), Özyürek ve ark., (2015)'nin bulduğu değerlerden yüksek, Özbaşer (2010) ve Doğan ve ark., (2013)'nin bulduğu değerden düşük, Mundan & Özbeyaz (2004) ve Yüceer ve ark. (2015)'nin bulduğu değerlere benzerlik göstermektedir. Şavak Akkaramanların memelerinin yere yakınlığının (28.0 cm) Anadolu Merinoslarından (Doğan ve ark., 2013) daha fazla (34.3 cm) olduğu görülmektedir. Memenin yere olan mesafesine (MBY, MAY) ait değerlerde laktasyonun sonuna doğru artış gözlenmiştir. Süt veriminin azalması ile memenin yere olan mesafesi artış eğilimi göstermiştir.

## SONUÇ ve ÖNERİLER

Şavak Akkaraman koyunlarının laktasyon ve sağımda kalma sürelerinin yeterli düzeyde olduğu söylenebilir. Ancak, uzun sayılabilecek sağım süresine kıyasla süt verimlerinin tatmin edici düzeyde olduğu söylenemez. Ayrıca, kontrol günü sağımlarında tespit edilen günlük süt verimi sağımların başlangıcından itibaren sağım dönemi boyunca hızlı bir azalma göstermesi ek tedbirler alınmasının zorunlu olduğu hususlardan biri olarak görülmüştür. Toplam süt verimi bakımından işletmeler arasında 20 kg'a ulaşan farklılıkların bakım-besleme-idare farklılıklarını yansıttığı kadar,

her bir işletmenin kendi bünyesindeki hayvanlarının en düşük ve en yüksek verimleri farkının işletmeler arası farklılıktan büyük olması ve özellikle bir işletmedeki farklılığın (113,6 kg) diğer işletmelerdekini (sırasıyla 53.3 ve 63 kg) iki katı kadar olması, işletmelerin kendi içinde işletmeler arası farklılıktan daha büyük bir varyasyonu barındırdığına işaret etmekte ve işletmeler arası varyasyondan ziyade işletme içi varyasyonun ön plana çıktığını göstermektedir. Aynı durum günlük süt verimleri içinde geçerlidir. Dolayısıyla, yararlanılabilecek bir varyasyon potansiyelinden söz edilebilir.

Şavak Akkaraman koyunlarının meme ölçülerinin laktasyonun farklı dönemlerinde gözlenen önemli ölçüdeki değişikliklerin daha ziyade süt verimindeki değişimlere bağlı kaldığı söylenebilir.

Meme ölçüleri arası korelasyonların hemen tamamının çok önemli düzeyde olması ve korelasyon katsayılarından birçoğunun oldukça yüksek bulunmasına dayanarak; meme yapısının simetrik olduğu, süt veriminin yüksek olduğu laktasyon döneminde orantılı şekilde büyüdüğü ve yere yaklaştığı, süt verimi azaldıkça yine orantılı biçimde memenin küçüldüğü, pörsüdüğü söylenebilir.

Çalışma, aynı bölgede yürütülen geniş tabanlı halk elinde ıslah projelerinden bir alt projenin temsile yeterli düzeyde bir örneği üzerinde yürütüldüğünden bazı genel yargılara ulaşılabilir. Bu bağlamda elde edilen bulgular, Şavak Akkaraman koyunlarının gerek diğer Akkaramanlardan gerekse Akkaraman varyetelerinden süt verimi ve meme özellikleri bakımından farklılıklar gösterdiğini ortaya koymuştur. Türkiye'nin yerli genetik kaynağı olan bu popülasyonda ortaya çıkan varyasyondan yararlanılabilmektedir. Ayrıca, yetiştiricilerin süt verimi yönünden uyguladıkları farklı bir yetiştirme sistemi olarak; doğum sonrasında koç katım dönemine kadar sürekli sağma gayreti, popülasyonun diğer özellikler bakımından da etkilendiğini göstermektedir. Yetiştiriciler, bu sağım geleneği sayesinde hem kuzu üretiminden hem de tulum peyniri üretiminden oldukça önemli düzeyde gelir elde etmektedirler. Bu sağım alışkanlığının diğer Akkaraman ve varyeteleri yetiştiriciliği yapan yetiştiricilere yaygınlaştırılması durumunda ekonomik katkılar sağlanabilir.

## TEŞEKKÜR

Bu çalışma; birinci yazarın doktora tezinden özetlenmiştir. Bu tez; Kahramanmaraş Sütçü İmam Üniversitesi, Bilimsel Araştırma Projeleri Koordinasyon Birimi (Proje No:2014/3-30 D) ve Tarımsal Araştırmalar ve Politikalar Genel Müdürlüğü, Erzincan İli Halk Elinde Şavak Akkaraman Koyunu Islahı Projeleri (Proje Kodu: 24SAV2011-01 ve 24SAV2012-02) kapsamında desteklenmiştir.



### 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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