Citation/Atıf: Özdemir M, Yıldızlar O. Analysis of pesticides, antibiotics, and heavy metal levels in honey produced in the Bayburt and upper Çoruh valley regions of Türkiye. U. Bee J. / U. Arı D. 2025; 25 (1): 1-18 DOI:10.31467/uluaricilik.1570326

ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

ANALYSIS OF PESTICIDES, ANTIBIOTICS, AND HEAVY METAL LEVELS IN HONEY PRODUCED IN THE BAYBURT AND UPPER ÇORUH VALLEY REGIONS OF TÜRKİYE

Türkiye'nin Bayburt ve Yukarı Çoruh Vadisi Bölgelerinde Üretilen Ballarda Pestisit, Antibiyotik ve Ağır Metal Düzeylerinin Analizi

Mustafa ÖZDEMİR[1*], Osman YILDIZLAR[2]

[1*]Department of Emergency Aid and Disaster Management, Faculty of Applied Sciences, Bayburt University, Bayburt, TÜRKİYE. Corresponding author / Yazışma yazarı E-mail: mozdemir@bayburt.edu.tr, ORCID No: 0000-0002-6067-2007,

[2]Department of Institute of Health Sciences, Avrasya University, Trabzon,TÜRKİYE, E-mail: oyldzlar@hotmail.com, ORCID No: 0000-0002-5485-8702

Received / Geliş: 19.11.2024 Accepted / Kabul: 04.12.2024 DOI:10.31467/uluaricilik.1570326

ABSTRACT

This study investigates pesticide, antibiotic, and heavy metal levels in Bayburt and the Upper Çoruh Valley honey. Thirteen honey samples were collected from different apiaries managed by stationary and migratory beekeepers. These samples were analysed for heavy metals (Fe, Cu, Zn, Cd, Pb), antibiotics, pesticides, and chemical residues such as naphthalene. The results revealed that lead (Pb) levels exceeded international food safety standards in 2 honey samples, while pesticide residues were detected in 5 samples. Additionally, antibiotic residues were found in 6 samples, including sulfamethazine, tetracycline, and streptomycin. However, no naphthalene was detected in any of the samples. These findings highlight the importance of stricter regulations and monitoring systems to control chemical use in beekeeping practices. Enhancing awareness among beekeepers regarding the risks associated with pesticide and antibiotic use is crucial for improving honey quality and ensuring the health of beekeepers and consumers. The adoption of safer practices and adherence to guidelines are necessary to mitigate these health hazards.

Keywords: Beekeeping, Honey residues, Heavy metals, Pesticides

ÖZ

Bu çalışma, Bayburt ve Yukarı Çoruh Vadisi'nde üretilen ballarda pestisit, antibiyotik ve ağır metal seviyelerini araştırmaktadır. Sabit ve gezgin arıcıların işlettiği 13 farklı arılıktan toplanan bal örneklerinde ağır metaller (Fe, Cu, Zn, Cd, Pb), antibiyotikler, pestisitler ve naftalin gibi kimyasal kalıntılar analiz edilmiştir. Analiz sonuçlarına göre, 2 bal örneğinde kurşun (Pb) seviyeleri uluslararası gıda güvenliği standartlarının üzerinde bulunmuş, 5 örnekte ise pestisit kalıntısına rastlanmıştır. Ayrıca, 6 bal örneğinde sulfamethazin, tetracycline ve streptomycin gibi antibiyotik kalıntıları tespit edilmiştir; ancak hiçbir örnekte naftalin kalıntısına rastlanmamıştır. Bu bulgular, arıcılık faaliyetlerinde kimyasal kullanımının kontrol edilmesi ve sıkı denetimlerin uygulanmasının önemini vurgulamaktadır. Arıcıların pestisit ve antibiyotik kullanımı konusunda bilinçlendirilmesi, bal kalitesinin artırılması ve tüketici sağlığının korunması için kritik öneme sahiptir. Güvenli uygulamaların teşvik edilmesi ve standartlara uygunluk, bu risklerin azaltılmasına katkı sağlayacaktır.

Anahtar kelimeler: Arıcılık, Balda kalıntılar, Ağır metaller, Pestisitler

GENİŞLETİLMİŞ ÖZET

Amaç: Bu çalışma, Bayburt ve Yukarı Çoruh Vadisi'nde faaliyet gösteren arıcıların ürettiği bal örneklerinde pestisit, antibiyotik ve ağır metal kalıntılarını belirlemeyi ve bu kalıntıların arıcılar ile tüketiciler üzerindeki potansiyel sağlık etkilerini değerlendirmeyi amaçlamaktadır. Özellikle sabit ve gezgin arıcıların işlettiği 13 farklı arılıktan toplanan bal örneklerinde yaygın olarak kullanılan kimyasalların kalıntı düzeyleri incelenmiş ve elde edilen bulgular ulusal ve uluslararası gıda güvenliği standartlarıyla karşılaştırılmıştır. Çalışma, kimyasal kalıntıların bal üretimi üzerindeki etkilerini ve bu kalıntıların potansiyel risklerini ortaya koymayı hedeflemektedir.

Gereç-Yöntem: Araştırma, kesitsel bir çalışma olarak planlanmış ve Ağustos-Eylül 2020 döneminde Bayburt ve Yukarı Çoruh Vadisi'nde faaliyet gösteren 13 arılıktan bal örnekleri toplanmıştır. Her arılıktan birer adet bal örneği alınarak, bu örneklerde ağır metaller (Fe, Cu, Zn, Cd, Pb), antibiyotikler (sulfamethazin, tetracycline ve streptomycin), pestisitler ve naftalin gibi kimyasal kalıntılar analiz edilmiştir. Ağır metal tayinleri ICP-AES cihazı ile pestisit ve antibiyotik kalıntı tayinleri ise HPLC-DAD ve floresan dedektörleri kullanılarak Bayburt Üniversitesi Merkezi Laboratuvarı'nda gerçekleştirilmiştir. Elde edilen sonuçlar, Türk Gıda Kodeksi ve Avrupa Birliği gıda standartları ile kıyaslanarak değerlendirilmiş ve her örneğin uygunluk durumu detaylı olarak analiz edilmiştir.

Bulgular: Analizler sonucunda, 13 bal örneğinden 2 tanesinde kurşun (Pb) seviyelerinin hem Türk Gıda Kodeksi hem de Avrupa Birliği standartlarının üzerinde olduğu belirlenmiştir. Kurşun kalıntıları, özellikle balın üretildiği bölgede bulunan çevresel faktörler ve arıların bu alanlarda temas ettiği kirleticiler nedeniyle yükselmiştir. Pestisit kalıntıları açısından, 5 bal örneğinde bu kalıntıların sınır değerlerinin aşıldığı tespit edilmiştir ve bu örnekler hem Türk Gıda Kodeksi hem de Avrupa Birliği standartlarına uygun bulunmamıştır. Antibiyotik kalıntılarında ise, 6 bal örneğinde sulfamethazin, tetracycline ve streptomycin kalıntılarına rastlanmıştır. Bu antibiyotik kalıntılarına sahip örneklerin 4'ü Türk Gıda Kodeksi'ne uygun bulunurken, 2'si kodekse uygunluk göstermemiştir. Avrupa Birliği standartlarına göre yapılan değerlendirmede ise, 8 bal örneğinin bu standartlara uymadığı, sadece 5 bal örneğinin uygunluk gösterdiği tespit edilmiştir. Buna ek olarak, analiz edilen bal örneklerinin hiçbirindenaftalin kalıntısına rastlanmamış olup, bu durum arıcıların bilinçlenme düzeyinin arttığını ve bu kimyasalın kullanımının azaldığını göstermektedir.

Sonuç: Çalışma sonuçları, Bayburt ve Yukarı Çoruh Vadisi'nde üretilen bal örneklerinde bazı ağır metal ve kimyasal kalıntıların bulunduğunu ve bunların bal kalitesi ile tüketici sağlığı üzerinde potansiyel riskler taşıdığını ortaya koymaktadır. Özellikle 2 bal örneğinde kurşun seviyelerinin yüksek olması ve 5 örnekte pestisit kalıntılarının sınırların üzerinde çıkması, arıcıların kimyasal kullanımı konusunda daha fazla bilinçlendirilmesi gerektiğini göstermektedir. Aynı şekilde, 6 örnekte tespit edilen antibiyotik kalıntıları, arıcıların ilaçlama uygulamalarında daha dikkatli ve kontrollü olmaları gerektiğini ortaya koymaktadır. Eğitim programlarının artırılması ve sıkı denetimlerin yapılması, arıcıların bilinç düzeyini yükselterek bal kalitesini artıracak ve tüketici sağlığını koruyacaktır. Sonuç olarak, bu çalışma, kimyasal kalıntıların kontrol altına alınması, güvenli arıcılık uygulamalarının teşvik edilmesi ve ulusal ve uluslararası standartlara uygun bal üretiminin sağlanması gerektiğini vurgulamaktadır. Özellikle bölgedeki arıcılık faaliyetlerinin sürdürülebilirliğini artırmak ve bal üretiminde kaliteyi korumak adına denetimlerin sıklaştırılması ve arıcılara yönelik eğitimlerin yaygınlaştırılması büyük önem arz etmektedir. Arıcıların pestisit ve antibiyotik kullanımı konusunda bilinçlendirilmesi, tüketici sağlığı ve ürün kalitesinin korunması açısından önemli rol oynayacaktır.

INTRODUCTION

Beekeeping is an activity that combines the use of plant resources, bees, and labour to produce products such as honey, royal jelly, bee venom, pollen, and propolis, which humans have utilised for nutrition, health protection, and treatment purposes since ancient times. In addition to these products, beekeeping also includes activities such as the production of queen bees, swarms, and package bees, which are significant sources of income. The vital role of bees in pollination is also of great importance for the agricultural sector. Beekeeping is the most nature-dependent livestock activity because the life cycle of honeybees and the raw material sources of the products obtained are directly tied to nature (Fıratlı et al. 2000).

Beekeeping is a globally widespread agricultural activity, with approximately 100.996 hives and 1.830,768 tons of honey produced, according to the 2022 FAO (Food and Agriculture Organization) statistics. The reasons for choosing beekeeping include low capital requirements, high return rates, low costs, relatively low labour needs, long shelf life of products, and opportunity for a hobby and additional income. Additionally, the fact that beekeeping does not require land makes it an attractive option for landless farmers (Gösterit and Gürel 2004).

Türkiye has highly favourable natural conditions for beekeeping. Utilising these natural advantages more consciously can contribute to the increased production of honey, an excellent food in every aspect, and other bee products (Gürcan and Soysal 2005).The antibiotics such as oxytetracycline and tylosin, widely used in beekeeping to manage bacterial brood diseases, have been shown to leave harmful residues in honey, raising health concerns for both beekeepers and consumers. For instance, residues of tylosin in honey have been detected at levels that can persist for months, potentially leading to antimicrobial resistance and contamination risks (Caldow et al. 2005).

Oxytetracycline, another commonly used antibiotic, has been found to degrade slowly in honey and brood nest areas, leading to long-term residue persistence, which could disrupt microbial flora in humans and potentially contribute to resistance development (Matsuka and Nakamura 1990).Similarly, pesticides such as amitraz and fluvalinate, used to control Varroa mites, can also leave residues in honey. Amitraz has been shown to have toxic effects on hormonal systems in humans when consumed over time (Kochansky 2004). Fluvalinate residues, detected in honey, have been associated with risks of chronic diseases due to their neurotoxic properties (Gilliam and Argauer 1981).

Pesticide residues are a significant factor in honey contamination. They occur through the direct contact of bees with pesticides or the indirect transfer of pesticides applied to plants in agricultural areas into the hives. It is known that even at low doses, pesticides can have harmful effects, accumulate in fat tissues, and cause carcinogenic and organ damage (Aygün 2020). In contrast, some types can damage nerve cells and cause cognitive disorders. This situation necessitates the conscious use of pesticides. Honeybees, as indicators of environmental pollution, are essential in detecting pesticide residues, as these residues can lead to harmful residues in bee products when medicines used against the varroa parasite are applied (Çakar 2019). Commonly used licensed drugs in Türkiye include active ingredients such as flumethrin, amitraz, and malathion. Chemical methods are often preferred for treating honeybee diseases, and antibiotics are widely used. Some antibiotics, such as chloramphenicol, have been banned in many countries (Sunay 2006).

Honey contamination with PAHs (Polycyclic Aromatic Hydrocarbons) can result from using naphthalene and sources like industrial facilities, while heavy metal contamination arises due to industrial pollution and improper beekeeping practices. Bees and bee products are considered effective bioindicators for detecting environmental pollution (Bogdanov et al. 2003, Gül et al. 2005, Lambert 2012, Morzycka 2002). The purpose of this study is to determine the pesticide, antibiotic heavy metal analyses of 13 different honey samples produced in Bayburt and Upper Coruh Valley Regions of Türkiye and to study the honeys contents comprehensively. It is thought that determining the contents of the honeys in these regions will be a precursor for future studies.

MATERIALS AND METHODS

Study Region

This study, planned as a cross-sectional research, involved the collection of thirteen honey samples from stationary and migratory beekeepers operating in the Çoruh Valley and Bayburt province. The aim was to detect commonly used chemicals and pesticides in the sector. The samples were collected from thirteen different apiaries in the same region, and the analysis for heavy metals (Fe, Cu, Zn, Cd, and Pb), as well as chemical residues (antibiotics, pesticides, and naphthalene residue tests), was conducted through a service obtained from the Central Laboratory of Bayburt University. The objective was to assess the potential health effects of these chemical residues, whether they enter the food chain affecting all consumers or pose risks to the beekeepers themselves, and to propose measures to mitigate these risks. Approximately 550-600 beekeepers are active in the region. Information regarding the locations where the honey samples were collected is presented in Figure 1 and Table 1.

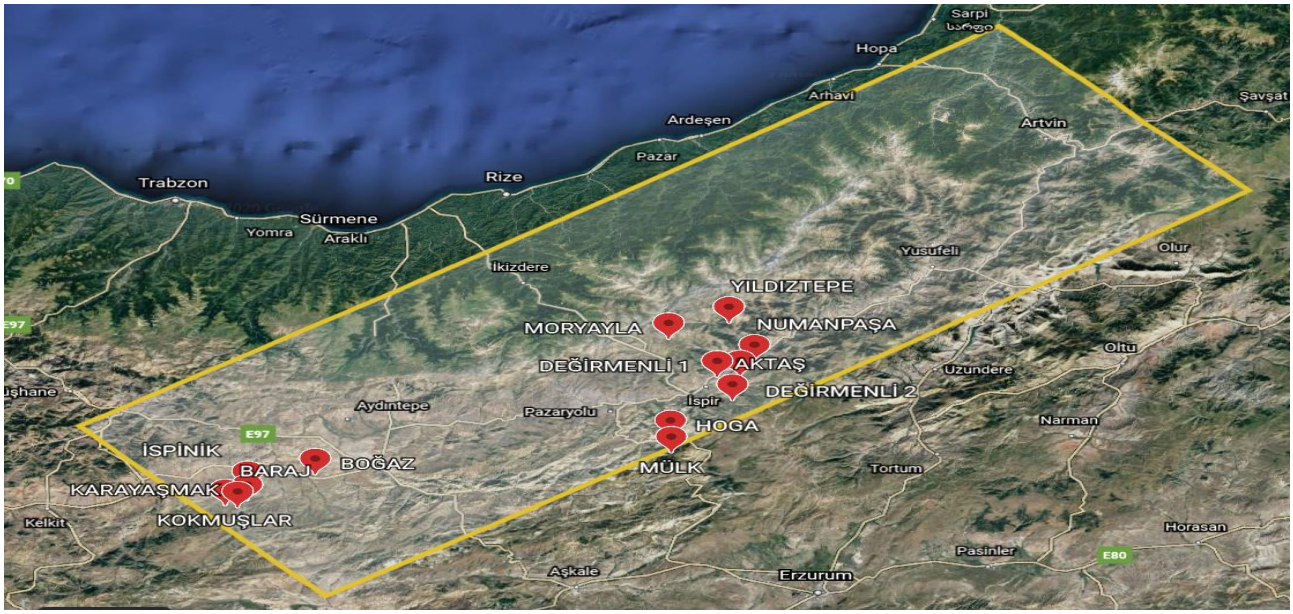


Figure-1. Regional distribution of examined apiaries

Table-1. Location information of examined apiaries

Apiary number	Region of the apiary	Coordinates	Date of examination
1	Mülk	40º 17’ 28º N - 40º 55’ 14º E	August - September 2020
2	Yıldıztepe	40º 39’ 20º N - 41º 03’ 46º E	August - September 2020
3	Değirmenli 1	40º 30’ 12º N - 41º 01’ 45º E	August - September 2020
4	Değirmenli 2	40º 30’ 18º N - 41º 05’ 01º E	August - September 2020
5	Moryayla	40º 36’ 36º N - 40º 54’ 49º E	August - September 2020
6	Aktaş	40º 26’ 17º N - 41º 03’ 56º E	August - September 2020
7	Numanpaşa	40º 32’ 56º N - 41º 07’ 05º E	August - September 2020
8	Karayaşmak	40º 09’ 41º N - 39º 54’ 47º E	August - September 2020
9	Kokmuşlar	40º 11’ 22º N - 39º 50’ 23º E	August - September 2020
10	Baraj	40º 07’ 53º N - 39º 53’ 23º E	August - September 2020
11	Boğaz	40º 13’ 42º N - 40º 04’ 31º E	August - September 2020
12	İspinlik	40º 11’ 19º N - 39º 54’ 44º E	August - September 2020
13	Hoga	40º 20’ 12º N - 40º 55’ 07º E	August - September 2020

The Çoruh Valley is known for its rich biodiversity due to its natural features. The valley is located in the Caucasus Ecological Region, one of the world's 200 most ecologically significant areas identified by the WWF (World Wildlife Fund), and it is one of nine essential plant areas on the Turkish side of this region. The basin, with an area of 19.748 km², lies between 39° 40’ and 42° 35’ longitude and 39° 52’ and 41° 32’ latitude, bordered by the Eastern Black Sea Mountains to the north, the Giresun Mountains to the west, Otlukbeli, Dumlu, Kargapazarı, Güllü, and Allahüekber Mountains to the south, and the Yanlızçam Mountains and Georgia to the east. As one moves inland from the Black Sea coast, the climate transitions from temperate to continental. The mountains surrounding the Çoruh River rise to 3.000 meters within 15 km, while the valley floor descends to 75 meters near the Georgian border (Erdoğan et al. 2014, Erdoğan and Erdoğan 2014). Bayburt, located within the study area, is situated in the Eastern Black Sea region of the Black Sea Region, between 40° 37’ north latitude and 40° 45’ east longitude, 39° 52’ south latitude and 39° 37’ west longitude. Bayburt, located along the Çoruh

River at an elevation of 1.550 meters above sea level, covers an area of 3.739 km². Geographically, it consists of a basin extending between mountain ranges to the north and south, and its topography includes mountains, plains, and valleys (Birinci 2013). Bayburt is a newly developing region in terms of beekeeping, with 72.266 hives recorded in 2018 and 408 tons of honey produced in the same year, according to Turkish Statistical Institute (TÜİK) data. Recently, migratory beekeeping activities have increased significantly in the region (Koday and Karadağ 2020).

Residue Analysis Method in Honey

Element Analysis Method: During element analysis, approximately 1 g of each honey sample was weighed into Teflon containers, and 10 mL of nitric acid was added. After tightly sealing the containers, they were placed in a microwave digestion unit. The honey samples were then digested using microwave radiation and cooled. The extracts obtained were filtered through blue band filter paper into 25 mL volumetric flasks and diluted to 25 mL with ultra-pure water (Demirezen and Aksoy 2005). An ICP-AES (Varian Model-Liberty Series II) device was used to determine metal concentrations. The results were calculated as mg/kg based on wet weight by measuring each element individually. When selecting each component of the honey samples, calibration curves obtained using ICP standards of known concentration (High-Purity standards) were utilised (Gül 2008).

Analysis Method for Medications Used by Beekeepers for Bee Diseases: In the honey samples collected in this study, residue analysis was conducted for sulfonamide, tetracycline, and streptomycin, medications commonly used by beekeepers for bee diseases. Screening analysis was performed using the Charm II 6600/7600 system, and the residue quantities were determined using HPLC-DAD (Diode Array Detector) and fluorescence detectors along with columns. To identify positive honey samples, all honey samples were first subjected to screening analysis using the Charm II device, and standard solutions were processed through the Charm II to establish control points. The presence of these medication residues in the honey samples was determined based on the defined control point. Honey samples found to contain residues were prepared for HPLC analysis. Further analysis in HPLC was performed to quantify the residue levels, considering the retention times and peak areas of standard solutions (Gül 2008).

Pesticide Residue Analysis Method: In the honey samples collected during the study, residues of organophosphate pesticides such as amitraz and coumaphos, which are used by some beekeepers against bee diseases, were analysed. To prepare the standard solutions, 10 mg of amitraz and coumaphos standards were separately dissolved in hexane in 10 mL volumetric flasks, resulting in a primary stock solution of 1,000 µl/mL (mg/kg). A separate 1/1 dilution (1 µL/mL) was then prepared from the primary stock solution. From the prepared primary stock solution, 100 µL was taken and diluted with acetonitrile in a 10 mL volumetric flask to obtain an intermediate stock solution of 10 µL/mL (1 mg/kg). Sequential dilutions of 5, 10, 25, 50, 100, and 200 µL/L (ppb) were prepared from the intermediate stock solution, and the peak areas were determined using a GC-MS (HP 6890 Series / 5972 A- GC-MS (Gas Chromatography-Mass Spectrometry) (Gül 2008).

Naphthalene Analysis Method: Naphthalene analysis in the honey samples collected during the study was performed using GC-MS (HP 6890 Series / 5972 A- GC-MS (Gas Chromatography-Mass Spectrometry) and a headspace sampler. For the analysis, 5 g of honey from each sample was weighed into 15 mL headspace vials and kept at 90 °C for 45 minutes. After heating, the samples were immediately placed into the headspace sampler for analysis (Gül 2008).

RESULTS

Heavy Metal Analysis Values in Honey Samples

The heavy metal residue analysis results for the 13 honey samples collected from the study area are presented in Table 2.

Table-2. Heavy metal analysis values in honey samples

Samp. No	Region	Cd (mg/kg)	Fe (mg/kg)	Cu (mg/kg)	Zn (mg/kg)	Pb (mg/kg)
1	Mülk	*N.D.	1.69±0.01	0.16±0.07	0.21±0.03	0.06±0.00
2	Yıldıztepe	*N.D.	1.77±0.01	0.17±0.02	0.23±0.04	0.04±0.00
3	Değirmenli 1	*N.D.	1.79±0.02	0.15±0.03	0.23±0.05	0.1±0.00
4	Değirmenli 2	*N.D.	1.33±0.01	0.15±0.01	0.20±0.01	0.6±0.01
5	Moryayla	*N.D.	1.25±0.03	0.16±0.03	0.19±0.07	0.16±0.01
6	Aktaş	*N.D.	1.41±0.04	0.14±0.01	0.22±0.01	0.13±0.08
7	Numanpaşa	*N.D.	1.07±0.01	0.10±0.01	0.17±0.09	0.12±0.07
8	Karayaşmak	*N.D.	0.95±0.01	0.09±0.00	0.19±0.01	0.11±0.00
9	Kokmuşlar	*N.D.	1.19±0.02	0.09±0.00	0.21±0.07	0.14±0.02
10	Baraj	*N.D.	1.86±0.03	0.17±0.06	0.23±0.01	2.01±0.01
11	Boğaz	*N.D.	1.95±0.02	0.19±0.01	0.25±0.06	2.0±0.03
12	İspinlik	*N.D.	1.97±0.03	0.16±0.07	0.25±0.01	1.94±0.09
13	Hoga	*N.D.	1.46±0.05	0.16±0.08	0.22±0.01	0.13±0.06

*N.D.: Not Detected

Residue Analysis Results in Honey Samples

The results of the residue analysis for medications used against bee diseases and pests in the 13 honey samples collected from the study area are presented in Table 3.

Table-3. Residue analysis values for abtibitics and organophosphate ınsecticides used against bee diseases and pests in honey samples

Samp. No	Region	Naphthalene (mg/kg)	Sulfamethazin (mg/kg)	Tetracycline (mg/kg)	Streptomycine (mg/kg)	Coumaphos (mg/kg)	Amitraz (mg/kg)
1	Mülk	*N.D.	*N.D.	*N.D.	*N.D.	*N.D.	*N.D.
2	Yıldıztepe	*N.D.	*N.D.	*N.D.	0.021	*N.D.	*N.D.
3	Değirmenli 1	*N.D.	*N.D.	*N.D.	*N.D.	*N.D.	*N.D.
4	Değirmenli 2	*N.D.	*N.D.	*N.D.	*N.D.	*N.D.	*N.D.
5	Moryayla	*N.D.	*N.D.	0.001	*N.D.	*N.D.	*N.D.
6	Aktaş	*N.D.	*N.D.	*N.D.	0.002	*N.D.	*N.D.
7	Numanpaşa	*N.D.	*N.D.	*N.D.	*N.D.	0.002±0.000	0.044±0.000
8	Karayaşmakk	*N.D.	0.007±0.000	*N.D.	*N.D.	*N.D.	*N.D.
9	Kokmuşlar	*N.D.	*N.D.	*N.D.	*N.D.	*N.D.	0.007
10	Baraj	*N.D.	0.001±0.000	*N.D.	*N.D.	*N.D.	0.006
11	Boğaz	*N.D.	*N.D.	0.001±0.000	*N.D.	0.06±0.001	0.004±0.000
12	İspinlik	*N.D.	0.010	*N.D.	*N.D.	*N.D.	0.035±0.000
13	Hoga	*N.D.	*N.D.	*N.D.	*N.D.	*N.D.	0.004±0.000

*N.D.: Not Detected

DISCUSSION

Evaluation of Heavy Metal Analysis

In this study, 13 honey samples collected from the Upper Çoruh Valley and Bayburt Region were analysed for heavy metals, including Iron (Fe), Copper (Cu), Cadmium (Cd), Zinc (Zn), and Lead (Pb). The data obtained from the analysis are presented in Table 2.

The analysis revealed that the lowest iron (Fe) concentration was found in sample 8, at 0.95 mg/kg, while the highest concentration was observed in sample 12, at 1.97 mg/kg. Türkiye, there is no specific standard for the amount of iron that may be

present in honey; however, as shown in Table 4, all honey samples were found to comply with the FAO-WHO Codex Alimentarius standards, which permit a maximum iron (Fe) concentration range of 1.5-15 mg/kg in food products (see Figure 2).

Table-4. Maximum permitted heavy metal levels in food according to fao-who codex alimentarius (WHO and FAO 1972)

Heavy metals	Maximum permissible levels in food (mg/kg)
Cadmium (Cd)	Not Allowed
Lead (Pb)	0.1-2.0
Copper (Cu)	0.1-5.0
Iron (Fe)	1.5-15
Zinc (Zn)	5
Fe + Cu + Zn	20

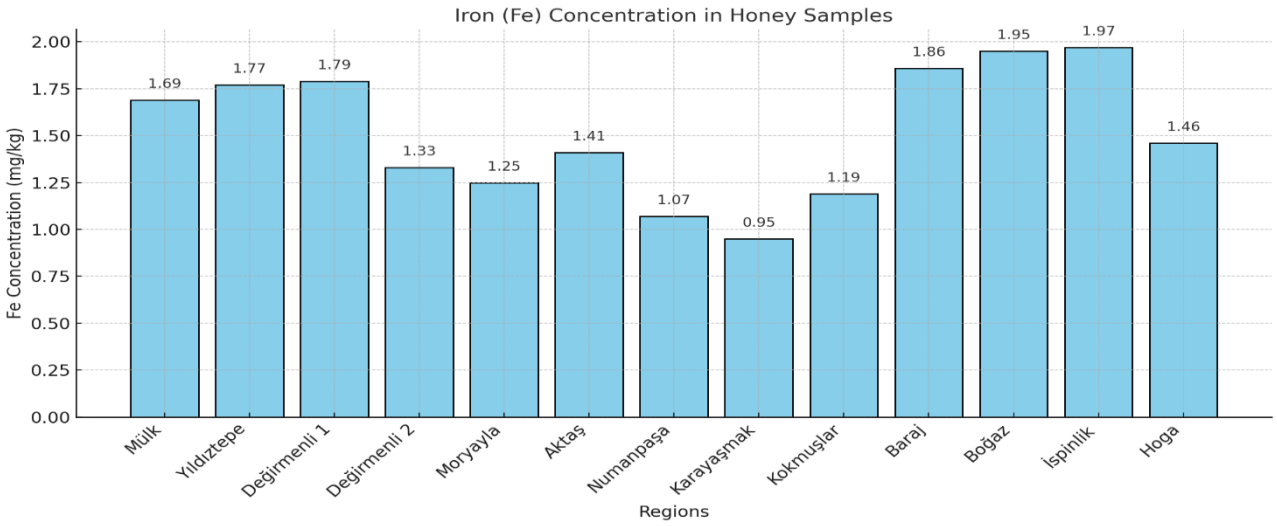


Figure-2. Iron (Fe) concentration in analyzed honey samples (mg/kg)

The lowest copper (Cu) concentration was found in samples 8 and 9 0.09 mg/kg, while the highest concentration was observed in samples 11 0.19 mg/kg. Türkiye has no specific standard for the amount of copper in honey. However, as shown in Table 4, all honey samples complied with the FAO-WHO Codex Alimentarius standards, which permit a maximum copper (Cu) concentration range of 0.1-5.0 mg/kg in food products (see Figure 3).

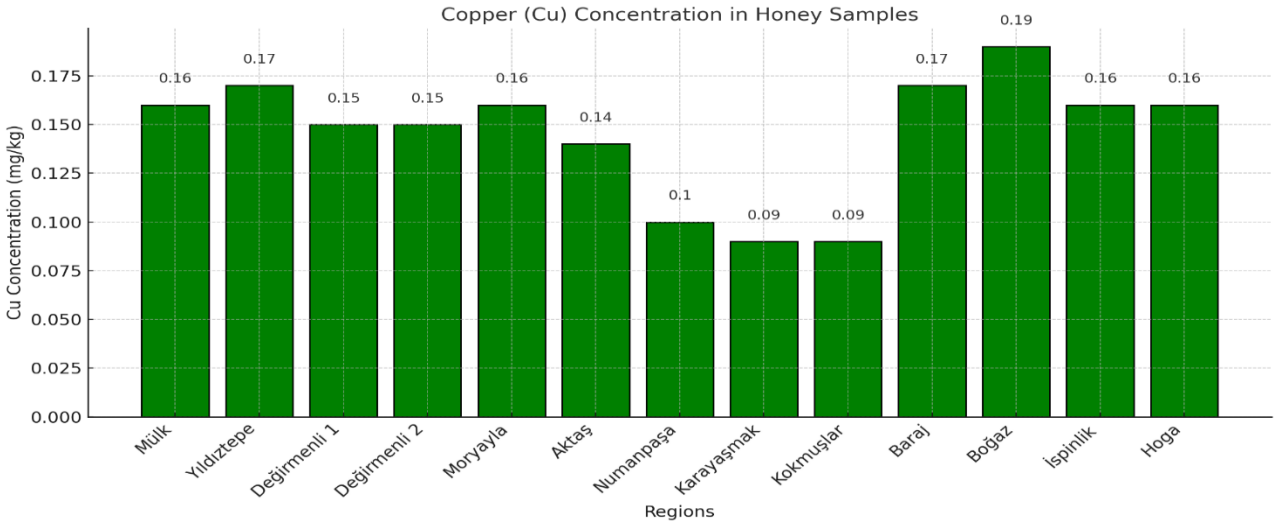


Figure-3. Copper (Cu) concentration in analyzed honey samples (mg/kg)

In the study, the lowest zinc (Zn) concentration was found in sample 7 0.17 mg/kg, while the highest concentration was observed in samples 11 and 12 0.25 mg/kg. In Türkiye, there is no specific standard for the amount of zinc that may be present in honey, as with other heavy metals. However, as shown in Table 4, all honey samples were found to comply with the FAO-WHO Codex Alimentarius standards, which permit a maximum zinc (Zn) concentration of 5 mg/kg in food products (see Figure 4).

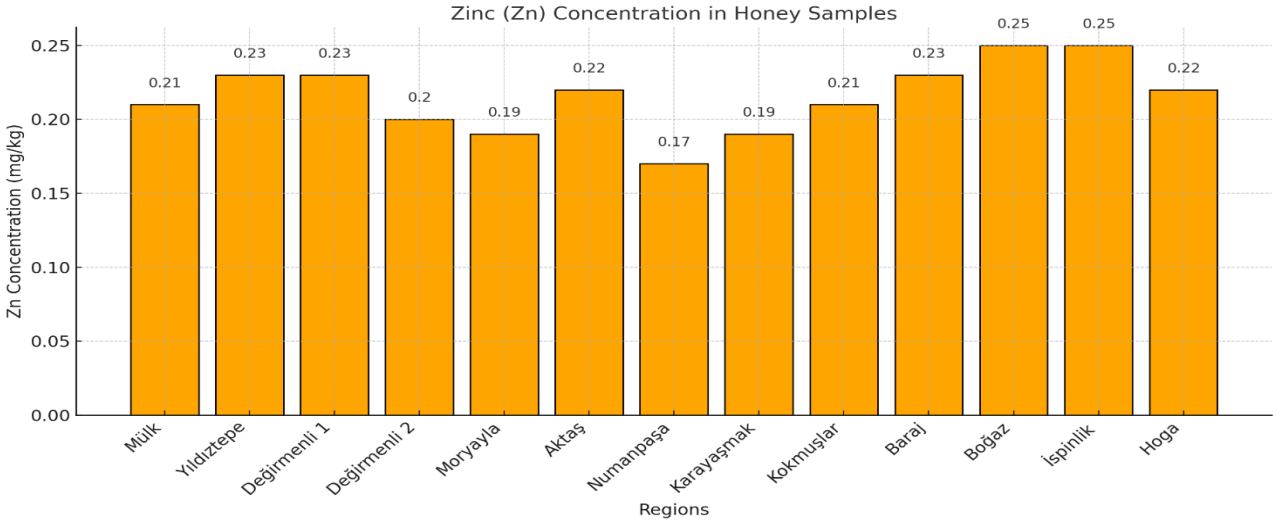


Figure-4. Zinc (Zn) concentration in analyzed honey samples (mg/kg)

Zinc and iron are essential elements for humans, animals, and plants. According to the National Academy of Science (NAS) and other sources, the recommended daily intake for an adult (aged 19-70) is 40 mg of zinc and 45 mg of iron. Considering the amounts that people can consume through their daily diet, the results of this study indicate that the levels of Zn and Fe found are significantly below these limits. Therefore, all honey samples analyzed in terms of these metals are considered safe (Demirezen and Aksoy 2005). In this study, cadmium (Cd) was not detected in any of the honey samples analyzed. In Türkiye, there is no specific standard for the amount of cadmium that may be present in honey; however, as shown in Table 4, all samples were found to comply with the FAO-WHO Codex Alimentarius standards, which state that cadmium (Cd) should not be present ('not detectable'). Various studies conducted in different regions of Türkiye have identified a wide range of Zn, Fe, and Cu levels in honey samples. For instance, in 25 honey samples collected from six different regions, Zn, Fe, and Cu levels were found to range between 1.1-12.7 mg/kg, 1.8-10.2 mg/kg, and 0.23-2.41 mg/kg, respectively (Tüzen et al. 2007). In 60 honey samples from Central Anatolia, Zn and Cu levels were determined to be 1.1-24.2 mg/kg and 0.25-1.10 mg/kg, respectively, which were higher than the results reported in the current study (Arslanbaş 2010). In 20 honey samples collected from the Black Sea Region, Zn, Fe, and Cu levels were reported as 0.47-6.57 mg/kg, 1.12-12.9 mg/kg, and 0.009-0.035 mg/kg, respectively, which were observed to be similar to the results of the present study (Silici et al. 2008). In samples obtained from provinces in Eastern Anatolia, the Fe, Zn, and Cu levels were measured as 9.799±5.615 mg/kg, 3.705±1.708 mg/kg, and 2.635±1.198 mg/kg, respectively, which were found to be lower than those in the current study (Güleç 2007). In 45 honey samples collected across Türkiye, Fe levels were determined to range between 3.71-5.43 mg/kg, while Zn levels were found to range between 6.24-11.53 mg/kg (Yarsan et al. 2007).

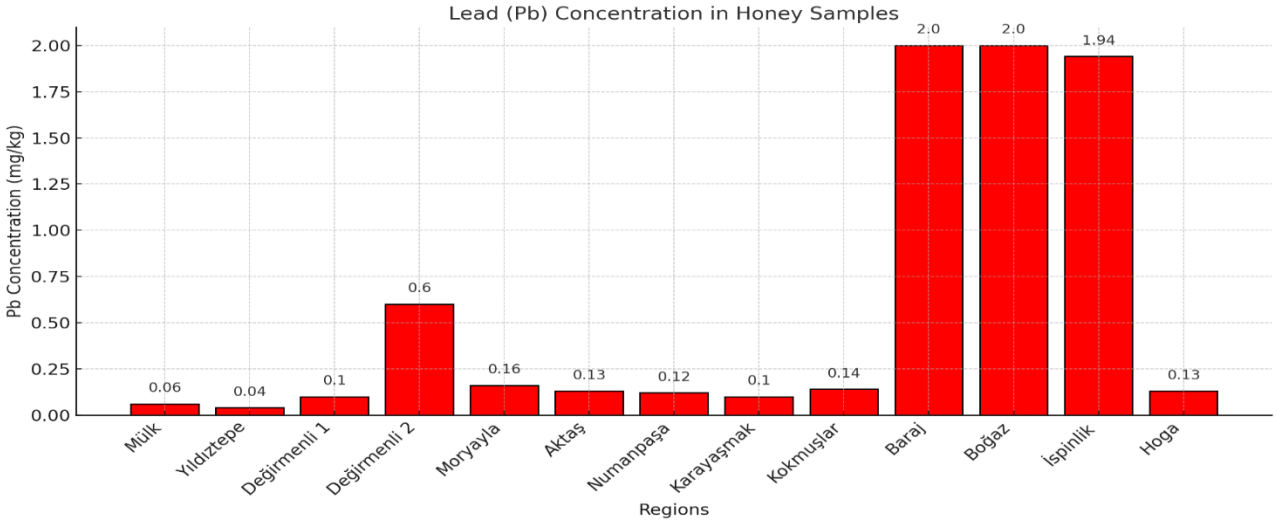


Figure-5. Lead (Pb) concentration in analyzed honey samples (mg/kg)

The lowest lead (Pb) concentration was found in sample 2 0.04 mg/kg, while the highest concentration was observed in sample 10 2.01 mg/kg. In Türkiye, there is no specific standard for the amount of lead that may be present in honey. However, as shown in Table 4, all honey samples, except for sample 10, were found to comply with the FAO-WHO Codex Alimentarius standards, which permit a maximum lead (Pb) concentration of '0.1-2.0' mg/kg. Sample 11 was found to be at the limit value of 2.0 mg/kg (see Figure 5). The Upper Çoruh and Bayburt regions, where the honey samples were collected, are generally not significant industrial areas in Türkiye. Therefore, the heavy metal content in the collected honey samples was not found to be high. Except for the lead (Pb) level in sample 10, all samples were within the limit values specified in the FAO-WHO Codex Alimentarius. The honey sample with a lead (Pb) level above the limit was collected from an apiary located approximately 300 meters

from the Bayburt Demirözü Irrigation Pond. This area has recently become a tourist destination with many visitors, leading to heavy vehicle traffic, especially in the summer months. It is considered that the bees and vegetation in this area may have been exposed to exhaust gases, resulting in contamination of bee products. Therefore, the honey and other bee products produced within 5 km of this area are not considered safe in terms of carcinogenic residue risk and are believed to potentially pose a health risk to the public. It is recommended to support tourism activities in this area and conduct beekeeping activities at least 5 km away from this region. Various studies conducted in Türkiye and other countries have compared Pb and Cd levels in honey samples, revealing a wide range of variability. In a study conducted in Türkiye by Tüzen et al. (2007), Pb and Cd levels were found to range between 0.0084-0.106 mg/kg and 0.0009-0.0179 mg/kg, respectively (Tüzen et al. 2007). In Poland, Pb and Cd levels in honey samples were reported as 0.025-0.071 mg/kg and 0.008-0.027 mg/kg, respectively, which were lower than the Pb levels but higher than the Cd levels in the current study (Przybyloeski and Wilczynska 2001). In 200 flower honey samples collected from Eastern Anatolia, average Pb and Cd levels were determined to be 0.131±0.081 mg/kg and 0.006±0.007 mg/kg, respectively, with Pb levels higher and Cd levels lower compared to the current study (Güleç 2007). Honey samples from the vicinity of Mount Erciyes in Kayseri showed Pb and Cd levels of 0.1-0.85 mg/kg and 0.11-0.18 mg/kg, respectively, both of which were lower than the results of the current study (Demirezen and Aksoy 2005). In honey samples from Kahramanmaraş, the average Cd level was reported as 0.32 mg/kg, which was lower than the current study's findings (Erbilir and Erdoğrul 2005). In France, honey samples available for sale were found to have Pb and Cd levels of 0.28-1.08 mg/kg and 0.08-0.25 mg/kg, respectively, with Pb levels higher and Cd levels lower than those of the current study (Devillers et al. 2002). In samples from Tenerife Island, Spain, Pb and Cd levels were measured as 0.03733 mg/kg and 0.00438 mg/kg, respectively, both of which were lower than the results of the current study (Frias et al. 2008).

Residue Analysis Evaluation

In the study, residue analysis was conducted for substances categorized as residues in the 13 honey samples collected from the Upper Çoruh Valley and Bayburt Region. These substances included residues from medications used by beekeepers to combat bee diseases and pests (sulfamethazine, tetracycline, and streptomycin), pesticide residues (amitraz and coumaphos), and naphthalene residues. The data obtained from these analyses are presented in Table 3. Standards for the levels of these substances in honey, as established by Türkiye and the European Union for medications used against bee diseases and pests, are shown in Table 5 (Sunay 2006).

Table-5. Permitted medication levels for bee diseases and pests according to the Turkish food codex honey communiqué

Medicine used for bee diseases and pests	Turkish food codex drug tolerance level (mg/kg)	European Union drug tolerance level (mg/kg)
Amitraz	0.02	0.02
Coumaphos	0.01	0.01
Streptomycine	0.02	Not found
Sulfonamid Group	0.01	Not found
Tetracycline Group	0.01	Not found

Evaluation of Medication Analyses Used for Bee Diseases and Pest Control

Evaluation of Sulfa Group Antibiotic Analyses

In this study, the amount of sulfamethazine, an antibiotic used by beekeepers for bee diseases and pests, was detected in samples 8., 10., and 12. at concentrations of 0.0071 mg/kg, 0.0098 mg/kg, and 0.102 mg/kg, respectively. Accordingly, samples 8. and 10. were found to comply with the limit value of '0.01 mg/kg' specified in the Turkish Food Codex Medication Tolerance Level, while sample 12. was found to exceed both the limit value specified in the Turkish Food Codex and the limit value of 'not detectable' specified by the EU Medication Tolerance Level (see Table 5 and Figure 6). In our study, only one of the three honey samples containing detectable levels of sulfa group antibiotics

was considered unsafe in terms of sulfa group antibiotic content. Currently, the most common honeybee diseases faced by beekeepers are brood diseases and nosema disease. To protect or treat bee colonies from these diseases, chemical methods are often used. Antibiotics such as streptomycin, tetracyclines, or sulfonamides are commonly applied, especially against brood diseases. Frequent and unconscious use of antibiotics can increase the resistance of bacteria causing these diseases, resulting in negative effects for both honeybees and bee products. It is considered that beekeepers need education on the correct dosage and conscious application of medications for bee diseases (Söğüt et al. 2019). In honey samples collected from 22 different regions of Türkiye in 2006, the presence of antibiotics from the sulfa, tetra, and strepto groups was investigated, and among sulfa antibiotics, only sulfadimidine was detected. Analysis revealed that 10% of the 1,714 samples contained this antibiotic, and 5% of the 91 samples showed streptomycin residues above 0.0177 mg/kg (Sunay 2006). In a study conducted on 536 honey samples collected between 2007 and 2009, sulfa group antibiotic analysis identified average residue levels of 0.102 mg/kg sulfanilamide, 0.597 mg/kg sulfamethazine, and lower levels of other sulfa antibiotics (Erdoğdu et al. 2011). The levels of sulfa antibiotics detected in this study were found to be lower than those reported in previous studies.

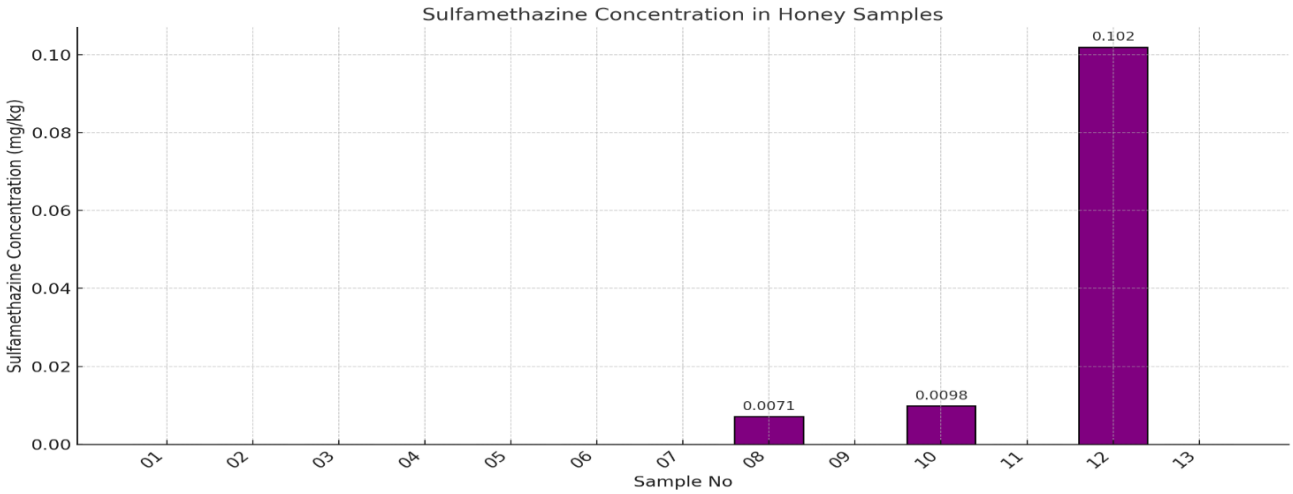


Figure-6. Sulfamethazine concentration in analyzed honey samples (mg/kg)

Evaluation of Tetra Group Antibiotic Analyses

In this study, tetracycline, an antibiotic used by beekeepers for bee diseases, was detected in samples 5 and 11 at concentrations of 0.0014 mg/kg and 0.0011 mg/kg, respectively. Both samples complied with the Turkish Food Codex limit of 0.01 mg/kg but did not meet the EU standard, which requires no detectable residue. Overall, all 13 samples were deemed safe regarding tetracycline levels (see Table 5 and Figure 7). In a study conducted in Greece, drug residues were detected in 29% of honey samples, with 20.3% attributed to tetracyclines and their derivatives. Residue levels ranged between 0.018-0.055 mg/kg, with some samples reaching up to 0.1 mg/kg (Saridaki et al. 2006). In Poland, an analysis of 178 honey samples revealed insecticide residues ranging from 0-0.06 mg/kg, attributed to environmental contamination (Wilczynska and Przybylowski 2007). In another study on honey produced in and imported to Belgium, 0.015 mg/kg streptomycin, 0.01 mg/kg sulfamethazine, 0.01 mg/kg penicillin, and 0.0001 mg/kg chloramphenicol were detected, with residues predominantly found in imported honey samples (Reybroec 2003). The levels of tetracycline group antibiotics detected in this study were found to be lower than those reported in the three aforementioned studies.

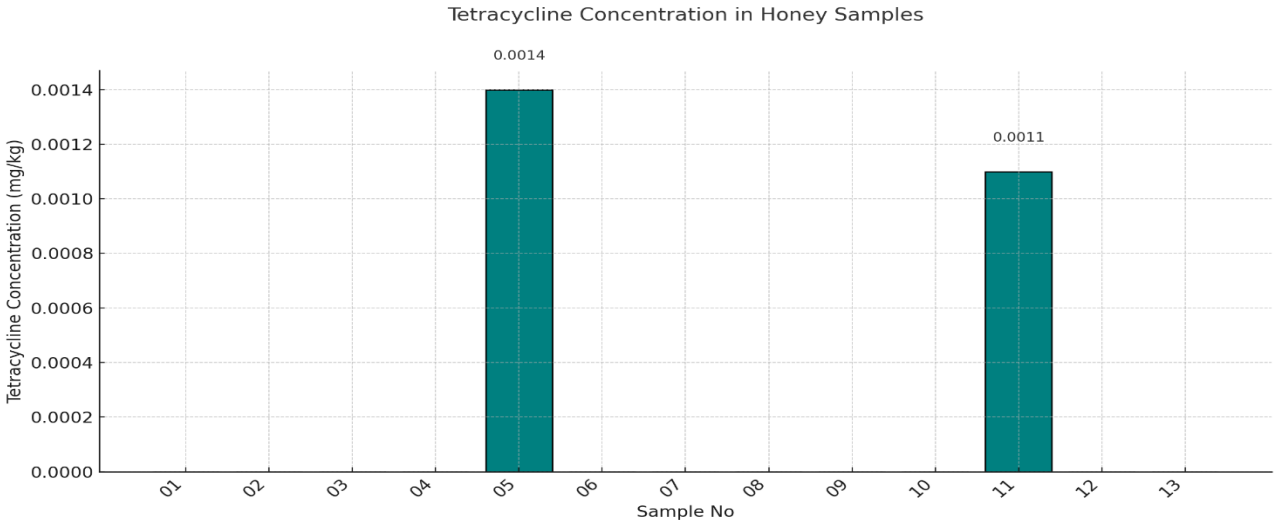


Figure-7. Tetracycline concentration in analyzed honey samples (mg/kg)

Evaluation of Strepto Group Antibiotic Analyses: In this study, streptomycin was detected in samples 2 and 6 at 0.00211 mg/kg and 0.00212 mg/kg, respectively. While both samples met the Turkish Food Codex limit of 0.02 mg/kg, they did not comply with the EU standard of 'not detectable' (see Table 5 and Figure 8). Nevertheless, all 13 samples were deemed safe regarding strepto group antibiotics. In a study conducted on 180 honey samples collected from the central and district areas of Ardahan province, streptomycin residues were detected in 37% of the samples, while sulfonamide residues were found in 52% of the samples. (Özkan et al. 2015). The levels of streptomycin group antibiotics detected in this study were found to be lower than the values reported in the previously mentioned studies.

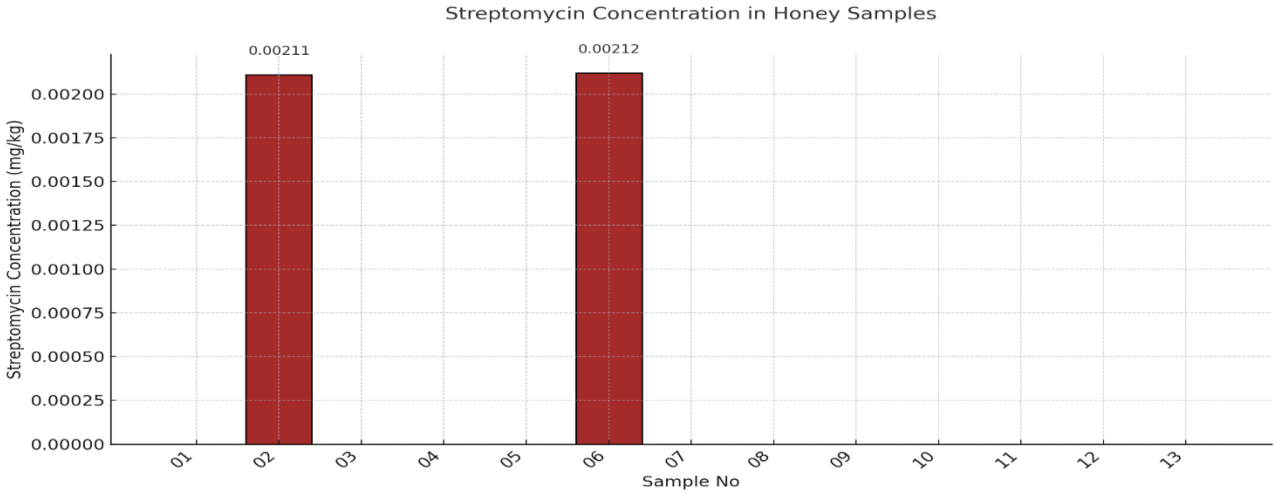


Figure-8. Streptomycin concentration in analyzed honey samples (mg/kg)

Evaluation of Pesticide Analyses

Evaluation of Coumaphos Analyses: Coumaphos, amitraz, and malathion are chemical pesticides widely utilized in beekeeping to control Varroa destructor, a parasitic mite that poses a severe threat to honeybee health and colony

survival. While these substances are effective in controlling the mites, their use raises concerns due to the potential for residues accumulating in bee hives. These residues can contaminate hive products such as honey, wax, and propolis, potentially affecting food safety and the health of bees and humans. Residue persistence may also interfere with the natural behavior of bees and the long-term sustainability of beekeeping practices. Thus, while these chemicals are valuable tools in mite management, their use requires careful regulation and adherence to safe application practices to mitigate environmental and health risks. In this study, the amount of coumaphos, a pesticide, was detected in samples 7 and 11 at concentrations of 0.0002 mg/kg and 0.06 mg/kg, respectively. Sample 7 complied with both the Turkish Food Codex and EU Medication Tolerance Levels, which set the limit at 0.01 mg/kg. However, sample 11 exceeded the limit set by both codices (see Table 5 and Figure 9).

One sample was found unsafe due to coumaphos content. Some honey samples had pesticide residues exceeding limits set by the Turkish Food Codex Honey Communiqué. The likely cause is the low education level of beekeepers in the sampled regions, leading to excessive and uncontrolled pesticide use and environmental contamination. Varroa mite populations rise in summer and peak in fall; honey samples were collected in August and September. Coumaphos, commonly used in Türkiye for varroa control, should be applied at proper doses in early spring and late fall, when hive brood activity is low, to minimize residue risk. In a study investigating coumaphos residues in honey and comb samples from different regions of Türkiye and Israel, it was found that coumaphos levels in 49 out of 55 honey samples from Türkiye averaged 0.0308 mg/kg, and in all 10 comb samples, the average was 0.0213 mg/kg. In Israel, 33 out of 38 honey samples contained an average of 0.0461 mg/kg coumaphos, while 60 out of 67 comb samples had an average of 0.0030 mg/kg (Barel et al. 2011).

In Spain, coumaphos residues ranging from 0.001-0.053 mg/kg were detected in 32 out of 221 honey samples (Garcia et al. 1996). The coumaphos levels detected in this study were lower than those reported in the aforementioned studies.

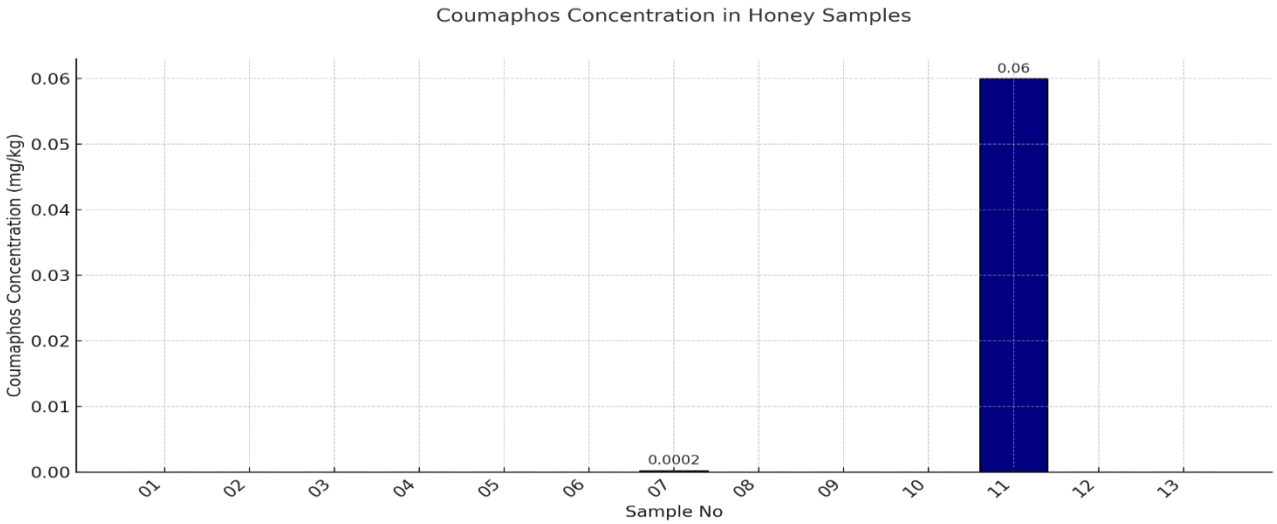


Figure-9. Coumaphos concentration in analyzed honey samples (mg/kg)

Evaluation of Amitraz Analyses: Amitraz, a commonly used pesticide for controlling the varroa mite due to its low cost, is typically applied in the evening when bees return to the hive, at temperatures between 15-20 ºC, over 4 consecutive days. When properly applied, it has shown effective results (Daş and Aksoy). In this study, amitraz was detected in samples 7, 9, 10, 11, 12, and 13 at concentrations of 0.0436 mg/kg, 0.0069 mg/kg, 0.0057 mg/kg, 0.0041 mg/kg, 0.0352 mg/kg, and 0.0044 mg/kg, respectively. Samples 9, 10, 11, and 13 complied with both the Turkish Food Codex and EU standards, which set the limit at 0.02 mg/kg. However, samples 7 and 12 exceeded these limits.

Of the six honey samples where amitraz was detected, two were considered unsafe regarding amitraz content. (see Table 5 and Figure 10). Between 1986 and 1990 in Germany, amitraz residues exceeding 0.05 mg/kg were detected in 8.5% of 330 honey samples (Hammerling et al. 1991). In studies conducted in Spain, residues of amitraz, bromoprophylate, coumaphos, and fluvalinate ranged from 0.001-0.04 mg/kg, with amitraz levels reported as high as 0.033-1.82 mg/kg in some samples (Garcia et al. 1995). In a study conducted in Türkiye, amitraz residues ranging from 0.0013-0.0334 mg/kg were detected in 25 of 135 honey samples (Bilgili and Selçukoğlu 2022). The levels of amitraz identified in this study were found to be lower than those reported in the aforementioned studies.

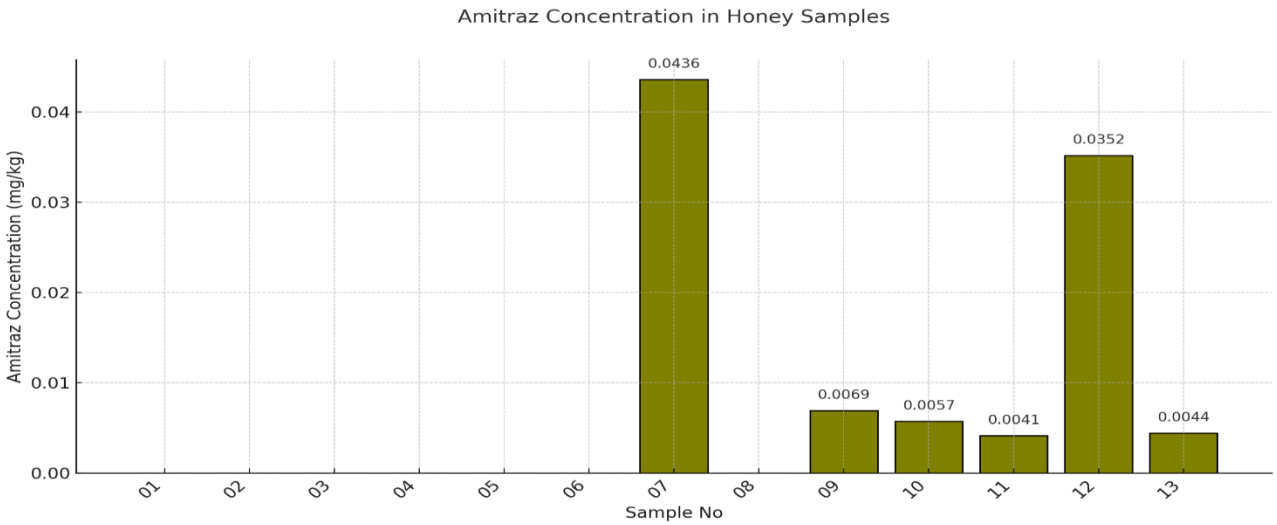


Figure-10. Amitraz concentration in analyzed honey samples (mg/kg)

Evaluation of PAH Analyses

Evaluation of Naphthalene Analyses: Beekeepers typically use naphthalene in the fall, after honey extraction, to combat wax moths in improperly stored combs. When naphthalene-treated combs are returned to the colony, the residues can transfer into the honey (Johnson et al. 2010).

In this study, none of the 13 honey samples collected showed any detectable naphthalene residues. It is believed that the lack of naphthalene residues in the honey is due to the increased information campaigns for beekeepers in recent years about the harmful effects of naphthalene. This outcome indicates the effectiveness of recent training efforts warning beekeepers against the use of naphthalene. Therefore, the honey samples collected from the study area are considered safe in terms of naphthalene residues. In a three-year study conducted in Greece to detect naphthalene residues in honey, 115 commercial honey samples and 1,060 beehive honey samples were analyzed. In the first year, higher levels of naphthalene were detected in commercial honey compared to honey obtained directly from beekeepers. A decrease in naphthalene levels was observed over the subsequent two years (Tananaki et al. 2006).

In Romania, honey samples collected from eight regions revealed naphthalene levels ranging from 0.17-0.665 mg/kg in areas near urban settlements, and from 0.027-0.068 mg/kg in areas near rural regions (Dobrinas et al. 2008). In Türkiye, as part of the "National Residue Monitoring Project" conducted by the Ministry of Agriculture and Rural Affairs' Directorate General for Protection and Control in 2002, naphthalene residues were detected in 22% of the 118 analyzed honey samples (Daş 2004). In contrast, no naphthalene residues were detected in any of the 13 honey samples collected within the scope of the present study.

Conclusion: "In the study, heavy metal analysis was conducted on 13 honey samples collected from the Upper Çoruh Valley and Bayburt Region,

focusing on Iron (Fe), Copper (Cu), Cadmium (Cd), Zinc (Zn), and Lead (Pb). Except for Cd, all other elements were detected in the samples; however, only 2 samples did not comply with the maximum allowable heavy metal levels set by the FAO-WHO Codex Alimentarius for food. It was also determined that there is no established standard for heavy metal residues in food in Türkiye. Regarding the analysis of residues from medications (sulfamethazine, tetracycline, and streptomycin), pesticides (amitraz and coumaphos), and naphthalene used by beekeepers to prevent and control bee diseases and pests, sulfamethazine residues were detected in 3 sample, tetracycline in 2 sample, streptomycin in 2 sample, coumaphos in 2 sample, and amitraz in 6 sample.

No naphthalene residues were detected in any of the samples. Based on the analyses conducted, it was determined that out of the 13 honey samples collected from the region, 2 samples (samples 11 and 12) exceeded the limit values specified in the Turkish Food Codex Honey Communiqué. Additionally, 8 samples (samples 2, 5, 6, 7, 8, 10, 11, and 12) did not comply with the values specified in the European Union Standard and Codex Standards. However, except for samples 11 and 12, all other samples were found to comply with the Turkish Food Codex Honey Communiqué. Overall, only two samples (11 and 12) did not comply with either codex.

As a general observation from the results obtained in the study, it was noted that some beekeepers in the region, although not in large numbers, were using medications for bee diseases and pests, as well as some pesticides, either unconsciously or illegally. This poses health risks for bees, beekeepers, and consumers. However, the absence of naphthalene and similar PAHs, which are major issues in honey exports, indicates that there is increased awareness among beekeepers on this matter. To address the problem of the use of unlicensed antibiotics, particularly against brood diseases, and other medications not licensed for beekeeping that are intended for poultry, small livestock, and cattle, control mechanisms need to be more effectively enforced. Harmonization efforts between EU and Turkish regulations should be accelerated, and beekeepers should be trained on beekeeping practices, the prevention and control of bee diseases and pests, and how to produce high-quality bee products.

Acknowledgement: This study is derived from a section of the doctoral thesis titled 'Investigation of Occupational Health and Safety Risks in the Beekeeping Sector: A Case Study of the Upper Çoruh Valley Region and Bayburt,' completed by Mustafa ÖZDEMİR under the supervision of Dr. Osman YILDIZLAR at the Institute of Health Sciences, Avrasya University.

Author Contribution: Mustafa Özdemir: investigation, analysis, writing–original draft, writing–review & editing; Osman Yıldızlar: research planning, writing–review & editing.

Data Availability: Data are available in the manuscript.

Declaration of interest: The authors declare that there is no conflict of interest.

Ethics: Ethics committee approval is not required for this study.

Financial support: No financial support was received for this study.

REFERENCES

Arslanbaş E. Türkiye'de organik ve geleneksel olarak üretilen bazı hayvansal ve bitkisel ürünlerdeki metal (kurşun, kadmiyum, bakır, çinko, demir) düzeylerinin karşılaştırılması. Ankara Üniversitesi, Sağlık Bilimleri Enstitüsü, Doktora tezi, , Ankara, 2010 (erişim tarihi.20.12.2023).

Aygün O. Elazığ'da üretilen balların bazı toksik ağır metal düzeyleri. Fırat Üniversitesi Mühendislik Bilimleri Dergisi. 2020;32(1):119-125, https://doi.org/10.35234/fumbd.593396

Barel S, Zilberman D, Aydin L, Girisgin O, Efrat H, Kamer Y, vd. Distribution of coumaphos residues in Turkish-Israeli hives: A collaborative study. Uludağ Arıcılık Dergisi. 2011;11(2):47-50.

Bilgili A, Selçukoğlu E. Çukurova bölgesinden toplanan bal örneklerinde amitraz ve fluvalinat kalıntılarının belirlenmesi. İzmir Democracy University Health Sciences Journal. 2022;5(3):805-813, https://doi.org/10.52538/iduhes.1191545

Birinci S. Bayburt ilinin coğrafyası. Atatürk Üniversitesi, Sosyal Bilimler Enstitüsü, Doktora tezi, Erzurum, 2013, (erişim tarihi:

20.01.2024), https://tez.yok.gov.tr/UlusalTezMerkezi

Bogdanov S, Ryll G, Roth H. Pesticide residues in honey and beeswax produced in Switzerland. Apidologie. 2003;34:484-485.

Caldow M, Stead S, Day J, Sharman M, Situ C, Elliott C. Development and validation of an optical SPR biosensor assay for tylosin residues in honey. Journal of Agricultural and Food Chemistry. 2005;53(19):7367-70, https://doi.org/10.1021/jf050725s

Daş YK, Aksoy A. Ballarda ilaç kalıntılarının sağlık üzerine etkileri. Orduda Gıda Güvenliği. 2008;2(4):6-13.

Daş YK. Türkiye'de üretilen ballarda bazı organik fosforlu ve sentetik piretroid insektisit kalıntılarının incelenmesi. Ankara Üniversitesi, Sağlık Bilimleri Enstitüsü, Doktora tezi, Ankara, 2004, (erişim tarihi: 20.01.2024), https://tez.yok.gov.tr/UlusalTezMerkezi

Demirezen D, Aksoy A. Determination of heavy metals in bee honey using by inductively coupled plasma optical emission spectrometry (ICP-OES). G.U. J Sci. 2005;18:569-575.

Devillers J, Doré JC, Marenco M, Poirier-Duchêne F, Galand N, Viel C. Chemometrical analysis of 18 metallic and nonmetallic elements found in honeys sold in France. Journal of Agricultural and Food Chemistry. 2002;50:5998-6007, https://doi.org/10.1021/jf020497r

Dobrinas S, Birghila S, Coatu V. Assessment of polycyclic aromatic hydrocarbons in honey and propolis produced from various flowering trees and plants in Romania. Journal of Food Composition and Analysis. 2008;21:71-77, https://doi.org/10.1016/j.jfca.2007.07.003

Erbilir F, Erdoğrul Ö. Determination of heavy metals in honey in Kahramanmaraş city, Turkey. Environmental Monitoring and Assessment. 2005;109:181-187, https://doi.org/10.1007/s10661-005-5848-2

Erdoğan Ü, Erdoğan Y. Çoruh Vadisinin Yabani Meyveleri. Biyoloji Bilimleri Araştırma Dergisi. 2014;7(1):49-52.

Erdoğan Y, Erdoğan Ü. Pollen analysis of honeys from the Çoruh Valley (Türkiye). International Journal of Plant Animal Environmental Sciences. 2014;4:5-13.

Erdoğdu AT, Coşkun Y, İspirli Güven S. Tüketime sunulan ballarda sülfonamid türevi antibiyotiklerin kalıntılarının belirlenmesi. Bornova Veteriner Bilimleri Dergisi. 2011;33(47):37-44.

FAO. Food and agricultural organization of the United Nations. FAOSTAT. http:/www.fao.org/faostat/en/#data/TM, 2021, (erişim tarihi: 02.04.2024).

Fıratlı Ç, Genç F, Karacaoğlu M, Gencer HV. Türkiye arıcılığının karşılaştırmalı analizi sorunlar öneriler. TMMOB Ziraat Mühendisleri Odası Türkiye Ziraat Mühendisliği V. Teknik Kongresi, Ankara-Türkiye, Bildiriler Kitabı, 2000, p.811-826.

Frias I, Rubio C, González-Iglesias T, Gutiérrez ÁJ, González-Weller D, Hardisson A. Metals in fresh honeys from Tenerife Island, Spain. Bulletin of Environmental Contamination and Toxicology. 2008;80:30-33, https://doi.org/10.1007/s00128-007-9301-9

Garcia M, Fernandez M, Melgar M. Contamination of honey with organophosphorus pesticides. Bulletin of Environmental Contamination and Toxicology. 1995;54:825-832, https://doi.org/10.1007/BF00197965

Garcia M, Fernandez M, Herrero L, Melgar M. Acaricide residue determination in honey. Bulletin of Environmental Contamination and Toxicology. 1996;56:881-887, https://doi.org/10.1007/PL00002971

Gilliam M, Argauer R. Oxytetracycline residues in surplus honey, brood nest honey, and larvae after medication of colonies of honey bees, Apis mellifera, with antibiotic extender patties, sugar dusts, and syrup sprays. Environmental Entomology. 1981;10:479-482, https://doi.org/10.1093/ee/10.4.479

Gösterit A, Gürel F. Türkiye arıcılığının yapısı ve sürdürülebilir arıcılık olanakları. 4. Ulusal Zootekni Bilim Kongresi, Isparta-Türkiye, Bildiriler Kitabı. 1-3 Eylül 2004, p.24-27.

Gül A. Türkiye'de üretilen bazı balların yapısal özelliklerinin gıda güvenliği bakımından araştırılması. Mustafa Kemal Üniversitesi, Fen Bilimleri Enstitüsü, Doktora tezi, Hatay,

2008. (erişim tarihi: 20.01.2024), https://tez.yok.gov.tr/UlusalTezMerkezi

Gül A, Şahinler N, Akyol E, Şahin A. Organik arı yetiştiriciliği. MKU Ziraat Fakültesi Dergisi. 2005;10(1-2):63-70.

Güleç M. Türkiye'nin Doğu Anadolu Bölgesi'ndeki bazı illerden toplanan bal örneklerinde metal düzeylerinin belirlenmesi. Ankara Üniversitesi, Sağlık Bilimleri Enstitüsü, Doktora tezi, Ankara, 2007, (erişim tarihi: 20.01.2024), https://tez.yok.gov.tr/UlusalTezMerkezi

Gürcan EK, Soysal Mİ. Tekirdağ ili arı yetiştiriciliği üzerine bir araştırma. Tekirdağ Ziraat Fakültesi Dergisi. 2005;2(2):161-165.

Hammerling B, Augustyniak C, Risto C. Gesammt-amitraz rückstande in bienenhonigen. Die Nahrung. 1991;35(10):1047-1052, https://doi.org/10.1002/food.19910351012

Johnson RM, Ellis MD, Mullin CA, Frazier M. Pesticides and Honey Bee Toxicity–USA. Apidologie. 2010;41(3):312-331, https://doi.org/10.1051/apido/2010018

Kochansky J. Degradation of tylosin residues in honey. Journal of Apicultural Research. 2004;43:65-68, https://doi.org/10.1080/00218839.2004.11101113

Koday Z, Karadağ H. Türkiye'deki Arıcılık Faaliyetleri ve Bal Üretiminin Bölgesel Dağılımı (2007-2018). Atatürk Üniversitesi Sosyal Bilimler Enstitüsü Dergisi. 2020;24(1):495-510.

Lambert O, Durand S, Marchand P, Le Bizec B, Piroux M, Puyo S, Thorin C, Delbac F, Pouliquen H. Polycyclic aromatic hydrocarbons: Bees, honey and pollen as sentinels for environmental chemical contaminants. Chemosphere. 2012;86:98-104, https://doi.org/10.1016/j.chemosphere.2011.09.025

Matsuka M, Nakamura J. Oxytetracycline residues in honey and royal jelly. Journal of Apicultural Research. 1990;29:112-117, https://doi.org/10.1080/00218839.1990.11101206

Morzycka B. Simple method for the determination of trace levels of pesticides in honeybees using matrix solid-phase dispersion and gas chromatography. Journal of Chromatography A. 2002;982:267-273, https://doi.org/10.1016/S0021-9673(02)01505-4

Özkan O, Eşsiz D, Yazıcı K, Erdağ D. Ardahan ilinde üretilen ballarda antibiyotik kalıntı düzeylerinin araştırılması. Atatürk Üniversitesi Veteriner Bilimleri Dergisi. 2015;10(2):88-92, https://doi.org/10.17094/avbd.31906

Przybyloeski P, Wilczynska A. Honey as an environmental marker. Food Chemistry. 2001;74:289-291, https://doi.org/10.1016/S0308-8146(01)00153-4

Reybroeck W. Residues of antibiotics and sulphonamides in honey on the Belgian market. Apiacta. 2003;38:23-30.

Saridaki-Papakonstadinou M, Andredakis S, Burriel A, Tsachev I. Determination of tetracycline residues in Greek honey. Trakia Journal of Science. 2006;4(1):33-36.

Selçukoğlu E. Çukurova bölgesinde toplanan bal örneklerinde amitraz ve fluvalinate kalıntılarının belirlenmesi. Ankara Üniversitesi, Sağlık Bilimleri Enstitüsü, Doktora tezi, , Ankara, 1999 (erişim tarihi: 20.01.2024), https://tez.yok.gov.tr/UlusalTezMerkezi

Silici S, Uluözlü ÖD, Tüzen M, Soylak M. Assessment of trace element levels in rhododendron honeys of Black Sea region, Turkey. Journal of Hazardous Materials. 2008;156:612-618, https://doi.org/10.1016/j.jhazmat.2007.12.065

Söğüt B, Şeviş HE, Karakaya E, İnci H, Yılmaz HŞ. Bingöl İlinde arıcılık faaliyetinin mevcut yapısı üzerine bir araştırma. Türk Tarım ve Doğa Bilimleri Dergisi. 2019;6(2):168-177, https://doi.org/10.30910/turkjans.556596

Sunay AE. Balda antibiyotik kalıntısı sorunu. Uludağ Arıcılık Dergisi. 2006;6(4):143-148.

Tananaki C, Thrasyvoulou A, Karazafiris E, Zotou A. Contamination of honey by chemicals applied to protect honeybee combs from wax-moth (*Galleria mellonela* L.). Food Additives and

Contaminants. 2006;23(2):159-163, https://doi.org/10.1080/02652030500350248

Tüzen M, Silici S, Mendil D, Soylak M. Trace element levels in honeys from different regions of Turkey. Food Chemistry. 2007;103:325-330, https://doi.org/10.1016/j.foodchem.2006.07.053

WHO, FAO. Evaluation of Certain Food Additives and the Contaminants Mercury, Lead and Cadmium. Sixteen Report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva; 1972, No. 505, p.11-24.

Wilczynska A, Przybylowski P. Residues of organochlorine pesticides in Polish honeys. Apiacta. 2007;42:16-24.

Yarsan E, Karaçal F, İbrahim İG, Dikmen B, Köksal A, Daş YK. Contents of some metals in honeys from different regions in Turkey. Bulletin of Environmental Contamination and Toxicology. 2007;79:255-258, https://doi.org/10.1007/s00128-007-9034-9.

Citation/Atıf: Tarekegn A. Identification, characterisation, and evaluation of honey bee flora in Benishangul Gumuz regional state, Ethiopia. U. Bee J. / U. Arı D. 2025; 25 (1): 19-31. DOI: 10.31467/uluaricilik.1587466

ARAŞTIRMA MAKALESI / RESEARCH ARTICLE

IDENTIFICATION, CHARACTERISATION, AND EVALUATION OF HONEY BEE FLORA IN BENISHANGUL GUMUZ REGIONAL STATE, ETHIOPIA

Benishangul Gumuz Bölgesel Eyaleti, Etiyopya'da Bal Arısı Florasının Tanımlanması, Karakterizasyonu ve Değerlendirilmesi

Alayu TAREKEGN

Ethiopian Institute of Agricultural Research, Assosa Agricultural Research Center, Assosa ETHIOPIA, Corresponding author / Yazışma Yazarı E-mail: alayutarekegn68@gmail.com, ORCID No: 0000-0001-6736-5264

Received / Geliş:18.11.2024 Accepted / Kabul:31.01.2025 DOI: 10.31467/uluaricilik.1587466

ABSTRACT

This study aimed to identify and evaluate the major and minor bee forage sources to recommend seasonal colony management practices. Conducted in the Bambasi, Homosha, and Mao Komo districts of Benishangul Gumuz Regional State in western Ethiopia, the research involved a survey of 90 beekeepers using semi-structured questionnaires from three peasant associations per district. Honey and pollen samples were collected from established colonies in each district for melissopalynological analysis. 71 plant species were identified as forage sources for honeybees in the study area. Two primary flowering periods were observed in 2020, 2021, and 2022 G.C., corresponding to the honey harvesting seasons. During the first season, key plants included *Pterocarpus lucens, Bidens prestinaria, Glycine* species*, Guizotia abyssinica, Guizotia scabra,* and several *Bidens* species*.* From February to May, the main sources of pollen and nectar in the second season were woody plants such as *Cordia africana, Syzygium guineense, Turraeanthus africanus,* and *Terminalia laxiflora*. In the first honey flow season*, Guizotia scabra* and *Guizotia abyssinica* made up 62.13% of monofloral honey*.* In the second season*, Turraeanthus africanus* and *Syzygium guineense* contributed 48.23%. In Benishangul Gumuz, beekeepers report that food scarcity peaks during the rainy season (late July to August) and the dry season (December to January) when flowering plants are limited. Providing supplemental food and water and conducting regular inspections is important to support the bee colonies during these times. Additionally, rapid biodiversity loss from deforestation and agricultural expansion reduces available bee forage in the region.

Keywords: Bee flora, Bee forage, Honey*, Guizotia* species*,* Melissopalynology

ÖZ

Bu çalışma, mevsimsel koloni yönetimi uygulamalarını tavsiye etmek için ana ve küçük arı yem kaynaklarını belirlemeyi ve değerlendirmeyi amaçlamıştır. Batı Etiyopya'daki Benishangul Gumuz Bölgesel Eyaleti'nin Bambasi, Homosha ve Mao Komo ilçelerinde yürütülen araştırma, ilçe başına üç köylü derneğinden yarı yapılandırılmış anketler kullanılarak 90 arıcı ile yapılan bir anketi içermektedir. Melissopalynolojik analiz için her ilçedeki yerleşik kolonilerden bal ve polen örnekleri toplanmıştır. Çalışma alanında 71 bitki türü bal arıları için yem kaynağı olarak tanımlanmıştır. Bal hasat mevsimlerine denk gelen 2020, 2021 ve 2022 yıllarında iki ana çiçeklenme dönemi gözlemlenmiştir. İlk sezonda, kilit bitkiler şunları içeriyordu Pterocarpus lucens, Bidens prestinaria, Glycine türleri, Guizotia abyssinica, Guizotia scabra ve birkaç Bidens türü içermektedir. Şubat ayından Mayıs ayına kadar, ikinci sezonda ana polen ve nektar kaynakları Cordia africana, Syzygium guineense, Turraeanthus africanus ve Terminalia laxiflora gibi odunsu bitkilerdir. İlk bal akış sezonunda, Guizotia scabra ve Guizotia abyssinica monofloral balın %62,13'ünü oluşturmuştur. İkinci sezonda, Turraeanthus africanus ve

Syzygium guineense %48,23 oranında katkı sağlamıştır. Benishangul Gumuz'da arıcılar, çiçekli bitkilerin sınırlı olduğu yağmur mevsimi (Temmuz sonundan Ağustos'a kadar) ve kurak mevsimde (Aralık'tan Ocak'a kadar) gıda kıtlığının zirve yaptığını bildirmektedir. Bu dönemlerde arı kolonilerini desteklemek için ek gıda ve su sağlamak ve düzenli denetimler yapmak önemlidir. Ayrıca, ormansızlaşma ve tarımsal genişlemeden kaynaklanan hızlı biyoçeşitlilik kaybı, bölgedeki mevcut arı yemini azaltmaktadır.

Anahtar Kelimeler: Arı florası, Arı yemleri, Bal, Guizotia türleri, Melissopalynoloji

GENİŞLETİLMİŞ ÖZET

Amaç: Bir çiçek takvimi oluşturmak, kovan ballığı eklemek ve bal akışını tahmin etmek gibi temel faaliyetleri düzenlemek için çok önemlidir. Bu nedenle, bu çalışma etkili mevsimsel koloni yönetimi uygulamaları önermek için temel arı yemlerini tanımlamayı, karakterize etmeyi ve değerlendirmeyi amaçlamaktadır.

Gereç ve yöntem: Çalışma, Batı Etiyopya'nın Homosha, Bambasi ve Benishangul Gumuz Bölgesel Eyaleti'nin Mao Komo özel bölgelerinde gerçekleştirilmiştir. Her ilçedeki üç köylü derneği, arıcılık potansiyellerine ve agroekolojik farklılıklarına göre seçildi. 2020'den 2022'ye kadar, çiçek yoğunluğu yüksek olan çeşitli bitkiler incelenmiştir. Bal ve polen örnekleri toplamak için dokuz bölgede arı kolonileri kurulmuştur. Her sahada beş kutu kovan kurulmuştur: ikisi polen yakalama ve üçü bal örnekleme için. Bal örnekleri Kasım-Aralık ayları arasındaki büyük ve küçük akış mevsimlerinde ve Nisan ayında botanik kökenlerini belirlemek üzere laboratuvar analizi için toplanmıştır. Sonuçlar, çalışma alanında bal arıları için yem kaynağı olarak yetmiş bir bitki türünün tespit edildiğini göstermektedir. Bu türlerin yüksek çeşitliliği, arıcılık için uygun olan yağlı tohumlar, tahıllar, bakliyat ve bahçe bitkileri de dahil olmak üzere doğal olarak oluşan bitkilerden ve ekili ürünlerden kaynaklanmaktadır. Tespit edilen bitki türlerinin 44'ü (%61,98) ağaç, 12'si (%16,90) ot, 9'u (%12,67) çimen, 3'ü (%4,23) çalı, (%2,81) tırmanıcı ve 1'i (%1,41) asmadır. Çalışılan üç ilçede de bal arısı bitkileri için iki ana çiçeklenme dönemi vardı ve bu da bölgedeki iki bal hasat dönemine karşılık geliyordu. Tespit edilen arı yemi türlerinin %70'i Eylül ve Kasım ayları arasında çiçeklenmiştir. İlk bal akışı sezonunda birincil ve en değerli arı yemi bitkileri Pterocarpus lucens, *Bidens* prestinaria, *Glycine* türleri, *Guizotia abyssinica*, *Guizotia scabra* ve diğer *Bidens* türlerini içeriyordu. Buna karşılık, Şubat-Mayıs ayları arasında gerçekleşen ikinci bal akışı sezonunda, *Cordia africana, Syzygium guineense* ve *Terminalia laxiflora* gibi odunsu bitkiler ana polen ve nektar kaynakları olarak öne çıkmaktadır. Çalışma alanında bitkilerin dağılımına bakıldığında en fazla türün yayla bölgesinden toplandığı görülmektedir.

Bulgular ve sonuç: Sonuçlar, ağaç arısı yemlerinin bolluğunun, ikinci bal akışı sezonu için hayati önem taşıyan kurak mevsimde daha yüksek olduğunu göstermiştir. Polen örnekleri çalışma alanının farklı bölgelerinden toplanmıştır *Guizotia* türleri ve Terminalia laxiflora çalışma alanlarında tespit edilen başlıca polen kaynağı bal arısı bitki türleridir. *Guizotia scabra* ve *Guizotia abyssinica* monofloral bal üretimi için önemli olup, %62,13 sıklıkta gözlenmiştir. Benishangul Gumuz'da arıcılar, gıda kıtlığının en yoğun olduğu dönemlerin Temmuz sonundan Ağustos'a kadar olan yağışlı sezonda ve Aralık'tan Ocak'a kadar olan kurak sezonda gerçekleştiğini gözlemlemiştir. Belirlenen polen ve nektar kaynaklarının besin değerini değerlendirmek için araştırma yapılması gerekmektedir. Tarım alanları içinde ve çevresinde arı dostu bitkileri entegre eden tarımsal ormancılık sistemlerinin teşvik edilmesi de önemlidir.

INTRODUCTION

Ethiopia has abundant natural and cultivated flora, along with diverse agro-ecological and climatic conditions that are ideal for beekeeping (Jacobs et al. 2006). Ethiopia produces 66221.82 tons of honey and 6,000 tons of wax (CSA 2018), showcasing its significant contributions to the honey and wax industries. The honey is exceptional for its distinct flavor, aroma, and color, all of which are influenced by the floral sources and geographical origin (Belay et al. 2017). However, many beekeepers still rely on traditional methods, which are less efficient and can lead to lower honey yields and quality.

Western Ethiopia is recognized as a promising area

for commercial and small-scale beekeeping due to its high vegetation density and a substantial population of honeybees (Addi and Bareke 2019). Benishangul Gumuz, one of the regional states in western Ethiopia, is particularly rich in natural resources and has favorable climatic conditions supporting beekeeping development. In this region, beekeeping is a significant income source for communities living near the forests (Abebe et al. 2016).

Success in beekeeping relies heavily on the availability and abundance of floral resources for bees (Addi et al. 2014). The development of beekeeping in any region requires a thorough understanding of the local flora, including the length of flowering periods, flowering phenology, and the nectar and pollen production of various bee plants (Wubie et al. 2014). There is a strong correlation between the seasonal cycles of honeybee colonies and the flowering calendar of bee plants (Haftom et al. 2014). This relationship can be utilized for effective seasonal management of bee colonies. It is essential to time management operations by the phenological patterns of local bee plants, as this is crucial for building up colony populations before the main nectar flow. While bees naturally increase their population when resources are abundant, beekeepers must take steps to ensure that the colony reaches its peak population size either before or during the nectar flow.

Different locations exhibit variations in plant species composition and flowering durations due to factors such as topography, climate, and cultural and agricultural practices (Reinhard and Admasu 1994). To achieve successful beekeeping, it is essential to have extensive knowledge of the types, population density, and quality of floral rewards, including nectar and pollen. The diversity of honey types produced in a specific area is influenced by the variety of nectar-producing plant availability (Silici and Gökceoglu 2007). This diversity can be identified through honey pollen analysis.

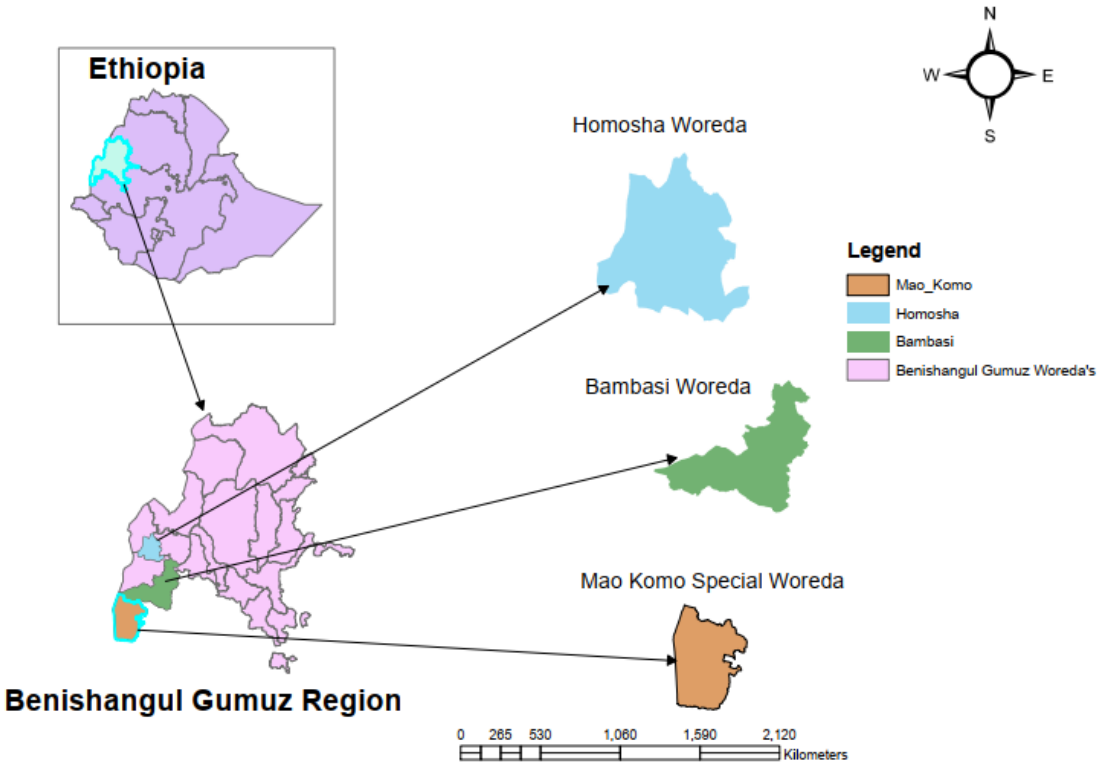
Melissopalynology is the definitive study of pollen grains found in honey (Louveaux et al., 1978). This analysis is crucial for determining the geographical and botanical origins of honey through microscopic examination of honey sediments. The study is a more reliable method than visual surveys for investigating honeybee forage, making it an essential tool for the development of regional apiculture (Begum et al. 2021). Honey can be classified as monofloral or multifloral. Monofloral honey is primarily derived from the pollen of a single plant species, while multifloral honey contains pollen from multiple species (Louveaux et al. 1978). A study conducted in southwest Ethiopia identified *Terminalia* spp., *Guizotia* spp., and *Bidens* spp. as the most important secondary pollen sources, with *Eucalyptus camaldulensis* being the predominant pollen type (Tulu et al. 2023). Furthermore, another study indicated that the leading pollen sources during the first honey harvesting season were *Guizotia* species, while *Coffea arabica* was predominant in the second harvesting season in the western Oromia region of Ethiopia (Tesfaye et al. 2023).

In Benishangul Gumuz, the assessment of bee forages is insufficient to identify high-performing plants that could enhance beekeeping practices. The major and minor bee plants identified through honey pollen analysis lack proper documentation, and their relationships to the seasonal colony management calendar are not well established. This oversight hinders the recognition of unique production opportunities, thereby limiting the potential for sustainable beekeeping that could benefit local economies. To improve honey production using the region's resources, it is essential to document economic bee forages and their flowering calendars. While creating a complete overview of all flowering plants may not be feasible, focusing on major bee forage plants and their flowering times will support effective beekeeping management. Additionally, by mapping floral resources, researchers can identify areas of high biodiversity and implement conservation measures to protect these valuable ecosystems. Establishing a floral calendar is crucial for organizing key activities, such as adding hive supers and predicting honey flow. Therefore, this study aims to identify, characterize, and evaluate key bee forages to recommend effective seasonal colony management practices.

MATERIALS and METHODS

The study was conducted in the Homosha, Bambasi, and Mao Komo special woredas of the Benishangul Gumuz Regional State in western Ethiopia, located between 9°30'N to 11°39'N latitude and 34°20'E to 36°30'E longitude. These districts represent lowland, midland, and highland agro-ecologies. Assosa, the regional capital, is 670 km west of Addis Ababa, with

Mao Komo 105 km south, Bambasi 40 km southeast, and Homosha 30 km north of Assosa (Fig. 1).
The region experiences a long uni-modal rainfall pattern (May to November), with annual precipitation ranging from 340.9 mm in 1996 to 2417.5 mm in 2000, averaging around 1146 mm. The temperatures in the area vary, with minimum average temperatures between 16.8 °C and 20.6 °C, and maximum averages ranging from 27 °C to 35.1 °C and elevation from 580 to 2731 masl (NMA 2017). The predominant vegetation consists of woodlands and shrubs, which cover 77% of the region, while grasslands and cultivated land account for 3% and 5%, respectively (AsARC 2006, unpublished).
The local farming system primarily involves mixed crop-livestock production (WBISPP 2003). The main crops grown in the area include sorghum, maize, haricot beans, soybeans, sweet potatoes, onions, mangoes, and various other fruits and vegetables. The key cash crops of the region are sesame, 'nug' (*Guizotia abyssinica*), and red pepper.



Figur 1. Map of the study area

Survey and inventory of bee forages

In each district, three peasant associations were chosen based on their potential for beekeeping and prior experience. From 2020 to 2022 G.C., a comprehensive survey was conducted to examine various plants, including trees, shrubs, grasses, crops, and weeds that exhibit high floral density. To collect primary data, semi-structured questionnaires were administered to 90 beekeepers selected from the nine peasant associations. Furthermore, secondary data was collected from district and zonal agricultural offices. Beekeepers categorise flowers into major and minor bee floras. Major bee floras were attracted a larger number of bees, while minor bee floras were visited by fewer bees and less often (Teklay 2011). This distinction was based on the quantity and frequency of bee visits to the flowers.

Participatory Rural Appraisal techniques were employed, which included focused group discussions, resource mapping, mental and social mapping, modeling, transects, and historical timelines, ranking, and scoring preferences, and observation with model beekeepers, development agents, bee technicians, and experts. These discussions aimed to identify local honeybee plants and their flowering seasons. Following these sessions, plant samples were collected for botanical identification, noting features such as leaves and

flowers, with assistance from a biodiversity expert from the Assosa biodiversity office. The area's bee flora was further validated through direct observation and published reports using taxonomic keys in Books of Flora of Ethiopia and Eritrea (Hedberg 1996) and Honey Bee Flora of Ethiopia (Reinhard and Admasu 1994).

Honey bee flora cover-abundance

The occurrence of the plant species in each agro-ecologies was determined by bee flora composition and diversity in the study area. The size of the quadrate used in the cultivated land and pasture was 25 m^2 (5 m*5 m), while for closed forest area used 400 m^2 (20 m*20 m), and for homestead land 100 m^2 (10 m*10 m) quadrate in a two-kilometer radius every 0.1 km from the hive. A total of 30 plots were taken from each district, representing different agro-ecosystems. At each quadrant observations of the plant were included whether the plant was visited by honeybees, flowering period, and food source. Then cover within a quadrat determined which was occupied by the above-ground parts of each bee plant species when viewed from above. Cover abundance was estimated visually as a percentage by stratification in multiple layering of vegetation types (trees, shrubs, and herbaceous). For recording the cover-abundance determination was used Braun-Blanquet scale from r (one or few individuals with less than 1% cover of the area) to 5 (75- 100% cover of the total area irrespective of the number of individuals) (Westhoff and Van der Maarel 1973).

Honey and pollen sample collection

For honey and pollen sample collection colonies were established in selected nine sites in the study area. In each site, five honey bee colonies were established in box hives (two for pollen trapping and three for honey sampling). To determine the botanical origin of the honey in the laboratory, fresh honey samples from each site were collected at major and minor honey flow seasons from November to December and April respectively.

To collect the pollen, honeybee colonies were fitted with pollen traps with 16% pollen trapping. Pollen loads were collected twice every seven days intervals. Pollen samples were dried and sorted by colour at Assosa Agricultural Research Center Laboratory and maintained until used for analysis. The collected honey and pollen samples were brought to the Holota Bee Research Centre Laboratory, using sterile glass cup honey containers for Melissopalynological analysis.

Pollen extraction from honey was carried out according to the method described by Louveaux et al. (1978). For this, 10 grams of honey was dissolved in 20 ml of warm distilled water and was stored at a temperature range of 20-40°C. The solution was centrifuged at 3800 rpm for 10 minutes and decanted the supernatant. Again, 20 ml of distilled water was added to completely dissolve the remaining sugar crystals and again centrifuged at 3800 rpm for 5 minutes and the supernatant was removed. The remaining precipitate was spread evenly on a microscope slide and the sample was exposed to air dry. Finally, one drop of glycerin jelly was added to the coverslip and examined under the light microscope (Zeiss AxioVert, Mg. Power 40*x*), and the morphological structure of selected pollen pictures were taken from each slide. The source of dominant pollen plants was then identified using reference slides and a pollen atlas (Adgaba 2007). Observation of fungal spores, soot particles, fine granular mass, and mineral particles were also included in the analysis.

The nomenclature of honey based on the frequency classes was determined by samples of percentage calculation, 500-1000 pollen grains had to be counted and the following terms have been adopted. The types of pollen were allocated to one of four frequency classes for nectar source plants: predominant pollen (>45%); accompanying pollen (16%-45%); important isolated pollen (4%-15%); and isolated pollen (3%) (Louveaux et al 1978). The percentages of pollen types in each honey sample were calculated based on the total number of different kinds of pollen grains counted for each honey sample. Then the honeys with predominant pollen types were considered as monofloral and if there was no predominant pollen then this kind of honey was classified as multifloral.

Richness and diversity of bee forage plants

The Shannon-Wiener diversity index, species richness, and Shannon's evenness were used to determine the diversity of bee forage plant species. Shannon index (H') =Ȼ (pi*lnpi), where H' = Shannon index, pi= proportion of individual species, and Ln= log base n; Evenness (J) = H'/ H'max = H'/INS, where H' =Shannon diversity index, H'max = lns where S was the number of species, Ln = logbase.

Data analysis

Descriptive statistics, including mean, frequency, percentage, and standard deviation, were used to

summarize primary and secondary data. Secondary sources were obtained from the Agricultural office and research center around the study area. SPSS (version 23) was used to analyze the data.

RESULTS

Honey bee forages

Seventy-one plant species were identified as forage sources for honeybees in the study area. The high diversity of these species results from naturally occurring plants and cultivated crops, including oilseeds, cereals, pulses, and horticultural crops, all suitable for beekeeping. Among the plant species identified, 44 (61.98%) were trees, 12 (16.90%) were herbs, 9 (12.67%) were grasses, 3 (4.23%) were shrubs, (2.81%) were climbers, and 1 (1.41%) was a vine (Table 1). In the study area, fifteen tree species were identified as having a high abundance among bee forage species. The major bee forage species during the first honey harvesting season (mid-November) included *Pterocarpus lucens, Acacia hecatophylla, Cordia africana, Croton macrostachyus, Ficus sycomorus,* and *Lannea welwitschii*, all of which were particularly important for honeybees. During the second honey flow season (April) beekeepers identified *Syzygium guineense* and *Terminalia laxiflora* as the most important honeybee plants. Notably, the beekeepers emphasized that *Syzygium guineense* and *Terminalia laxiflora* were major sources of forages for honeybees and play a critical role in honey production due to their abundant flowers, which provide excellent nutrition for the bees.

The beekeepers identified several key herbaceous and grass species that were frequently visited by bees for pollen and nectar. Among these abundant species, compared to other herbs and grasses were *Bidens prestinaria, Guizotia abyssinica, Guizotia scabra, Sorghum bicolor,* and *Bidens pilosa*. *Bidens* species*, Guizotia scabra,* and *Plantago larceolata* were found in both forested and cultivated areas, playing a crucial role during the honey flow season (Table 1).

Table 1: Honeybee forage species, identified based on survey and field observation

Habit	Botanical Name	Local Name	Pollen (P) /nectar (N)	Flowering Time	Source	Abundance
Tree	*Accacia hecatophylla*	Qudo	P and N	Sept-Feb	Major	+
	Cordia africana	Wanza	P and N	Oct-March	Major	r
	Croton macrostachyus	Bekanisa	P and N	June-Sept	Major	+
	Ficus sycomorus	Shola	P and N	May-Sept	Major	r
	Lannea welweschi	Quwa	P and N	Sept-Nov	Major	r
	Pterocurpus lucens	Amiraro	P and N	Nov - Dec	Major	+
	Syzigium guineense	Dokma	N	March-May	Major	2a
	Terminalia laxiflora	Ashure	P and N	Dec-May	Major	2b
	Turraeanthus africanus	Yechaka	P and N	March-April	Major	r
	Acacia seyal	Kesh	P and N	Sept-Nov	Minor	2a
	Mangifera indica	Mango	P and N	Dec-April	Minor	2a
	Annona senegalensis	Adegela	P and N	March-June	Minor	1
	Boswellia papyrifera	Etan zaf	P and N	Sept-Dec	Minor	1
	Breonadia salicina	Debesa	P and N	August-Dec	Minor	2a
	Catha edulis	Chat	P and N	Throughout	Minor	1
	Citrus somensis	Burtukan	P and N	May-Sept	Minor	+
	Coffea arabica	Coffee	P and N	Feb- August	Minor	1
	Combretum molle	Ageraei	P and N	April-July	Minor	1
	Dalbergia boehmii	Tseba	P and N	Feb-May	Minor	r
	Erythrina abyssinica	Ambelsh	P and N	Oct-Jan	Minor	2a
	Erythrina brucei	Embelsh	P and N	Oct-Jan	Minor	2a
	Eucalyptus saligna	Bahrzaf	P and N	Sept- Dec	Minor	1
	Faurea speciosa	Atete	P and N	Nov-Feb	Minor	2a
	Ficus ingens	Bambegle	P and N	Feb-Sept	Minor	2a
	Ficus lutea	Warka	P and N	August-Oct	Minor	r

	Ficus sycomorus	Arbu	P and N	July-Oct	Minor	1
	Ficus vasta	Hodo	P and N	July-Oct	Minor	r
	Gardenia ternifolia	Ankeda	P and N	Sept-Nov	Minor	1
	Gardenia volkensi	Gambela	P and N	Sept-Jan	Minor	2a
	Grewia mollis	Aroresa	P and N	July-Sept	Minor	1
	Kotschya africana	Sseqe	P and N	March-June	Minor	r
	Loncocarpus laxiflorus	Afud	P and N	Dec-May	Minor	1
	Manilkara butuji	Butiji	P and N	Jan-April	Minor	2a
	Maytenus senegalensis	kombolch	P and N	Nov-April	Minor	2a
	Mytenus senegalensis	Agero	P and N	Nov-April	Minor	2a
	Persea americana	Avocado	P and N	Sept-Dec	Minor	r
	Phoenix reclinata	Zenbaba	P and N	June-Oct	Minor	r
	Pilostigma thunningii	Megel	P and N	May-Oct	Minor	2a
	Psidium guajava	Zeytuna	P and N	Oct-Nov	Minor	r
	Securidaca longepedanculata	Sheqet	P and N	March-June	Minor	r
	Strychnos innocua	Abunbuqo	P and N	April-July	Minor	1
	Vepris spp.	Zafi	P and N	Oct-Nov	Minor	2a
	Vitex doniana	kurkura	P and N	June-Oct	Minor	2a
	Ziziphus abyssinica	Qurqura	P and N	August-Nov	Minor	r
Shrubs	*Phoenix dactylifera*	Zenbaba	P and N	June-Sept	Minor	r
	Vernonia amygdolina	Grawa	P and N	Dec-Feb	Minor	2
	Ximenia americana	Bibi	P and N	August-Nov	Minor	2
Vines	*Cesalpinea* spp.	Ashi	P and N	Throughout	Minor	2
Climbers	*Cuccurbita pepo*	Duba	P and N	July-August	Minor	2
	Saba comorensis	Bishqore	P and N	August-Feb	Minor	2
Herbs	*Bidens pilosa*	Adeketsiya	P and N	Sept-Nov	Major	3
	Bidens prestinaria	Abumerery	P and N	Sept-Nov	Major	3
	Glycine spp.	Akuri	P and N	August-Oct	Major	1
	Guizotia abyssinica	Nug	P and N	Oct-Nov	Major	2b
	Guizotia scabra	Ada	P and N	Sept-Oct	Major	3
	Plantago larceolata	Arem	P	June-Oct	Major	3
	Solanum dasyphyllum	adro	P and N	June-Sept	Major	3
	Abelmoscus esculantus	Qeneqse	P and N	August-Oct	Minor	1
	Acanthus polystachius	Sokoru	P and N	Oct-Jan	Minor	1
	Amaranthus hybridus	Tsunda/Tika	P and N	June-Oct	Minor	r
	Capsicum minimum	Mitimita	P and N	May-July	Minor	r
	Vigna subterranea	Almdmese	P and N	August-Oct	Minor	r
Grasses	*Cyndon dectylon*	Serdo	P	May-Oct	Major	1
	Sorghum bicolar	Mashila	P	Oct-Dec	Major	2a
	Zea mays	Bekolo	P	July-Sept	Major	2b
	Andropogon schirensis	Abandu	P and N	May-Sept	Minor	1
	Cyndon nlemfuensis	Mergagogor	P and N	May-Oct	Minor	1
	Hyparrhenia diplandra	Cheto	P and N	Sept-Nov	Minor	1
	Hyperhina spp.	Muja	P and N	May-Sept	Minor	1
	Oxythenantra abyssinica	Bamboo	P and N	Sporadic	Minor	1
	Rhodus spp.	Yekoksar	P and N	July-Sept	Minor	+

Codes of cover-abundance: r= One or few individuals, += Occasional and less than 5%, 1= Abundant and with very low cover or less abundant but with higher cover; in any case less than 5% cover of total area, 2a= Very abundant and less than 5% cover, 2b= 5–12.5% cover, irrespective of number of individuals, 2c= 12.5–25% cover of total area, irrespective of number of individuals, 3= 25–50% cover of total area, irrespective of number of individuals, 4= 50–75% cover of total area, irrespective of number of individuals, 5= 75–100% cover of total area, irrespective of number of individuals

Season of flowering

In all three districts studied, there were two main flowering periods for honeybee plants, corresponding to two honey harvesting periods in the area. Seventy percent of the identified bee forage species flowered between September and November. The primary and most valuable bee forage plants during the first honey flow season included *Pterocarpus lucens, Bidens prestinaria, Glycine* species*, Guizotia abyssinica* (Fig 2)*, Guizotia scabra,* and other *Bidens* species.

Figur 2. Nug (*Guizotia abyssinica*)

In contrast, the second honey flow season, which was occurred from February to May, features woody plants such as *Cordia africana* (Fig 3)*, Syzygium guineense,* and *Terminalia laxiflora* as the main sources of pollen and nectar. The discussions and individual responses from beekeepers revealed that in the first season, flowered species yielded honey of varying quality, with more brood produced and poorer quality compared to the honey harvested in April of the second season.

Figur 3. Wanza (*Cordia africana*)

Species diversity, richness, and evenness of honeybee plant species

In the study area regarding the distribution of plants, the highest number of species has been collected from the highland area (Table 2). The results indicated that the abundance of tree bee forages was higher during the dry season which was vital for the second honey flow season. The value of the Shannon-Weaver diversity index indicated that in the highland area of the region, the honey bee flora species were more evenly distributed than the midland and lowland (Table 2). Moreover, the Shannon evenness value were ranged from 0.72-0.80 showing that the honeybee plant species counted in the study areas were evenly distributed in the sample plots and sites.

Table 2: Species diversity and species richness and evenness

Factors	Agro ecologies		
	Lowland	Midland	Highland
Species richness	63	55	71
Shannon species diversity	3.2	2.89	3.41
H'max (lns) Shannon	4.14	4.00	4.26
Evenness	0.77	0.72	0.80

Bee pollen sources

The pollen samples were collected in different sites of the study area *Guizotia* species and *Terminalia laxiflora* were the major pollen-source honeybee plant species identified in the study areas (Table 3). On the other hand, *Plantago larceolata, Zea mays,* and *Bidenis* spp. were the secondary pollen sources of honey bee plant species, as the present findings indicated.

Table 3. Pollen sources for Bee Foraging in the study areas

N	Districts	Predominant pollen source	Secondary pollen source	Important minor pollen source	Minor pollen source
4	Bambasi	*Guizotia* spp.	*Plantago lanceolata*		
4	Bambasi	*Guizotia* spp.	*Plantago lanceolata*		
4	Bambasi	*Terminalia laxiflora*	*Zea mays, Bidenis* spp.	*Guizotia* spp.	
4	Homosha	*Terminalia laxiflora*	*Annona senegalensis*	*Syzigium guineense*	
4	Homosha	*Guizotia* spp.	Grass		
4	Homosha	*Guizotia*, spp.	*Turraeanthus africanus*	Grass species	
4	Mao komo	*Guizotia* spp.			
4	Mao komo	*Terminalia laxiflora*	*Plantago lanceolata*		
4	Mao komo	*Turraeanthus africanus*	*Plantago lanceolata*	*Guizotia* spp	

Botanical name of honey

The honey pollen analysis was revealed a diverse array of 21 plant species that serve as nectar sources for honey production. Among these, *Guizotia scabra* and *Guizotia abyssinica* were emerged as the most significant contributors to monofloral honey, boasting an impressive frequency percentage of 62.13%. This indicated their prevalence and importance in the region's honey production. Furthermore, the melissopalynological analysis of honey samples highlighted the dominant pollen types present, pinpointed *Syzygium guineense* and *Turraeanthus africanus* as the primary tree plant species involved in the formation of monofloral honey (48.23%) in the study area. Their abundance reflected not only their role as essential nectar sources but also their ecological relevance within the local environment.

Dearth period for honeybees

In Benishangul Gumuz, beekeepers have observed that the peak periods of food scarcity were occurred during the rainy season, from late July to August, and during the dry season, from December to January. During these times, there are limited flowering plants, which means fewer sources of pollen and nectar for the bees. To help support the bee colonies through these two challenging periods, it was crucial to provide them with supplemental food and water, as well as to conduct regular inspections of the colonies. The group discussions also revealed that smallholder farmers in the study areas were the primary users of pesticides, which negatively impacts both the honey bees and the plant species they rely on, leading to increased cases of honey bee colony absconding. Furthermore, the region has experienced a rapid biodiversity loss in bee forage due to deforestation and the expansion of cultivated land.

DISCUSSION

The findings from the study conducted in Benishangul Gumuz, western Ethiopia, reveal a robust ecological framework supporting honeybee foraging, characterized by a diverse array of flora. The identification of seventy-one plant species as forage sources for honeybees underscores the ecological richness of the region. This diversity is attributed to both naturally occurring flora and cultivated crops, including oilseeds, cereals, pulses, and horticultural varieties, which collectively enhance the beekeeping potential in the area. The results align with previous research by Addi and Bareke (2019), who documented seventy-four honeybee forage species in southwestern Ethiopia, indicating a regional consistency in floral diversity that supports apiculture. Such findings highlight the importance of maintaining ecological integrity in agricultural landscapes to sustain honeybee populations and, by extension, local biodiversity.

The value of the Shannon-Weaver diversity index in this study provides critical insights into the distribution patterns of honeybee forage species across different ecological zones. The higher

evenness observed in the highland areas compared to midland and lowland regions suggests that the floral resources available to honeybees are more uniformly distributed, thereby potentially enhancing pollination efficiency and honey production. This observation corroborates the work of Wubie et al. (2014), who noted greater species diversity and richness in highland agro-ecological systems. While the evenness values ranging from 0.72 to 0.80 indicate a relatively balanced distribution of honeybee forage species, they are lower than those reported by Debissa and Amsalu (2006), which ranged from 0.79 to 0.89. This difference may reflect variations in sampling methods, ecological conditions, or the specificities of regional flora. However, the values are notably higher than those documented by Addi and Bareke (2019), underscoring the uniqueness of the Benishangul Gumuz ecosystem.

The present study's findings highlight the significant role of various herbaceous and woody plant species as forage resources for honeybees in the study area. Notably, *Bidens prestinaria*, *Guizotia abyssinica*, *Guizotia scabra*, *Sorghum bicolor*, and *Bidens pilosa* were identified as abundant species that contribute to the floral diversity essential for honey production. The presence of *Bidens spp*., *Guizotia scabra*, and *Plantago lanceolata* in both forested and cultivated landscapes underscores their ecological importance during the first honey flow season, corroborating the observations made by Degaga (2017) in the Jimma Zone, Southwest Ethiopia. During the second honey flow season in April, beekeepers indicated that *Syzygium guineense* and *Terminalia laxiflora* serve as critical forage sources due to their profusion of flowers, which provide vital nutritional resources for honeybees. This observation aligns with existing literature that recognizes the importance of these species in enhancing honey production, particularly during periods of resource scarcity. The increased abundance of tree bee forages during the dry season is particularly noteworthy, as it supports the notion that the availability of floral resources is closely tied to seasonal variations. This finding is consistent with Abebe et al. (2016), who documented that shrubs, crops, forbs, and certain woody plants constitute the primary bee forage from October to December, while the flowering of woody plants predominates as a source of pollen and nectar from February to May in the Assosa, Homosha, and Mao Komo districts of Benishangul Gumuz.

Furthermore, the belief among local beekeepers that the flowering period of *Syzygium guineense* serves as an indicator for honeybee colonies to prepare for honey production highlights the interconnectedness of ecological knowledge and beekeeping practices in the region. Research has established *Syzygium guineense* as a significant nectar source for *Apis mellifera*, thereby reinforcing its role in supporting honey production (Storrs 1995). Similar studies have also identified *Syzygium guineense* as a predominant nectariferous plant species in the Sudano-Guinean transition zone in Benin, emphasizing its importance in honey accumulation within hives (Yedomonhan 2009).

The primary flowering period for honey plants in Ethiopia, occurring from September to November and again from April to May, reflects the influence of the country's bimodal rainfall pattern (Teferi 2018). However, the unique climatic conditions of Benishangul Gumuz, characterized by a singular long rainy season lasting from May to November, necessitate a reevaluation of these patterns in the context of honey production. The findings of this study not only corroborate previous research but also emphasize the need for adaptive management strategies that consider the specific ecological and climatic characteristics of the region to optimize honey production.

The identification of 70% of the bee forage species flowering between September and November reinforces the significance of this time frame for beekeeping activities in the study area. This observation is consistent with Wubie et al. (2014), who reported a peak in flowering activity from August through October, particularly in August and September. The findings indicate that the first flowering season yields honey of varying qualities, with beekeepers noting that honey produced during this period tends to have more brood but lower quality than the honey harvested in April from woody plants. This suggests that floral resources' nutritional composition and availability during different seasons may significantly affect honey quality and overall beekeeping productivity.

While Wubie et al. (2014) documented a broader variety of pollen source plant species, our study highlights the importance of specific species such as *Guizotia* spp. and *Terminalia laxiflora* as primary pollen sources within the study area. This discrepancy may be attributed to differences in sampling methodologies or ecological variances

between regions. The melissopalynological analysis conducted by Tulu et al. (2023) further supports this notion, revealing a predominance of multifloral honey samples with significant contributions from *Eucalyptus camaldulensis* and other lowland bee forages. The comparative analysis reveals that while certain species like *Terminalia* spp. and *Guizotia* spp. serve as critical pollen sources, their classification as secondary in other studies underscores the need for localized assessments to capture the dynamics of bee forage availability.

Moreover, the seasonal patterns observed from late July to August and during the dry season highlight the complexities of honeybee foraging behavior and the adaptability of beekeeping practices to local environmental conditions. The reliance on diverse pollen sources across different agroecological zones emphasizes the need for beekeepers to be aware of the specific flowering patterns and forage availability in their respective areas.

In discussing the findings of our research, it's clear that seasonal dynamics play a critical role in the health and stability of bee populations in the study area. The limited availability of flowering plants from late July to August and the dry season from December to January creates a challenging environment for bees. During these months, the lack of pollen and nectar sources significantly weakens bee colonies. This aligns with previous studies highlighting how resource scarcity directly impacts bee health and colony survival (Teklay 2011).

The situation gets even more complicated with the indigenous practice of burning forest areas from January to March. While this may serve certain agricultural or ecological purposes, it seems to push many bee colonies to abscond. It's interesting to note that this behavior is a survival strategy for bees when their habitat is compromised. Previous research has shown similar patterns, where habitat destruction leads to increased rates of colony abandonment (Belsky and Joshi 2019).

Moreover, the role of smallholder farmers using pesticides cannot be overlooked. Our discussions revealed that these farmers are major contributors to the pesticide problem in the region. The fact that many farmers apply pesticides multiple times during a growing season, as noted by Deressa and Alemu (2022), raises serious concerns. The negative impact of pesticides on both honey bees and the flowering plants they depend on is well-documented. It creates a vicious cycle: pesticides weaken bee colonies, which in turn affects pollination and the health of local flora, ultimately leading to fewer resources for the bees.

Conclusıon: In the Benishangul Gumuz regional state of Ethiopia, beekeeping plays a crucial role. It contributes significantly to household cash income, which is used for taxes, clothing, and school fees. The study identified 71 plant species that serve as forage sources for honeybees, with two main flowering periods corresponding to the honey harvesting seasons. In the region, the most abundant honeybee flora includes *Pterocarpus lucens, Bidens prestinaria, Guizotia species, Cordia africana, Syzygium guineense,* and *Terminalia laxiflora*. Approximately 70% of the bee forage species bloom from September to November, with key plants such as *Pterocarpus lucens, Bidens prestinaria, and Guizotia* species contributing significantly during the first honey flow season. In the second season, from February to May, woody plants like *Cordia Africana, Syzygium guineense,* and *Terminalia laxiflora* become the primary sources of pollen and nectar. *Guizotia scabra* and *Guizotia abyssinica* are significant for monofloral honey production, observed with a frequency of 62.13%. Research is needed to evaluate the nutritional value of the identified species of pollen and nectar sources. Promoting agroforestry systems that integrate bee-friendly plants within and around agricultural fields is also important. Furthermore, to support bee colonies during critical food shortages, we must provide supplemental food and water and conduct regular inspections. Finally, rapid biodiversity loss due to deforestation and the expansion of cultivated land poses a significant threat to the region's forage availability for bees.

Acknowledgment: The author expresses gratitude to the livestock researchers and field assistants at the Assosa Agricultural Research Center, as well as the Holleta Bee Research team. They would also like to extend their heartfelt thanks to the Ethiopian Biodiversity Institute Assosa Biodiversity Center for their support in identifying bee forage both in the field and the herbarium.

Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethical statement: The ethical statement is not applicable to this study and does not involve any animals that would require approval from the ethics committee.

Funding: This project is funded by the Ethiopian Institute of Agriculture Research and the authors acknowledged the financial support.

Statements and Declarations: The author declares they have no competing interests or personal relationships that could influence this work.

REFERENCE

Abebe A, Tadesse Y, Equar Y, Faji M. and Alebachew H. Analysis of honey production systems in three agro-ecologies of Benishangul-Gumuz, Western Ethiopia. Journal of Agricultural Extension and Rural Development, 2016; 8(3): 29-38. DOI: https://doi.org/10.5897/jaerd2014.0705.

Addi A and Bareke T. Floral resources diversity and vegetation types important for honeybees in Ethiopia. Asian Journal of Forestry; 2019; 3(2). DOI:https://doi.org/10.13057/asianjfor/r030203.

Addi A, Wakjira K, Kelbessa E. and Bezabeh A. Honeybee forages of Ethiopia. Holeta Bee Research Center, Addis Ababa, Ethiopia, 2014.

Adgaba N. Atlas of pollen grains of major honeybee flora of Ethiopia. Holetta bee research centre, Holetta, Ethiopia, 2007.

Assosa Agricultural Research center (AsARC). Results of farming system survey in Benishangul Gumuz regional state. Ethiopian institute of agricultural research; Assosa Agricultural Research Center, Assosa, 2006. (Unpublished).

Begum HA, Iqbal J. and Aziz A. Characterization of pollen profile of *Apis mellifera* L. in arid region of Pakistan. Saudi journal of biological sciences, 2021; *28*(5): 2964-2974. https://doi.org/10.1016/j.sjbs.2021.02.035.

Belay A, Haki GD, Birringer M, Borck H, Lee YC, Cho CW, Kim KT, Bayissa B, Baye K. and Melaku S. Sugar profile and physicochemical properties of Ethiopian monofloral honey. International Journal of Food Properties, 2017; *20*(11): 2855-2866. https://doi.org/10.1080/10942912.2016.1255898.

Belsky J and Joshi NK. Impact of biotic and abiotic stressors on managed and feral bees. Insects, 2019; *10*(8): 233. https://doi.org/10.3390/insects10080233.

CSA. 2018. CSA (Central Statistical Authority). Agricultural Sample Survey: Report on Livestock, and Livestock Characteristics, II, CSA, Addis Ababa, Ethiopia (2018), 2017; 28-29, No 587.

Debissa L and Amsalu B. August. Botanical inventory and phenology of bee plants in rift valley regions of East Showa Zone. The Role of Agricultural Universities/Colleges in Transforming Animal Agriculture in Education. In Research and Development in Ethiopia: Challenges & Opportunities. Proceedings of the 13th annual conference of the Ethiopian Society of Animal Production (ESAP) held in Addis Ababa, Ethiopia, 2006.

Degaga AH. Identification of honey source bee floras during major and minor honey harvesting seasons in Jimma Zone, Southwest Ethiopia. Journal of Environment and Earth Science, 2017; *7*(3): 25-32.

Deressa DA and Alemu K. Assessment of Pesticide Use by Farmers in Assosa District, Benishagul Gumuz National Regional State of Ethiopia. Journal of Health and Environmental Research, 2022; 7(1): 37-42. https://doi.org/10.11648/j.jher.20220801.16.

Haftom Gebremedhn HG, Zelealem Tesfay ZT, Girmay Murutse GM and Awet Estifanos AE. Seasonal honeybee forage availability, swarming, absconding and honey harvesting in Debrekidan and Begasheka watersheds of Tigray, Northern Ethiopia, 2013. http://www.lrrd.org/lrrd25/4/haft25061.htm.

Hedberg I. Flora of Ethiopia and Eritrea. In The Biodiversity of African Plants: Proceedings XIVth AETFAT Congress 22–27 August 1994, Wageningen, The Netherlands, 1996, :802-804. Springer Netherlands. https://doi.org/10.1007/978-94-009-0285-5_104.

Jacobs FJ, Simoens C, Graaf D and Deckers J. Scope for non-wood forest products income generation from rehabilitation areas: focus

on beekeeping. Journal of the Drylands, 2006; 1(2): 171-185. http://hdl.handle.net/1854/LU-376496.

Louveaux J, Maurizio A and Vorwohl G. Methods of melissopalynology. Bee world, 1978; *59*(4): 139-157. https://doi.org/10.1080/0005772x.1978.11097714.

National Metrological Agency (NMA). Rainfall and Temperature historical data in Asosa area, Benishangul Gumuz Regional State, Ethiopia, 2017.Unpuplished.

Silici S and Gökceoglu M. Pollen analysis of honeys from Mediterranean region of Anatolia. Grana, 2007; 46(1): 57-64. https://doi.org/10.1080/00173130601138783.

Storrs AE. Know your trees. Some common trees found in Zambia. Zambia: Regional Soil Conservation Unit (RSCU), 1995. https://books.google.com.et/books?id=-24pPwAACAAJ.

Reinhard F and Admasu A. Honeybee flora of Ethiopia. DED-Weikersheim: Margraf, Germany, 1994. http://catalog.hathitrust.org/api/volumes/oclc/31749347.html.

Teferi K. Status of Beekeeping in Ethiopia-a review. Dairy and Vet Sci J, 2018; *8*(4): 555-743. https://doi.org/10.19080/jdvs.2018.08.555743.

Teklay A. Seasonal availability of common bee flora in relation to land use and colony performance in Gergera Watershed Atsbi Wemberta District, Eastern Zone of Tigray, Ethiopia (Doctoral dissertation, Hawassa University), 2011.

Tesfaye O, Mekonen E and Disa A. Botanical origin and physicochemical composition of *Apis mellifera* L. Honey from western Oromia, Ethiopia. Asian Journal of Bio Science, 2023; 16(1): 40-51. https://doi.org/10.3923/ajbs.2023.40.51.

Tulu D, Aleme M, Mengistu G, Bogale A, Bezabeh A and Mendesil E. Melissopalynological analysis and floral spectra of *Apis mellifera* scutellata Lepeletier bees in different agroecologies of southwest Ethiopia. Heliyon, 2023; 9(5). https://doi.org/10.1016/j.heliyon.2023.e16047.

Westhoff V, Van der Maarel E. The Braun-Blanquet approach. In Classification of Plant Communities: Whittaker, R.H., Ed: Springer: Hague, 617-726, Netherlands, 1973: https://doi.org/10.1007/978-94-010-2701-4_20.

Woody Biomass Inventory and Strategic Planning Project (WBISPP). Report on natural grazing lands and livestock feed resources, Benishangul Gumuz Regional State. 2003.

Wubie AJ, Bezabeh A and Kebede K. Floral phenology and pollen potential of honey bee plants in North-East dry land areas of Amhara region, Ethiopia. IOSR Journal of Agriculture and Veterinary Science, 2014; 7(5): 36-49. https://doi.org/10.9790/2380-07513649.

Yedomonhan H. Honey plants and potential for honey production in Guinean and Sudano-Guinean areas of Benin. Doctoral thesis from the University of Abomey-Calavi (Benin), FAST/UAC, 2009.

Citation/Atıf: Özkök A, Ceren Özkul C, Golshan Zare G, Kadriye Sorkun K. Chemical profile and antimicrobial activity of important honey plants *Thymus nummularius* M. Bieb. and *Vaccinium myrtillus* L. U. Bee J. / U. Arı D. 2025; 25 (1): 32-.42 DOI: 10.31467/uluaricilik.1597080

ARAŞTIRMA MAKALESI / RESEARCH ARTICLE

CHEMICAL PROFILE AND ANTIMICROBIAL ACTIVITY OF IMPORTANT HONEY PLANTS *THYMUS NUMMULARIUS* M. BIEB. AND *VACCINIUM MYRTILLUS* L.

Önemli Bal Bitkileri *Thymus nummularius* M. Bieb. ve *Vaccinium myrtillus* L.'nin Kimyasal Profili ve Antimikrobiyal Aktivitesi

Aslı ÖZKÖK[1*], Ceren ÖZKUL[2], Golshan ZARE[3], Kadriye SORKUN[4]

[1]Hacettepe University, Science Faculty, Biology Department, Beytepe, Ankara, TÜRKİYE, Corresponding author / Yazışma Yazarı E-mail: aozkok@hacettepe.edu.tr. ORCID No: 0000-0002-7336-2892

[2]Hacettepe University, Faculty of Pharmacy, Department of Pharmaceutical Microbiology, Sıhhiye, Ankara, TÜRKİYE, E-mail: cerenozkul@hacettepe.edu.tr, ORCID No: 0000-0002-0921-5863

[3]Hacettepe University, Faculty of Pharmacy, Department of Pharmaceutical Botany, Sıhhiye, Ankara, TÜRKİYE, E-mail: golshanzare@gmail.com, ORCID No: 0000-0002-5972-5191

[4]Hacettepe University, Science Faculty, Biology Department, Beytepe, Ankara, TÜRKİYE, E-mail: kadriye@hacettepe.edu.tr, ORCID No: 0000-0003-3224-7748

Received / Geliş:06.12.2024 Accepted / Kabul:04.02.2025 DOI: 10.31467/uluaricilik.1597080

ABSTRACT

The aim of this study was to determine the chemical and antimicrobial activity of two taxa which are important nectar and pollen plants for honey bees. *Thymus nummularius* M. Bieb. and *Vaccinium myrtillus* L. which produced by local people of Anzer. For this purpose, chemical compounds scanned by GC–MS system and antimicrobial activities were tested against major oral pathogens including *Porphyromonas gingivalis* ATCC 33277, *Streptococcus salivarius* DSM 13084, *Streptococcus mitis*, *Streptococcus mutans* and *Candida albicans* ATCC 90028 by agar well diffusion and broth microdilution. Aldehydes, alcohols, carboxylic acids and esters, ketones, terpenes, fatty acids and esters, acetic acids and esters and other chemical components were detected in different percentages in both *T. nummularius*. and *V. myrtillus* plants. A total of 30 chemical components were detected in *T. nummularius*. while 26 chemical components were found in *V. myrtillus*. Carvacrol, lauryl acetate, and thymol were detected 20.34%, 6.31% and 3.36%, respectively at high levels in *T. nummularius*. On the other hand, diethyl succinate, hexanoic acid, lauryl acetate and benzoic acid were determined 12.68%, 11.29%, 10.89% and 10.16% respectively predominantly in *V. myrtillus*. As a result of antimicrobial activity of two taxa, *V. myrtillus*. exhibited more potent antimicrobial activity when compared to *T. nummularius*. Antimicrobial activity of *V. myrtillus* against viridans streptococci and *C. albicans* was promising and maybe used for the treatment of cariogenic microorganisms or oral Candidiasis in future studies.

Key words: Antimicrobial activity, Chemical compounds, Honey plants, *Thymus nummularius*, *Vaccinium myrtillus*

ÖZ

Bu çalışmanın amacı, Anzer yöresi halkı tarafından üretilen ve bal arıları için önemli nektar ile polen kaynağı bitkiler olan *Thymus nummularius* M. Bieb. ve *Vaccinium myrtillus* L. adlı iki taksonun kimyasal ve antimikrobiyal aktivitesini belirlemektir. Bu amaçla, kimyasal bileşikler GC-MS sistemi ile

taranmış ve antimikrobiyal aktiviteleri *Porphyromonas gingivalis* ATCC 33277, *Streptococcus salivarius* DSM 13084, *Streptococcus mitis*, *Streptococcus mutans* ve *Candida albicans* ATCC 90028 dahil olmak üzere başlıca oral patojenlere karşı agar kuyu difüzyon ve sıvı mikrodilüsyon ile test edilmiştir. Aldehitler, alkoller, karboksilik asitler ve esterler, ketonlar, terpenler, yağ asitleri ve esterler, asetik asitler ve esterler ve diğer kimyasal bileşenler hem *T. nummularius* hem de *V. myrtillus* bitkilerinde farklı yüzdelerde tespit edilmiştir. *T. nummularius*'ta toplam 30 kimyasal bileşen tespit edilirken, *V. myrtillus*'ta 26 kimyasal bileşen tespit edilmiştir. Karvakrol, lauril asetat ve timol *T. nummularius*'ta sırasıyla %20,34, %6,31 ve %3,36 oranında yüksek seviyelerde tespit edilmiştir. Öte yandan, dietil süksinat, hekzanoik asit, lauril asetat ve benzoik asit *V. myrtillus*'ta baskın olarak sırasıyla %12,68, %11,29, %10,89 ve %10,16 oranlarında tespit edilmiştir. İki taksonun antimikrobiyal aktivitesinin bir sonucu olarak, *V. myrtillus* bitkisi *T. nummularius* ile karşılaştırıldığında daha güçlü antimikrobiyal aktivite göstermiştir. *V. myrtillus*'un özellikle viridans streptokoklara ve *C. albicans*'a karşı antimikrobiyal aktivitesinin olması ileriki çalışmalarda karyojenik mikroorganizmaların veya oral Kandidiyazis'in tedavisinde kullanılabilme potansiyeli olduğunu göstermektedir.

Anahtar Kelimeler: Antimikrobiyal aktivite, Kimyasal bileşenler, Ballı bitkiler, *Thymus nummularius*, *Vaccinium myrtillus*

GENİŞLETİLMİŞ ÖZET

Amaç: Bu çalışmanın amacı, Anzer yöresi halkı tarafından üretilen ve bal arıları için önemli nektar ile polen kaynağı bitkiler olan *Thymus nummularius* M. Bieb. ve *Vaccinium myrtillus* L. adlı iki taksonun kimyasal ve antimikrobiyal aktivitesini belirlemektir. Bu amaçla Anzer Bölgesi'nden toplanan *Thymus nummularius* M. Bieb. (Kekik, Anzer çayı, Anuk) ve *Vaccinium myrtillus* L. (Yaban mersini, Maviyemiş, Likapa) bitkilerinin kimyasal analizleri ve oral patojenlere karşı antimikrobiyal aktiviteleri belirlenmiştir.

Gereç ve yöntem: *Thymus nummularius* M. Bieb. ve *Vaccinium myrtillus* L. bitkilerinin ekstraktları methanol ile yapılarak Gaz Kromatografisi ve Kütle Spektrometresi (GC-MS) cihazında kimyasal bileşenleri tespit edilmiştir. Ayrıca antimikrobiyal aktiviteleri *Porphyromonas gingivalis* ATCC 33277, *Streptococcus salivarius* DSM 13084, *Streptococcus mitis* (klinik izolat), *Streptococcus mutans* (klinik izolat) ve *Candida albicans* ATCC 90028 dahil olmak üzere başlıca oral patojenlere karşı agar kuyu difüzyon ve sıvı mikrodilüsyon yöntemi ile test edilmiştir.

Bulgular ve tartışma: GC-MS cihazında yapılan çalışma sonucunda hem *T. nummularius* bitkisinde hem de *V. myrtillus* bitkilerinde farklı yüzdelerde aldehitler, alkoller, karboksilik asitler ve esterler, ketonlar, terpenler, yağ asitleri ve esterler, asetik asitler ve esterler ve diğer kimyasal bileşenler tespit edilmiştir (Tablo 1). *T. nummularius*'ta toplam 30 kimyasal bileşen tespit edilirken, *V. myrtillus*'ta 26 kimyasal bileşen tespit edilmiştir. Karvakrol, lauril asetat ve timol *T. nummularius*'ta sırasıyla %20,34, %6,31 ve %3,36 oranında yüksek seviyelerde tespit edilmiştir (Tablo 1). Ertas vd. (2015) *T. nummularius*'te timol (%60,38) ve terpinil-asetat (%10,49), Gerçek vd. (2022) ise timol (%38,91), linalool (%13,12) ve geraniol (%6,51) bulmuşlardır. Bizim çalışmamızda ise bu çalışmalardan farklı olarak yüksek oranda karvakrol tespit ettik. Karvakrol ve timolün kekik için önemli belirteç bileşenler olduğu belirtilmiştir. Küçükbay vd. (2014) tarafından karvakrol ve timolün yüksek antioksidan aktiviteye sahip olduğu bildirilmiştir. Ayrıca Tepe vd. (2011) karvakrolün antifungal, timolün ise antiseptik etkiye sahip olduğunu söylemişlerdir. Ayrıca, Shah vd. (2020) lauril asetatın antioksidan özellikte olduğunu bildirmişlerdir. Diğer yandan, dietil süksinat, hekzanoik asit, lauril asetat ve benzoik asit *V. myrtillus*'ta baskın olarak sırasıyla %12,68, %11,29, %10,89 ve %10,16 oranlarında tespit edilmiştir (Tablo 1). Aranega-Bou vd. (2014) hekzanoik asidin antifungal aktivitesini bildirmiştir. Ayrıca, Özkök vd. (2016) tarafından benzoik asidin balgam söktürücü, analjezik ve antiseptik aktivitelere sahip olduğu bildirilmiştir. Bununla birlikte, Elkıran ve Avşar (2020) Türkiye'nin Sinop ilinden toplanan *V. myrtillus*'ta 1,8-sineol (%38,6), α-pinen (%21), linalool (%19,5), α-terpineol (%5,8) bulmuştur. Çalışmamızda farklı bileşenler bulunmuş ve bu durum kimyasal içeriğin bölgelere göre değişkenlik olabileceğini göstermiştir.

Ayrca, *T. nummularius* ve *V. myrtillus*'un başlıca oral patojenlere karşı antimikrobiyal aktivitesini agar

kuyusu difüzyonu (Tablo 2, Şekil 2) ve et suyu mikroseyreltme testi (Tablo 3) ile araştırılmıştır. Sonuçlarımıza göre, *V. myrtillus*, test edilen tüm mikroorganizmalara karşı *T. nummularius*'tan daha etkili çıkmıştır. Bununla birlikte, *V. myrtillus, C. albicans*'a karşı güçlü bir antimikrobiyal aktivite sergilemiştir. *V. myrtillus*'un *C. albicans*'a karşı ümit verici antimikrobiyal aktivitesi, nispeten daha yüksek karboksilik asitlere ve esterlere (%23,59), özellikle benzoik aside atfedilebilir. Benzoik asidin öncelikle mantarlara karşı antimikrobiyal aktiviteye sahip olduğu gösterilmiştir (Teodoro vd. 2015).

Sonuç: Bu çalışmada, kimyasal bileşik analizleri her iki bitki türünün de yüksek antioksidan bileşenlere sahip olduğunu göstermiştir. *V. myrtillus*, *T. nummularius* ile karşılaştırıldığında daha güçlü antimikrobiyal aktivite göstermiştir. *V. myrtillus*'un viridans streptokoklarına ve *C. albicans*'a karşı antimikrobiyal aktivitesi ümit vericidir ve gelecekteki çalışmalarda karyojenik mikroorganizmaların veya oral Kandidiyazis'in tedavisinde kullanılabilir.

INTRODUCTION

Local people and traditional healers have long history in using of plants to prevent or cure bacterial infections. Nowadays, in many parts of the world, 70 -95% of people use plants as a primary form of medicine, and many countries have integrated traditional plant-based medicines (Willis 2017). Plants are the main source of a wide variety of secondary metabolites, such as alkaloids, flavonoids, tannins, and terpenoids, which have been determined in vitro to have antimicrobial properties (Cowan 1999).

Thymus L. (Lamiaceae) are well-known genera of the family that is used as folk medicine (Ozen and Demirtas 2015). This species belongs to the group of plants commonly called “kekik” in Turkish (Baytop 1999). For centuries, these taxa have traditionally been used to flavour foods and treatment of various diseases due to the high percentage of their essential oils (Baytop 1999, Lukas et al. 2010, Sezik et al. 1992). *Thymus* species have been used traditionally for bronchitis, coughs, asthma, rheumatism, colic, diarrhoea, and arteriosclerosis (Tammar et al. 2018).

Thymus nummularius M. Bieb. (synonym *Thymus pseudopulegioides* Klokov & Des. Shots.) is distributed widely in the Caucasian area (Güner 2012). It is known as “Anzer tea” (Rize) and “Anuk” (Trabzon) by local people (Günaydın et al. 2017) and also honey bees collect nectar and pollen from this plant. Especially this plant’s antimicrobial bioactive compounds “thymol” and “carvacrol” was found in the honey. Thymol is an important acaricide and is used in the fight against the bee parasite *Varroa destructor*. Therefore, this type of thyme containing thymol is also an important plant in terms of use in bee health (Demirezen 2019, Turkun 2016).

T. nummularius is a perennial shrub, growing wild in the Black Sea Region of Turkiye. Its herbal parts are consumed as tea, condiments, and herbal remedies for gastrointestinal disorders. It is also known that extracts of these plant materials involve bioactive compounds that can be helpful to health such as diuretics, circulation regulators, and sedatives. There have been restricted studies assessing the chemical constituents and biological activity of *T. nummularius* (Baser et al. 1999).

In previous studies (Baser et al. 1999, Gül et al. 2022, Sunar et al. 2009), various *Thymus* species (including *T. nummularius*) were evaluated for their chemical profiles and biological activity. Results revealed significant differences at intra- and interspecific levels. However, a survey of the literature confirmed that *T. nummularius* antibacterial activity was evaluated by Gül et al. 2022.

Vaccinium myrtillus L. (Yaban mersini) from Ericaceae family known as Yaban mersini, Maviyemiş and Likapa in black see regions of Turkiye (Baytop 1999, Güner 2012) and also honey bees collect nectar and pollen from this plant. Four species contain *Vaccinium arctostaphylos* L., *Vaccinium myrtillus* L., *Vaccinium vitisidea* L., *Vaccinium uliginosum* L. grown naturally in Turkiye. *V. myrtillus* is a perennial shrub which grows in coniferous forests. This taxon blooms from April through June have edible spheroidal fruit of blue/black colour with many seeds (Davis 1982).

Using the fruit of this plant in different way such as the decoction of dried fruits has a long history in the human diet. Bilberry commercially is observed as fresh, frozen, and dried berries, in addition to inside the shape of preserves, jams, juices, and liquid or powdered concentrates as meal supplements (Chu et al. 2011).

Vaccinium contains a variety of phenolic compounds, including flavonols, tannins, ellagitannins, and phenolic acids but mostly known

as a rich source of anthocyanins (Seeram 2008, Upton 2001). Anthocyanins in the last years have gained research attention due to their numerous functions and applications in mind diet and neuroprotective effects (Chu et al. 2011). These polyphenolics are responsible their blue/black colour and high antioxidant content, and they are believed to be the key bioactive responsible for the many reported health benefits of bilberry (Chu et al. 2011, Upton 2001,). Although most of the researche has been concentrated on the antioxidant properties of anthocyanins other biological activities of them involve cell-signalling pathways, gene expression, DNA repair, and anticancer effects, as well as antimicrobial and antineoplastic effects (Karakaş et al. 2022, Kowalczyk et al. 2003, Seeram 2008, Zafra-Stone et al. 2007).

Some the berry fruit and their phenolics, have been pronounced to reveal antimicrobial results in opposition to human pathogens. The results of numerous berries in opposition to numerous organisms are different. This suggested different taxa extracts may have high potential as antimicrobials (Puupponen-Pimià et al. 2005).

Anzer Region is an important region where Turkiye's aromatic plants are found, and Anzer honey produced here is a world-famous honey. For this reason, the properties of the plants found here are also important for human health. This research attempted to elucidate the chemical and antimicrobial activity of two taxa *T. nummularius* M. Bieb. and *V. myrtillus* L. which are used by local people of Anzer. For this purpose, chemical compounds scanned by GC–MS system and antimicrobial activities were determined.

MATERIAL AND METHODS

Collection of plant material

The research area Anzer Region is placed in the borders of İkizdere district of the Rize province. Bayburt and Trabzon provinces in the west, Erzurum province inside the south, Kalkandere and Rize vital districts inside the north, Çayeli and Çamlıhemşin districts inside the east (Erata et al. 2021) (Figure 1).

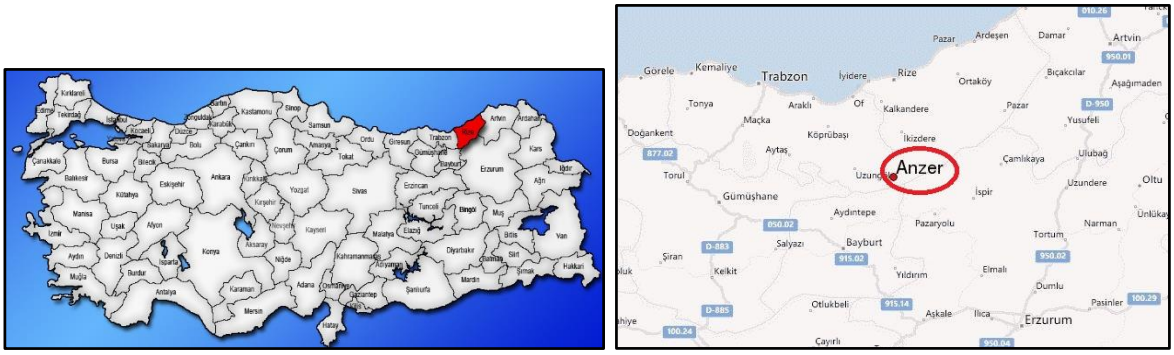


Figure 1. Rize province and Anzer region

The fresh plant material of the *T. nummularius* M. Bieb. and *V. myrtillus* L. were collected from the Anzer Region which is the north-east region of Turkiye. The plants were identified based on the Flora of Turkiye and East Aegean Island (Davis 1982).

Extraction procedure of plant material

Aerial parts of the plants were air-dried in shade condition and powdered. Aerial parts of *T. nummularius* were extracted by methanol. Methanol extracts were prepared by the maceration method and 10 g of cured drug were extracted in 100 mL of solvent (10% m/v) for 24 h with occasional shaking. At the end of the extraction, the solution was filtered, the process was repeated two times, and the filtrates were combined and collected. Finally, it was evaporated to dryness under low pressure.

The liquid segment becomes separated from the stable residue with the aid of using filtering via Whatman No four filter paper and the organic solvent is eliminated with a rotary evaporator (Büchi

Rotavapor, Büchi Labortechnik AG, Flawil, Switzerland). The extracts were lyophilized.

Chemical profile analysis

The chemical profiles of *T. nummularius* and *V. myrtillus* were determined by Gas Chromatography-Mass Spectrometry (GC–MS) analysis. For this analysis, Agilent 6890N GC system coupled with a mass selective detector MS5973 was used. Lyophilized plant extracts were dissolved in methanol and 1 µl of plant extract was injected into the GC-MS system to screen the chemical compounds. For the GC-MS experimental conditions Temiz et al. (2011) method followed. According to this, a DB 5MS capillary column (30 m x 0.25 mm x 0.25 µm) changed into used and the flow rate of mobile phase (He) changed into a set at 0.7 mL/min. In the gas chromatography part, the temperature changed into stored at 50 °C for 1 min. After this period, the temperature changed extended to 150 °C with a 10 °C/min heating ramp after which stored at 150 °C for two min. Finally, the temperature changed extended to 280 °C with a 20°C/min heating ramp after which stored at 280 °C for 30 min. Chemical compounds have been diagnosed via way of means of pc seek the use of reference The Wiley Registry/NIST Mass Spectral Library, which's to be had inside the records acquisition machine of GC-MS.

Antimicrobial activity analysis

Microorganisms and culture conditions: Antimicrobial activity of two taxa *T. nummularius*, and *V. myrtillus* were tested against main oral pathogens including *Porphyromonas gingivalis* ATCC 33277, *Streptococcus salivarius* DSM 13084, *Streptococcus mitis* (clinical isolate) and *Streptococcus mutans* (clinical isolate) and *Candida albicans* ATCC 90028 by agar well diffusion and broth microdilution. *P. gingivalis* was cultured on Brucella Agar (5% sheep blood agar supplemented with vitamin K1 and hemin) under anaerobic conditions at 37 °C for 5 days. Black-pigmented colonies were confirmed after the incubation period. Viridans streptococci were cultured on blood agar (5% CO2, 95% humidified air, at 37 °C) and *C. albicans* was cultured on Sabouraud Dextrose agar (SDA) at 37 °C overnight.

Agar well diffusion assay: Plant extracts were dissolved in dimethyl sulphoxide (DMSO) prior to antimicrobial activity screening. Agar well diffusion assay was performed by harvesting cells from fresh cultures and a standard suspension became organized with the aid of adjusting the turbidity of the suspension to suit the 0.5 McFarland ($1.5X10^8$ cfu/mL). Five-millimeter diameter wells were prepared in Mueller-Hinton Agar (MHA) with 2% glucose for *C. albicans*, MHA with 5% sheep blood for viridans streptococci and Brucella agar for *P. gingivalis*, and the bacterial suspensions were inoculated into corresponding media. After the incubation period, the inhibition zones were measured and means and standard deviations were calculated.

Broth microdilution assay: The minimum inhibitory concentrations (MIC) of the extracts were determined using broth microdilution assay in sterile 96-well microplates. Briefly, two-fold serial dilutions of the extracts were prepared ranging from 1024 to 0.5 µg/mL using Brain Heart Infusion (BHI) medium for viridans streptococci, BHI medium supplemented with vitamin K1 (0.1 µg/mL) and hemin (5 µg/mL) for *P. gingivalis* and Roswell Park Memorial Institute(RPMI) 1640 Medium for *C. albicans*. The inoculum containing 10^6 CFU/mL of each bacterium was added to each well. After the incubation period bacterial growth was evaluated by observing the presence of turbidity. MIC was defined as the lowest concentration of the samples with no bacterial growth.

RESULTS

Chemical compounds of *T. nummularius*, and *V. myrtillus* were given in Table 1.

As seen in Table 1, aldehydes, alcohols, carboxylic acids and esters, ketones, terpenes, fatty acids and esters, acetic acids and esters and other chemical components were detected in different percentages in both *T. nummularius*, and *V. myrtillus* plants. A total of 30 chemical components were detected in *T. nummularius*. while 26 chemical components were found in *V. myrtillus*. While the terpenes group was found most in *T. nummularius*. with 27.13%, carboxylic acids and esters were found most in *V. myrtillus* with 23.59%.

Also, present study investigated the antimicrobial activity of *T. nummularius*. and *V. myrtillus* against major oral pathogens by agar well diffusion (Table 2, Figure 2, Figure 3) and broth microdilution assay (Table 3).

Table 1. Chemical compounds of *T. nummularius*. and *V. myrtillus*

Chemical compounds	*Thymus nummularius* M.Bieb.	*Vaccinium myrtillus* L.
Aldehydes (%)		
trans, trans-2, 4-Hexadienal	1.40	-
Benzaldehyde	1.22	-
Phenylacetaldehyde	-	0.19
Phenylacetaldehyde dimethyl acetal	0.30	-
Cis-6-Nonenal	2.45	-
Total	***5.37***	***0.19***
Alcohols (%)		
3-Octanol	0.83	0.42
2-Pentanol	0.92	-
Isobutyl alcohol	1.16	-
Alpha-Terpineol	0.53	-
Isopropyl alcohol	-	0.37
Total	***3.44***	***0.79***
Carboxylic acids and esters (%)		
Hexanoic acid	-	11.29
Benzoic acid	4.09	10.16
Propionic acid	0.54	-
Pyruvic acid	1.81	-
2-Methyl-2-pentanoic acid	-	0.10
4-Methylpentanoic acid	0.29	1.13
3-Hexenoic acid	-	0.91
Total	***6.73***	***23.59***
Ketones (%)		
Homofuronol	3.20	8.91
6-Methyl-3,5-heptadien-2-one	1.24	-
Methyl-2-pyrrolyl ketone	1.14	-
Total	***5.58***	***8.91***
Terpenes (%)		
Linaloloxide	0.51	0.19
Carvacrol	20.34	-
Thymol	3.36	-
Citronellol	1.97	-
Alpha-terpinene	0.95	-
Isoborneol	-	0.53
Total	***27.13***	***0.72***
Fatty acids and esters (%)		
Myristic acid	-	0.95
Stearic acid	-	5.11
Decanoic acid	2.08	0.62
Octanoic acid	2.81	-
Palmitic acid	2.45	-
Total	***7.34***	***6.68***
Acetic acids and esters (%)		
Lauryl acetate	6.31	10.89
Isopropyl acetate	-	0.45
n-Propyl acetate	-	1.48
Hexyl acetate	0.53	0.73
n-Butyl acetate	-	0.30
Total	***6.84***	***13.85***

Chemical compounds	*Thymus nummularius* M.Bieb.	*Vaccinium myrtillus* L.
Others (%)		
Quinoline	0.77	1.14
Ethyl-p-anisate	0.45	-
Acetanisole	3.68	-
Methyl benzoate	-	0.81
Isobutyl propionate	-	0.58
4-Methylacetophenone	1.11	-
Phenol	3.02	-
Dimethyl anthranilate	-	0.26
Neroloxide	-	0.47
2-Methoxy-3-methylpyrazine	-	0.89
Diethyl succinate	-	12.68
Total	***9.03***	***16.83***

Table 2. Agar well diffusion assay indicating inhibition zones (mm) of *Thymus nummularius* and *Vaccinium myrtillus* on various oral pathogens

	Thymus nummularius M.Bieb (51.2 µg/mL)	*Vaccinium myrtillus* L. (51.2 µg/mL)
S. mutans (clinical isolate)	9.00±0	11.33±0.58
S. mitis (clinical isolate)	9.00±0	14.33±0.58
S. salivarius DSM 13084	No zone	11.33±0.58
P. gingivalis ATCC 33277	8.00±0	9.33±0.58
C. albicans ATCC 90028	No zone	18.67±0.58

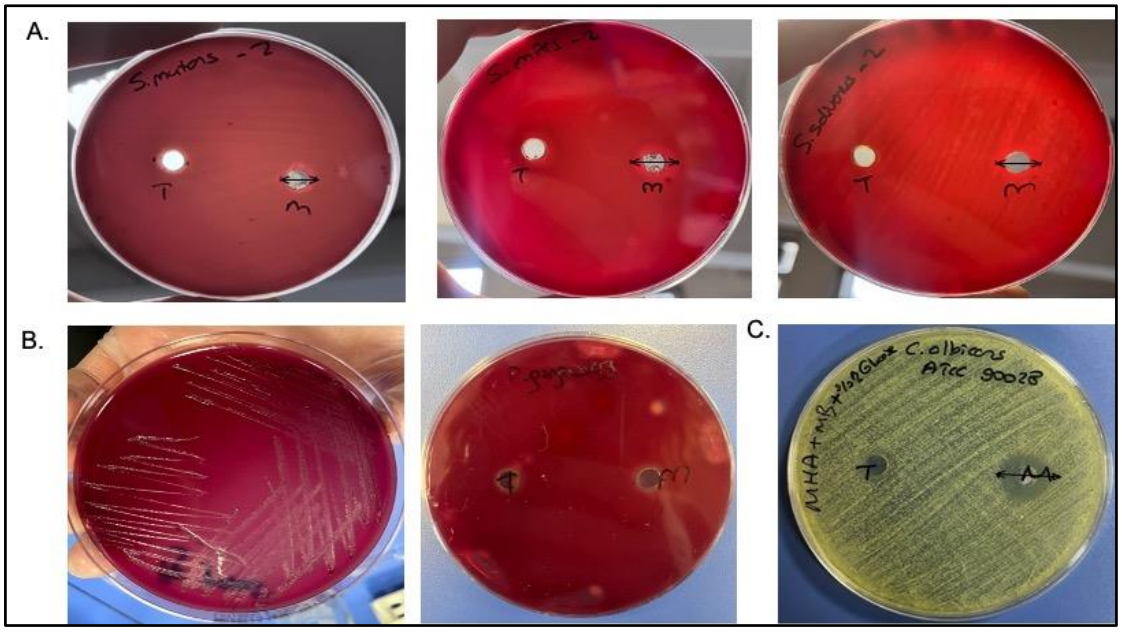


Figure 2. Agar well diffusion results for *V. myrtillus* L. and *T. nummularius* M. Bieb against tested oral microorganisms. A) Inhibition zones for viridans streptococci. B) Black pigmented colonies of *P. gingivalis* ATCC 33277 on Brucella Agar and agar well diffusion results. C) Agar well diffusion results for *C. albicans* ATCC 90028.

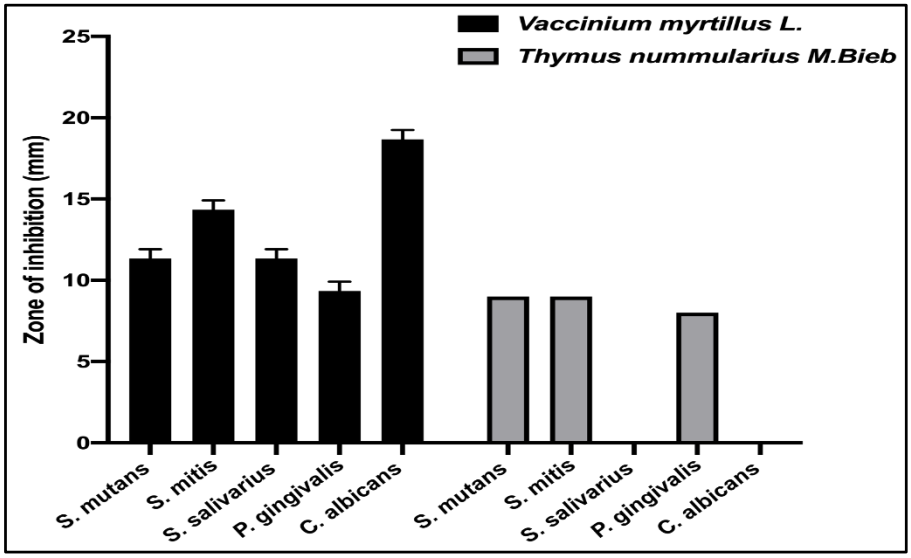


Figure 3. Inhibition zones for *V. myrtillus* L. and *T. nummularius* M. Bieb against tested oral microorganisms.

Table 3. Minimum inhibitory concentrations (µg/ml) of *T. nummularius* and *V. myrtillus* against tested oral pathogens

	***Thymus nummularius* M.Bieb**	***Vaccinium myrtillus* L.**
S. mutans (clinical isolate)	>1024	512
S. mitis (clinical isolate)	1024	256
S. salivarius DSM 13084	>1024	512
P. gingivalis ATCC 33277	>1024	>1024
C. albicans ATCC 90028	1024	128

DISCUSSION

As a result of GC-MS analysis, carvacrol, lauryl acetate, and thymol were detected 20.34%, 6.31% and 3.36% respectively at high levels in *T. nummularius*. On the other hand, Ertas et al. (2015) found thymol (60.38%) and terpinyl-acetate (10.49%) and Gercek et al. (2022) were determined thymol (38.91%), linalool (13.12%) and geraniol (6.51%) in *T. nummularius*. In present study, it found that differently from them carvacrol predominantly. Carvacrol and thymol, are considered important marker components for thyme. It was reported by Küçükbay et al. (2014) that carvacrol and thymol have high antioxidant activity. In addition, Tepe et al. (2011) said that carvacrol has an antifungal effect and thymol has an antiseptic effect. At the same time, thymol can be used against *Varroa destructor*, an important bee parasite. It is also preferred because it does not leave residue in bee products (Demirezen 2019, Turkun 2016). In a study conducted by Demirezen (2019), a total of 36 colonies were studied, with 9 colonies in each group (flumethrin, oxalic acid, thymol and control). In the study results; significant statistical differences were found between the varroa numbers falling according to the spring and autumn periods, according to the days, and according to the days within the same season. According to the percentage change formula, drug efficiencies were found as; flumethrin 85%, oxalic acid 80%, thymol 87% in the autumn period and flumethrin 75%, oxalic acid 68%, thymol 84% in the spring period. The results of this study showed that flumethrin was successful in long-term, oxalic acid and thymol were successful in short-term varroa control. On the other hand, Shah et al. (2020) reported the antioxidant properties of lauryl acetate. Lauryl acetate, also known as dodecyl acetate, has a floral odor and is useful as a perfume additive.

Diethyl succinate, hexanoic acid, lauryl acetate and benzoic acid were determined 12.68%, 11.29%, 10.89% and 10.16% respectively predominantly in *V. myrtillus*. Aranega-Bou et al. (2014) reported antifungal activity of hexanoic acid. Also, it was reported by Özkök et al. (2016) that benzoic acid has an expectorant, analgesic, and antiseptic activity. On the other hand, Elkıran and Avşar (2020) found 1,8-cineole (38.6%), α-pinene (21%), linalool (19.5%), α-terpineol (5.8%) in *V. myrtillus*, which was collected Sinop province of Turkiye. In this present study, it found of different components and it can be related and changed according to regions.

Antimicrobial outcomes of plants and natural products may be through inhibition of bacterial binding (adhesion) to cell walls, direct antimicrobial killing, or by effects that potentiate antibiotics, as evidenced with the aid of using diminished minimal inhibitory concentration (MIC) of antibiotics inside the presence of a plant in comparison to that of the antibiotic alone. Several natural products have been determined to have antimicrobial outcomes (Lee et al. 2006). Cranberry (*V. macrocarpon* Ait.) has effective antiadhesion properties (Dao et al. 2012).

According to antimicrobial results, *V. myrtillus* was more effective than *T. nummularius* against all tested microorganisms. Moreover, *V. myrtillus* exhibited a potent antimicrobial activity against *C. albicans*. The promising antimicrobial activity of *V. myrtillus* against *C. albicans* can be attributed to relatively higher carboxylic acids and esters (23.59%), especially benzoic acid. Benzoic acid has been shown to possess antimicrobial activity primarily against fungi (Teodoro et al. 2015).

Conclusion: In this study, chemical compound analyses showed that both plant species have high antioxidant components. *Vaccinium myrtillus* exhibited more potent antimicrobial activity when compared to *Thymus nummularius*. The antimicrobial activity of *V. myrtillus* against viridans streptococci and *C. albicans* was promising and may be used for the treatment of cariogenic microorganisms or oral Candidiasis in future studies. On the other hand, *T. nummularius* can use against to *Varroa destructor* because of thymol ingredient. At the same time, it is planned to study the contents of monofloral honey produced from these plants in further studies and to introduce these honeys to Turkish beekeeping. Therefore, it is essential to protect these plants.

Acknowledgements: We would like to thank İSHAK YILMAZ, the owner of Paşanzer and BeeMyosotis companies, who collected the plants used in this study and supported the field work.

Author Contributions: AO and KS conceived and designed the study. AO, CO, GS performed experiments and analysed results and wrote the manuscript. All authors read and approved the manuscript.

Conflict of interest: All authors declare no conflict of interest.

Data availability: Data are available on request due to privacy or other restrictions.

Ethics: No animal or human experiments were performed during the conduct of the study.

Financial Support: All authors declared that this study received no financial support.

REFERENCES

Aranega-Bou P, de la O Leyva M, Finiti I, García-Agustín P, González-Bosch C. Priming of plant resistance by natural compounds. Hexanoic acid as a model. Frontiers in Plant Science, 2014; 5(488) 1-12. https://doi.org/10.3389/fpls.2014.00488

Baser KHC, Kürkçüoglu M, Ermin N, Tümen G, Malyer H. Composition of the essential oil of *Thymus pseudopulegioides* Klokov et Des.-Shost from Turkey. Journal of Essential Oil Research, 1999; 11(1):86-88. https://doi.org/10.1080/10412905.1999.9701080

Baytop T. Türkiyede Bitkiler ile Tedavi, Publishing house: Ankara Nobel Tıp Kitabevleri, Istanbul, 1999.

Chu WK, Cheung SCM, Lau RAW, Benzie IFF. Bilberry (*Vaccinium myrtillus* L.) in Herbal Medicine: Biomolecular and Clinical Aspects. Benzie IFF, Wachtel-Galor S, editors. Boca Raton (FL): 2011. 2nd edition.CRC Press/Taylor & Francis, 2011.

Cowan MM. Plant products as antimicrobial agents. Clinical Microbiology Reviews, 1999; 12(4):564-582. https://doi.org/10.1128/cmr.12.4.564

Dao CA, Patel KD, Neto CC. Phytochemicals from the fruit and foliage of cranberry (Vaccinium macrocarpon)-potential benefits for human health. In *Emerging trends in dietary components for preventing and combating disease* (pp. 79-94). American Chemical Society, 2012.

Davis PH. Flora of Turkey and The East Aegean Islands, vol 6-7. Edinburgh University press, 1982.

Demirezen YY. *Varroa destructor* ile doğal enfeste bal arısı (Apis mellifera) kolonilerinde sentetik piretroid, organic asit ve esansiyel yağ asit etkinliğinin karşılaştırılması, Yüksek Lisans Tezi, Aydın Adnan Menderes Üniversitesi, Aydın, 2019.

Elkıran O, Avşar C. Chemical composition and biological activities of the essential oil from the leaves of *Vaccinium myrtillus* L. Bangladesh Journal of Botany, 2020; 49(1):91-96.

Erata H, Batan N, Abay G, Özdemir T. Anzer Vadisi ve Çevresinin Briyofit Florası (İkizdere, Rize). Anatolian Bryology, 2021;7(2):131-145. https://doi.org/10.26672/anatolianbryology.993644.

Ertas A, Boga M, Yilmaz MA, Yesil Y, Tel G, Temel H, Hasimi N, Gazioglu I, Ozturk M, Ugurlu, P. A detailed study on the chemical and biological profiles of essential oil and methanol extract of *Thymus nummularius* (Anzer tea): Rosmarinic acid. Industrial Crops and Products, 2015; 67:336-345. https://doi.org/10.1016/j.indcrop.2015.01.064.

Gerçek YC, Bayram S, Çelik S, Canlı D, Mavaldi MH, Boztas K, Bastürk FN, Kırkıncı S, Yesil Y, Kösesakal T, Öz GC, Bayram NE. Characterization of essential oil and wastewater from *Thymus nummularius* M. Bieb. and micromorphological examination of glandular trichomes. Journal of Essential Oil Bearing Plants, 2022; 25(3):690-706. https://doi.org/10.1080/0972060X.2022.2107403.

Gül LB, Özdemir N, Gül O, Çon A. Evaluation of *Thymus pseudopulegioides* plant extracts for total phenolic contents, antioxidant and antimicrobial properties. European Food Science and Engineering, 2022; 3(1):1-4. https://doi.org/10.55147/efse.1091864.

Günaydın M, Laghari AH, Bektaş E, Sökmen M. Sökmen A. Accumulation of phenolics in natural and micropropagated plantlets of *Thymus pseudopulegioides* Klokov & Des.-Shost. with their antioxidant potentials. Turkish Journal of Biology, 2017; 41(5):754-764. https://doi.org/10.3906/biy-1704-9.

Güner A. Türkiye Bitkileri Listesi (Damarlı Bitkiler), Nezahat Gökyiğit Botanik Bahçesi Yayınları, Istanbul, 2012.

Karakaş N, Okur ME, Sağır T, Uludağ D, Polat DÇ, Karadağ AE. Antioxidant activity and anti-cancer effects of bilberry (*Vaccinium myrtillus* L.) fruit extract on gastric cancer, AGS cell line. Journal of Faculty of Pharmacy Ankara, 2022; 46(3):781-792. https://doi.org/10.33483/jfpau.1069607.

Kowalczyk C, Kxzesmski P, Kura M, Szmigiel B, Blaszczyk J. Anthocyanins in medicine. Polish Journal of Pharmacology, 2003; 55(5):699-702.

Küçükbay FZ, Kuyumcu E, Azaz AD, Arabacı T, Yücetürk SÇ. Chemical composition of the essential oils of three Thymus taxa from Turkey with antimicrobial and antioxidant activities. Records of Natural Products, 2014; 8(2):110-120. https://hdl.handle.net/20.500.12462/8546.

Lee SO, Han SM, Kim HM, Jeung SK, Choi JY, Kang IJ. Chemical components and antimicrobial effects of Corni fructus. Journal of the Korean Society of Food Science and Nutrition, 2006; 35(7):891-896. https://doi.org/10.3746/jkfn.2006.35.7.891.

Lukas B, Schmiderer C, Mitteregger U, Novak J. Arbutin in marjoram and oregano. Food Chemistry, 2010; 121(1):185-90. https://doi.org/10.1016/j.foodchem.2009.12.028.

Ozen T, Demirtas I. Antioxidative properties of *Thymus pseudopulegioides*: comparison of different extracts and essential oils. Journal of Essential Oil Bearing Plants, 2015;18(2):496-506.https://doi.org/10.1080/0972060X.2014.998719.

Özkök A, Sorkun K, Salih B. The Microscopic and GC-MS Analysis of Turkish Honeydew Pine Honey. Hacettepe Journal of Biology and Chemistry, 2016; 44(4):375-383.

Puupponen-Pimià R, Nohynek L, Alakomi HL, Oksman-Caldentey KM. The action of berry phenolics against human intestinal pathogens. Biofactors, 2005; 23(4):243-51. https://doi.org/10.1002/biof.5520230410.

Seeram NP. Berry fruits: Compositional elements, biochemical activities, and the impact of their intake on human health, performance, and disease. Journal of Agricultural and Food Chemistry, 2008; 56(3):627-629. https://doi.org/10.1021/jf071988k.

Sezik E, Zor M, Yesilada E. Traditional medicine in Turkey II. Folk medicine in Kastamonu. International Journal of Pharmacognosy, 1992; 30(3):233-9. https://doi.org/10.3109/13880209209054005.

Shah MD, Seelan JSS, Iqbal M. Phytochemical investigation and antioxidant activities of methanol extract, methanol fractions and essential oil of *Dillenia suffruticosa* leaves. Arabian Journal of Chemistry, 2020; 13(9):7170-7182. https://doi.org/10.1016/j.arabjc.2020.07.022.

Sunar S, Aksakal O, Yildirim N, Guleray A, Medine G, Sahin F (2009). Genetic diversity and relationships detected by FAME and RAPD analysis among Thymus species growing in eastern Anatolia region of Turkey. Romanian Biotechnological Letters, 2009; 14(2):4313-4318.

Tammar S, Salem N, Bettaieb Rebey I, Sriti J, Hammami M, Khammassi S, Msaada K. Regional effect on essential oil composition and antimicrobial activity of *Thymus capitatus* L. Journal of Essential Oil Research, 2018; 31(2):129-137. https://doi.org/10.1080/10412905.2018.1539415.

Temiz A, Şener A, Tüylü Özkök A, Sorkun K, Salih B. Antibacterial activity of bee propolis samples from different geographical regions of Turkey against two foodborne pathogens, *Salmonella enteritidis* and *Listeria monocytogenes*. Turkish Journal of Biology, 2011; 35(4):503-511. https://doi.org/10.3906/biy-0908-22.

Teodoro GR, Ellepola K, Seneviratne CJ, Koga-Ito CY. Potential use of phenolic acids as anti-Candida agents: A review. Frontiers in Microbiology, 2015; 6:1420. https://doi.org/10.3389/fmicb.2015.01420.

Tepe B, Sarıkürkcü C, Berk Ş, Alim A, Akpulat HA. Chemical composition, radical scavenging and antimicrobial activity of the essential oils of *Thymus boveii* and *Thymus hyemalis*. Records of Natural Products, 2011; 5(3):208-220. https://hdl.handle.net/20.500.12809/4480.

Tutkun E. Arı Akarı (*Varroa destructor*) mücadelesinde timolün kullanılması, Arıcılık Araştırma Dergisi, 2016; 8(1): 1-5.

Upton R. Bilberry fruit *Vaccinium myrtillus* L. *Standards of analysis, quality control, and therapeutics. Santa Cruz, CA:* American Herbal Pharmacopoeia and therapeutic compendium, 2001.

Willis KJ. State of the World's plants, Kew, London: England: Royal Botanic Gardens, 2017.

Zafra-Stone S, Taharat Y, Bagchi M, Chatterjee A, Vinson JA, Bagchi D. Berry anthocyanins as novel antioxidants in human health and disease prevention. Molecular Nutrition & Food Research, 2007; 51(6):675-683. https://doi.org/10.1002/mnfr.200700002.

Citation/Atıf: Çolak M, E Taş E. Investigation of the antimicrobial effect of honeybee venoms (Apitoxin) from *Apis mellifera caucasica* and *Apis mellifera carnica*. U. Bee J. / U. Arı D. 2025; 25 (1): 43-52. DOI: 10.31467/uluaricilik.1593886

ARAŞTIRMA MAKALESI / RESEARCH ARTICLE

INVESTIGATION OF THE ANTIMICROBIAL EFFECT OF HONEYBEE VENOMS (APITOXIN) FROM *APIS MELLIFERA CAUCASICA* AND *APIS MELLIFERA CARNICA*

Apis mellifera caucasica ve *Apis mellifera carnica* Irklarına Ait Arı Zehirlerinin (Apitoxin) Antimikrobiyal Etkisinin Araştırılması

Mehmet ÇOLAK[1]*, Esra TAŞ[2]

[1]*Department of Forest Entomology and Protection, Forest Engineering, Faculty of Forestry, Karabuk University, Merkez, Karabuk, TÜRKIYE. Corresponding author / Yazışma yazarı E-posta: mehmetcolak@karabuk.edu.tr, ORCID No: 0000-0001-6879-6701,

[2] Faculty of Medicine, Samsun University Canik Campus Gürgenyatak District Merkez Sokak No:40-2/1 Canik/Samsun, TÜRKIYE, E-posta: esra.tas@samsun.edu.tr, ORCID No: 0000-0003-0652-5847

Received / Geliş: 30.11.2024 Accepted / Kabul: 01.03.2025 DOI: 10.31467/uluaricilik.1593886

ABSTRACT

The discovery of new therapeutic agents is crucial in the fight against antimicrobial resistance. The antimicrobial potential of apitoxin from *Apis mellifera caucasica* and *A. m. carnica* (Hymenoptera: Apidae) was tested in vitro against Gram-positive (*Staphylococcus aureus* ATCC-25923, *Enterococcus faecalis* ATCC-29212), Gram-negative (*Escherichia coli* ATCC-25922, *Pseudomonas aeruginosa* ATCC-27853) bacterial strains and a fungal pathogen (*Candida albicans* ATCC-10231). Using an electro stimulation technique, Apitoxin was extracted from honey bee colonies under standardized conditions between May 2022 and April 2023. The antimicrobial activity was evaluated using the disk diffusion method and the results were compared with standard antibiotics (ampicillin, vancomycin, trimethoprim-sulfamethoxazole, itraconazole) to calculate the antibiotic equivalence of the apitoxins. Apitoxin from both subspecies showed dose-dependent inhibitory effects against all microorganisms tested. The highest activity was observed against *E. coli*, with inhibition zone diameters of 16.6±0.2 mm for *A. m. caucasica* and 17.0±0.2 mm for *A. m. carnica* ($p<0.05$). No significant differences were found between subspecies in their effects on *E.coli*, *E.faecalis*, and *P.aeruginosa* ($p>0.05$). The results indicate that apitoxin has a broad spectrum of antimicrobial activity and could be used as a therapeutic agent.

Keywords: Apitoxin, Antimicrobial activity, *Apis mellifera caucasica*, *Apis mellifera carnica*

ÖZ

Antimikrobiyal dirençle mücadelede yeni terapötik ajanların keşfi önem taşımaktadır. Bu çalışmada, *Apis mellifera caucasica* ve *A. m. carnica* (Hymenoptera: Apidae) alt türlerinden elde edilen apitoksinin antimikrobiyal potansiyeli, Gram-pozitif (*Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212), Gram-negatif (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853) bakteri suşları ve bir fungal patojen (*Candida albicans* ATCC 10231) mikroorganizmalar üzerinde *in vitro* olarak değerlendirilmiştir. Mayıs 2022-Nisan 2023 tarihleri arasında standardize koşullarda yetiştirilen arı kolonilerinden elektrostimülasyon tekniğiyle apitoksin ekstrakte edilmiştir. Antimikrobiyal aktivite disk difüzyon yöntemiyle değerlendirilmiş ve sonuçlar standart antibiyotiklerle (ampisilin, vankomisin, trimetoprim-sülfametoksazol ve itrakonazol) karşılaştırılarak apitoksinlerin

antibiyotik eşleniği hesaplanmıştır. Her iki alt türden elde edilen apitoksin, test edilen tüm mikroorganizmalara karşı doza bağımlı inhibitör etki göstermiştir. En yüksek etki *E. coli*'ye karşı gözlemlenmiş olup, inhibisyon zon çapları *A. m. caucasica* için 16,6 ± 0,2 mm ve *A. m. carnica* için 17,0 ± 0,2 mm olarak ölçülmüştür (p<0.05). *E. coli*, *E. faecalis* ve *P. aeruginosa* üzerindeki etkilerde alt türler arasında anlamlı fark bulunmamıştır (p>0.05). Sonuçlar, apitoksinin geniş spektrumlu antimikrobiyal aktiviteye sahip potansiyel bir terapötik ajan olarak değerlendirilebileceğini göstermektedir.

Anahtar Kelimeler: Apitoksin, Antimikrobiyal aktivite, *Apis mellifera caucasica, Apis mellifera carnica*

GENİŞLETİLMİŞ ÖZET

Amaç: Bu araştırmanın amacı, *Apis mellifera caucasica* ve *A. m. carnica* alt türlerinden izole edilen apitoksinlerin antimikrobiyal potansiyelinin karşılaştırmalı olarak değerlendirmektir. Çalışma kapsamında apitoksinlerin Gram-pozitif ve Gram-negatif bakteriler ile fungal patojenlere karşı etkinliği incelenmiş, ayrıca standard antimikrobiyal ajanlarla karşılaştırmalı analizleri yapılarak antibiyotik eşdeğerlik değerleri belirlenmiştir.

Gereç ve Yöntem: Araştırma, Mayıs 2022-Nisan 2023 periyodunda standardize koşullarda yetiştirilen arı kolonileri üzerinde yürütülmüştür. Apitoksin ekstraksiyonu, modifiye elektrostimülasyon tekniği kullanılarak 15 dakikalık periyotlar halinde ve 1/15 günlük aralıklarla altı ardışık uygulama şeklinde gerçekleştirilmiştir. Elde edilen apitoksin örnekleri fizyolojik tuz çözeltisinde (0,9% NaCl) 8 mg/mL konsantrasyonunda süspanse edilmiş ve 0,22 µm por çaplı membran filtrasyonla sterilize edilmiştir. Antimikrobiyal aktivite testlerinde referans suşlar olarak *Staphylococcus aureus* ATCC-25923, *Enterococcus faecalis* ATCC-29212, *Escherichia coli* ATCC-25922, *Pseudomonas aeruginosa* ATCC-27853 ve *Candida albicans* ATCC-10231 kullanılmıştır. Antimikrobiyal etkinlik, Kirby-Bauer disk difüzyon yöntemi ile değerlendirilmiş ve kontrol ajanları olarak standart antibiyotikler (ampisilin, vankomisin, trimetoprim-sülfametoksazol, itrakonazol) kullanılmıştır. Verilerin istatistiksel analizi tek yönlü varyans analizi (ANOVA) ve post hoc Tukey testi ile gerçekleştirilmiş, p<0.05 değeri istatistiksel anlamlılık sınırı olarak kabul edilmiştir.

Bulgular: Çalışmada test edilen her iki apitoksin preparatı, tüm mikroorganizmalara karşı doza bağımlı inhibitör etki göstermiştir. Maksimum antimikrobiyal aktivite *E. coli* üzerinde kaydedilmiş olup en yüksek konsantrasyonda inhibisyon zon çapları *A. m. carnica* için 17.0±0.2 mm ve *A. m. caucasica* için 16.6±0.2 mm olarak ölçülmüştür (p<0.05). Antimikrobiyal aktivitenin seyreltme oranı ile ters orantılı olduğu gözlenmiştir. Mikroorganizmaların apitoksine karşı duyarlılıkları değerlendirildiğinde, en yüksek duyarlılığın *E. coli*'de olduğu, bunu sırasıyla *E. faecalis*, *S. aureus* ve *C. albicans*'ın izlediği, en düşük duyarlılığın ise *P. aeruginosa*'da olduğu belirlenmiştir. Standart antibiyotiklerle karşılaştırmalı analizlerde, apitoksinlerin antibiyotik eşlenek değerleri şu şekilde saptanmıştır: *E. coli* için trimethoprim-sülfametoksazol eşleniği *A. m. carnica* ve *A. m. caucasica*'da sırasıyla 0,6 mg/mL ve 0,62 mg/mL; *S. aureus* için vankomisin eşleniği 0,72 mg/mL ve 0,84 mg/mL; *E. faecalis* için ampisilin eşleniği 0,36 mg/mL ve 0,38 mg/mL olarak belirlenmiştir. *P. aeruginosa*'ya karşı trimetoprim-sülfametoksazol eşleniği *A. m. carnica* ve *A. m. caucasica* için sırasıyla 0,48 mg/mL ve 0,52 mg/mL, ampisilin eşleniği ise 0,1 mg/mL ve 0,6 mg/mL olarak hesaplanmıştır. *C. albicans* için itrakonazol eşleniği *A. m. carnica*'da 0,3 mg/mL, *A. m. caucasica*'da 0,24 mg/mL olarak tespit edilmiştir.

Sonuç: Bu araştırma, farklı *A. mellifera* alt türleri arasında apitoksinlerin antimikrobiyal aktivitelerinin karşılaştırmalı analizini sunan ilk çalışmadır. Elde edilen veriler, apitoksinin geniş spektrumlu antimikrobiyal aktivite gösterdiğini ve terapötik ajan olarak potansiyel değer taşıdığını ortaya koymaktadır. Alt türler arasında spesifik patojenlere karşı etkinlik farklılıkları tespit edilmiştir. Antimikrobiyal aktivite analizlerinde *A. m. carnica*'dan elde edilen apitoksin *S. aureus*'a karşı sayısal olarak daha yüksek inhibisyon göstermiş ancak bu fark istatistiksel olarak anlamlı bulunmamıştır (p>0.05). *A. m. caucasica*'dan elde edilen apitoksin ise *C. albicans*'a karşı istatistiksel olarak anlamlı düzeyde daha güçlü antifungal etki sergilemiştir (p<0.05). Apitoksinin antimikrobiyal potansiyelinin tam olarak karakterize edilebilmesi için farklı coğrafi bölgelerden ve mevsimlerden elde edilen örneklerin değerlendirildiği, çevresel faktörlerin etkilerinin incelendiği ve *in vivo* etkinliğin araştırıldığı ileri çalışmalara ihtiyaç duyulmaktadır.

INTRODUCTION

Antimicrobial resistance (AMR) is seen as an increasing threat worldwide. Drug-resistant infections have become a critical challenge to human health due to the inadequacy of existing chemotherapeutic agents and the challenge of developing new antimicrobial agents. The World Health Organization has reported that the mortality rate associated with antimicrobial resistance is expected to be higher than cancer-related mortality by 2050 (O'Neill 2014, WHO 2022). This situation has necessitated the development of novel and effective antimicrobial agents. Endogenous antimicrobial peptides (AMPs) have been identified as bioactive molecules with high therapeutic potential as they are recognized as important components of the innate immune system of various organisms (Aşkar and Aşkar 2017). Apitoxin, which is extracted from *Apis mellifera*, has been identified as a collection of natural compounds with biological activity used for therapeutic purposes. (Tanuğur-Samancı and Kekeçoğlu 2021).

The antimicrobial, hepatoprotective, antioxidant, anti-inflammatory, antineoplastic, antiarthritic, radioprotective, cytoprotective and neuroprotective properties of apitoxin have been demonstrated *in vitro* and *in vivo* studies (Mizrahi and Lensky 1997, Münstedt and Bogdanov 2009). Apitoxin, which plays a crucial role in the defense mechanism of bee colonies and is synthesized in venom glands and stored in the venom sac, with one worker bee containing an average of 0.15-0.30 mg of venom (Crane 1990, Çaprazlı and Kekeçoğlu 2021, Schumacher *et al*. 1989). Apitoxin consists of various peptides, proteins, amino acids, enzymes, carbohydrates, essential oils and mineral components, with melittin (40-60% of dry weight) and phospholipase A2 (10-12% of dry weight) being the main components, along with apamin, histamine, dopamine and epinephrine. The chemical composition of apitoxin is known to vary depending on parameters such as bee subspecies, nutritional factors, ecological conditions and extraction methods (Karimi *et al*. 2012, Moreno and Giralt 2015, Wehbe *et al*. 2019).

Melittin is characterized as a peptide with bacterial cell membrane destabilizing properties and is the predominant active constituent in the apitoxin content. This peptide is characterized by its broad spectrum of antimicrobial, antifungal, antiviral, anticancer and neuromodulatory properties (Bogdanov 2015, Ownby *et al*. 1997, Park *et al*. 2010, Wang *et al*. 2009). The complex composition and versatile biological activity of apitoxin suggest a broad therapeutic potential. However, a gap was identified in the literature regarding the comparative analysis of the antimicrobial activities of apitoxins from different subspecies of *A. mellifera*. The aim of this study was to compare the antimicrobial activity of toxins (apitoxin) from *Apis mellifera caucasica* and *A. m. carnica* (Hymenoptera: Apidae) against various pathogenic gram-negative and gram-positive bacteria and fungi, including *Escherichia coli*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans*, and to compare these effects with the antibiotics commonly used in the treatment of these pathogens (ampicillin, vancomycin, trimethoprim-sulfamethoxazole and itraconazole).

MATERIALS AND METHODS

This study was conducted in two phases between May 2022 and April 2023. In the first phase, bees of *A. m. caucasica* and *A. m. carnica* (Hymenoptera: Apidae) breeds were obtained from the Karabuk Province Beekeepers Association, which was authorized by the Ministry of Agriculture and Forestry. The specimens were housed in separate hives which were maintained throughout the summer season and their venom was collected. Venom extraction was carried out using a low-current electrostimulation method. In the second phase, the antimicrobial efficacy of the venoms against *E. coli*, *E. faecalis*, *P. aeruginosa*, *S. aureus* and *C. albicans* was investigated using the disk diffusion method. The colonies of *A. m. caucasica* and *A. m. carnica* were placed in standard Langstroth hives with southern exposure. The taxonomic analysis of the colonies was based on wing vein patterns and metric body measurements (Ruttner 1988).

Apitoxin extraction was carried out between June and August 2022 using a modified version of the electrostimulation method described by Benton *et al*. (1963). A weak electric current (3.0 mA, 1 Hz) from a 12 V DC source was applied to a fine wire mesh placed at 0.5 cm intervals on a glass plate (20x30 cm) and the device was positioned in the hive. Each extraction session lasted 15 minutes and was repeated six times at 1/15 day intervals. To minimize the risk of contamination, the glass plates were

covered with stretch film before extraction (Fakhim 1998).

The crystallized apitoxin on the glass plates was taken to the laboratory and collected mechanically with a spatula. The apitoxins from different bee breeds were separately placed in Eppendorf tubes and dissolved in 0.9% NaCl solution to achieve a final concentration of 8mg/mL. The solution was sterilized through a membrane filter with a pore diameter of 0.22 µm and stored under cryogenic conditions at -20 °C until use. Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212), Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853) and the fungus *Candida albicans* ATCC 60192 were used for the antimicrobial activity tests. The standard strains were obtained from the American Type Culture Collection (ATCC, Rockville, Maryland). The bacterial strains were cultured with Mueller-Hinton Broth and Mueller-Hinton Agar (Merck), while *C. albicans* was cultured with Sabouraud Dextrose Agar (Difco) and Sabouraud Dextrose Broth (Oxoid). The apitoxins of *A. m. caucasica* and *A. m. carnica* were weighed with analytical precision to 8mg/mL and placed in separate Eppendorf tubes. Each tube was filled with 1 mL of sterile physiological saline (0.9% NaCl) and homogenized by vortexing for 10-12 seconds. Serial dilutions of 10^{1} to 10^{10} were then prepared for each apitoxin preparation (Patel *et al*. 2015). The antimicrobial activity was evaluated using the Kirby-Bauer disk diffusion method (CLSI 2012). The microbial suspensions were adjusted to 0.5 McFarland standard (10^{8} CFU/mL). Bacterial and yeast suspensions (100 µL) were plated in Petri dishes on Mueller-Hinton agar (for bacteria) or Sabouraud dextrose agar (for *C. albicans*). Sterile paper disks (6 mm diameter) were placed on the agar surface and each apitoxin sample (1 mg/mL) was applied at 15 µL/disk. Standard antimicrobials (ampicillin, vancomycin, trimethoprim-sulfamethoxazole, itraconazole) were used as positive controls and 0.9% NaCl as negative control. Specifically, trimethoprim-sulfamethoxazole (SXT25) and ampicillin (AM10) were used for *P. aeruginosa*, vancomycin (VA30) for *S. aureus*, ampicillin (AM10) for *E. faecalis*, trimethoprim-sulfamethoxazole (SXT25) for *E. coli* and itraconazole (ITC10) antibiotic plates for C. albicans. The bacterial plates were incubated at 37 °C for 24 hours, while the fungal plates were incubated at 30°C for 48 hours. The inhibition zones formed after incubation were measured in millimeters using a digital calliper. All experiments were performed in three independent replicates and results were expressed as arithmetic mean. The calculation of the equivalence of the antimicrobial substances was based on the logarithmic relationship between the diameters of the inhibition zones and the concentrations. The following formula was used for this calculation: $E = (\log C_2 - \log C_1) / (R_2 - R_1)$. *E: Equivalence; C_1: Concentration of the antibiotic (µg); C_2: Concentration of the apitoxin (µg);; R_1: Diameter of the inhibition zone of the antibiotic (mm); R_2: Diameter of the inhibition zone of the apitoxin (mm).* The equivalent concentration between two antimicrobial substances was determined using the known concentration and the resulting zone of inhibition of the reference substance and the concentration and the resulting zone of inhibition of the test substance (Andrews 2001, Barry *et al*. 1976).

Statistical analysis was performed using IBM SPSS Statistics 20 software. One-way analysis of variance (ANOVA) and post hoc Tukey tests were performed to assess differences between groups. p-value <0.05 was considered statistically significant. Further statistical analysis was performed to evaluate the magnitude of differences between the two subspecies using effect size calculations (Cohen's d) with 95% confidence intervals (CI).

RESULTS

It was found that the apitoxins obtained from the subspecies *Apis mellifera caucasica* and *A. m. carnica* showed concentration-dependent inhibitory effects against all microorganisms tested (Table-1). Of the microorganisms tested, the highest antimicrobial activity was found against *Escherichia coli*, and this effect was statistically significant compared to the other microorganisms tested ($p<0.05$, one-way ANOVA).

When the apitoxin concentration was lowered from 10^{0} to 10^{10} in *E. coli*, the diameter of the inhibition zone decreased from 17.0 mm to 6.6 mm in *A. m. carnica* and from 16.6 mm to 6.6 mm in *A. m. caucasica*. This observation shows that the effect of apitoxin on *E. coli* is strongly concentration-dependent. At the highest concentration (10^{0}), *E. coli* showed the largest zone of inhibition (*A. m. carnica*: 17.0 mm, *A. m. caucasica*: 16.6 mm), while the smallest zone of inhibition was shown by *P. aeruginosa* (*A. m. carnica*: 8.8 mm, *A. m. caucasica*:

9.4 mm at 10º dilution, Cohen's d = 3.00, 95% CI [1.19, 4.81])).

These results show that *E. coli* is the most sensitive and *P. aeruginosa* the the lowest sensitive microorganism to apitoxin. In the ranking of the sensitivity of the tested microorganisms to apitoxin, *E. coli* was identified as the most sensitive microorganism, followed by *E. faecalis*, *S. aureus* and *C. albicans*, while *P. aeruginosa* was identified as the least sensitive microorganism among the tested microorganisms. Against *E. coli*, the apitoxins of *A. m. carnica* and *A. m. caucasica* produced inhibition zones of 17.0 ± 0.2 mm and 16.6 ± 0.2 mm, respectively (at 10º dilution; d = 2.00, 95% CI [0.48, 3.52]). Both apitoxin preparations showed similar efficacy against *E. coli*, and the difference between the apitoxins was found to be statistically non-significant (p>0.05, Student's t-test). *Staphylococcus aureus and Enterococcus faecalis* showed moderate susceptibility to both apitoxins. The apitoxin of *A. m. carnica* showed higher antimicrobial activity against *S. aureus* than the apitoxin of *A. m. caucasica* (12.4 ± 0.2 mm vs. 11.4 ± 0.2 mm at 10º dilution; Cohen's d = 5.00, 95% CI [2.48, 7.52]). However, this difference did not prove to be statistically significant (p>0.05, Student's t-test).

The apitoxin of *A. m. carnica* remained effective against E. *faecalis* even at a dilution of 10^9 (d = 1.00, 95% CI [-0.31, 2.31]), while its activity against *S. aureus* was limited to a dilution of 10^4. This indicates that it has higher efficacy compared to *E. faecalis*. The apitoxin from *A. m. caucasica* showed higher antifungal activity against *Candida albicans*. A more pronounced zone of inhibition was measured with the apitoxin from *A. m. caucasica* (12.0 ± 0.2 mm versus 10.4 ± 0.2 mm, at 10º dilution; (Cohen's d = 8.00, 95% CI [4.28, 11.72]), and this difference was found to be statistically significant (p<0.05, Student's t-test).

Table-1: Inhibition zones produced by the apitoxins of *A. m. carnica* and *A. m. caucasica* at different dilutions (mm)

	Dilutions	**10^0**	**10^1**	**10^2**	**10^3**	**10^4**	**10^5**	**10^6**	**10^7**	**10^8**	**10^9**	**10^{10}**
A. m. carnica	*E. coli*	17.0	13.4	10.8	10.2	9.2	7.8	7.4	7.4	6.6	-	-
	S. aureus	12.4	10.6	9.4	8.2	8.0	-	-	-	-	-	-
	E. faecalis	12.4	10.6	10.8	10.0	9.4	8.8	8.0	7.6	6.4	6.2	-
	P. aeruginosa	8.8	8.0	8.4	6.8	7.4	8.0	8.0	6.8	8.2	-	-
	C. albicans	10.4	9.4	7.8	6.8	-	-	-	-	-	-	-
A. m. caucasica	*E. coli*	16.6	12.8	11.0	10.2	9.6	9.0	8.4	8.2	7.6	7.0	6.6
	S. aureus	11.4	11.0	9.6	8.8	8.4	8.2	8.0	-	-	-	-
	E. faecalis	12.2	11.0	9.4	8.6	8.4	7.8	7.6	7.4	7.2	6.8	-
	P. aeruginosa	9.4	8.4	9.0	6.4	7.0	6.8	6.6	7.0	6.8	-	-
	C. albicans	12.0	8.8	7.8	7.2	6.8	-	-	-	-	-	-

8 mg venom dissolved in 1 ml of 0.9% NaCl; ± SD (SD = 0.2 mm for all measurements)

The antibiotics used to compare antimicrobial efficacy (ampicillin, vancomycin, trimethoprim-sulfamethoxazole and itraconazole) produced the expected zones of inhibition against all microorganisms tested. The zones of inhibition were measured as follows: Trimethoprim-sulfamethoxazole (SXT) and ampicillin (AM10), used for *P. aeruginosa,* produced zones of 15.6 mm and 8.2 mm, respectively; vancomycin (VA30), used for *S. aureus*, produced a zone of 20 mm; ampicillin (AM10), used for *E. faecalis* (AM10) produced a 28.8 mm zone; the sulfamethoxazole (SXT25) antibiotic disk used for *E. coli* produced a 37.6 mm zone; and the itraconazole (ITC10) disk used for *C. albicans* produced a 14 mm zone of inhibition.

As expected, no zones of inhibition were produced with the 0.9% NaCl solution used as a negative control (Table-2). Calculation of the equivalent concentrations of apitoxins to the antibiotics tested yielded the following results: against *P. aeruginosa*, *A. m. carnica* showed apitoxin at a concentration of 0.48 mg/mL and *A. m. caucasica* apitoxin at a concentration of 0.52 mg/mL for trimethoprim-sulfamethoxazole (SXT25) showed an equivalent effect, while for ampicillin (AM10) effective concentrations of 0.1 mg/mL for *A. m. carnica* apitoxin and 0.6 mg/mL for *A. m. caucasica* apitoxin were determined.

When the zones generated with standard antibiotic disks (SXT-AM) were compared with those of the

apitoxins, it was found that both apitoxins were more effective against *P. aeruginosa* than ampicillin ($p<0.01$). Equivalent activity against *E. coli* was shown by *A. m. carnica* apitoxin at 0.6 mg/mL and *A. m. caucasica* apitoxin at 0.62 mg/mL for sulfamethoxazole (SXT25). For vancomycin (VA30), which is used against *S. aureus,* effective concentrations of 0.72 mg/mL for *A. m. carnica* apitoxin and 0.84 mg/mL for *A. m. caucasica* apitoxin were determined. For ampicillin (AM10) used against *E. faecalis*, equivalent effects were observed at concentrations of 0.36 mg/mL for *A. m. carnica* apitoxin and 0.38 mg/mL for *A. m. caucasica* apitoxin.

Although higher apitoxin concentrations were required for efficacy against *E. coli*, *S. aureus* and *E. faecalis* compared to standard antibiotics, significant antimicrobial activity was still demonstrated ($p<0.01$). Against *Candida albicans*, *A. m. carnica* apitoxin at a concentration of 0.3 mg/mL and *A. m. caucasica* apitoxin at a concentration of 0.24 mg/mL showed comparable activity to itraconazole (ITC10) (Table-2).

Table-2. Inhibition zones produced by standard antibiotic disks and equivalent concentrations of *A. m. carnica* and *A. m. caucasica* apitoxins to the antibiotics tested

Microorganisms	Antibiotic	Inhibition Zone (mm)	Equivalent Concentration (mg/mL)	
			A. m. carnica	*A. m. caucasica*
E. coli	Trimethoprim-Sulfamethoxazole	37.6	0.6	0.62
S. aureus	Vancomycin	20.0	0.72	0.84
P. aeruginosa	Trimethoprim-Sulfamethoxazole	15.6	0.48	0.52
P. aeruginosa	Ampicillin	8.2	0.1	0.6
E. faecalis	Ampicillin	28.8	0.36	0.38
C. albicans	Itraconazole	14.0	0.3	0.24
Negative control	%0.9 NaCl	0	-	-

DISCUSSION

Infectious diseases are considered a major health problem, especially due to the emergence of drug resistance. Therefore, the development of effective new antimicrobial agents with novel mechanisms of action is considered essential. Bee venom (apitoxin) has been identified as an important defense mechanism of honeybees and is considered a promising natural agent for the treatment of cancer and other diseases due to its high biological activity potential. The antibacterial, antifungal and antiviral effects of bee venoms and their therapeutic potential have been demonstrated in numerous studies (El-Seedi *et al*. 2020, Hwang *et al*. 2022, Jadhav *et al.* 2024, Memariani and Memariani 2020).

The antimicrobial efficacy of bee venom is directly related to the composition of its bioactive components. Many studies in the literature have shown that major peptides, particularly melittin and phospholipase A_2, are responsible for this activity and that the differences in efficacy between subspecies are due to variations in the amount and ratios of these components. Previous studies investigating the composition of venoms of different Apis mellifera subspecies showed natural differences in the profiles of bioactive components between subspecies (El Mehdi *et al*. 2021, Małek *et al*. 2022). Therefore, these differences in composition underline the differences in antimicrobial efficacy we observed. While different antimicrobial activities have been reported for different Apis species such as *Apis cerana*, *A. dorsata* and *A. florea* (Surendra *et al.* 2011), there is insufficient data in the literature for a comparative analysis of the antimicrobial activities of apitoxins obtained from *Apis mellifera* subspecies. The antimicrobial activity of bee venoms from *Apis mellifera caucasica* and *Apis mellifera carnica* breeds was investigated against selected Gram-positive and Gram-negative pathogenic microorganisms, and differences in antimicrobial efficacy between the breeds were examined. According to our results, both apitoxin preparations showed concentration-dependent inhibitory effects against all microorganisms tested.

Tanuwidjaja *et al*. (2021) reported that the tested bee venom exhibited broad-spectrum antibacterial activity against all tested potentially pathogenic Gram-positive and Gram-negative bacteria. In

addition, Leandro *et al*. (2015) reported proportional inhibitory effects of apitoxin and its components against oral pathogens with increasing concentration. The concentration-dependent inhibitory effect of phospholipase A_2 contained in bee venom was documented by Boutrin *et al*. (2008).

These studies have shown that apitoxin has a broad spectrum and dose-dependent antimicrobial activity. The highest antimicrobial activity was observed against *Escherichia coli* ($p<0.05$). As reported by Isidorov *et al*. (2023), although bee venom shows high activity against both Gram-positive and Gram-negative bacteria, *E. coli* showed increased sensitivity to its antimicrobial effect. This increased sensitivity of *E. coli* to apitoxin is attributed to the membrane permeabilizing properties of its major components melittin, mast cell degranulation peptide (MCD) and phospholipase A_2, especially on the outer membrane of Gram-negative bacteria (Tanuwidjaja *et al*. 2021).

The apitoxins showed significant antimicrobial activity against *E. coli*, *S. aureus* and *E. faecalis* ($p<0.01$), albeit at higher concentrations than standard antibiotics. This result is consistent with other studies in literature. In addition, Boutrin *et al*. (2008) found that bee venom components, such as phospholipase A_2, showed significant antimicrobial activity against Gram-negative bacteria, especially *E. coli*, albeit at higher effective doses than antibiotics. Hegazi *et al*. (2017) reported strong antimicrobial effects of honey against *E. coli* and *S. aureus*, albeit at higher concentrations than standard antibiotics. These studies indicate the potential of apitoxin as an alternative agent against pathogenic microorganisms but also emphasize the need for further research on the optimal dosage and method of application. In our study, both apitoxins were found to be more effective against *P. aeruginosa* than ampicillin ($p<0.01$). Similarly, Dosler and Karaaslan (2014) reported synergistic antimicrobial effects against multidrug-resistant *P. aeruginosa* strains.

Al-Ani *et al*. (2018) reported that propolis extracts exhibited strong antimicrobial activity against *P. aeruginosa*, comparable to conventional antibiotics. These results suggest that apitoxin should be considered as an alternative or complementary agent for the treatment of *P. aeruginosa* infections, especially against resistant strains. The molecular mechanisms underlying the antimicrobial activity of bee venom primarily involve the disruption of membranes and the inhibition of cellular processes. The main component, melittin, interacts with bacterial cell membranes through its amphipathic structure and forms transmembrane pores that disrupt membrane potential and ion homeostasis, leading to cell death. Phospholipase A_2 catalyzes the hydrolysis of membrane phospholipids and acts synergistically with melittin to increase membrane permeability. In addition, melittin acts on intracellular targets by inhibiting DNA/RNA synthesis and ATP production, while other components such as apamin and MCD peptide contribute by modulating ion channels and inhibiting cell wall synthesis. These multiple mechanisms make it difficult for bacteria to develop resistance (Carpena *et al*. 2020, Stela *et al.* 2024).

While numerous studies have demonstrated the antimicrobial activity and therapeutic potential of bee venoms (El-Seedi *et al.* 2020, Hwang *et al.* 2022, Jadhav *et al*. 2024, Memariani and Memariani 2020), there is insufficient data in the literature for a comparative analysis of the antimicrobial activities of apitoxins extracted from *Apis mellifera* subspecies. Our study revealed certain differences in the antimicrobial activity of apitoxins from *A. m. caucasica* and *A. m. carnica* subspecies. The apitoxin from *A. m. carnica* showed higher antimicrobial activity against S. aureus, while the apitoxin from *A. m. caucasica* showed higher activity against *C. albicans* at the same dilution factor ($p<0.05$).

Our study underlines not only the potential use of apitoxin as an alternative agent against pathogenic microorganisms, but also the importance of selecting bee subspecies. Although the antimicrobial efficacy of various bee products, including honey, propolis and perga, against pathogenic microorganisms has been extensively studied, few studies have been conducted on the antimicrobial efficacy of bee venom. This study was the first to investigate the different antimicrobial efficacy of bee breeds with respect to their venom composition. The lack of LC-MS or HPLC analysis in our study can be considered a limitation. These analysis could have revealed detailed compositional differences between the subspecies. However, the role of bioactive components in antimicrobial efficacy, which is well described in the literature, and the natural variations between subspecies explain the biochemical basis of the differences in efficacy we observed.

Further studies with different bee breeds and microorganisms still need to be conducted. In addition, comparative analysis of the antimicrobial efficacy of bee venoms from different geographical regions and seasons should be conducted to understand the effects of environmental factors. Moreover, analysis of protein composition by LC-MS or SDS-PAGE would be crucial to identify possible variations in protein profiles between different apitoxin samples. Furthermore, the antimicrobial efficacy of bee venoms needs to be investigated in vivo models to contribute to the growing literature on alternative antimicrobial agents.

Acknowledgment: This study was supported by the Karabuk University Scientific Research Project Coordination Unit (project number: KBUBAP-22-ABP-032).

Author Contributions: In this study, M.Ç. was involved in conceptualization, design and planning, project supervision and consultancy, providing resources and materials, data collection and processing. Analysis, interpretation, and literature review were carried out jointly by E.T. and M.Ç. The manuscript was written collaboratively by M.Ç. and E.T., with critical review performed by M.Ç.

Conflict of Interest Statement: The authors declare no conflict of interest with reviewers or editors.

Data Availability: The data used in this study can be obtained from the corresponding author upon reasonable request. Raw data from bee venom samples and antimicrobial test results are available in laboratory records.

Declaration: We declare that this manuscript has not been published elsewhere and has not been submitted for publication elsewhere. The authors are responsible for the accuracy of all information provided in this study.

Ethics Statement: This study does not require ethical approval as it only involves antimicrobial activity tests conducted under laboratory conditions.

Financial Support: This study was supported by the Karabuk University Scientific Research Project Coordination Unit (project number: KBUBAP-22-ABP-032).

REFERENCES

Al-Ani I, Zimmermann S, Reichling J, Wink M. Antimicrobial Activities of European Propolis Collected from Various Geographic Origins Alone and in Combination with Antibiotics. Medicines (Basel). 2018;3;5(1):2, doi.org/10.3390/medicines5010002

Andrews J M. Determination of minimum inhibitory concentrations. Journal of antimicrobial Chemotherapy. 2001;48:5-16, doi.org/10.1093/jac/48.suppl_1.5

Aşkar Ş, Aşkar TK. Antimikrobiyel proteinler ve bağışıklıktaki önemi. Balıkesir Sağlık Bilim Derg, 2017;6(2):82-86, doi.org/10.5505/bsbd.2017.13002

Barry AL, Hoeprich PD, Saubolle MA. The antimicrobic susceptibility test: principles and practices. Lea&Febiger. 1976, doi.org/10.7326/0003-4819-87-3-388_1

Benton AW, Morse RA, Stewart JD. Venom collection from honey bees. Science. 1963;142(3589):228–230, doi.org/10.1126/science.142.3589.228

Bogdanov S. Bee venom: composition, health, medicine: a review. Peptides. 2015, p.1-20

Boutrin MC, Foster HA, Pentreath VW. The effects of bee (*Apis mellifera*) venom phospholipase A2 on Trypanosoma brucei brucei and enterobacteria. Experimental parasitology. 2008;119(2):246-251, doi.org/10.1016/j.exppara.2008.02.002

Carpena M, Nuñez-Estevez B, Soria-Lopez A, Simal-Gandara J. Bee Venom: An Updating Review of Its Bioactive Molecules and Its Health Applications. Nutrients. 2020;12(11):3360, doi: 10.3390/nu12113360

CLSI. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; twenty-second informational supplement (CLSI Document M100-S22). Clinical and Laboratory Standards Institute. 2012

Chen J, Guan SM, Sun W, Fu H. Melittin, the major pain-producing substance of bee venom. Neuroscience bulletin. 2016;32:265-272, doi:10.1007/s12264-016-0024-y

Crane E. Bees and beekeeping: Science, Practice and World Resources. Cornstock Publ., Ithaca, NY., USA. 1990,pp1-593, doi.org/10.1080/0005772X.1953.11094786

Çaprazlı T, Kekeçoğlu M. Bal Arısı Zehrinin Kompozisyonunu ve Üretim Miktarını Etkileyen Faktörler. U. Arı D./U. Bee J. 2021;21:132-145, doi.org/10.31467/uluaricilik.901279

Dosler S, Karaaslan E. Inhibition and destruction of *Pseudomonas aeruginosa* biofilms by antibiotics and antimicrobial peptides. Peptides. 2014;62:32-7, doi.org/10.1016/j.peptides.2014.09.021

El Mehdi I, Falcão SI, Harandou M, Boujraf S, Calhelha RC, Ferreira ICFR, Anjos O, Campos MG, Vilas-Boas M. Chemical, Cytotoxic, and Anti-Inflammatory Assessment of Honey Bee Venom from *Apis mellifera intermissa*. Antibiotics (Basel). 2021;10(12):1514, doi.org/10.3390/antibiotics10121514

El-Seedi H, Abd El-Wahed A, Yosri N, Musharraf SG, Chen L, Moustafa M, Zou X, Al-Mousawi S, Guo Z, Khatib A, *et a*l. Antimicrobial Properties of *Apis mellifera*'s Bee Venom. Toxins. 2020;12(7):451, doi.org/10.3390/toxins12070451

Fakhim ZK. Improved device for venom extraction. Bee World. 1998;79(1):52–56

Hegazi AG, Guthami FM, Gethami AF, Allah FM, Saleh AA, Fouad EA. Potential antibacterial activity of some Saudi Arabia honey. Vet World. 2017;10(2):233-237, doi.org/10.14202/vetworld.2017.233-237

Hwang YN, Kwon IS, Na HH, Park JS, Kim K C. Dual cytotoxic responses induced by treatment of A549 human lung cancer cells with sweet bee venom in a dose-dependent manner. Journal of Pharmacopuncture.2022;25(4):390, doi.org/10.3831/KPI.2022.25.4.390

Isidorov V, Zalewski A, Zambrowski G, Swiecicka I. Chemical Composition and Antimicrobial Properties of Honey Bee Venom. Molecules. 2023;28(10):4135, doi.org/10.3390/molecules28104135.

Jadhav VR, Aher JS, Bhagare AM, Kardel A, Lokhande D. One-Pot Hydrothermal Synthesis of Bio-Active Bee Venom Nanoparticles with Potent Anticancer Activity. 2024, doi.org/10.2139/ssrn.4756813

Karimi A, Ahmadi F, Parivar K, Nabiuni M, Haghighi S, Imani S, Afrouzi H. Effect of honey bee venom on lewis rats with experimental allergic encephalomyelitis, a model for multiple sclerosis. Iran J Pharm Res. 2012;11(2):671-8, doi.org/10.22037/ijpr.2012.1181

Lad PJ, and Shier W T. Activation of microsomal guanylate cyclase by a cytotoxic polypeptide: Melittin, Biochemical and Biophysical Research Communications. 1979;89(1):315-321, ISSN 0006-291X, doi.org/10.1016/0006-291X(79)90980-X

Leandro LF, Mendes CA, Casemiro LA, Vinholis AH, Cunha WR, de Almeida R, Martins C H. Antimicrobial activity of apitoxin, melittin and phospholipase A_2 of honey bee (*Apis mellifera*) venom against oral pathogens. Anais da Academia Brasileira de Ciências. 2015;87(1):147-155, doi.org/10.1590/0001-3765201520130511

Małek A, Kocot J, Mitrowska K, Posyniak A, Kurzepa J. Bee venom effect on glioblastoma cells viability and gelatinase secretion. Frontiers in Neuroscience. 2022;16:792970, doi.org/10.3389/fnins.2022.792970

Memariani H, Memariani M. Anti-fungal properties and mechanisms of melittin. Appl Microbiol Biotechnol. 2020;104:6513–6526, doi.org/10.1007/s00253-020-10701-0

Mizrahi A, Lensky Y. (Eds.). Bee products: properties, applications, and apitherapy. Springer Science & Business Media. 1997. https://books.google.com.tr/books?id=x0PjBwAAQBAJ&printsec=frontcover&hl=tr

Moreno M. Giralt E. Three valuable peptides from bee and wasp venoms for therapeutic and biotechnological use: melittin, apamin and mastoparan. Toxins. 2015;7:1126–1150. doi.org/10.3390/toxins7041126

Münstedt K, Bogdanov S. Bee products and their potential use in modern medicine. Journal of ApiProduct and ApiMedical Science. 2009;1(3):57-63,. doi: 10.3896/IBRA.4.01.3.01

O'Neill J. Antimicrobial Resistance: Tackling a crisis for the health and wealth of nations. Review on Antimicrobial Resistance. 2014. https://wellcomecollection.org/works/rdpck35v/items

Ownby CL, Powell JR, Jiang MS, Fletcher JE. Melittin and phospholipase A2 from bee (*Apis mellifera*) venom cause necrosis of murine skeletal muscle in vivo. Toxicon. 1997;35(1):67-80, doi: 10.1016/s0041-0101(96)00078-5

Park JH, Kim KH, Kim SJ, Lee WR, Lee KG, Park KK. Bee Venom Protects Hepatocytes from Tumor Necrosis Factor-alpha and Actinomycin D. Archives of Pharmacal Research. 2010;33(2):215-223, doi.org/10.1007/s12272-010-0205-6

Patel JB, Cockerill FR, Bradford PA, Eliopoulos GM, Hindler JA, Jenkins SG & Zimmer, BL. M07-A10 Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard. Clinical Laboratory Standards Institute. 2015; 35(2)

Ruttner F. Biogeography and Taxonomy of Honeybees. Springer-Verlag, Berlin, 1988, doi.org/10.1007/978-3-642-72649-1_6

Schumacher MJ, Schmidt JO, Egen WB. Lethality of "killer" bee stings. Nature. 1989;337:413, doi.org/10.1038/337413a0

Stela M, Cichon N, Spławska A, Szyposzynska M, Bijak M. Therapeutic Potential and Mechanisms of Bee Venom Therapy: A Comprehensive Review of Apitoxin Applications and Safety Enhancement Strategies. Pharmaceuticals. 2024;17(9):1211, doi.org/10.3390/ph17091211

Surendra NS, Jayaram GN and Reddy MS. Antimicrobial activity of crude venom extracts in honeybees (*Apis cerana, Apis dorsata*, *Apis florea*) tested against selected pathogens. African Journal of Microbiology Research. 2011;5(18):2765-2772, doi.org/10.5897/AJMR11.593

Tanuğur-Samancı A.E, Kekeçoğlu M. An evaluation of the chemical content and microbiological contamination of Anatolian bee venom. PLoS One. 2021;16(7): e0255161, doi.org/10.1371/journal.pone.0255161

Tanuwidjaja I, Svečnjak L, Gugić D, Levanić M, Fuka MM. Chemical Profiling and Antimicrobial Properties of HoneyBee (*Apis mellifera* L.) Venom. Molecules. 2021;26(10):3049, https://doi.org/10.3390/molecules26103049

Wang C, Chen T, Zhang N, Yang M, Li B, Lü X, and Ling C. Melittin, a major component of bee venom, sensitizes human hepatocellular carcinoma cells to tumor necrosis factor-related apoptosis inducing ligand (TRAIL)-induced apoptosis by activating CaMKII-TAK1-JNK/p38 and inhibiting IκBα kinase-NFκB. Journal of Biological Chemistry. 2009;284(6):3804-3813, doi.org/10.1074/jbc.M807191200

Wehbe R, Frangieh J, Rima M, Obeid D El, Sabatier JM, Fajloun Z. Bee venom: Overview of main compounds and bioactivities for therapeutic interests. Molecules. 2019;24(16):2997.24, doi.org/10.3390/molecules24162997

WHO. World Health Organization. Global Antimicrobial Resistance and Use Surveillance System (GLASS) Report 2022. Geneva: World Health Organization. https://www.who.int/publications/i/item/9789240062702, 2022, (accessed: 11 October 2024) .

Citation/Atıf: Güzel A, Taşçı GT. Artvin yöresinde bal arılarında varroosis enfestasyonlarına karşı *coriandrum sativum l.* (kişniş otu) ekstraktının etkinliğinin araştırılması (Investigation on effectiveness of *Coriandrum sativum L*. (Coriander Herb) extract against Varroosis infestations in honey bees in Artvin Region-Extended Abstract in English). U. Bee J. / U. Arı D. 2025; 25 (1): 53-60. DOI: 10.31467/uluaricilik.1631987

ARAŞTIRMA MAKALESI / RESEARCH ARTICLE

ARTVİN YÖRESİNDE BAL ARILARINDA VARROOSİS ENFESTASYONLARINA KARŞI *CORIANDRUM SATIVUM L.* (KİŞNİŞ OTU) EKSTRAKTININ ETKİNLİĞİNİN ARAŞTIRILMASI

Investigation on Effectiveness of *Coriandrum sativum L*. (Coriander Herb) Extract Against Varroosis Infestations in Honey Bees in Artvin Region

Aşkın GÜZEL[1], Gencay Taşkın TAŞÇI[2*]

[1]Aşkın Veteriner Kliniği, Arhavi, Artvin, TÜRKİYE. E-posta: lazzz08@hotmail.com, ORCID No: 0000-0002-6365-658X

[2]Kafkas University, Faculty of Veterinary Medicine, Department of Preclinical Studies, Parasitology, Kars, TÜRKİYE. Corresponding author / Yazışma yazarı E-posta: taskintasci@hotmail.com, ORCID No: 0000-0002-8590-1101

Received / Geliş: 03.02.2025 Accepted / Kabul: 24.03.2025 DOI: 10.31467/uluaricilik.1631987

ÖZ

Bu araştırma, Artvin yöresinde arılarda varroosis tedavisinde kullanılan ticari bir preparat ile *Coriandrum sativum L.* (kişniş otu) bitki ekstraktının %5 ve %20'lik yoğunlukta hazırlanmış solusyonlarının erken ilkbahar ve geç sonbaharda etkinliğinin karşılaştırılması amacıyla yapılmıştır. Her iki mevsimde de arılı 7 kovana ilaç verilmemiş, 7 kovana %5'lik *Coriandrum sativum L.*, 7 kovana %20'lik *Coriandrum sativum L.* ve 7 kovana da 500mg Amitraz şerit uygulanmıştır. Uygulamalar sonrasında kovandaki Varroa etkenleri toplanmıştır. İlkbahar uygulamasında %5'lik *Coriandrum sativum L.* solusyonunun %16.07, %20'lik solusyonun ise %49.02 oranında etkili olduğu görülmüştür. Sonbaharda bu oran %5'lik solusyonda %11.91, %20'lik solüsyonda ise %54.12 olarak belirlenmiştir. Bu çalışmanın sonuçları *Coriandrum sativum L.* bitki ekstraktının varroasid etki gösterdiğini ortaya koymaktadır.

Anahtar Kelimeler: Amitraz, Artvin, *Coriandrum sativum, Varroa destructor*

ABSTRACT

This study was conducted to compare the efficacy of a commercial preparation used in the treatment of varroosis on bees with 5% and 20% solutions of *Coriandrum sativum L.* (coriander) plant extract during early spring and late autumn in Artvin. In both seasons, no treatment was applied to 7 beehives, while 7 beehives were treated with 5% *Coriandrum sativum L.,* 7 beehives with 20% *Coriandrum sativum L.,* and 7 beehives with 500 mg Amitraz strips. Following the applications, Varroa mites in the hives were collected. In the spring application, the 5% *Coriandrum sativum L.* preparation was found to have an efficacy rate of 16.07%, while the 20% preparation exhibited an efficacy of 49.02%. In the autumn application, the efficacy rates were 11.91% for the 5% preparation and 54.12% for the 20% preparation. The results of this study indicate that *Coriandrum sativum L.* plant extracts exhibit varroacidal effects.

Keywords: Amitraz, Artvin, *Coriandrum sativum, Varroa destructor*

EXTENDED ABSTRACT

Introduction: Turkey is one of the leading countries in beekeeping due to its rich flora, diverse climate, and natural conditions. However, the average yield per hive remains below global standards. A significant portion of this low productivity is attributed to bee diseases. One of the most common diseases affecting bees is varroosis infestation in Turkey since 1976. Chemical synthetic acaricides are commonly used against varroosis. However, over time, resistance to these drugs develops, and residues accumulate in bee products. Many plant extracts have been used as alternatives in varroosis control. In this study, the effect of *Coriandrum sativum L*. plant extract on varroosis infestations in bees in Artvin region was investigated.

Materials and Methods: The study was conducted in a total of 28 hives during early spring and late autumn as the first phase. Before the treatment applications, an average of 200 bees were collected from the outer frames of the hives using the powdered sugar method, and the Varroa infestation rate was determined. *Coriandrum sativum L.* was commercially obtained in dried and powdered form. Essential oil extraction was performed using the steam distillation method. The extracted *Coriandrum sativum L.* oil was suspended in pure olive oil, and 5% and 20% solutions were prepared for the study. These solutions were impregnated onto cardboard strips and placed inside the hives. The hives in the apiary were divided into four groups: Control group (no treatment), Amitraz-treated group, Experiment 1 (5% *Coriandrum sativum L*.), Experiment 2 (20% *Coriandrum sativum L*.). In both seasons when Amitraz and *Coriandrum sativum L.* extract were applied, white paper was placed at the bottom of the hives, and *Varroa* mites falling onto the hive drawer were counted on days 1, 3, 7, 14, 21, and 28 post-application. The effectiveness of the applied treatments was calculated using the Henderson–Tilton formula. The collected data were analyzed using one-way variance analysis (ANOVA) with SPSS 22.0 software.

Results: The results indicated that coriander extract was effective at certain doses in controlling varroosis during both early spring and late autumn.

In Early Spring**,** before treatment applications, the *Varroa destructor* infestation rates were determined as follows: Control group: 12.47%, Amitraz group: 12.31%, Experiment 1 (5% *Coriandrum sativum L*.): 11.63%, Experiment 2 (20% *Coriandrum sativum L*.): 10.68%. After treatment, the total number of *V. destructor* mites observed was: Control group: 10 mites, Amitraz group: 317 mites, Experiment 1: 110 mites, Experiment 2: 167 mites. The effectiveness rates were calculated as follows: Amitraz: 84.47% ± 2.16, 20% *Coriandrum sativum L*. extract: 49.02% ± 1.89, 5% *Coriandrum sativum L.* extract: 16.07% ± 1.52. Statistical analysis showed no significant difference between the groups ($P > 0.05$, $p = 0.327$).

In Late Autumn**,** before treatment applications, *V. destructor* infestation rates were determined as follows: Control group: 21.77%, Amitraz group: 21.81%, Experiment 1 (5% *Coriandrum sativum L*.): 19.42%, Experiment 2 (20% *Coriandrum sativum L*.): 23.15%. After treatment, the total number of *V. destructor* mites observed was: Control group: 18 mites, Amitraz group: 824 mites, Experiment 1: 170 mites, Experiment 2: 382 mites. The effectiveness rates were calculated as follows: Amitraz: 81.38% ± 1.01, 20% *Coriandrum sativum L.* extract: 54.12% ± 2.13, 5% *Coriandrum sativum L.* extract: 11.91% ± 1.74. Statistical analysis showed no significant difference between the groups ($P > 0.05$, $p = 0.207$).

Conclusion: Previous studies have reported that varroosis infestation rates in hives can reach up to 100%. Before conducting this study in Dülgerli Village, Arhavi, Artvin, all the examined hives were found to be infested with *V. destructor*. This study investigated the prevalence of *V. destructor* in bees and the effectiveness of *Coriandrum sativum L.* extract as a treatment. Although the effectiveness of this plant extract was found to be low, it is considered potentially beneficial due to the following reasons**:** it has no adverse effects, leaves no residues in bee products, and does not lead to resistance development in mites. Therefore, increasing the concentration of *Coriandrum sativum L.* extract may be a viable approach in varroosis control**.**

GİRİŞ

Türkiye iklimi ve doğası sayesinde arıcılıkta dünyanın sayılı ülkelerinden biri haline gelmiştir. Ancak arı ürünlerindeki verim oldukça düşük kalmıştır. Bu verim düşüklüğünün büyük bir kısmı arı hastalıklarından ileri gelmektedir. Arılarda en çok görünen hastalıklardan biri de varroosis enfestasyonudur. Varroosis etkenleri arıların en patojen hastalık etkenlerinden olup, hemolenf ve vücut yağı ile beslenirler. Hastalık etkeni ilk kez 1904

yılında Java Adası'nda Hint arısı olarak bilinen *Apis cerana*'da tanımlanmış ve türe önce *Varroa jacobsoni* (Oudemans 1904), ilerleyen yıllarda *Varroa destructor* adı verilmiştir (Anderson ve Trueman 2000). Türkiye'de 1976 senesinde Trakya'da tespit edilen varroosis, 1982 yılı itibariyle ülke çapında yayılım göstermiştir. Varroosis dünya üzerinde ilacına en yüksek bütçe ayrılan enfestasyonlardan biridir (Aydın 2013, Tutkun ve İnci 1985). Varroosise *Varroa jacobsoni*, *V. underwoodi, V. rindereri* ve *V. destructor* gibi akarlar neden olmaktadır (Lindquist vd. 2009). Bu akarların en patojeni Türkiye'nin yanı sıra dünya genelinde yaygınlık gösteren *V. destructor*'dur. Varroosis enfestasyonu arıların pupa ve erişkin evrelerine tutunarak arıda %25 ağırlık kaybına, kanat deformasyonlarına, yaşam süresinde azalmaya, koloni çökmesine sebebiyet verebilmektedir (Girişgin ve Aydın 2010).

V. destructor, yavru gözlerini arılarla beraber kullanıp pupanın vücut sıvısını emer, taşıdığı diğer patojenleri kovana nakleder, arıların kış salkımı yapmasına engel olur veya salkımın erken sonlanmasına sebep olur (Muz ve Muz 2009, Muz vd. 2012). Hem ekonomik hem de uygulaması kolay olduğu için varroosise karşı Dünya genelinde daha çok flumetrin, amitraz, coumaphos ve fluvalinate gibi kimyasal sentetik akarisitler tercih edilmektedir. Ancak bilinçsiz arıcıların yanlış uygulamaları akarlarda direnç gelişmesine neden olmakta ve bu akarisitler arı ürünlerinde kalıntı oluşturmaktadır (Bogdanov vd. 1998, Kayode vd. 2014, Kochansky vd. 2001, Martin 2004, Pettis 2004, Wallner 1999).

Varroa mücadelesinde; yapay oğul alarak tuzaklama, petek tellerine elektrik uygulanması, polen tuzağı, kovandaki ısının artırılması, her yıl genç kraliçe arı kullanımı, erkek yavru gözlerinin sınırlandırılması gibi yöntemlerin (Akkaya ve Vuruşaner 1996, Aydın 2013, Webster vd. 2001, Zeybek 1991) yanı sıra, ceviz yaprağı, propolis, ardıç katranı, kekik, sarımsak, kamfur, çörek otu, aloe vera, karanfil, hop beta asitleri gibi bitki ekstraktları kullanılmıştır (Cakmak vd. 2002, Coskun vd. 2008, DeGrandi Hoffman vd. 2012, Fouly ve Al-Dehhari 2009, Garbaczewska vd. 2010, Garedew vd. 2003, Girişgin vd. 2007, Girisgin vd. 2014, Rasool vd. 2017 Wang vd. 2009). Kişniş (*Coriandrum sativum L.*) Apiaceae familyasından olup, M.Ö. 1500'lerden beri parfümeride, yemeklerde ve ilaç sektöründe terapötik ajan olarak kullanılmaktadır (Ulutaş Deniz vd. 2018). Bu araştırmada, Artvin yöresinde arılarda *Coriandrum sativum L.* bitki ekstraktının varroosis enfestasyonlarına etkisinin olup olmadığı araştırılmıştır.

GEREÇ ve YÖNTEM

Araştırma Odaklarında Varroosis Tespiti

Araştırmanın ilk etabı 4 Kasım 2020-2 Aralık 2020, ikinci etap ise 5 Mart 2021-2 Nisan 2021 tarihleri arasında Artvin'in Arhavi ilçesine bağlı Dülgerli köyünde arılı 28 adet kovanda gerçekleştirilmiştir.

Coriandrum sativum L. Esansiyel Yağ Eldesi

Kişniş otu, kurutulmuş toz halinde temin edilmiş, uygulamaya kadar +4°C'ta saklanmıştır. Buhar distilasyon yöntemiyle kişniş otundan esansiyel yağ ekstraksiyonu yapılmıştır. Bu yöntemde 2 L distile suya 100 gr bitki numunesi eklenmiş, clevenger aparatında 100°C'ta 4 saat işlem yapılmıştır. Aparatın haznesinde biriken esansiyel yağ buradan 10 ml'lik deney tüpü içerisine alınmıştır. Tüpe 0,1 gr anhidroz sodyum sülfat eklenmiş, 20 dk sonra suyundan arındırılan yağ farklı bir tüpe alınmıştır (Jimenez-Carmona vd. 1999).

Kovanlara *Coriandrum sativum L.* ve Amitaz (Beeraz) Uygulanması

Bitki ekstraktı uygulanmadan önce, kovanlardan pudra şekeri yöntemiyle (Dietemann vd. 2013) ortalama 200 arı alınmış ve Varroosis oranı tespit edilmiştir. Kovanlardaki *Varroa* sayıları eşitlenmiştir. Kişniş otu yağı saf zeytinyağı ile karıştırılmış ve %5 lik ve %20 lik süspansiyonlar elde edilmiştir. Süspansiyonlar 23 cm x 3,5 cm x 1,25 mm'lik kovan içi şerit boyutlarındaki özel emici kartonlara emdirilerek kovan içerisine yerleştirilmiştir.

Arılıktaki kovanlar 7'şerli olmak üzere 4 gruba ayrılmıştır.

a) Kontrol: Su ve şeker dışında herhangi bir şey verilmemiştir.

b) Amitraz: 500 mg amitraz (Beeraz-Santavet) ihtiva eden şerit yerleştirilmiştir.

c) Deneme 1: %5'lik kişniş otu ekstraktı emdirilmiş 3-4 karton yerleştirilmiştir.

d) Deneme 2: %20'lik kişniş otu ekstraktı emdirilmiş 3-4 karton yerleştirilmiştir.

Uygulamaların yapıldığı iki dönemde de kovan zeminine beyaz kâğıt konulmuş, 1., 3., 7., 14., 21. ve 28. günlerde çekmecedeki akarlar sayılmıştır.

İlaç Etkinliğinin Hesaplanması

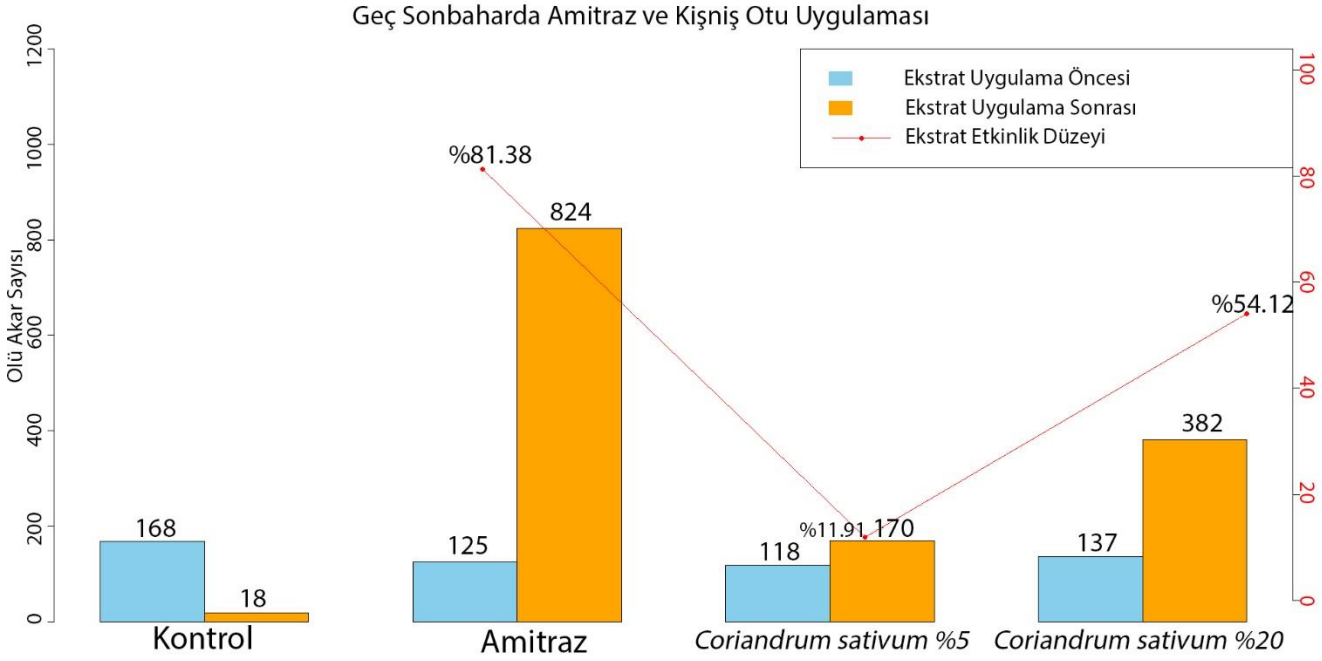
Sonuçlar Henderson-Tilton Formülü ile değerlendirilmiş (Herderson ve Tilton 1955), ilaç etkinliği aşağıdaki gibi hesaplanmıştır:
% Düzeltilmiş = [1 – {n1 x n2} / {n3 x n4}] x 100
n1: Kontrol grubundaki tedavi öncesi akar sayısı
n2: Amitraz veya *Coriandrium sativum L* verilen grupta uygulama sonrası akar sayısı
n3: Kontrol grubundaki tedavi sonrası akar sayısı
n4: Amitraz veya *Coriandrium sativum L* verilen grupta uygulama öncesi akar sayısı

İstatistiksel Analiz

Çalışma verileri SPSS 22.0 programıyla yorumlanmış, gruplar arası farklar tek yönlü varyans analizi (ANOVA) ile değerlendirilmiş, anlamlı farklılıklar Tukey testi ile değerlendirilmiştir.

BULGULAR

Çalışma sonrasında elde edilen veriler, kişniş otunun farklı konsantrasyonlardaki uygulamalarının *V. destructor* üzerine etkilerini ortaya koymuştur. Erken ilkbahar ve geç sonbahar dönemlerinde uygulamalar sonrasında arılarda herhangi bir olumsuz durum ve davranışa rastlanmamış, yapılan ölçümlerde kişniş otu ekstraktının varroosis mücadelesinde belirli dozlarda etkili olduğu görülmüştür. Erken ilkbaharda Amitraz ve kişniş otu uygulama öncesi ve 1, 3, 7, 14, 21 ve 28. günlerde uygulama sonrasında kovanlarda etkisiz hale gelen *Varroa* sayıları ile etken maddelerin etki düzeyleri Grafik-1.'de gösterilmiştir.

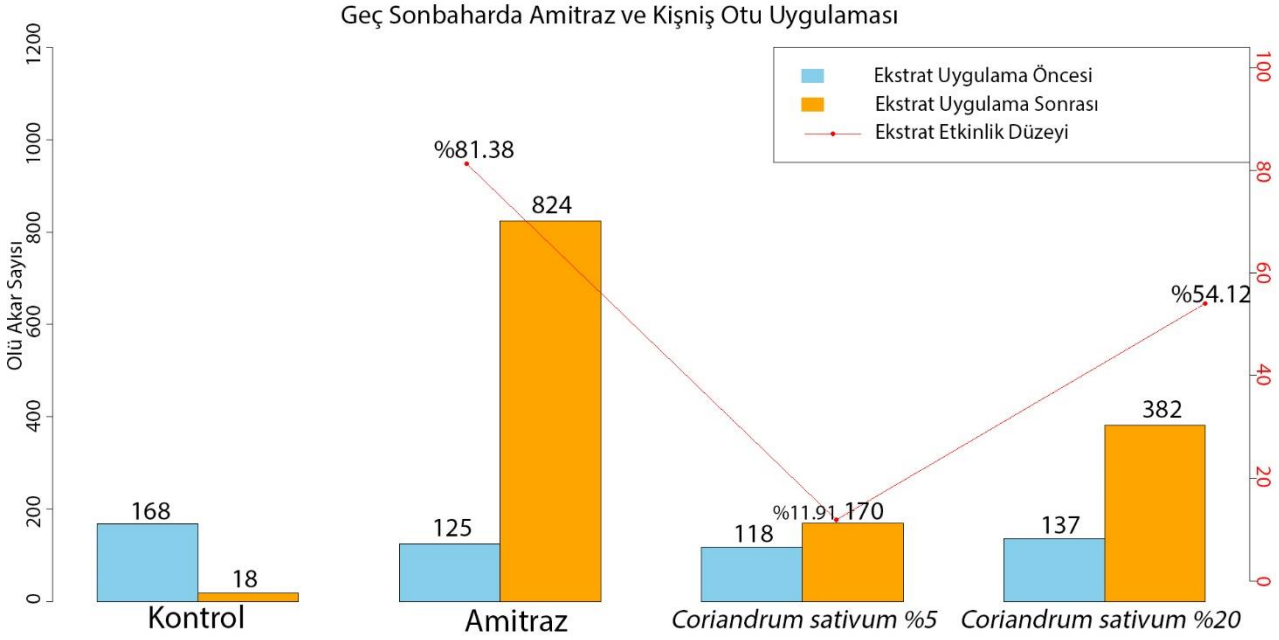


Grafik-1. Erken ilkbaharda Amitraz ve kişniş otu uygulama öncesi ve sonrasında kovanlarda etkisiz hale gelen *Varroa* sayıları ile etken maddelerin etki düzeyleri.

Figure-1. The number of ineffective *Varroa* mites detected in hives and the effectiveness levels of the active ingredients before and after the application of Amitraz and coriander during the early Spring period,

Erken İlkbaharda Amitraz ve kişniş otu uygulamaları öncesinde; 567 arının bulunduğu 7'şer kovandan oluşan Kontrol grubunda 72 (ortalama %12.47), 690 arının bulunduğu Amitraz grubunda 88 (ortalama %12.31), 462 arının bulunduğu Deneme 1 grubunda 63 (ortalama %11.63) ve 613 arının bulunduğu Deneme 2 grubunda ise 67 (ortalama %10.68) adet akar sayılmıştır. Aynı dönemde ilaç uygulamaları sonrasında yapılan sayımlar neticesinde ise Kontrol grubunda 10, Amitraz grubunda 317, Deneme 1

grubunda 110 ve Deneme 2 grubunda 167 adet *V. destructor*'un etkisiz hale geldiği görülmüştür. Amitraz uygulamalarının etkinlik düzeyi ilgili formüle göre %84.47±2.16, *C. sativum L* %20'lik ekstraktının uygulamaları sonrasında etkinlik düzeyi %49.02±1.89 ve *C. sativum L* %5'lik ekstraktının uygulama sonrası etkinliği %16.07±1.52 olarak tespit edilmiştir. Yapılan istatistiksel analizler neticesinde gruplar arasında anlamlı bir fark olmadığı (P>0.05, p = 0.327) görülmüştür. Geç sonbaharda Amitraz ve kişniş otu uygulama öncesi ve 1, 3, 7, 14, 21, ve 28. günlerde uygulama sonrasında kovanlarda etkisiz hale gelen *Varroa* sayıları ile etken maddelerin etki düzeyleri Grafik-2.'de gösterilmiştir.



Grafik-2. Geç sonbaharda Amitraz ve kişniş otu uygulama öncesi ve sonrasında kovanlarda etkisiz hale gelen *Varroa* sayıları ile etken maddelerin etki düzeyleri.

Figure-2. The number of ineffective *Varroa* mites detected in hives and the effectiveness levels of the active ingredients before and after the application of Amitraz and coriander during the late Autumn period,

Geç sonbaharda Amitraz ve kişniş otu uygulamaları öncesinde; 764 arının bulunduğu 7'şer kovandan oluşan Kontrol grubunda 168 (ortalama %21.77), 582 arının bulunduğu Amitraz grubunda 125 (ortalama %%21.81), 596 arının bulunduğu Deneme 1 grubunda 118 (ortalama %19.42) ve 593 arının bulunduğu Deneme 2 grubunda ise 137 (ortalama %23.15) adet akar sayılmıştır. Aynı dönemde ilaç uygulamaları sonrasında yapılan sayımlar neticesinde Kontrol grubunda 18, Amitraz grubunda 824, Deneme 1 grubunda 170 ve Deneme 2 grubunda 382 adet *V. destructor*'un etkisiz hale geldiği görülmüştür. Bu dönemde yapılan *Coriandrum sativum L.* %20'lik ekstraktının uygulamaları sonrasında etkinlik düzeyi %54.12±2.13 ve %5'lik ekstraktının uygulamaları sonrasında etkinlik düzeyi % 11.91±1.74, Amitraz'ın ise %81.38±1.01 olarak tespit edilmiştir. Yapılan istatistiksel analizler neticesinde gruplar arasında anlamlı bir fark olmadığı (P>0.05, p = 0.207) görülmüştür.

TARTIŞMA

Artvin ilinin Karadeniz Bölgesi'nin doğusunda engebeli bir arazide konumlanması, tarımsal üretimin ve hayvancılığın kısıtlı imkanlarla yapılmasına neden olmaktadır. Yörenin bitki örtüsü endemik bitkiler açısından zengin olup, bal üretimine önemli katkı sağlamaktadır. Arıcılık bölgede yoğun olarak ilgilenilen bir sektör olmakla birlikte talep edilen verim alınamamaktadır. Bu verim kaybının başlıca nedenleri arıcılığın geleneksel yöntemlerle

yapılıyor olması ve bazı patojen hastalık etkenleridir. Bu hastalıkların en önemlisi varroosis'dir. Kovandaki *Varroa* yoğunluğu yıldan yıla değişebilmektedir. Dolayısıyla sürekli takip gerekmektedir (Fries vd. 1994). Yapılan çalışmalarda kovanlarda varroosis enfestasyonunun %100'lere ulaşabildiği bildirilmiştir (Çakmak 2017, Önk 2003). Artvin ilinin Arhavi ilçesine bağlı Dülgerli köyünde yapılan bu araştırmada kovanların tamamında *V. destructor* görülmüştür. Arı ürünlerinde görülen en önemli kirlilik, insan sağlığını da ciddi anlamda tehdit eden sentetik kimyasal akarisit kalıntılarıdır. Bu problemi giderebilmek amacıyla organik asitler, uçucu yağlar ve bitki ekstrakları kullanılmıştır (Alparslan 2019, Aydın vd. 2007, Cakmak vd. 2002, Damiani vd. 2011, Girisgin ve Aydın 2010, Kütükoğlu vd. 2012, Rasool vd. 2017, Yücel ve Duran 2004).

Yapılan bir çalışmada karanfil %62, sarımsak %51, kamfur %47, çörek otu %43 ve aloe vera bitkisi %34 oranında varoosis mücadelesinde etkin bulunmuş (Fouly ve Al-Dehhari 2009), nane ekstraktının kovandaki *Varroa*'ların %97'sinden fazlasını öldürdüğü (Ariana vd. 2002) tespit edilmiştir. *Varroa* bulaşıklık oranı %36-38 arasında olan arı kolonilerine Mersin bitkisi (*Myritus communis L*.) verilmiş ve oranın %12-13'e düştüğü görülmüştür (Turhan ve Şengül 2020). Van yöresindeki bir araştırmada; bazı bitki ekstraklarının %25 yoğunluktaki süspansiyonlarının *Varroa*'lara etkinliği sonbaharda ceviz yaprağının %87, ardıç yaprağının %86, kekikin %83 ve nanenin %68 bulunurken, ilkbaharda bu oranlar sırasıyla %63, %61, %66 ve %55 olarak kaydedilmiştir (Alparslan 2019).

Varroosis ile mücadelede ceviz yaprağı (Cakmak vd. 2002, Garbaczewska vd. 2010, Rasool vd. 2017, Wang vd. 2009), propolis (Garedew vd. 2003), ardıç katranı (Girişgin vd. 2007), kekik (Coskun vd. 2008), karanfil (Girisgin vd. 2014), hop beta asitleri (DeGrandi Hoffman vd. 2012) gibi bitki ekstrakları da kullanılmıştır. Yapılan bu araştırmada, daha önce Dünya'da ve Türkiye'de *Varroa* veya başka bir zararlı etkene karşı arıcılıkta herhangi bir çalışmada kullanılmamış olan kişniş otu ekstraktının etkinlik düzeyi ilkbaharda %5'lik solusyonda %16.07, %20'lik solusyonda %49.02, sonbaharda %5'lik solusyonda %11.91 ve %20'lik solusyonda %54.12 oranında belirlenmiştir. Belirlediğimiz oranlar arasındaki farklılığın ekstrakların uygulandığı mevsimlerden ve kovanlardaki arı sayılarının farklı olmasından kaynaklandığı düşünülmektedir. Ayrıca bu oranların diğer araştırmalardan düşük olması, kişniş otunun %5 ve %20'lik süspansiyonlarının varroosise karşı etkinliğinin diğer bitki ekstraktlarına kıyasla yeterli düzeyde olmadığını göstermiştir. Bitki ekstraktlarının varrosis mücadelesindeki etkinliğini nem, sıcaklık gibi çevresel faktörlerin etkileyebileceği kaydedilmektedir (Alparslan 2019). Çalışmanın yapıldığı Artvin yöresi yıl boyunca yağış almaktadır. Kişniş otu ekstraktının etkinliğinin düşük bulunmasına gerekçe olarak ekstrakt uygulama zamanları ve sürekli yağan yağmurun gösterilebileceği kanısına varılmıştır. Araştırmanın yapıldığı zaman dilimlerinde hava sıcaklığı ortalaması Mart'ta 18^{o}C, Kasım'da 17^{o}C olarak tespit edilmiştir. *Varroa* etkenleriyle mücadele edilirken ilacın nasıl uygulandığı oldukça önemlidir. Bir araştırmada; kovanlara karton dumanı ve ardıç katranı körükte yakılarak iki farklı yöntem uygulanmış, karton dumanı %2.64 (±0.78) ve ardıç katranı dumanı %3.61 (±4.51) etkin bulunmuştur (Girişgin vd. 2007).

Yaptığımız bu araştırmada kişniş otu ekstraktı kartona emdirilerek kovanlara yerleştirilmiştir. Püskütme veya damlalık uygulamaların daha etkili olacağı düşünülmektedir. Sonuçta; bu araştırmada Artvin ilinin Arhavi ilçesine bağlı Dülgerli köyünde bal arılarında *V. destructor*'un yaygınlığı ve bu akar ile mücadelede kişniş otu ekstraktının etkinlik düzeyi araştırılmıştır. Bu araştırmada; arıcılığın en önemli sorunlarının başında yer alan varroosisle mücadelede kişniş otu ekstraktının etkinlik düzeyi her ne kadar düşük bulunsa da, önceki yıllarda yapılan çalışmalarda da (Cakmak vd. 2002, Coskun vd. 2008, DeGrandi Hoffman vd. 2012, Garbaczewska vd. 2010, Girisgin vd. 2014, Rasool vd. 2017) belirtildiği üzere, diğer bitki ekstraktlarında olduğu gibi kişniş otu ekstraktının da kalıntı bırakmaması veya az kalıntı bırakması, arıya herhangi bir zararlı etkisinin olmaması, direnç oluşturmaması gibi avantajlardan dolayı faydalı olabileceği, hatta yoğunluğu arttırıldığı takdirde *Varroa* etkenleriyle mücadelede etkin olabileceği düşünülmektedir.

Çıkar Çatışması: Yazarlar arasında herhangi bir tartışmalı durum söz konusu değildir.

Etik Durumu: Çalışma için gerekli olan izinler TC Tarım ve Orman Bakanlığı (21.01.2020 tarih ve 32342694-325.99-E200182 sayılı yazı) ile Kafkas Üniversitesi Hayvan Deneyleri Yerel Etik Kurulu (28.04.2020 tarih ve KAÜ-HADYEK/2020-069 sayılı yazı)'ndan alınmıştır.

Mali Kaynak: Bu çalışma ilk isim yazarın Yüksek Lisans tezinden özetlenmiş olup, Kafkas Üniversitesi

Bilimsel Araştırma Projeleri Koordinatörlüğü (Proje No: 2021-TS-64) tarafından desteklenmiştir.

Yazar Katkıları: Yazarlar makaleye eşit oranda katkı sağlamıştır.

KAYNAKLAR

Akkaya H, Vuruşaner C. Bal arısı hastalıkları ve zararları, Teknik Yayın, İstanbul, 1996, s.131.

Alparslan B. Van gölü havzasında bal arısı (*Apis mellifera L*.) kolonilerinde varroosise karşı tıbbi bitki ekstrelerinin etkinliğinin araştırılması, Ege Üniversitesi, Sağlık Bilimleri Enstitüsü, Doktora Tezi, İzmir, 2019, (erişim tarihi.11.01.2025), https://tez.yok.gov.tr/UlusalTezMerkezi/tezDetay.

Anderson DL, Trueman JWH. *Varroa jacopsoni* (Acari: Varroidea) is more than one species. Exp Appl Acarol. 2000;24(3):165-189, DOİ: 10.1023/a:1006456720416.

Ariana A, Ebadi R, Tahmasebi G. Laboratory evaluation of some plant essences to control *Varroa destructor* (Acari: Varroidae). Exp App Acarol. 2002; 27(4):319-327, DOİ: 10.1023/a:1023342118549.

Aydın L, Güleğen E, Çakmak İ, Girişgin O. The occurrence of *Varroa destructor* Anderson and Trueman, 2000 on honey bees (*Apis mellifera*) in Turkey. Turk J Vet Anim Sci. 2007;31(3):189-191.

Aydın L. Balarılarının Paraziter Hastalıkları ve Zararları. Ed. Özcel M.A ‘’Veteriner Hekimliğinde Parazit Hastalıkları’’ Türkiye Parazitoloji Derneği Yayın No:24, İzmir, 2013, s.1347-1352.

Bogdanov S, Imdorf A, Kilchenmann V. Residues in wax and honey after apilife VAR® treatment. Apidologie. 1998;29(6): 513-524.

Cakmak I, Aydin L, Camazine S, Wells H. Pollen traps and walnut leaf smoke for *Varroa* control. American Bee J. 2002;142(5): 367-37.

Coskun S, Girisgin O, Kürkcüoglu M, Malyer H, Girisgin AO, Kırımer N vd. Acaricidal efficacy of *Origanum onites L*. essential oil against *Rhipicephalus turanicus* (Ixodidae). Parasitol Res. 2008;103(2): 259-261, DOİ: 10.1007/s00436-008-0956-x.

Çakmak SS. Balıkesir Marmara adasındaki bal arısı (*Apis Mellifera Anatoliaca*) kolonilerinde Pudra Şekeri yöntemi ile *Varroa* (*Varroa destructor*) bulaşıklık seviyesinin belirlenmesi. Ankara Üniversitesi, Fen Bilimleri Enstitüsü, Biyoloji Anabilim Dalı Yüksek Lisans Tezi, Ankara, 2017, (erişim tarihi.11.01.2025), https://tez.yok.gov.tr/UlusalTezMerkezi/tezDetay.

Damiani N, Gende LB, Maggi MD, Palacios S, Marcangeli JA, Eguaras MJ. Repellent and acaricidal effects of botanical extracts on *Varroa destructor*. Parasitol Res. 2011;108(1):79-86, DOİ: 10.1007/s00436-010-2043-3.

Degrandi-Hoffman G, Ahumada F, Probasco G, Schantz L. The effects of beta acids from hops (*Humulus lupulus*) on mortality of *Varroa destructor* (Acari: Varroidae). Exp App Acarol. 2012;58(4): 407-421, DOİ: 10.1007/s10493-012-9593-2.

Dietemann V, Nazzi F, Martin SJ, Anderson DL, Locke B, Delaplane KS vd. Standard methods for *Varroa* research. J Apicultural Res. 2013;52(1):1-54, DOİ:10.3896/IBRA.1.52.1.09.

Fouly AH, Al-Dehhairi MA. Evaluation of infestation levels of the ectoparasitic mite *Varroa destructor* infesting honeybee *Apis mellifera* and its control using essential oil in Qassim Region, Saudi Arabia. J Ent. 2009;6:135-144, DOİ: 10.3923/je.2009.135.144.

Fries I, Wallner K, Rosenkranz P. Effects on *Varroa jacobsoni* from acaricides in beeswax. J Apicultural Res. 1994;37(2): 85-90, DOİ:10.1080/00218839.1998.11100959.

Garbaczewska S, Kazimierczak J, Lysik A, Londzin W. Ecological mite control agents, particularly against *Varroa destructor*. Pesticides. 2010;1(4): 73-77.

Garedew A, Schmolz E, Lamprecht I. Microcalorimetric and respirometric investigation of the effect of temperature on the antivarroa action of the natural bee product-propolis. Thermochimica Acta. 2003;399(1-2): 171-180, DOİ: 10.1016/S0040-6031(02)00453-7.

Girisgin AO, Barel S, Barzilai DZ, Girisgin O. Determining the stability of clove oil (eugenol) for use as an acaricide in beeswax. Isr J Vet Med. 2014;69(4):192-196.

Girişgin O, Çakmak İ, Çakmak SS, Aydın L. *Varroa*'ya karşı ardıç katranı dumanı etkili mi?. Uludağ Arıcılık Derg, 2007;4,132-134.

Girişgin O, Aydın L. *Varroa destructor* ile doğal enfeste bal arılarında organik asitlerin kullanımı ve etkinliği. Kafkas Üniv Vet Fak Derg. 201016(6): 941-945, DOİ: 10.9775/kvfd.2010.1965.

Henderson CF, Tilton EW. Tests with acaricides against the brown wheat mite. J Econ Entomol. 1955;48(2):157-161, DOİ: 10.1093/jee/48.2.157.

Jimenez-Carmona MM, Ubera JL, Luque de Castro MD. Comparison of continuous subcritical water extraction and hydrodistillation of marjoram essential oil. J Chromatogr A. 1999;855(2): 625-632, DOİ: 10.1016/s0021-9673(99)00703-7.

Kayode L, Lizette D, Johnson RM, Siegfried BD, Ellis MD. Effect of amitraz on queen honey bee egg and brood development. Mellifera. 2014;14, 33-40.

Kochansky J, Wilzer K, Feldlaufer M. Comparison of the transfer of coumaphos from beeswax into syrup and honey. Apidologie. 2001;32(2):119-125, DOİ: 10.1051/apido:2001117.

Kütükoglu F, Girisgin AO, Aydin L. Varroacidal efficacies of essential oils extracted from *Lavandula officinalis*, *Foeniculum vulgare*, and *Laurus nobilis* in naturally infested honeybee (*Apis mellifera L.*) colonies. Turk J Vet Anim Sci. 2012;36: 554-559, DOİ: 10.3906/vet-1104-12.

Lindquist EE, Krantz GW, Walter DE. Order mesostigmata. Eds. Krantz GW, Walter DE ''A Manual of Acarology''. 3th ed. Texas Tech University Pres, USA, 2009, P:124-232.

Martin SJ. Acaricide (pyrethroid) resistance in *Varroa destructor*. Bee World. 2004;85(4):67-69, DOİ: 10.1080/0005772X.2004.11099632.

Muz D, Muz MN. Survey of the occurrence of Deformed Wing Virus and multiple parasites of queens (*Apis mellifera L.*) in apiaries with collapsed colonies in Hatay, Turkey. J Apicultural Res and Bee World. 2009;48(3): 204-208, DOİ:10.3896/IBRA.1.48.3.09.

Muz MN, Solmaz H, Yaman M, Karakavuk M. Kış salkımı erken bozulan arı kolonilerinde paraziter ve bakteriyel patojenler. YYU Vet Fak Derg. 2012;23:147-150.

Oudemans AC. Note VIII. On a new genus and species of parasitic Acari, 216, 222, 1904.

Önk K. Kars Yöresindeki Bal Arılarında (*Apis mellifera*) Varroosis'in Yaygınlığı, Kafkas Üniversitesi, Sağlık Bilimleri Enstitüsü, Yüksek Lisans Tezi, Kars, 2003.

Pettis JS. A scientific note on *Varroa destructor* resistance to coumaphos in the United States. Apidologie. 2004;35(1): 91-92, DOİ:10.1051/apido:2003060.

Rasool K, Ahad I, Rasool. Efficacy of various botanicals and chemicals on ectoparasitic mite, *Varroa destructor* feeding on European honey bee, *Apis mellifera.* J Entomol Zool Stud. 2017;5(5): 589-595, DOİ: 10.22271/j.ento.

Turhan M, Şengül T. Bal arısı kolonilerinde *Varroa* mücadelesinde Mersin bitkisinin (*Myritus communis L.*) kullanılma imkânları. Türk Tarım ve Doğa Bil Derg. 2020;7(4): 928-939, DOİ:10.30910/turkjans.760897.

Tutkun E, Inci A. Balarılarında zarar yapan arı akarı (*Varroa jacobsoni* Oudemans)'nın tanınması, yayılışı, biyolojisi ve mücadelesi. Türkiye Kalkınma Vakfı Entegre Arıcılık Projesi Yayını, 1985.

Ulutaş Deniz E, Yeğenoğlu S, Sözen Şahne B, Gençler Özkan AM. Kişniş (*Coriandrum sativum L.*) üzerine bir derleme. Marmara Pharm J. 2018;22 (1) : 15-28.

Wallner K. Varroacides and their residues in bee products. Apidologie. 1999;30(2-3): 235-248, DOİ: 10.1051/apido:19990212.

Wang YN, Wang HX, Shen ZJ, Zhao LL, Clarke SR, Sun JH vd. Methyl palmitate, an acaricidal compound occurring in green walnut husks. J Econ Entomol. 2009;102(1): 196-202, DOİ.10.1603/029.102.0128.

Webster TC, Delaphane KS. Mites of the Honey Bee. Dadant and Sons publish. Ohio, 2001.

Yücel B, Duran G. *Varroa* (*Varroa jacobsoni Q.*) ile mücadelede organik asitlerin ve bitkisel maddelerin kullanımı. Hasad. 2004;2, 22-25.

Zeybek H. Arı Hastalıkları ve Zararlıları. T.K.B. Hayvan Hastalıkları Araştırma Müdürlüğü, Etlik, Ankara, 1991. S:96.

Citation/Atıf: Huseynova A, Asadov E, Boulebd H, Akkal S, Novruzova L. Phenolic composition and antioxidant properties of *Ranunculus arvensis* l. Flower pollen: in vitro and silico insights. U. Bee J. / U. Arı D. 2025; 25 (1): 61-72. DOI:10.31467/uluaricilik.1631570

ARAŞTIRMA MAKALESI / RESEARCH ARTICLE

PHENOLIC COMPOSITION AND ANTIOXIDANT PROPERTIES OF *RANUNCULUS ARVENSIS* L. FLOWER POLLEN: IN VITRO AND SILICO INSIGHTS

Ranunculus arvensis L. Çiçek Polenlerinin Fenolik Bileşimi ve Antioksidan Özellikleri: in Vitro ve Siliko Görüşler

Aziza HUSEYNOVA[1*], Elsever ASADOV[1], Houssem BOULEBD[2], Salah AKKAL[3], Leman NOVRUZOVA[1]

[1]Nakhchivan State University, Faculty of Medical, Nakhchivan, AZERBAIJAN, Corresponding author / Yazışma Yazarı E-mail: azizahuseynova@ndu.edu.az, ORCID No: 0000 0003 0943 5214, E-mail: asadoves1974@gmail.com, ORCID No: 0000 0003 2892 2974, E-mail: leman.novruzova.1990@gmail.com, ORCID No: 0009 0008 4269 3219

[2]Laboratory of Synthesis of Molecules with Biological Interest, Frères Mentouri Constantine 1 University, 25000 Constantine, ALGERIA, E-mail: boulebd.houssem@umc.edu.dz, ORCID No: 0000 0002 7727 8583

3Unit for the Valorization of Natural Resources, Faculty of Exact Sciences, Department of Chemistry, Bioactive Molecules and Physico-Chemical and Biological Analyzes, Mentouri University of Constantine 1, ALGERIA, E-mail: salah4dz@yahoo.fr, ORCID ID: 0000 0002 4999 5673

Received / Geliş:02.02.2025 Accepted / Kabul:15.04.2025 DOI:10.31467/uluaricilik.1631570

ABSTRACT

***Ranunculus arvensis* L., although classified as a poisonous plant, holds significant value in medicine, food, and apiculture. Its pollen is actively collected by various bee species, including honeybees, and stored as a vital nutritional reserve for their larvae. This study investigates the polyphenol and flavonoid content, as well as the antioxidant properties, of *R. arvensis* flower pollen. The antioxidant activity of the pollen, sourced from the Nakhchivan Autonomous Republic, was measured as 179.102 ± 1.5919 *µ*mol $FeSO_4 \cdot 7H_2O$/g dw using the FRAP method and 0.137 ± 0.015 mg/mL using the DPPH method. The phenolic content of the methanolic extract was determined to be 17.952 ± 0.160 mg/g, while the flavonoid content was 5.660 ± 0.055 mg/g. Phenolic profiling via HPLC identified six key compounds: ferulic acid (521.163 *µ*g/g), caffeic acid (170.119 *µ*g/g), p-hydroxybenzoic acid (46.529 *µ*g/g), protocatechuic acid (22.377 *µ*g/g), chrysin (11.353 *µ*g/g), and pinocembrin (10.953 *µ*g/g). Quantum chemistry calculations revealed that ferulic acid and caffeic acid, the most abundant phenolic compounds, exhibited the most favorable profiles for antioxidant activity. These findings suggest that these two compounds are the primary contributors to the antioxidant potential of the pollen extract. Given the nutritional and pharmacological significance of bee products, continued investigation into the phytochemical composition of flower pollen is essential to better understand its functional properties and applications.**

Keywords: *Ranunculus arvensis,* Plant pollen, Polyphenol, Flavonoid, Antioxidant, Bee food

ÖZ

***Ranunculus arvensis* L., zehirli bir bitki olarak sınıflandırılmasına rağmen, tıp, gıda ve arıcılıkta önemli bir değere sahiptir. Poleni, bal arıları da dahil olmak üzere çeşitli arı türleri tarafından aktif olarak toplanır ve larvaları için hayati bir besin rezervi olarak depolanır. Bu çalışma, R. arvensis çiçek poleninin polifenol ve flavonoid içeriğinin yanı sıra antioksidan özelliklerini araştırmaktadır. Nahçıvan Özerk**

Cumhuriyeti'nden elde edilen polenin antioksidan aktivitesi FRAP yöntemi kullanılarak 179,102 ± 1,5919 µmol $FeSO_4$-$7H_2O$/g dw ve DPPH yöntemi kullanılarak 0,137 ± 0,015 mg/mL olarak ölçülmüştür. Metanolik ekstraktın fenolik içeriği 17.952 ± 0.160 mg/g, flavonoid içeriği ise 5.660 ± 0.055 mg/g olarak belirlenmiştir. HPLC yoluyla yapılan fenolik profilleme altı temel bileşiği tanımlamıştır: ferulik asit (521.163 µg/g), kafeik asit (170.119 µg/g), p-hidroksibenzoik asit (46.529 µg/g), protokateşuik asit (22.377 µg/g), krisin (11.353 µg/g) ve pinokembrin (10.953 µg/g). Kuantum kimyası hesaplamaları, en bol bulunan fenolik bileşikler olan ferulik asit ve kafeik asidin antioksidan aktivite için en uygun profilleri sergilediğini ortaya koymuştur. Bu bulgular, bu iki bileşiğin polen ekstraktının antioksidan potansiyeline birincil katkıda bulunan bileşikler olduğunu göstermektedir. Arı ürünlerinin besinsel ve farmakolojik önemi göz önüne alındığında, çiçek poleninin fitokimyasal bileşiminin sürekli araştırılması, fonksiyonel özelliklerinin daha iyi anlaşılması için gereklidir.

Anahtar Kelimeler: *Ranunculus arvensis*, Bitki poleni, Polifenol, Flavonoid, Antioksidan, Arı besini

GENİŞLETİLMİŞ ÖZET

Amaç: *Ranunculus* L. cinsine ait bitkiler tıp, beslenme ve arıcılıkta çeşitli uygulamalarıyla bilinmektedir, ancak bu bitkiler aynı zamanda toksik özellikleri nedeniyle de sınıflandırılmaktadır (Al-Snafi, 2022). Zehirli olmasına rağmen hem bal arıları hem de diğer arı türleri *Ranunculus* bitkisinden polen toplar ve kovanlarında depolarlar. Ancak araştırmacılar, *Ranunculus* türlerinin polenlerinin düşük miktarda toksik madde protoanemioin içerdiğini ve bu nedenle arı larvaları üzerinde yıkıcı bir etkisinin olmadığını bulmuşlardır (Sedivy ve ark., 2012). Bu çalışmanın amacı, arılar tarafından çok sevilen *Ranunculus* cinsine ait *Ranunculus arvensis* L. poleninin arı larvalarının gelişimi açısından yararlı olan biyoaktif maddeleri ve antioksidan özelliklerini değerlendirmektir.

Gereç-Yöntem: *Ranunculus arvensis* L.'nin çiçekleri çiçeklenme evresinin başlangıcında toplanmış, polenler taç yapraklarından ayrılmış, kurutulmuş ve %98'lik metanol ile ekstrakte edilmiştir. Metanol ekstraktındaki (ME) toplam fenolik madde içeriği Folin-Ciocalteu reaktifi kullanılarak belirlenmiştir. Karışımın absorbansı Thermo Scientific Evolution TM 201 UV-VIS spektrofotometresi kullanılarak 760 nm'de ölçülmüşdür. Toplam fenolik içerik (TPC), gram kuru polen başına miligram gallik asit eşdeğeri (GAE) olarak ifade edilmiştir (Slinkard ve diğerleri, 1997). Toplam flavonoid içeriği (TFC), Fukumoto ve Mazza tarafından geliştirilen kolorimetrik yöntem kullanılarak 415 nm'de ölçülmüş. TFC, numunenin kurutulmuş ağırlığının (dw) gramı başına kuersetin eşdeğeri (QUE) miligramı olarak ifade edilmiştir (Fukumoto ve Mazza, 2000).

Polen özütünün toplam antioksidan kapasitesi Benzie ve Strain (1996) ve Pulido ve ark. tarafından belirlenmiştir. (2020) bitki özütlerinin antioksidan aktivitesinin belirlenmesi için uyarlanmış bir demir indirgeyici antioksidan gücü (FRAP) testi ile değerlendirilmişdir. Serbest radikal temizleme aktivitesi, Molyneux (2004) ve Erdogan ve ark. (2012) açıklanan yöntemle belirlenmiştir.

HPLC analizi, PDA dedektörü ve C18 kolonu ile donatılmış Shimadzu LC-20AT HPLC sistemi kullanılarak gerçekleştirilmişdir. Bu analizde 25 adet fenolik standart eş zamanlı olarak analiz edilmişdir.
Polen ekstraktındaki polifenollerin moleküler geometrisi ve antioksidan mekanizmaları, yoğunluk fonksiyonel teorisi (DFT) hesaplamaları kullanılarak analiz edilmişdir. Bu hesaplamalar için, ampirik değişim korelasyonlarını içeren yüksek parametreli bir yöntem olan M06-2X fonksiyoneli ve 6-31+G(d,p) baz seti uygulanmışdır (Galano & Alvarez-Idaboy, 2014). Hidrojen atom transferi (HAT) için bağ ayrışma entalpisi (BDE) ve tek elektron transferi (SET) için iyonlaşma potansiyeli (IP) gibi antioksidan mekanizmalarla ilişkili termodinamik parametreler Boulebd ve arkadaşları (2022) ve Boulebd (2022) tarafından önerilen yöntemlere göre hesaplanmıştır.
Bulgular: Nahçıvan Özerk Cumhuriyeti'nde yaygın olarak yetişen *R.arvensis* L. bitkisinin poleninin polifenol içeriği ve antioksidan aktivitesi ilk kez araştırılmışdır. toplam fenolik içerik -17,952±0,160

mg GAE/g kuru ağırlık; toplam flavonoid içeriği 5.660±0.055mg QUE/g dw; toplam antioksidan kapasitesi: FRAP - 179,102±1,591 µmol $FeSO_4 \cdot 7H_2O$/g dw; DPPH SC_{50}'nin 137±0,015 mg/mL olduğu belirlenmişdir.

R. arvensis poleninin metanol ekstraktındaki fenolik bileşikler 25 standart kullanılarak analiz edilmış ve bunlardan 6'sının miktarları tahmin edilmiştir: protokatekuik asit-22,377 µg/g, p-OH benzoik asit-46,529 µg/g, kafeik asit-170,119 µg/g, ferulik asit-512,163 µg/g, krizin-11,353 µg/g, pinocembrin-10,953 µg/g.

Hem HAT hem de SET mekanizmalarının bulgularına dayanarak, *R. arvensis* özütünde en aktif antioksidanların kafeik asit ve ferulik asit olduğu sonucuna varılabilir ve antioksidan aktiviteleri belirlenebilir.

Sonuç: Çiçek polenlerinden elde edilen arı poleninin sağlık açısından çok çeşitli faydaları olduğu biliniyor. Çalışmamızda, arı kolonilerinin gelişimi ve arı ürünlerinin kalitesinin artırılması amacıyla çiçek polenlerinin fenolik bileşenleri ve antioksidan aktivitesinin incelenmesi amaçlanmaktadır. *Ranunculus arvensis* çiçek poleninin biyokimyasal bileşiminde yararlı bileşenlerin incelenmesi literatüre ve araştırmacılara önemli katkı sağlaybileceyi düşünülmektedir.

INTRODUCTION

Beekeeping, which has an ancient history, has generated great interest in studying flowering plants' chemical composition, nutritional value and pharmacological benefits. Honeybees produce honey, bee pollen, bee bread, propolis, etc. from the nectar, pollen and resinous substances they collect from plants, and the chemical composition of these products largely depends on the plants used to collect them. Although the chemical composition of plants that are important for beekeeping has been widely studied, very few studies have been conducted analyzing the chemical composition of pollen. As you know, pollen is the main food source for bee colonies (Abd El-Wahab 2016).

15 genera and 57 species represent the family of Ranunculaceae Adans. in the Nakhchivan Autonomous Republic. These species are considered useful plants in medicine, food and beekeeping, but are also known to be poisonous plants. *Ranunculus arvensis* L., belonging to the genus *Ranunculus* L. section, is widespread in the middle mountain zone of the autonomous republic. It is an annual mesophytic plant with a height of 10-40 cm, a branching stem, yellow flowers and abundant pollen. According to its geographical type, it is considered a Mediterranean-Iranian-Turanian plant. The plant blooms in May-June (Talibov & Ibrahimov 2008).

Plants belonging to the genus *Ranunculus* L. are considered to be useful for medicine, food and beekeeping, but they are also known to be poisonous plants (Al-Snafi 2022). All bee genera and also honeybees collect the pollen of plants belonging to the *Ranunculus* L. family, which are toxic, and store them in the hives to feed their larvae. The toxic effect of these plant pollens on the development of bee larvae has long been a matter of debate. However, it is clear from some studies that protoanemoin, the substance that causes plant toxicity, is found in large amounts in the buds of the plant and in small amounts in the pollen. It was also established that the amount of protoanemoin in bee pollen was drastically reduced as protoanemoin was detoxified and turned into harmless substances as a result of enzymatic processes. For this reason, the pollen of plants of the *Ranunculus L*. genus contained in bee pollen was found to have no adverse effect on the bee larvae development (Sedivy et al, 2012). Although the plant belongs to the group of poisonous plants, it is also widely used in pharmacology because it is non-toxic after drying (Kurkin 2004).

In terms of chemical composition, the plant is rich in proteins, amino acids, carbohydrates, glycosides, saponins, polyphenols, and flavonoids (Boroomand et al. 2018). This plant also contains ranunculus glycoside, the enzymatic breakdown of which produces a toxic substance called protoanemonin. For this reason, the plant is considered poisonous. However, since the dried form or infusion of the plant does not contain protoanemonin, it does not have a toxic effect (An et al. 2018; Jürgens & Dötterl 2004). The flavonoid-rich plant *R. arvensis* has strong antioxidant and antimicrobial effects (Hachelaf et al. 2015; Al-Snafi 2022).

This study aimed to evaluate the phenolic compounds and antioxidant properties of *Ranunculus arvensis* L. pollen, which serves as an important food source for bees in the Nakhchivan Autonomous Republic.

MATERIALS AND METHODS

Plant Materials

Ranunculus arvensis L. (Order: Ranunculales/Family: Ranunculaceae) was collected from the Anagut village in the Ordubad district of the Nakhchivan Autonomous Republic for research purposes. Anagut is located at an altitude of 38°58'53" N. E. 45°57'51" N. U. At the end of May, During the period of full flowering of the species *R. arvensis*, parts of flowers were collected, the stamens were separated and dried in a shaded and ventilated place.

Pollen Extraction

A methanolic extract of the dried flower pollen was prepared by weighing 1 g of pollen and adding 50 mL of 98% methanol. The mixture was left to stand for 24 h, then sequentially filtered using Whatman No. 4 and Whatman No. 1 filter papers. The resulting extract was subsequently stored in a deep freezer at -18°C. The extracted pollen was analyzed for its total phenolic content, total flavonoid content, and antioxidant activity.

Total Phenolic Content (TPC)

The Folin-Ciocalteu reagent was used to measure the total phenol concentration in the methanol extract (ME). 400 µL of 0.5 N Folin-Ciocalteu reagent was mixed with 20 µL of the prepared methanol extract (*R.arvensis*). 680 µL of distilled water was added to dilute the mixture. Folin-Ciocalteu reagent is a strong oxidizing agent that reacts with the extract's phenolic compounds to form a blue complex. The mixture was incubated for 3-4 min. to allow the initial reactions between the reagent and phenolic compounds in the extract to occur. Then 400 µL of Na_2CO_3 (10%) was added and kept at room temperature for 2 h. Using a Thermo Scientific Evolution™ 201 UV-VIS spectrophotometer, the absorbance of the mixture was measured at a wavelength of 760 nm. The total phenolic content was expressed as milligrams of gallic acid equivalents (GAE) per gram of dry pollen (Slinkard et al. 1997). The absorbance of the solution was then measured at 760 nm using a Thermo Scientific Evolution TM 201 UV-VIS spectrophotometer. The total phenolic content was expressed as milligrams of gallic acid equivalents (GAE) per gram of dry pollen (Slinkard et al. 1997).

Total Flavonoid Content (TFC)

The total flavonoid content (TFC) was determined using the colorimetric assay developed by Fukumoto and Mazza. In this procedure, 25 µL of the pollen extract was mixed with 50 µL of 10% aluminum nitrate [$Al(NO_3)_3$] solution and 50 µL of 1.0 M ammonium acetate (NH_4CH_3COO) solution. The aluminum ions (Al^{3+}) are responsible for forming complexes with the flavonoid molecules in the extract. After incubating the mixture at room temperature for 45 minutes, its absorbance was measured at 415 nm. Quercetin standards were utilized to express TFC as milligrams of quercetin (QUE) per gram of dried weight (dw) of the sample (Fukumoto & Mazza 2000).

Total Antioxidant Capacity

The ferric-reducing antioxidant power (FRAP) assay was employed to evaluate the total antioxidant capacity of the pollen extract., as developed by Benzie and Strain (1996) and adapted by Pulido et al. (2020) for plant extract antioxidant activity determination. To prepare the FRAP reagent, 2.5 mL of 10 mM TPTZ, 2.5 mL of 20 mM $FeCl_3$, and 25 mL of 300 mM acetate buffer (pH 3.6) were combined. The reaction mixture, consisting of 3 mL FRAP reagent and 100 µL pollen extract, was incubated at 37°C for 4 minutes. During this incubation, Fe^{3+} ions in the ferryl tripyridyltriazine reagent are reduced to Fe^{2+} ions, causing the reagent to turn dark blue. The absorbance was then measured at 595 nm. The FRAP values were expressed as micromoles of $FeSO_4 \cdot 7H_2O$ per gram of dry weight of the sample.

DPPH (2,2- diphenyl-1-picrylhydrazyl) Radical Scavenging Activity

Free radical scavenging activity was measured using the method described by Molyneux (2004) and Erdogan et al. (2012). Briefly, 750 µL of the sample extract was combined with 750 µL of DPPH radical solution and incubated in the dark at 25°C for 45 min. The absorbance was subsequently recorded at 517 nm. Scavenging activity was quantified as SC_{50}, with lower SC_{50} values indicating stronger radical scavenging ability.

RP-HPLC-PDA (Reversed-Phase High-Performance Liquid Chromatography) Analysis and Determination of Phenolic Derivatives

The chromatographic analyses were carried out utilizing a Shimadzu LC-20AT high-performance liquid chromatography system, which was outfitted with a photodiode array (PDA) detector and a C18 reversed-phase column (dimensions: 250 mm by 4.6 mm, particle size 5 µm; manufactured by GL Sciences). Separation was achieved through

gradient elution with two mobile phases: Mobile Phase A consisting of 10% acetonitrile in ultrapure water, and Mobile Phase B containing 2% acetic acid diluted in water. The system operated at a flow rate of 1 mL/min, and a 20 *µ*L sample volume was injected. Detection occurred at wavelengths of 250, 280, 320, and 360 nm, with the column temperature maintained at 30ºC. The gradient program was as follows: initial composition of 95% solvent A and 5% solvent B, transitioning to 15% A/85% B by 8 min., 21% A/79% B at 10 min., 52% A/48% B at 20 min., 67% A/33% B at 35 min., 90% A/10% B at 50.5 min., 50% A/95% B at 50.1 min., and concluding with 5% A/95% B at 60 min. Prior to analysis, all samples were filtered through 0.45 *µ*m membranes (Kolayli et al., 2024; Zehra et al., 2015).

Standard Phenolics

A total of 25 phenolic standards were analyzed simultaneously, including gallic acid, protocatechuic acid, p-OH benzoic acid, m-OH benzoic acid, caffeic acid, syringic acid, p-coumaric acid, ferulic acid, apigenin, myricetin, chlorogenic acid, quercetin, resveratrol, daidzein, t-cinnamic acid, epicatechin, hesperetin, rutin, luteolin, rhamnetin, pinocembrin, chrysin, CAPE, curcumin, and ellagic acid.

DFT calculations

The molecular geometry and antioxidant mechanisms of the polyphenols in the pollen extract were analyzed using density functional theory (DFT) calculations. The M06-2X functional, a highly parameterized method that includes empirical exchange-correlation, was applied for all computations, alongside the 6-31+G(d,p) basis set. Among the approaches used for thermodynamic calculations of radical reactions, M06-2X is considered one of the most reliable (Galano & Alvarez-Idaboy, 2014). To simulate the effects of ethanol as a solvent, Truhlar's SMD solvation model was used. The thermodynamic parameters associated with the antioxidant mechanisms, such as bond dissociation enthalpy (BDE) for hydrogen atom transfer (HAT) and ionization potential (IP) for single electron transfer (SET), were calculated according to the methods outlined by Boulebd et al. (2022) and Boulebd (2022).

$$BDE = H(HZ\text{–}N^{\cdot}) + H(H^{\cdot}) - H(HZ)$$

$$IP = H(HZ^{+\cdot}) + H(e^{-}) - H(HZ)$$

In this expression, *H(HZ)*, *H(HZ–N·)*, *H(HZ·+)*, *H(e-)*, and *H(H+)* denote the enthalpies corresponding to the neutral molecule, radical species, radical cation, electron, and proton, respectively. All calculations were performed using Gaussian09 software (Frisch et al. 2009). The analysis and visualization of the results were conducted using Multiwfn and VMD software (Lu & Chen 2012; Humphrey et al. 1996).

Statistical Analysis

The results from three separate experimental replicates were statistically analyzed using SPSS 20.0 (SPSS Inc., Chicago, IL, USA). To compare the total phenolic content (TPC), total flavonoid content (TFC), FRAP, and DPPH parameters across the different species, one-way ANOVA followed by Tukey's test was applied.

RESULTS

We studied the content of polyphenols and antioxidant activity of the pollen of the *R.arvensis* plant, common in the Nakhchivan Autonomous Republic, for the first time. Since methanol is the best solvent for most polyphenol derivatives, 98% methanol was used to prepare the extract. Table 1 displays the results of the quantitative analysis of total phenol and flavonoid concentrations and antioxidant activity of the *R. arvensis* pollen.

Table 1. Total phenolic content, total flavonoid content, and antioxidant capacity of the *R.arvensis* pollen

	***R.arvensis* flower pollen extract (Mean ±st)**
Total phenolic content (mg GAE/g dw)	17.952±0.160
Total flavonoid content (mg QUE/g dw)	5.660±0.055
Total antioxidant capacity (FRAP) (*µ*mol $FeSO_4 \cdot 7H_2O$/g dw)	179.102±1.591
DPPH SC_{50} (mg/mL)	0.137±0.015

Phenolic compounds in the methanolic extract of *R.arvensis* pollen were analyzed using 25 standards. Of these, the amount of 6 substances was rated quite highly. The results of the RP-HPLC-PDA analysis are presented in Table 2.

Table 2. Phenolic composition of the *R.arvensis* pollen based on spectrophotometry and high-performance liquid chromatography

Phenolic acids (*µ*g/g)		Flavonoids (*µ*g/g)	
Gallic Acid	nd*	Resveratrol	nd
Protocatechuic Acid	22.377	Daidzein	nd
Chlorogenic Acid	nd	Luteolin	nd
p-OH Benzoic Acid	46.529	Quercetin	nd
t-Cinnamic Acid	nd	Epicatechin	nd
Caffeic Acid	170.119	Apigenin	nd
Syringic Acid	nd	Hesperidin	nd
m-OH Benzoic Acid	nd	Rhamnetin	nd
p-Coumaric Acid	nd	Chrysin	11.353
Ellagic Acid	nd	Pinocembrin	10.953
Ferulic Acid	512.163	CAPE	nd
		Curcumin	nd
		Rutin	nd
		Myricetin	nd

nd*: Not detected*

According to the HPLC chromatogram, the amount of pinocembrin was lower (10.953 *µ*g/g), while the amount of ferulic acid was higher (521.163 *µ*g/g). The antioxidant function of each of the resulting phenol derivatives has been studied since they are substances widely used in the food, pharmaceutical, and cosmetic industries.

In silico studies

The main phenols found in the *R.arvensis* extract, which are protocatechuic acid, p-hydroxybenzoic acid, caffeic acid, ferulic acid, chrysin, and pinocembrin, were individually investigated for their antioxidant capacity using DFT calculations.

The molecular geometries of the compounds were optimized using the M06-2X/6-31+G(d,p) level of theory, with ethanol (EtOH) as the solvent to simulate the environment of the in vitro experiments. The most stable molecular geometries are presented in **Figure 1**. Except for pinocembrin, all compounds exhibit a planar geometry, reflecting significant electron delocalization throughout their molecular structures. Pinocembrin, which differs from the others by the saturation of the pyran ring, displays a non-planar geometry. In this case, the benzene moiety is inclined at an angle of 87° from the molecular plane. The distribution of HOMO and LUMO orbitals, also shown in **Figure 1**, further highlights the extensive electron delocalization in these compounds. Analysis of the HOMO energy levels reveals that caffeic acid and ferulic acid have the highest values (-7.21 to -7.26 eV compared to -7.63 to -7.96 eV for other compounds), indicating superior electron-donating capacities. Additionally, the HOMO-LUMO energy gap analysis demonstrates that caffeic acid and ferulic acid exhibit the smallest gaps (6.12–6.16 eV versus 6.38–7.49 eV for other derivatives), suggesting their higher chemical reactivity relative to the other compounds.

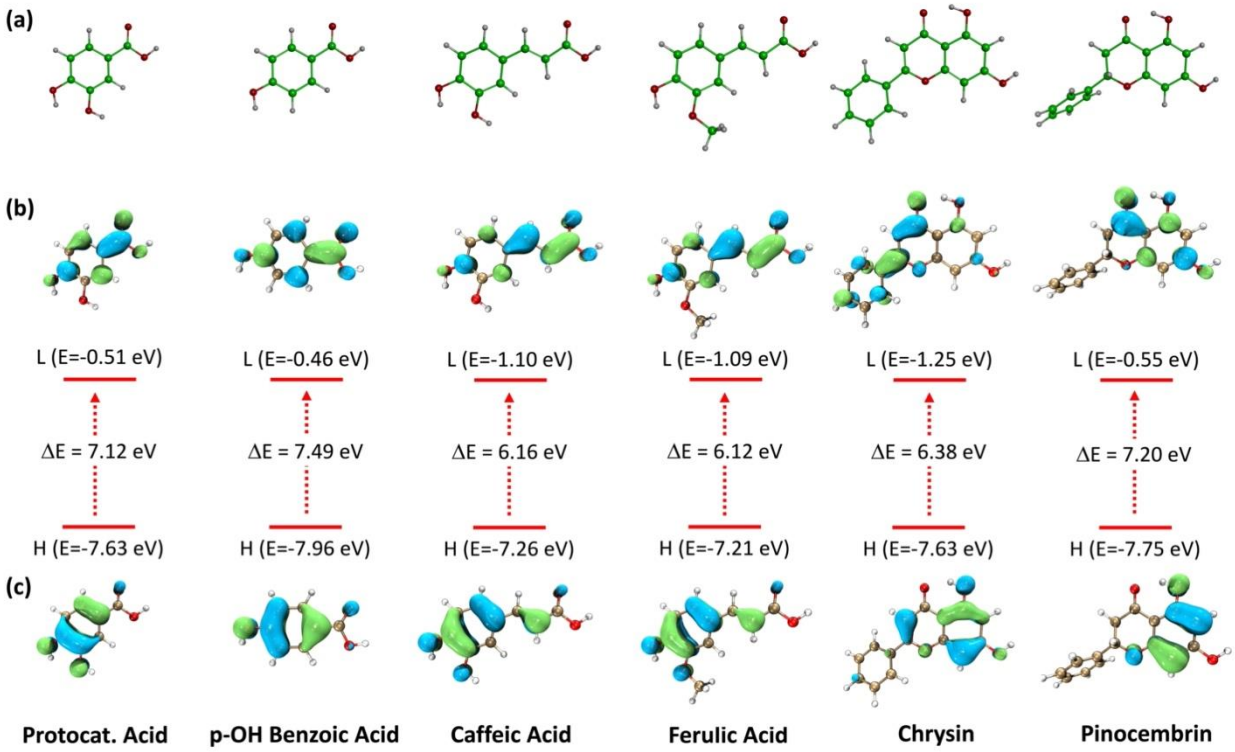


Figure 1. Molecular geometry (a), LUMO (b), and HOMO (c) of the main polyphenols of *R.arvensis* extract obtained at M06-2X/6-31+G(d,p) level in EtOH.

The two main mechanisms, hydrogen atom transfer (HAT) and single electron transfer (SET), which govern the experimental assays, were investigated (Lü et al. 2010; Spiegel 2022). In the HAT mechanism, the antioxidant transfers a hydrogen atom to the DPPH radical, transforming it into a more stable radical species (Boulebd, 2023). This mechanism is characterized by the bond dissociation enthalpy (BDE) of the active OH bond. For the SET mechanism, the antioxidant donates an electron to the Fe ion or a free radical, neutralizing it (Boulebd 2024). This mechanism is characterized by the ionization potential (IP) of the antioxidant. The BDE and IP values calculated for the HAT and SET mechanisms, respectively, of the compounds studied are shown in Figure 2. Regarding the HAT mechanism, the lowest BDE value was obtained for the 4-OH group of caffeic acid (79.9 kcal/mol), followed by protocatechuic acid and ferulic acid, which showed approximately the same values of 83.3 and 83.4 kcal/mol, respectively. These results indicate that caffeic acid, protocatechuic acid, and ferulic acid are the most active compounds in the HAT mechanism. On the other hand, the analysis of IP values also shows the lowest values for caffeic acid and ferulic acid (112.4 and 111.7 kcal/mol, respectively), indicating that these molecules may also exhibit the highest electron-donating capacity compared to the other phenolic derivatives. Based on the findings from both the HAT and SET mechanisms, we can conclude that caffeic acid and ferulic acid are the most active antioxidants in the *R.arvensis* extract and determine their antioxidant activity.

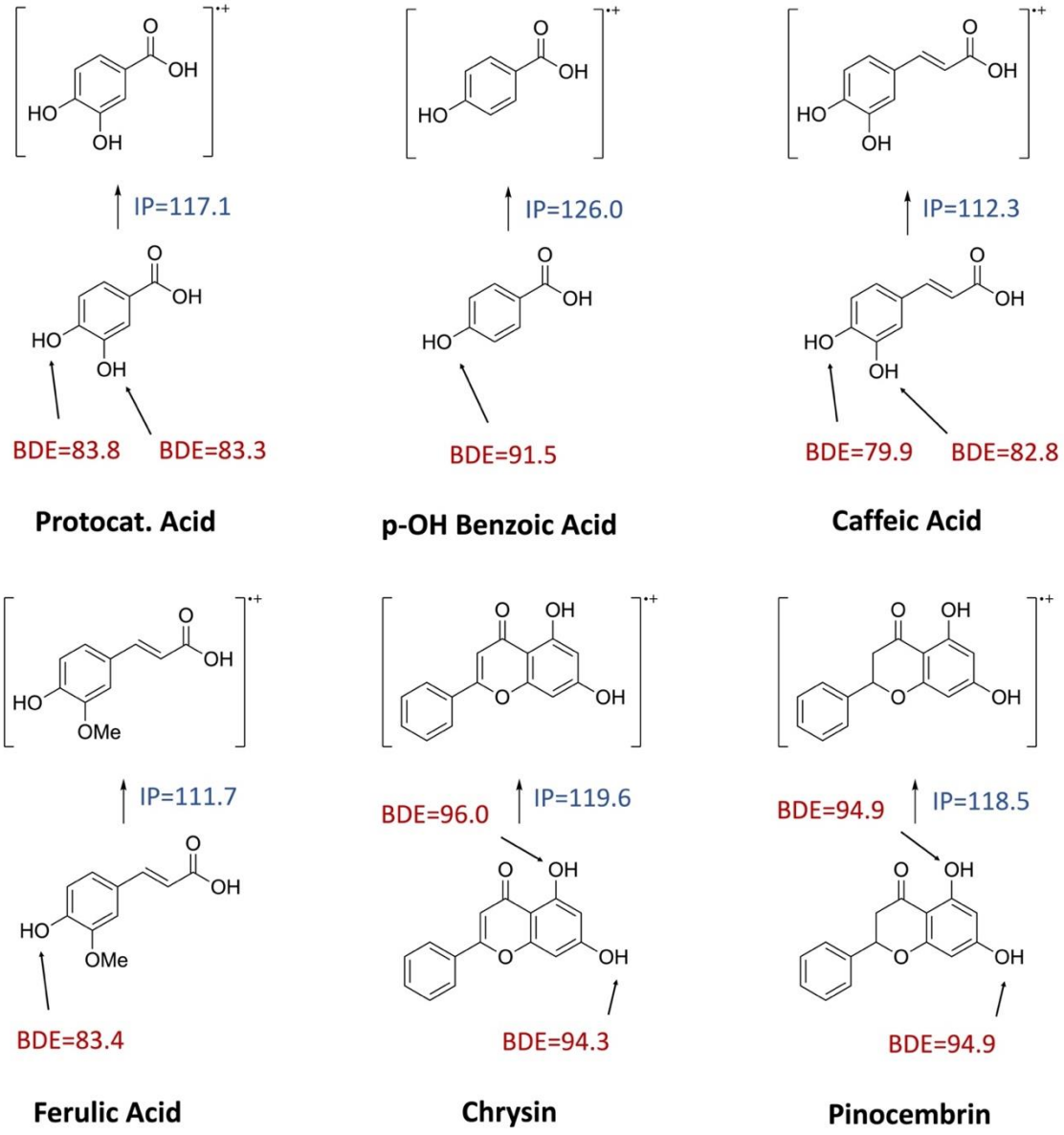


Figure 2. Computed BDE and IP kcal/mol of the main polyphenols and antioxidant mechanisms (d) of the main compounds of the *R.arvensis* extract obtained at M06-2X/6-31+G(d,p) level in EtOH.

DISCUSSION

There are no studies on phytochemical analysis of *R.arvensis* pollen extract in scientific databases. In this study, the phytochemical composition of *R.arvensis* pollen was compared with the composition of flower pollens of other plants that are food sources for bees. Such comparisons help to better understand the biologically active components of different types of pollens for the development of bee colonies and the benefits of bee products.

Kostic et al. (2021) investigated the phytochemical profile of pollen collected by honeybees from the artichoke (*Cynara scolymus*) plant in the Belgrade area. This research focused on phenolic compounds and their concentrations within the pollen. The findings showed that the Total Phenolic Content

(TPC) in artichoke pollen was 5.3 mg/g GAE dw, and the Total Flavonoid Content (TFC) was 0.81 mg/g QE dw. Using HPLC DAD MS/MS analysis, 10 phenolic compounds were identified, with Isorhamnetin 3-O-glucoside showing the highest concentration (49,171 mg/kg), comprising more than 70% of the total phenolic content (Kostic et al., 2021). In comparison, the analysis of *R.arvensis* pollen revealed TPC and TFC values that were 3-5 times higher than those in artichoke pollen. The predominant phenolic compound in *R.arvensis* pollen was ferulic acid, which represented over 60% of the total phenolic content.

De-Melo et al. (2018) investigated the phenolic and flavonoid contents as part of their study on, along with the antioxidant properties, of eight monofloral bee pollen samples collected from the state of Brasilia were analyzed. These samples consisted of more than 90% pollen from a single plant species. The Total Phenolic Content (TPC) varied depending on the plant species, ranging from 5.6 to 29.7 mg GAE/g, while the flavonoid content ranged from 0.3 to 19.0 mg GAE/g. The antioxidant activity, measured using the DPPH method, showed values between 10.3 and 110.8 *μ*mol TE/g. HPLC-MS analysis identified quercetin, kaempferol 3-O-glycosides, and isorhamnetin flavonoid glycosides as the major compounds in the samples (De-Melo et al., 2018).

In the study conducted by Sadeq et al. (2021), the phytochemical composition, along with the antioxidant and antibacterial properties of pollens from Micromeria fruticosa, Achillea fruticosa grantissima, and Phoenix dactylifera-plants native to the Palestinian Territory-were thoroughly investigated. Among the samples, *Micromeria fruticosa* pollen exhibited the highest results across all parameters. Micromeria fruticosa pollen extract was reported to contain a high level of phenolic compounds (TPC: 56.78 ± 0.49 mg GAE/g) and flavonoids (TFC: 2.48 ± 0.05 and 8.03 ± 0.01 mg QE/g). Its antioxidant capacity was confirmed by low IC_{50} values of 0.047 mg/mL in the DPPH assay and 0.039 mg/mL in the FRAP assay, indicating potent free radical scavenging activity (Sadeq et al., 2021). The study of plant pollens, which serve as food sources for honey bees, provides valuable insights into both the growth of bee colonies and the nutritional and pharmacological benefits of bee products. *Micromeria fruticosa* (Lamiaceae) is one of the key nectar- and pollen-producing plants for honey bees (Albaba, 2015).

These analyses show that *R.arvensis* pollen, a species of *Ranunculus* L., which is widespread in forests and meadows, is a source of phenolic and flavonoids for honey bees.

Quantum chemical calculations using the DFT method at the M06-2X/6-31+G(d,p) level revealed significant differences in the reactivity of the extract's primary polyphenols. Caffeic acid and ferulic acid emerged as the most reactive compounds, following both hydrogen atom transfer (HAT) and single electron transfer (SET) pathways. These results are consistent with the potent and well-documented antioxidant activity of these compounds, making *Ranunculus arvensis* L. flower pollen a rich source of antioxidants.

Ranunculus arvensis L., which has a mass flowering period in April-May in many regions of the Nakhchivan Autonomous Republic, has been observed to be used as a food source by honey bees during the active beekeeping season. X et al. Y et al. etc. studies indicate that monofloral bee pollen, which constitutes 90-100% of *R.arvensis* plant pollen, is obtained. This shows that *R. arvensis* is a plant loved by bees and its pollen is used as food. In this study, the TPC, TFC and antioxidant content of *R.arvensis* flower pollen is studied to understand the value of this plant for the development of bee colonies. *R.arvensis*, which has a higher TPC, TFC and antioxidant value than most flower pollens, is antimicrobial for bees. It has anti-inflammatory and antioxidant effects. This also means the healthy development of bee colonies.

We have observed the use of Ranunculus arvensis L., which has a mass flowering period in April-May in many regions of the Nakhchivan Autonomous Republic, as a food source by honey bees during the active beekeeping season. The lack of literature data on the biochemical analysis of Ranunculus arvensis flower pollen is a factor that emphasizes the originality and importance of this study. Thus, the fact that these analyses were performed by us for the first time not only presents a new approach scientifically, but also creates a basic database for other studies to be conducted in this field in the future. Such studies are also of particular importance in terms of their contribution to the development of plant pollens and the field of beekeeping. The results of biochemical analyses of *Ranunculus arvensis* flower pollen show that, unlike other plant pollens loved by bees, it has rich phenolic components. Phenolic components play an important role in the

healthy development of bee colonies and in leading a healthy lifestyle in general. Thanks to the antioxidant properties of phenols, they strengthen the immune system of bees and increase their resistance to diseases. The result confirms the idea that pollen plays an important role in the development of beekeeping. This also supports the development of the beekeeping industry and has a positive impact on its productivity.

This topic opens up an interesting area for further research into apitherapy and the role of bees in the ecosystem. A more extensive biochemical analysis of the relationship between bee health and plant pollen composition may offer new approaches to protecting bees in agriculture and natural ecosystems.

Conclusion: The present study highlights the significance of *Ranunculus arvensis* as a valuable pollen source for honey bees during the mass flowering period in April-May within the Nakhchivan Autonomous Republic. Comprehensive phytochemical, antioxidant, and HPLC analyses revealed that ferulic acid and caffeic acid are abundant phenolic components in the pollen. DFT calculations further identified these compounds as key contributors to the antioxidant activity of the extract. The bioactive-rich pollen of *R. Arvensis* underscores its potential to support the healthy development of bee colonies. Furthermore, the transfer of diverse phenolic compounds from pollen to bee-derived products enhances their nutritional and medicinal value, providing a strong foundation for future applications in apiculture and human health.

Acknowledgments: The supercomputing resources used in this work were supported by the HPC of UCI-UFMC (Unité de Calcul Intesif of the University Fréres Mentouri Constantine 1).

Author contribution: AH: Project adminstration; EA: Designed the hypotheses, statistical analysis, evaluated the results; AH, EA, LN: Metogologiya and TPC, TFC, antioksidant, HPLC analysis; HB and SA: DFT calculations.

Conflict of interest: There is no conflict of interest between the authors

Data availability: The data can be found within the manuscript.

Ethics: No animal or human experiments were performed during the conduct of the study.

Financial resources: There is no financial support

REFERENCES

Abd El-Wahab, T.E., Ghania, A.M.M. and Zidan, E.W. Assessment a new pollen supplement diet for honey bee colonies and their effects on some biological activities./ Journal of Agricultural Technology, 2016; Vol. 12(1):55-62

Albaba, İ. A List of Important Honeybee Nectariferous and Polleniferous Plant Species in the West Bank Governorates, Palestine / Journal of Agricultural Science and Technology A. 2015; 5(2), 114-121 doi: 10.17265/2161-6256/2015.02.005

Al-Snafi, A. E. Pharmacological and toxicological effects of the Ranunculus species (Ranunculus arvensis and Ranunculus sceleratus) grown in Iraq. *Int. J. Biol. Pharm. Sci. Arch*, 2022; 3, 64-72. Article DOI: https://doi.org/10.53771/ijbpsa.2022.3.1.0032

An, İ., Ucmak, D., Esen, M., & Gevher, O. D. Phytocontact dermatitis due to Ranunculus arvensis: Report of three cases. *North. Clin. Istanb*, 2019, *6(1)*, 81-84. doi: 10.14744/nci.2018.93695

Benzie, I. F., & Strain, J. J. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical biochemistry*, 1996, *239*(1), 70-76. https://doi.org/10.1006/abio.1996.0292

Boroomand, N., Sadat-Hosseini, M., Moghbeli, M., & Farajpour, M. Phytochemical components, total phenol and mineral contents and antioxidant activity of six major medicinal plants from Rayen, Iran. *Natural product research*, 2018, *32*(5), 564-567. http://dx.doi.org/10.1080/14786419.2017.1315579

Boulebd, H. Insights on the antiradical capacity and mechanism of phytocannabinoids: H-abstraction and electron transfer processes in physiological media and the influence of the acid-base equilibrium. *Phytochemistry***,** 2023; *208*, 113608. https://doi.org/10.1016/j.phytochem.2023.113608

Boulebd, H. Is cannabidiolic acid an overlooked natural antioxidant? Insights from quantum chemistry calculations. *New Journal of Chemistry***,** 2022; *46* (1), 162-168. DOI: 10.1039/D1NJ04771J.

Boulebd, H. Mechanistic Insights into the Antioxidant and Pro-oxidant Activities of Bromophenols from Marine Algae: A DFT Investigation. *The Journal of Organic Chemistry***,** 2024; *89* (11), 8168-8177. https://doi.org/10.1021/acs.joc.4c00851.

Boulebd, H.; Pereira, D. M.; Amine Khodja, I.; Hoa, N. T.; Mechler, A.; Vo, Q. V. Assessment of the free radical scavenging potential of cannabidiol under physiological conditions: Theoretical and experimental investigations. *Journal of Molecular Liquids***,** 2022; *346*, 118277. DOI: 10.1016/j.molliq.2021.118277.

De-Melo AAM., Estevinho LM., Moreira MM., Delerue-Matos C., Freitas AS., Barth OM., Almeida-Muradian LB. Phenolic profile by HPLC-MS, biological potential, and nutritional value of a promising food: Monofloral bee pollen/ J Food Biochem.; 2018; 1-21 https://doi.org/10.1111/jfbc.12536

Erdogan, T. F., Kivcak, B., Onur, M. A., & Braca, A. Chemical constituents and cytotoxic activity of Ranunculus pedatus subsp. pedatus. *Chemistry of Natural Compounds*, 2012; *48*, 486-488. DOI: 10.1007/s10600-012-0284-3.

Frisch M. J.; Trucks G. W.; Schlegel H. B.; Scuseria G. E.; M. A. Robb; J. R. Cheeseman; G. Scalmani et al. *Gaussian 09, Gaussian, Inc., Wallingford CT,* 2009.

Fukumoto, L. R., & Mazza, G. Assessing antioxidant and prooxidant activities of phenolic compounds. *Journal of agricultural and food chemistry*, 2000; *48*(8), 3597-3604. https://doi.org/10.1021/jf000220w.

Galano, A.; Alvarez-Idaboy, J. R. Kinetics of radical-molecule reactions in aqueous solution: A benchmark study of the performance of density functional methods. *Journal of Computational Chemistry*, 2014; *35* (28), 2019-2026. DOI: 10.1002/jcc.23715.

Hachelaf, A., Touil, A., Zellagui, A., & Rhouati, S. Antioxidant and antibacterial activities of essential oil extracted from Ranunculus arvensis L. *Der Pharma Chem*, 2015, 7(9):170-173.

Humphrey, W.; Dalke, A.; Schulten, K. VMD: Visual molecular dynamics. *Journal of Molecular Graphics***,** 1996; *14* (1), 33-38. https://doi.org/10.1016/0263-7855(96)00018-5.

Jürgens, A., & Dötterl, S. Chemical composition of anther volatiles in Ranunculaceae: genera-specific profiles in Anemone, Aquilegia, Caltha, Pulsatilla, Ranunculus, and Trollius species. *American Journal of Botany*, 2004; *91*(12), 1969-1980. DOI: 10.3732/ajb.91.12.1969.

Kolaylı, S., Asadov, E., Huseynova, A., Rahimova, S., & Kara, Y. Phenolic composition and antioxidant properties of black mulberry (Morus nigra l.) fruits and leaves. *Journal of Wildlife and Biodiversity*, 2024; 8(2), 355-364. DOI: https://doi.org/10.5281/zenodo.11078022.

Kostic, A., Milincic, D.D., Nedic, N., Gašic, U.M., Trifunovic, B., Vojt, D. et al. Phytochemical Profile and Antioxidant Properties of Bee-Collected Artichoke (*Cynara scolymus*) Pollen. Antioxidants- 2021; 10(7), 1091. https://doi.org/10.3390/antiox10071091.

Kurkin, V. Pharmacognosy. Textbook for students of pharmaceutical universities (faculties). Samara: OFORT LLC, *State Educational Institution of Higher Professional Education* "Sam GMU", 2004; 1180 p.

Lu, T.; Chen, F. Multiwfn: A multifunctional wavefunction analyzer. *Journal of Computational Chemistry***,** 2012; *33* (5), 580-592. DOI: 10.1002/jcc.22885.

Lü, J.-M.; Lin, P. H.; Yao, Q.; Chen, C. Chemical and molecular mechanisms of antioxidants: experimental approaches and model systems. *Journal of Cellular and Molecular Medicine***,** 2010; 14 (4), 840-860. DOI: 10.1111/j.1582-4934.2009.00897.x

Molyneux, P. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin J. sci. technol*, 2004; *26*(2), 211-219.

Pulido, R., Bravo, L., & Saura-Calixto, F. Antioxidant activity of dietary polyphenols as determined

by a modified ferric reducing/antioxidant power assay. *Journal of agricultural and food chemistry*, 2000; *48*(8), 3396-3402. DOI: 10.1021/jf9913458.

Sadeq, O.; Mechchate, H.; Es-safi, I.; Bouhrim, M.; Jawhari, F.z.; Ouassou, H.; Kharchoufa, L. et al. Phytochemical Screening, Antioxidant and Antibacterial Activities of Pollen Extracts from Micromeria fruticosa, Achillea fragrantissima, and Phoenix dactylifera. Plants, 2021; 10, 676. https://doi.org/ 10.3390/plants10040676.

Sedivy, C., Piskorski, R., Müller, A., & Dorn, S. Too low to kill: concentration of the secondary metabolite ranunculin in buttercup pollen does not affect bee larval survival. *Journal of chemical ecology*, 2012. *38*, 996-1002. DOI 10.1007/s10886-012-0153-3

Slinkard, K., & Singleton, V. L. Total phenol analysis: automation and comparison with manual methods. *American journal of enology and viticulture*, 1997; *28*(1), 49-55. DOI:10.5344/ajev.1977.28.1.49.

Spiegel, M. Current Trends in Computational Quantum Chemistry Studies on Antioxidant Radical Scavenging Activity. *Journal of Chemical Information and Modeling***,** 2022; *62* (11), 2639-2658. https://doi.org/10.1021/acs.jcim.2c00104.

Talibov, T. H., & Ibrahimov, A. S. Taxonomic spectre of the flora of Nakhchivan Autonomous Republic. *Nakhchivan: Ajamy*, 2008, *364*.

Zehra, C., Yildiz, O., Şahin, H., Asadov, A., & Kolayli, S. Phenolic profile and antioxidant potential of propolis from Azerbaijan. *Mellifera*, 2015, *15*(1), 16-28.

Citation/Atıf: Abd Alla AE, Shehata MG, Masry SHD. Profiling and antimicrobial activity of Egyptian loofah honey as an unconventional bee honey: a comprehensive study. U. Bee J. / U. Arı D. 2025; 25 (1): 73-88. DOI: 10.31467/uluaricilik.1647070

ARAŞTIRMA MAKALESI / RESEARCH ARTICLE

PHYSICOCHEMICAL PROFILING AND ANTIMICROBIAL ACTIVITY OF EGYPTIAN LOOFAH HONEY AS AN UNCONVENTIONAL BEE HONEY: A COMPREHENSIVE STUDY

Geleneksel Olmayan Bir Arı Balı Olarak Mısır Lif Kabağı Balı'nın Fizikokimyasal Görünüşü ve Antimikrobiyal Aktivitesi: Kapsamlı Bir Çalışma

Asmaa E. ABD ALLA[1]*, Mohamed G. SHEHATA[2,3], Saad H. D. MASRY [4,5]

[1]Economic Entomology and Pesticides Department, Faculty of Agriculture, Cairo University, 12613 Giza, EGYPT, Corresponding author / Yazışma Yazarı E-mail: Supersemsema@gmail.com, ORCID No: 0000-0001-6140-8446

[2]Food Research Section, Applied Research and Capacity Building Division, Abu Dhabi Agriculture and Food Safety Authority (ADAFSA), Abu Dhabi 52150, UNITED ARAB EMIRATES, E-mail: gamalsng@gmail.com, ORCID No: 0000-00030760-5945,

[3]Food Technology Department, Arid Lands Cultivation Research Institute, City of Scientific Research and Technological Applications (SRTA-City), Alexandria 21934, EGYPT

[4]Abu Dhabi Agriculture and Food Safety Authority (ADAFSA), Al Ain 52150, UNITED ARAB EMIRATES, E-mail: sahmasry72@gmail.com, ORCID No: 0000-0002-9741-0238

[5]Department of Plant Protection and Biomolecular Diagnosis, Arid Lands Cultivation Research Institute (ALCRI), City of Scientific Research and Technological Applications, New Borg El-Arab City P.O. Box 21934, EGYPT

Received / Geliş: 26.02.2025 Accepted / Kabul: 15.04.2025 DOI: 10.31467/uluaricilik.1647070

ABSTRACT

This investigation focuses on exploring the physicochemical characteristics and antimicrobial activity of loofah honey in the Egyptian governorates of Kafr El-Shaikh and El-Beheira. A novel variety of honey, designated as a supplementary resource, has been identified as a means of sustenance for bees during periods of scarcity. Pollen analysis of the examined honey samples revealed its natural origin from various plant sources in trace amounts. The physicochemical analysis produced noteworthy results, with estimated reducing sugars ranging from 61.10±0.20 to 69.29±0.12 g/100g and pH values varying between 3.53±0.01 and 3.74±0.01. There were notable variations amongst the samples in terms of free acidity, total lactone, and total acidity, while no significant distinctions were noted in ash content. The study further identified the highest recorded values for H_2O_2, DN, and HMF as 76.80±0.01 mg/kg, 12.50±0.06 U/kg, and 5.35±0.01 mg/kg, respectively. Additionally, the maximum levels of phenols, flavonoids, and DPPH were determined as 210.56±0.01 mg/kg, 52.84±0.01 mg/kg, and 83.33±0.01 %, respectively. In terms of antimicrobial activity, all samples exhibited efficacy against *Bacillus subtilis* and *Klebsiella pneumoniae*, except for one sample that demonstrated antimicrobial activity against all six tested microorganisms' types.

Keywords: Loofah honey, Physicochemical characteristics, Pollen analysis, Antimicrobial activity

ÖZ

Bu araştırma, Mısır'ın Kafr El-Shaikh ve El-Beheira vilayetlerinde lif kabağı balının fizikokimyasal özelliklerini ve antibakteriyel aktivitesini araştırmaya odaklanmaktadır. Ek bir kaynak olarak belirlenen yeni bir bal çeşidi, kıtlık dönemlerinde arılar için bir beslenme aracı olarak tanımlanmıştır. İncelenen bal örneklerinin polen analizi, eser miktarda çeşitli bitki kaynaklarından gelen doğal kökenini ortaya

koymuştur. Fizikokimyasal analizler, 61,10±0,20 ile 69,29±0,12 g/100g arasında değişen tahmini indirgen şekerler ve 3,53±0,01 ile 3,74±0,01 arasında değişen pH değerleri ile kayda değer sonuçlar vermiştir. Örnekler arasında serbest asitlik, toplam lakton ve toplam asitlik açısından önemli farklılıklar bulunurken, kül içeriğinde önemli bir farklılık gözlenmemiştir. Çalışmada ayrıca H2O2, DN ve HMF için kaydedilen en yüksek değerler sırasıyla 76,80±0,01 mg/kg, 12,50±0,06 U/kg ve 5,35±0,01 mg/kg olarak belirlenmiştir. Ayrıca, maksimum fenol, flavonoid ve DPPH seviyeleri sırasıyla 210.56±0.01 mg/kg, 52.84±0.01 mg/kg ve %83.33±0.01 olarak belirlenmiştir. Antimikrobiyal aktivite açısından, test edilen altı mikroorganizma türünün tümüne karşı antimikrobiyal aktivite gösteren bir örnek dışında, tüm örnekler *Bacillus subtilis* ve *Klebsiella pneumoniae*'ya karşı etkinlik göstermiştir.

Anahtar Kelimeler: Lif kabağı balı, Fizikokimyasal özellikler, Polen analizi, Antimikrobiyal aktivite

GENİŞLETİLMİŞ ÖZET

Giriş: Oldukça besleyici bir gıda olan bal, çeşitli faktörlerden etkilenen fizikokimyasal özellikler sergiler. Bu çalışmanın amacı, Haziran'dan Ekim'e kadar üretilen yeni bir ikincil bal türü olarak potansiyeline odaklanarak lif kabağı balının özelliklerini araştırmaktır. Bileşimini, kalitesini ve potansiyel faydalarını inceleyerek, bu çalışma lif kabağı balının farklı özellikleri hakkında değerli bilgiler sağlamayı ve gıda ve ilaç endüstrilerinde uygulanabilirliğinin daha iyi anlaşılmasına katkıda bulunmayı amaçlamaktadır.

Gereç ve Yöntem: Bu araştırma, Mısır'ın Kafr El-Shaikh ve El-Beheira vilayetlerinde lif kabağı balının fizikokimyasal özelliklerini ve antibakteriyel aktivitesini araştırmaya odaklanmaktadır. Alışılmadık ve nispeten yeni bal türlerinden biri olan bu bal, arıların kıtlık zamanlarında kullandığı ek bir kaynak olarak bilinmektedir. Beş bal örneği, bitkilerin çiçeklenme döneminde Haziran ve Kasım 2021 tarihleri arasında iki ildeki farklı arılıklardan toplanmıştır. Üç örnek El-Beheira'dan, iki örnek ise Kafr El-Shaikh'ten alınmıştır. Her biri üç kopyadan oluşan örnekler, daha sonra kimyasal bileşim açısından analiz edilene kadar Kahire Üniversitesi Ziraat Fakültesi Deney İstasyonu'nun arı kovanı bahçesindeki laboratuvarında -28±2ºC'de saklanmıştır. Kimyasal analiz; şeker, nem içeriği, pH, serbest asitlik, hidroksimetilfurfural (HMF), toplam fenoller, toplam flavonoidler, 2,2-difenil-1-pikrilhidrazil (DPPH), C vitamini, diastaz aktivitesi, hidrojen peroksit (H_2O_2) ve iletkenliğin değerlendirilmesinin yanı sıra melissopalinoloji ve antimikrobiyal aktivitenin incelenmesini de içermektedir. Fizikokimyasal analiz, 61,10±0,20 ila 69,29±0,12 g/100g arasında değişen tahmini indirgen şekerler ve 3,53±0,01 ila 3,74±0,01 arasında değişen pH değerleri ile kayda değer sonuçlar vermiştir.

Bulgular: Fizikokimyasal analizler, 61.10±0.20 ile 69.29±0.12 g/100g arasında değişen tahmini indirgen şekerler ve 3.53±0.01 ile 3.74±0.01 arasında değişen pH değerleri ile kayda değer sonuçlar vermiştir. Örnekler arasında serbest asitlik, toplam lakton ve toplam asitlik açısından önemli farklılıklar bulunurken, kül içeriğinde önemli bir farklılık gözlenmemiştir. Çalışmada ayrıca H2O2, DN ve HMF için kaydedilen en yüksek değerler sırasıyla 76,80±0,01 mg/kg, 12,50±0,06 U/kg ve 5,35±0,01 mg/kg olarak belirlenmiştir. Ayrıca, maksimum fenol, flavonoid ve DPPH seviyeleri sırasıyla 210.56±0.01 mg/kg, 52.84±0.01 mg/kg ve %83.33±0.01 olarak belirlenmiştir. Antimikrobiyal aktivite açısından, test edilen altı mikroorganizma türünün hepsine karşı antimikrobiyal aktivite gösteren bir örnek dışında, tüm örnekler *Bacillus subtilis* ve *Klebsiella pneumoniae'ya* karşı etkinlik göstermiştir. Geleneksel olmayan balın özelliklerinin incelenmesi, özellikle ana ürünlerin kıt olduğu zamanlarda hayati önem taşımaktadır. Geleneksel olmayan bal, arılar için önemli nektar kaynakları olan birkaç yeni bitkinin geliştirilmesinin bir sonucu olarak üretilmektedir. Son olarak, tüm yıl boyunca nektar bitkileri yetiştirmek önemlidir çünkü bu, arılara bal üretimini artıran sabit bir besin kaynağı sağlar.

Sonuç: Bu çalışma, son zamanlarda ortaya çıkan en yeni ve en tuhaf bal türlerinden biri olan lif kabağı balının özelliklerini daha iyi anlamak için yapılmıştır. Sonuçlar, lif kabağı poleninin test edilen tüm bal türlerinde ikincil bir kaynak olarak ortaya çıktığını vurgulamıştır. Bu türler, bitki kökeni, iklim koşulları, arı muameleleri ve depolama koşulları dahil olmak üzere çok çeşitli değişkenlere bağlı olarak yüksek nem içeriği, normal monosakkarit içeriği, sükroz içeriği ve pH ile karakterize edilmiştir. Arı balının

fizikokimyasal özellikleri bir bölgeden diğerine değişmektedir. Geleneksel olmayan balın özelliklerinin incelenmesi, özellikle ana ürünlerde kıtlık yaşandığında hayati önem taşımaktadır. Geleneksel olmayan bal, arılar için önemli nektar kaynakları olan birkaç yeni bitkinin geliştirilmesinin bir sonucu olarak üretilmektedir. Son olarak, tüm yıl boyunca nektar bitkileri yetiştirmek önemlidir çünkü bu, arılara bal üretimini artıran sabit bir besin kaynağı sağlar.

INTRODUCTION

Bees collect nectar from flowers or the secretions of sap-sucking insects, transform it through enzymatic processes, and store it in honeycombs, resulting in bee honey-a naturally sweet and flavourful product (Codex Alimentarius Commission 2001). It is well established that honey has been used by both ancient and modern civilisations for its therapeutic properties. It is a natural remedy for a variety of illnesses due to its antimicrobial, antioxidant and wound-healing abilities (Karabagias et al. 2014). Its extensive application spans both pharmaceutical and food industries, where it is valued not only as a functional food but also as a natural medicine. Furthermore, its pleasant taste and ease of digestion make it particularly beneficial for patients, the elderly and pregnant women, offering both nutritional and medicinal benefits (Bihonegn and Begna 2021). Honey is a supersaturated solution of sugars; it consists mainly of the sugar's fructose (~38%) and glucose (~31%), along with other 200 ingredients such as water, traces of organic acids, minerals, proteins, ashes, enzymes, amino acids, vitamins, antioxidants, phenol compounds, and flavonoids (Pasias et al. 2017, Da Silva et al. 2016, Ouchemoukh et al. 2006).

Honey exhibits remarkable therapeutic properties and is widely used in traditional medicine due to its ability to combat pathogenic bacteria (Israili 2014). Its antimicrobial efficacy is primarily attributed to several mechanisms involving both enzymatic and non-enzymatic components. For instance, the high acidity of honey, with a typical pH range of 3.2 to 4.5, creates an inhospitable environment for many microorganisms. Additionally, hydrogen peroxide produced by the enzymatic action of glucose oxidase, acts as a potent antimicrobial agent by generating reactive oxygen species (ROS) that damage bacterial cells. Osmosis, resulting from the high sugar concentration in honey, dehydrates bacterial cells, leading to their inhibition (Combarros-Fuertes et al. 2020, Snowdon & Cliver 1996).

Nonperoxide compounds, such as phenolic acids and flavonoids, further enhance honey's antimicrobial activity through specific mechanisms. Phenolic acids and flavonoids disrupt bacterial cell membrane integrity, impairing vital processes such as nutrient transport and energy production. These bioactive compounds also induce oxidative stress by increasing ROS within bacterial cells, which damages proteins, lipids, and DNA. Furthermore, phenolics and flavonoids inhibit bacterial enzymes essential for replication and survival, such as those involved in quorum sensing and energy metabolism (Bucekova et al. 2018 and Israili 2014).

Beyond its antimicrobial properties, honey also demonstrates significant anti-inflammatory and antioxidant effects. It modulates inflammatory pathways by reducing pro-inflammatory cytokines and enhancing the expression of anti-inflammatory mediators, contributing to its use in wound healing. Honey's high phenolic and flavonoid content scavenges free radicals, mitigating oxidative stress and promoting tissue repair. In wound healing, honey accelerates tissue regeneration, stimulates the formation of granulation tissue, and reduces inflammation, leading to faster recovery and improved outcomes (Martinotti et al. 2019).

The quality of honey is influenced by a range of factors, including its type, characteristics, composition, geographical and plant origins, the season of collection, local climate, improper beekeeping techniques, and storage conditions (García et al. 2020, El Sohaimy et al. 2015). These factors collectively shape the physicochemical properties, nutritional value, and therapeutic potential of honey, highlighting the importance of careful management and monitoring to ensure high-quality production. Since honeybees gather nectar from various blooms, honey can be either monofloral, derived primarily from one plant species with distinct characteristics, or polyfloral, sourced from multiple species, resulting in diverse flavours and bioactive compounds. These variations highlight the influence of plant diversity on honey's quality and properties. Due to increasing global trade and the higher economic values associated with specific types of honey, these products are especially vulnerable to adulteration, honey mixing, and misleading or dishonest labelling of honey of lower value. Honey's authenticity is assessed through

methods like melissopalynological analysis, chemical profiling (e.g., HMF levels and diastase activity), isotopic analysis for sugar adulteration, and advanced spectroscopic techniques (Soares et al. 2017).

Melissopalynology, a subfield of palynology (the study of pollen and spores), is one of the best techniques for categorizing different kinds of honey because it focuses on microscopic studies of bee honey (Attia El-Sofany et al. 2020). By analysing the pollen content in honey, this method can identify the geographical origin and the plant species from which the nectar was collected. For example, specific pollen grains serve as markers for certain regions or floral sources, allowing precise tracing of honey's botanical and geographical origins, which is crucial for ensuring authenticity and understanding its unique properties (EL-Metwally 2015).

The three primary flowering honey crop seasons in Egypt—citrus fruits in March and April, Egyptian alfalfa in April to June, and cotton in July and August—are well-documented. However, loofah honey (Luffa Egyptiac), emerging as a secondary type of honey, serves as a critical resource during off-season periods of scarcity. Loofah blooms, which persist from June to October, provide an abundant and consistent nectar supply that supports the growth and sustenance of bee colonies. This is particularly beneficial for beekeepers in regions with limited alternative floral resources, allowing them to sustain production and maintain colony health. Additionally, loofah honey is gaining attention due to its distinct physicochemical properties and health benefits, making it a potential candidate for both local markets and international export (Taha et al. 2019). Therefore, the aim of this work is to investigate the characteristics of loofah honey, focusing on its potential as a new secondary type of honey produced from June to October. By examining its composition, quality, and potential benefits, this study seeks to provide valuable insights into the distinct properties of loofah honey, contributing to a better understanding of its applicability in the food and pharmaceutical industries.

MATERIALS AND METHODS

Bee honey samples

Five honey samples were collected from different apiaries in two governorates between June and November 2021, during the blooming period of the plants. Three samples were obtained from El-Beheira, and two samples were taken from Kafr El-Shaikh. The samples, each consisting of three duplicates, were stored at the apiary yard laboratory of Cairo University's Faculty of Agriculture, Experimental Station, at -28±2 ºC until they were later analysed for chemical composition.

Examination of melissopalynology

The pollen grains from each examined honey sample were analysed using the methodology described by Louveaux et al. (1978). Ten grams of honey were dissolved in 20 millilitres of warm water and centrifuged at 3500 revolutions per minute for 10 minutes. The liquid was then decanted, replaced with fresh water, and centrifuged again for an additional 10 minutes. The sediment was gently dried by heating it to 40°C, then placed on a microscope slide and spread evenly over an area of approximately 20 × 20 mm. Glycerin gelatin was applied to the sediment, which was subsequently examined under a light microscope. Pollen grain frequency was classified as follows: "Very frequent" for grains constituting more than 45%, "Frequent" for grains comprising 16–45%, "Rare" for grains ranging from 3 to 15%, and "Sporadic" for grains constituting less than 3% of the total grains, based on the criteria outlined by El Sohaimy et al. (2015).

Physiochemical analysis of loofah honey

Chemical analysis, which included the assessment of sugars, moisture content, pH, free acidity, hydroxymethylfurfural (HMF), total phenols, total flavonoids, 2,2-diphenyl-1-picrylhydrazyl (DPPH), vitamin C, diastase activity, hydrogen peroxide (H_2O_2), and conductivity, was thoroughly performed at Cairo University's Faculty of Agriculture, Food Safety, and Quality Control Laboratory in Giza, Egypt. The quantification of sugars, specifically fructose, glucose, and sucrose, was accomplished through high-performance liquid chromatography (HPLC). The analysis employed a Phenomenex Luna NH2 column (250×4.6 mm), with the column temperature maintained at a constant 30º C. The mobile phase consisted of acetonitrile and HPLC-grade water in a ratio of 80:20 (v: v). Detection of the sugars was achieved using a refractive index (RI) detector, and data integration was performed through ClarityChrom software. Calibration of the system was carried out using standard sugar solutions, and the detection limits for the sugars were determined to ensure accurate quantification (El Sohaimy et al. 2015).

Measurement of Hydroxymethylfurfural (HMF): The determination of hydroxymethylfurfural (HMF) was carried out using a combination of UV/V spectrophotometry and a modified White technique. To begin, five grams of honey were weighed and homogenized with distilled water. HMF stabilization was achieved by adding 10 mL of 2% w/v sodium bisulfite solution, followed by a 15-minute incubation. Acid hydrolysis was performed by adding 10 mL of 4N hydrochloric acid, with a subsequent 30-minute incubation in a controlled-temperature water bath (60-70°C). After cooling, HMF was extracted using 10 mL of acetone, and the solution was filtered for clarity. UV/Vis spectrophotometric measurements were taken at 284 nm. HMF quantification was conducted using a calibration curve created with standard HMF solutions. The curve equation was derived from the linear relationship between known HMF concentrations and their absorbance. The method was validated using certified reference materials, ensuring precision in duplicate analyses and confirming its reliability for measuring HMF levels in honey samples (Pasias et al. 2017).

Moisture content: Water content was determined with a digital refractometer at 20 ºC according to AOAC 1990.

Electrical conductivity: The conductivity was measured using a conductivity meter for a 20% honey volumetric weight in a water-based solution at 200 ºC, where the honey dry matter was represented by 20% (FiveEasy, Mettler Toledo, Switzerland) (AOAC 1990).

pH value: The pH value of the honey samples was measured using a pH meter from Boeco, Germany. The meter was calibrated using buffer solutions with pH values of 4, 7, and 10, ensuring accurate readings according to international standards.

Free acidity: Free acidity was determined using the equivalence point titration method, as specified in the Codex Alimentarius (2001), ensuring that the procedure adheres to internationally recognized food quality standards.

Hydrogen peroxide assay (H_2O_2): The hydrogen peroxide (H_2O_2) assay was performed utilizing peroxidase (HRP). In the presence of 3.5-dichloro-2-hydroxybenzenesulfonic acid (DHBS) and 4-aminophenazone (AAP), H_2O_2 reacts to produce a chromophore. The enzymatic reaction, catalysed by HRP, leads to the formation of a quinone imine dye along with the generation of four molecules of water. This method, as described by Lehmann et al. (2019), provides a reliable means of assessing hydrogen peroxide levels.

Diastase activity: This was assessed to obtain the diastatic number (DN) following a period of shading. The resulting measurement is expressed in Gothe units (Lehmann et al. 2019). The method was conducted in accordance with international standards, specifically the Codex Alimentarius and the AOAC (2010) guidelines.

Total phenols content: The total phenols content was quantified calorimetrically using the Folin-Ciocalteu reagent, following the method outlined by Singleton and Rossi (1965). For the analysis, 10 g of honey were dissolved in 50 mL of distilled water to prepare the sample extract. A standard curve was constructed using gallic acid solutions with concentrations ranging from 10 to 300 mg gallic acid equivalent per kilogram (mg GAE/kg). The regression equation of the standard plot ($y = 101.71x - 0.4181$, $R^2 = 0.9979$) was used to calculate the total phenolic content. The results were expressed as milligrams of gallic acid equivalents per kilogram of honey (mg GAE/kg). The analysis was performed using a UV/Vis spectrophotometer from Jenway, England.

Total flavonoid content: The total flavonoid content was determined using the aluminium chloride colorimetric technique. A solution was prepared by dissolving 10 g of honey in 50 mL of distilled water. The following mixture was then prepared: 1 mL of the prepared honey extract, 3 mL of methanol, 0.2 mL of 10% aluminium chloride, 0.2 mL of 1M potassium acetate, and 5.6 mL of distilled water. After allowing the mixture to stand at room temperature for 30 minutes, absorbance was measured at 420 nm. Rutin was used as the standard, with concentrations ranging from 10 to 100 mg rutin equivalent per kilogram (mg RE/kg) to construct the standard curve. The regression equation of the standard plot ($y = 365.26x - 6.1589$, $R^2 = 0.9978$) was used to calculate the total flavonoid content. The results were expressed as milligrams of rutin equivalents per kilogram of honey (mg RE/kg). The analysis was conducted based on the method described by Abu Safe et al. (2023).

Antioxidant Activity by DPPH: The activity of DPPH radical scavenging was determined by preparing concentrations ranging from 1% to 5% with 50% methanol from each sample extract (100 µL). To this, 100 µl of DPPH radical solution (0.2

mM) dissolved in methanol was added. After stirring the mixture, it was left in the dark for fifteen minutes. Subsequently, the absorbance was measured using a UV/Vis spectrophotometer (Jenway, England) at 517 nm in comparison to a blank. The scavenging impact percentage was calculated using the formula: [(A0 - A1) / A0] × 100, where A0 represents the absorbance of the control (without sample), and A1 is the absorbance in the presence of the sample. (Shehata et al. 2023)

Examination of water-soluble vitamins: Water-soluble vitamins were analysed using an Agilent 1260 Infinity HPLC (Agilent, USA) equipped with a Quaternary pump and a HyperClone BDS C18, 100 mm × 4.6 mm, 3 µm column (Phenomenex, USA). The instrument was operated at a temperature of 35°C. Separation was achieved using a binary linear elution gradient with mobile phase A consisting of 25 mM NaH2PO4 at pH 2.5 and mobile phase B consisting of methanol. The gradient started with 95% A and 5% B, gradually transitioning to 75% A and 25% B over 20 minutes, and then to 50% A and 50% B for column equilibration. The flow rate was set at 1.0 mL/min, and the injected sample volume was 20 µL. Detection was performed using a VWD detector set at 270 nm, with humidity maintained at 38% RH and the temperature at 25°C. Samples were filtered through a 0.45 µm syringe filter prior to injection. The method was based on the work of Abd El-Aziz et al. (2021).

Antimicrobial activity

Using the agar well diffusion method, the honey extracts' antibacterial activity was assessed (Shehata et al. 2017). Nine species, regarded as harmful to humans, including *Escherichia coli* BA 12296, *Bacillus subtilis* DB 100 host, *Candida albicans* ATCC MYA-2876, *Klebsiella pneumoniae* ATCC12296, *Salmonella senftenberg* ATCC 8400, *Staphylococcus aureus* NCTC 10788, were used. Each microorganism's media was combined with 100 µL of the inoculum (1 x 10^8 CFU/mL) before being added to the Petri plate. The honey extracts were added to the well in a volume of 100 µL. After 48 hours of overnight incubation at 37 °C, the diameter (mm) of the resultant zone of inhibition was measured on the plates.

Statistical analysis

Data were analyzed using IBM SPSS software package version 16.0 (Kirkpatrick & Feeney 2013). Quantitative data were described using means and standard deviation. For normally distributed data, comparisons between the different studied inhibitors were performed using F-test (ANOVA). The significance of the obtained results was determined by p-value ($p < 0.05$) (Kotz et al. 2006).

RESULT

Sugar Content

The sugar profile of the honey samples from different regions exhibited significant variations ($P \leq 0.05$). Fructose, glucose, and sucrose concentrations were determined for all samples, as shown in Table 1. Fructose content ranged from 33.50±0.12 g/100 g to 39.90±0.06 g/100 g, with Sample 5 from Kafr El-Shaikh showing the highest fructose concentration, while Sample 2 from El-Beheira recorded the lowest.

Glucose concentrations varied significantly across the samples, with the highest value detected in Sample 1 from El-Beheira (32.29±0.12 g/100 g) and the lowest in Sample 5 from Kafr El-Shaikh (27.60±0.12 g/100 g). Sucrose content ranged from 0.81±0.12 g/100 g in Sample 5 to 5.03±0.01 g/100 g in Sample 1, revealing significant inter-sample differences.

Water Content and Sugar Ratios

The water content of the honey samples, as detailed in Table 1, showed significant differences ($P \leq 0.05$), ranging from 20.80±0.06% in Sample 1 to 22.40±0.06% in Sample 4, both from Kafr El-Shaikh. The glucose-to-water (G/W) ratio exhibited a significant difference ($P \leq 0.05$) across samples, with Sample 1 having the highest ratio of 1.55±0.01 and Sample 4 the lowest at 1.23±0.01. This ratio is important as it indicates the relative concentration of glucose to water, which influences the honey's viscosity and potential for fermentation. The fructose-to-glucose (F/G) ratio also varied significantly ($P \leq 0.05$), with Sample 5 from Kafr El-Shaikh recording the highest value of 1.46±0.01, while Sample 1 from El-Beheira had the lowest ratio at 1.15±0.01. The F/G ratio is significant because it reflects the sweetness and overall composition of the honey, which can affect its flavor profile and crystallization tendencies.

Table 1. Sugar spectrum, water content, and G/W and F/G ratios of examined honey samples

Honey samples	Fructose g/100 g	Glucose g/100 g	Estimated reducing sugars g/100 g	Sucrose g/100 g	Water content %	G/W ratio	F/G ratio
1	37.00± 0.12^{B}	32.29± 0.12^{A}	69.29± 0.12^{A}	0.81± 0.12^{E}	20.80±0.06^{D}	1.55±0.01^{A}	1.15± 0.01^{D}
2	33.50±0.12^{E}	27.60±0.12^{C}	61.10±0.20^{E}	1.07±0.01^{D}	21.00±0.05^{C}	1.31±0.01^{C}	1.21 ±0.01^{C}
3	35.75±0.12^{D}	29.26±0.12^{B}	65.01±0.20^{C}	5.03±0.01^{A}	21.20±0.06^{B}	1.38 ±0.01^{B}	1.22±0.01^{C}
4	36.28± 0.01	27.47±0.12^{DC}	63.75±0.12^{D}	2.50±0.12^{C}	22.40± 0.06^{A}	1.23±0.01^{D}	1.32±0.01^{B}
5	39.90±0.06^{A}	27.30±0.01^{D}	67.20± 0.06^{B}	3.10± 0.01^{B}	21.00±0.06^{C}	1.30±0.01^{C}	1.46±0.01^{A}

The data represent the mean values (±standard deviation) obtained from three replicate measurements at two different time points. abcde Means in the same column followed by different superscript letters are significantly different (P < 0.05). G/W: glucose/water and F/G: fructose/glucose.

Physicochemical Properties

The physicochemical properties of the honey samples showed significant variations (P ≤ 0.05) in pH, free acidity, total lactone, and total acidity, as summarized in Table 2. The pH values of the samples ranged from 3.53±0.11 in Sample 5 to 3.74±0.01 in Sample 3. Free acidity levels varied between 28.25±0.12 meq/kg in Sample 5 and 56.25±0.11 meq/kg in Sample 3. Total acidity values ranged from 40.75±0.01 meq/kg to 73.75±0.01 meq/kg, with two samples exceeding the Codex Alimentarius limit of 50 meq/kg for total acidity. These variations highlight the differences in the honey's acid content, which can influence its taste, preservation, and overall quality.

Table 2. The physicochemical characteristics of the tested honey samples

Honey samples	pH	Free acidity meq/kg	Total lactone meq/kg	Total acidity meq/kg	Ash %	Conductivity ms/cm
1	3.67±0.01^{ab}	56.25±0.11^{a}	17.50± 0.05^{d}	73.75±0.01^{a}	0.23±0.11^{a}	0.306±0.57^{b}
2	3.62± 0.01^{ab}	37.25±0.11^{c}	14.00± 0.06^{c}	51.25±0.11^{c}	0.08± 0.01^{a}	0.214± 0.57^{d}
3	3.74±0.01^{a}	28.25±0.12^{e}	12.50±0.12 ^{e}	40.75± 0.01^{e}	0.05±0.01^{a}	0.219±0.58^{c}
4	3.53±0.11^{b}	54.75± 0.01^{b}	15.00±0.06^{b}	69.75±0.01^{b}	0.05±0.01^{a}	0.363±0.58^{a}
5	3.70± 0.01^{ab}	32.25±0.02^{d}	13.00±0.11^{d}	45.25±0.01^{d}	0.11±0.01^{a}	0.214± 0.58^{d}

The data represent the mean values (±standard deviation) obtained from three replicate measurements at two different time points. abcde Means in the same column followed by different superscript letters are significantly different (P < 0.05).

Ash Content and Electrical Conductivity

The ash content and electrical conductivity values of the honey samples are summarized in Table 2. Ash content ranged from 0.05% in Sample 3 to 0.23% in Sample 4, with no significant differences (P > 0.05) between the samples. Electrical conductivity showed significant variation (P ≤ 0.05), with the highest value

recorded for Sample 4 (0.363±0.577 ms/cm) and the lowest for Samples 1 and 5 (0.214±0.577 ms/cm). According to the Codex Alimentarius, the maximum allowable ash content for honey is typically 0.6%, and the electrical conductivity should generally not exceed 0.8 ms/cm. Based on these standards, all honey samples in this study fall within the acceptable limits for both ash content and electrical conductivity.

Diastase Number and Enzyme Activity

The diastase number (DN), which reflects the enzyme activity in the honey samples, varied significantly ($P \leq 0.05$) across the samples, as shown in Figure 1. Sample 1 from El-Beheira had the highest DN (12.50±0.06 U/kg), while Sample 5 from Kafr El-Shaikh exhibited the lowest DN (3.33±0.01 U/kg). According to the Codex Alimentarius, the minimum allowable diastase number for honey is typically 8 U/kg, and honey with a DN lower than this may be considered substandard. Based on these criteria, Sample 5 falls below the acceptable limit, while all other samples meet the Codex standard for enzyme activity.

Hydrogen Peroxide and HMF Content

Hydrogen peroxide (H_2O_2) concentrations varied significantly ($P \leq 0.05$) across the samples, with Sample 5 showing the highest concentration (76.80±0.01 mg/kg) and Sample 3 exhibiting the lowest (35.20±0.01 mg/kg). HMF (hydroxymethylfurfural) content, which is an indicator of honey quality and freshness, ranged from 1.20±0.06 mg/kg to 5.35±0.01 mg/kg, as shown in figure 1. According to the Codex Alimentarius, the maximum allowable HMF content for honey is 40 mg/kg, beyond which honey may be considered of poor quality or adulterated. Based on this standard, all honey samples in this study fall well below the Codex limit for HMF.

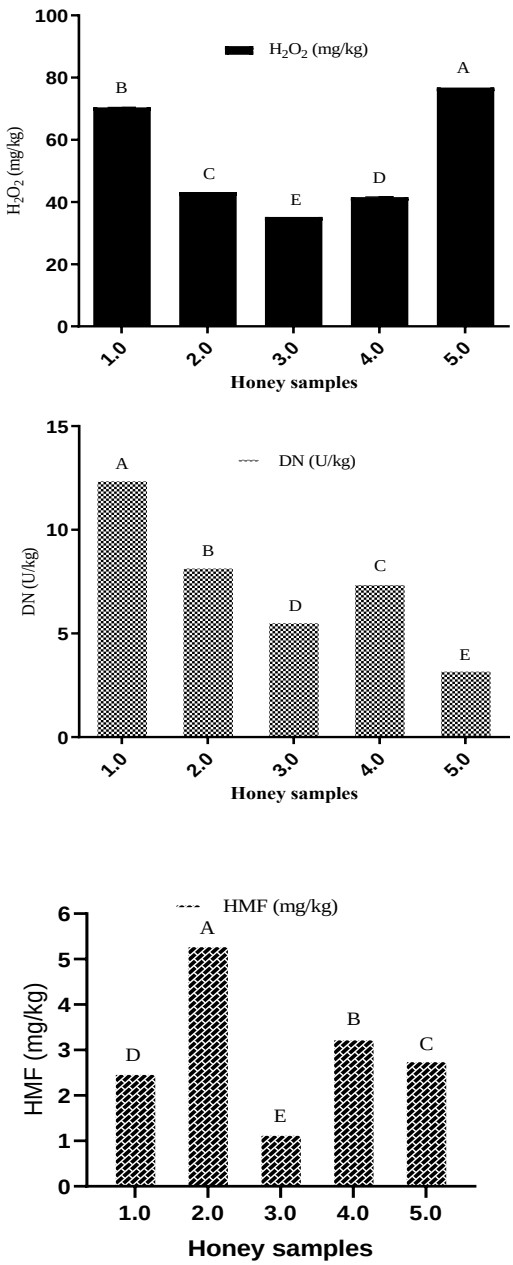


Figure 1: The Parameters of H_2O_2, DN, and HMF in the tested honey samples. The data represent the mean values (±standard deviation) obtained from three replicate measurements at two different time points. abcde Means in the same column followed by different superscript letters are significantly different ($P < 0.05$). H_2O_2: Hydrogen Peroxide; DN: diastatic number; HMF: hydroxymethylfurfural

Phenolic, flavonoid Content and Antioxidant Activity

The total phenolic content of the honey samples showed significant differences (P ≤ 0.05), with Sample 4 from Kafr El-Shaikh having the highest value (210.56±0.01 mg/kg) and Sample 5 the lowest (18.61±0.01 mg/kg). Flavonoid content ranged from 32.76±0.01 mg/kg to 52.84±0.01 mg/kg, with Sample 2 from El-Beheira recording the highest flavonoid concentration, as shown in Table 3.

Antioxidant activity, as measured by the DPPH radical scavenging assay, ranged from 61.46±0.01% in Sample 3 to 83.33±0.01% in Sample 4. Vitamin C content showed significant variations (P ≤ 0.05), with values ranging from 10.12±0.01 to 22.56±0.01 mg/100 g.

Table 3. Phenols, flavonoids, DPPH, and V.C content in the tested honey samples.

Honey samples	polyphenols		DPPH (%)	Vitamin C (mg/kg)
	Total Phenolic content (mg gallic acid equivalent/100g)	Total Flavonoids content (mg rutin equivalent /100g)		
1	28.21±0.01^{c}	50.53±0.01b^{B}	80.21±0.01^{b}	1.72±0.01^{bc}
2	19.66± 0.01^{d}	52.84± 0.01^{a}	68.06± 0.01^{d}	1.73± 0.01^{ab}
3	169.01±0.01^{b}	32.76±0.01^{e}	61.46±0.01^{e}	1.71±0.01^{c}
4	210.56±0.01^{a}	33.11±0.01^{d}	83.33±0.01^{a}	1.73±0.01^{ab}
5	18.61±0.01^{e}	46.78±0.01^{c}	75.35±0.01^{c}	1.74±0.01^{a}

The data represent the mean values (±standard deviation) obtained from three replicate measurements at two different time points. abcde Means in the same column followed by different superscript letters are significantly different (P < 0.05).

Melissopalynological Analysis

The melissopalynological analysis revealed that Eucalyptus was the dominant pollen type in the examined sample, along with the presence of other pollen types in lower concentrations, as shown in Table 4. Sample 1 from El-Beheira contained 23.18% loofah pollen (*Luffa aegyptiaca*), while Sample 4 from Kafr El-Shaikh had 31.25% clover pollen (*Trifolium alexandrinum*). However, it should be noted that only Eucalyptus sp. qualifies as a dominant pollen type based on its percentage, while *Luffa aegyptiaca* and *Trifolium alexandrinum* are better described as secondary or minor pollen contributors. This clarification ensures consistency with the frequency-based classification of pollen types.

Table 4. The melissopalynological examination of pollen grains in the tested honey samples.

Melissopalynological analysis	Pollen types %				
	1	2	3	4	5
Family: Fabaceae ***Trifolium alexandrinum***	8.34 ± 0.04^{d}	26.31 ± 0.04^{b}	20.27 ± 0.05^{c}	31.35 ± 0.02^{a}	0
Family: Arecaceae ***Phoenix dactylifera***	21.08 ± 0.03^{b}	5.26 ± 0.06^{d}	16.27 ± 0.03^{c}	30.15 ± 0.02^{a}	4.50 ± 0.04^{e}
Family: Chenopodiaceae ***Chenopodium sp.***	17.04 ± 0.01^{c}	26.31 ± 0.05^{b}	37.01 ± 0.06^{a}	0	0
Family: Compositae ***Helianthus annuus***	4.50 ± 0.01^{a}	0	4.05 ± 0.02^{b}	0	0
Family: Myrtaceae ***Eucalyptus spp.***	8.38 ± 0.04^{c}	1.09 ± 0.04^{d}	0	10.47 ± 0.03^{b}	60.53 ± 0.02^{a}
Family: Umbelliferae	7.34 ± 0.05^{b}	0	4.00 ± 0.02^{c}	3.12 ± 0.03^{d}	7.58 ± 0.02^{a}
Family: Casuarinaceae ***Casuarina sp.***	10.14 ± 0.05^{a}	4.20 ± 0.55^{b}	2.70 ± 0.01^{c}	0	10.39 ± 0.03^{a}
Family: Cucurbitaceae ***Luffa aegyptiaca***	23.18 ± 0.05^{b}	23.15 ± 0.03^{b}	15.70 ± 0.02^{d}	25.00 ± 0.04^{a}	17.00 ± 0.03^{c}
Family: Cucurbitaceae ***Cucurbita sp.***	0	13.68 ± 0.03^{a}	0	0	0

Antimicrobial Activity

The antimicrobial activity of the honey samples was evaluated against six bacterial strains, as detailed in Table 5. Sample 2 from El-Beheira exhibited the strongest antibacterial activity, particularly against *Bacillus subtilis* (12.76±1.35 mm inhibition zone) and *Escherichia coli* (12.53±1.23 mm inhibition zone). Moderate antibacterial effects were also observed against *Salmonella senftenberg* and *Klebsiella pneumoniae*, with significant differences ($P \leq 0.05$) noted between the samples.

Table 5. Inhibition zone of six pathogen's microorganisms for the examined honey samples.

Pathogenic microorganisms	Diameter of inhibition zone (mm)				
	Honeybee samples				
	El-Beheira 1	El-Beheira 2	El-Beheira 3	Kafr El-Shaikh 4	Kafr El-Shaikh 5
***Escherichia coli* BA 12296**	7.09±0.49^{b}	12.53±1.23^{a}	7.46±1.22^{b}	ND	ND
***Bacillus subtilis* DB 100 host**	7.37±0.91^{c}	12.76±1.35^{a}	8.73±0.32^{bc}	9.20±0.70^{a}	8.63±0.96^{bc}
***Candida albicans* ATCC MYA-2876**	ND	8.13± 0.21^{a}	ND	ND	ND
***Klebsiella pneumoniae* ATCC12296**	9.21±0.35^{ab}	10.13±0.65^{a}	8.45±0.93^{b}	7.23±0.70^{c}	6.80±0.46^{c}
***Salmonella senftenberg* ATCC 8400**	5.63±0.61^{b}	10.53±0.94^{a}	ND	6.50±0.88^{b}	ND
***Staphylococcus aureus* NCTC 10788**	ND	5.87±0.24^{a}	ND	ND	ND

The data represent the mean values (±standard deviation) obtained from three replicate measurements at two different time points. abcde Means in the same column followed by different superscript letters are significantly different ($P < 0.05$).

Principal Component Analysis (PCA)

Principal Component Analysis (PCA) was performed to examine the relationship between antimicrobial activity, total phenols, flavonoids, and DPPH activity (Fig. 2). The first two principal components (PC1 and PC2) accounted for a significant proportion of the total variance, with PC1 explaining X% and PC2 explaining Y% of the variance, collectively accounting for Z%. The PCA plot revealed a clear clustering of antimicrobial activity with total phenols and flavonoids, suggesting a positive correlation between these variables. Samples with higher phenolic and flavonoid contents showed stronger antimicrobial properties. However, DPPH activity, which measures antioxidant potential, displayed a weaker correlation with antimicrobial activity, though it was still somewhat associated with phenols and flavonoids. This indicates that antioxidant activity, while not directly contributing to antimicrobial activity, shares some overlap with phenolic compounds in the dataset.

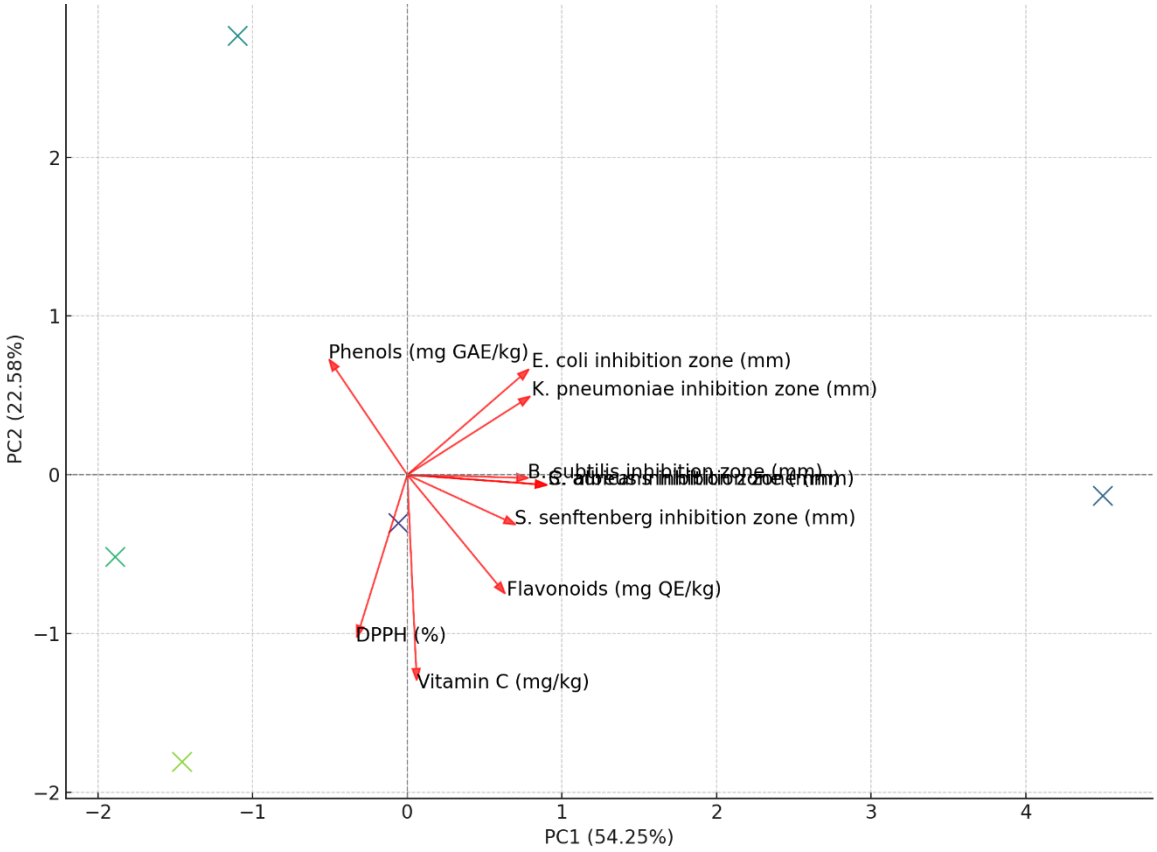


Fig. 2. Principal Component Analysis (PCA) Biplot showing the relationships between antimicrobial activity, total phenols, flavonoids, and DPPH activity. The first two principal components (PC1 and PC2) account for X% and Y% of the total variance, respectively.

DISCUSSION

The current study focused on the physicochemical properties, sugar content, and bioactive compounds in honey samples from different regions of Egypt, including El-Beheira and Kafr El-Shaikh governorates. The results provide valuable insights into the variations in honey composition depending on geographical origin, and these findings align with the established literature while also contributing new data to the field.

The observed differences in fructose and glucose contents among the honey samples are consistent with previous studies that have reported similar variations based on geographical and botanical origins. For instance, Persano Oddo et al. (2004) highlighted that the fructose/glucose ratio (F/G) is a critical determinant in honey crystallization. Our findings, which show a higher fructose content in Kafr El-Shaikh samples compared to El-Beheira, corroborate earlier studies by White (1975) and Siddiqui (1970), which indicated that honey with higher fructose levels tends to crystallize more slowly. This is supported by Draiaia et al. (2015), who found that honey samples with an F/G ratio greater than 1.0 crystallize more slowly, which is consistent with the slow crystallization observed in our samples.

The glucose-to-water (G/W) ratio is another significant factor influencing honey crystallization. The highest G/W ratio observed in El-Beheira sample 1 aligns with findings from Escuredo et al. (2014), who noted that higher G/W ratios increase the tendency of honey to crystallize. However, our data suggest that the honey samples from Kafr El-Shaikh, with lower G/W ratios, may have a reduced crystallization tendency. This observation is crucial for honey producers and consumers, as

crystallization affects the texture and marketability of honey.

The pH values of the tested honey samples fell within the range specified by Codex Alimentarius (2001), indicating freshness and quality. The slight variations observed in pH values between samples are in line with the results reported by Borawska, & Socha (2020) and El Sohaimy et al. (2015), who found that honey pH can vary depending on the floral source and environmental conditions. The moderate acidity levels detected in the honey samples are attributed to the presence of organic acids, which are known to influence honey's flavor and preservation qualities. This is consistent with the findings of Karabagias et al. (2014), who suggested that the fermentation of sugars into organic acids by bee enzymes plays a significant role in honey's acidity.

The variation in total acidity across different samples, with some exceeding the Codex Alimentarius limits, suggests that certain environmental factors or floral sources may contribute to higher acid levels. This observation is supported by Ndife et al. (2013) and Borawska, & Socha (2020), who reported that total acidity in honey could vary widely based on botanical and geographical factors. The higher total acidity observed in some samples may be indicative of the fermentation process or the presence of specific organic acids, as discussed by Diafat et al. (2017).

The ash content, which is indicative of the mineral content in honey, demonstrated no significant differences among the samples, aligning with the findings of previous studies by Živkov Baloš et al. (2018). These researchers suggested that ash content reflects the inorganic mineral composition and is a marker for the botanical and geographic origin of honey. The electrical conductivity values, however, varied significantly between samples, with Kafr El-Shaikh samples showing higher conductivity. This finding is consistent with the research by Rysha et al. (2021), who noted that higher ash and acid contents in honey are directly related to increased electrical conductivity. The higher conductivity in Kafr El-Shaikh samples suggests a richer mineral content, potentially due to the specific floral sources in that region.

The significant variations in hydrogen peroxide (H_2O_2) levels among the samples align with studies by Martinotti et al. (2019) and Bucekova et al. (2018), who reported that H_2O_2 is a major factor in the antimicrobial properties of honey. The highest H_2O_2 levels observed in Kafr El-Shaikh sample 5 could be attributed to the specific floral sources in that region, which may enhance the production of glucose oxidase, the enzyme responsible for H_2O_2 generation in honey.

The diastase number (DN), which serves as an indicator of honey freshness and enzymatic activity, showed significant differences among the samples. These findings are consistent with Tadesse et al. (2021) and El Sohaimy et al. (2015), who reported that DN can vary based on storage conditions, floral sources, and the physiological state of the bee colony. The lower DN observed in some samples may suggest longer storage periods or exposure to higher temperatures, which could degrade the enzymatic activity, as noted by Da Silva et al. (2016).

The hydroxymethylfurfural (HMF) content in all tested samples was within the acceptable limits set by Codex Alimentarius, indicating that the honey samples were fresh and had not undergone significant heat treatment. This is consistent with findings by Pasias et al. (2017), who stated that HMF levels increase during storage and heat treatment due to the Maillard reaction. The low HMF content in our samples suggests minimal processing and good storage conditions.

The significant variations in phenolic content among the honey samples are consistent with the findings of Saeed et al. (2021) and Al-Mamary et al. (2002), who reported that the phenolic content in honey is highly dependent on its botanical source, color, and geographical origin. The higher phenolic content observed in Kafr El-Shaikh samples may be attributed to the specific floral sources in that region, which are known to be rich in phenolic compounds. The variations in flavonoid content among the samples further support this, as flavonoids are also influenced by the floral origin of honey.

The antioxidant activity, as measured by DPPH, varied significantly among the samples, with Kafr El-Shaikh samples showing higher antioxidant activity. This finding aligns with the literature, which suggests that honey's antioxidant properties are primarily due to its phenolic and flavonoid content (Saeed et al. 2021). The higher antioxidant activity in Kafr El-Shaikh samples may be indicative of the region's richer floral diversity, which contributes to the higher levels of bioactive compounds in honey.

The melissopalynological analysis revealed significant differences in the pollen content among

the honey samples, indicating diverse floral sources. The dominance of loofah pollen in the El-Beheira samples is consistent with the findings of Taha et al. (2019), who reported that certain regions have characteristic pollen profiles that reflect the local flora. The presence of eucalyptus pollen in Kafr El-Shaikh samples suggests that these honey samples may have unique antimicrobial properties, as noted by Cortopassi-Laurino and Gelli, (1991) in their study on the antibacterial properties of eucalyptus honey.

The antimicrobial activity of the honey samples against various pathogens is a critical finding, particularly the higher activity observed in El-Beheira sample 2. This aligns with studies by Stefanis et al. (2023) and Kwakman and Zaat (2012), who reported that the antimicrobial properties of honey are influenced by its phenolic and flavonoid content, as well as the presence of hydrogen peroxide, methylglyoxal, and other bioactive compounds. The higher antimicrobial activity in El-Beheira sample 2 suggests that the floral sources in this region contribute to the production of honey with enhanced bioactive properties.

The PCA results indicate that total phenols and flavonoids are key contributors to antimicrobial activity, supporting previous research highlighting their bioactive roles in inhibiting microbial growth (Boy et al. 2021). The positive correlation observed between these compounds and antimicrobial activity suggests that phenolic compounds, through mechanisms such as enzyme inhibition and membrane disruption, may enhance the antimicrobial potential of the samples. On the other hand, the relatively weak association between DPPH activity and antimicrobial properties suggests that antioxidants like phenolics may protect against oxidative stress in microbial cells but are not the primary agents behind antimicrobial effects. This aligns with existing studies which have shown that while antioxidants and antimicrobial properties both provide protective benefits, they operate through distinct mechanisms (Rammali et al. 2024, Gupta et al. 2022). The variability in DPPH activity could also stem from differences in the antioxidant capacity of individual phenolic compounds, further complicating the direct link between antioxidant and antimicrobial functions.

Conclusion: This study was done to gain a better understanding of the properties of loofah honey, one of the newest and most peculiar types of honey to appear recently. The results highlighted that the loofah pollen came as a secondary source in all tested honey types. These types were characterized by high moisture content, normal content of monosaccharides, sucrose content, and pH depending on a wide range of variables, including the plant origin, climatic conditions, bee treatments, and storage conditions. The physicochemical properties of bee honey vary from one region to another. Studying the properties of nontraditional honey is vital, particularly when the main crops are in shortage. Nontraditional honey is produced as a result of the development of several new plants, which are significant sources of nectar for bees. Finally, it is important to grow nectar crops all year round because it guarantees bees a steady supply of food, which boosts the production of honey.

Acknowledgement: The authors sincerely thank Dr. El-Kazafy A. Tahaa, Professor at the Department of Economic Entomology, Faculty of Agriculture, Kafr El-Sheikh University, Egypt, for his assistance in sample selection from Kafr El-Sheikh governorate.

Authors' Contributions: AEA, MGSH, and SHD: planning, data collection, methodological planning, experimenting, and setting up the study. All authors read and approved the final manuscript.

Consent to participate: Not applicable**.**

Consent for publication: Not applicable**.**

Competing interests: The authors declare no competing interests.

Data availability statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Funding: The authors declare that no funds, grants, or other support were received during the preparation of this manuscript

REFERENCES

Abd El-Aziz N M, Eldin Awad O M, Shehata M G & El-Sohaimy S A, Antioxidant and anti-acetylcholinesterase potential of artichoke phenolic compounds. Food Biosci. 2021;41, 101006. https://doi.org/10.1016/j.fbio.2021.101006.

Abu Safe F A, Badr A N, Shehata M G & El-Sayyad G S. Antimicrobial and anti-aflatoxigenic

activities of nanoemulsions based on *Achillea millefolium* and Crocus sativus flower extracts as green promising agents for food preservatives. BMC Microbiol. 2023;23, 289. https://doi.org/10.1186/s12866-023-03033-2.

Al-Mamary M, Al-Meeri A & Al-Habori M. Antioxidant activities and total phenolics of different types of honey. Nutr. Res. 2002; 22(9): 1041-1047. https://doi.org/10.1016/S0271-5317(02)00406-2.

AOAC. Official Methods of Analysis of Association of Official Analytical Chemists. 18th Edition, 2010; Washington, DC.

AOAC. Official Methods of Analysis, 15th ed. Association of Official Analytical Chemists, 1990; Inc., Arlington.

Attia El-Sofany A, Al Naggar Y, Naiem E, Giesy J P & Seif A. Authentication of the botanical and geographic origin of Egyptian honey using pollen analysis methods. J. Apic. Res. 2020; 946-955. https://doi.org/10.1080/00218839.2020.1720950.

Bihonegn A & Begna D. Beekeeping Production System, Challenges, and Opportunities in Selected Districts of South Wollo Zone, Amhara, Ethiopia. Adv. Agric. 2021;(1): 2278396. https://doi.org/10.1155/2021/2278396.

Borawska MH & Socha K. Modern methods for assessing the quality of bee honey and botanical origin identification. Foods 2020; 9(8): 1028. https://doi.org/10.3390/foods9081028.

Boy FR, Casquete R, Martínez A, Córdoba MG, Ruíz-Moyano S & Benito MJ. Antioxidant Antihypertensive and Antimicrobial Properties of Phenolic Compounds Obtained from Native Plants by Different Extraction Methods. *Int. J. Environ. Res. Public Health,* 2021;18(5): 2475. https://doi.org/10.3390/ijerph18052475.

Bucekova M, Buriova M, Pekarik L, Majtan V & Majtan J. Phytochemicals-mediated production of hydrogen peroxide is crucial for high antibacterial activity of honeydew honey. Sci. Rep., 2018: 8:9061. https://doi.org/10.1038/s41598-018-27449-3.

Codex Alimentarius Commission. Revised Codex standard for honey, Codex STAN 12-Rev.2 (2001). Food and Agriculture Organization of the United Nations (FAO).

Combarros-Fuertes P, Fresno JM, Estevinho, MM, Sousa-Pimenta M, Tornadijo ME & Estevinho LM, Another alternative in the fight against antibiotic resistant bacteria? Antibiotics 2020; 9:774. https://doi.org/10.3390/antibiotics9110774.

Cortopassi-Laurino M & Gelli DS. Analyse pollinique, propriétés physico-chimiques et action antibactérienne des miels d'abeilles africanisées *Apis mellifera* et de Méliponinés du Brésil. Apidologie 1991; 22: 61-73. https://doi.org/10.1051/apido:19910108.

da Silva PM, Gauche C, Gonzaga LV, Costa AC & Fett R. Honey: Chemical composition, stability and authenticity. Food Chem. 2016; 196: 309-323. https://doi.org/10.1016/j.foodchem.2015.09.051.

Diafat AEO, Benouadah A, Bahloul A, Meribai A & Mekhalfi H. Physicochemical properties and pollen analyzes of some Algerian honeys. Int. Food Res. J. 2017; 24: 1453-1459. http://www.ifrj.upm.edu.my/24%20(04)%202017/(15).pdf.

Draiaia R, Chefrour A, Dainese N, Borin A, Manzinello C, Gallina A & Mutinelli F. Physicochemical parameters and antibiotics residuals in Algerian honey. Afr. J. Biotechnol. 2015;14(14):1242-1251. https://doi.org/10.5897/AJB2015.14456.

EL-Metwally, A.A.E. (2015). Factors Affecting the Physical and Chemical Characteristics of Egyptian Beehoney. Ph. D. Thesis, Faculty of Agriculture, Cairo University, Egypt. 320p. **DOI:**10.13140/RG.2.2.22564.30086

El Sohaimy S, Masry S & Shehata M. Physicochemical characteristics of honey from different origins. Ann. Agric. Sci. 2015; 60(2): 279-287. https://doi.org/10.1016/j.aoas.2015.10.015.

Escuredo O, Dobre I, Fernández-González M. & Seijo M C. Contribution of botanical origin and sugar composition of honeys on the crystallization phenomenon. Food Chem. 2014; 149: 84-90.

https://doi.org/10.1016/j.foodchem.2013.10.097.

García S, Troncoso JM & Rondanelli-Reyes M. Study of honey according to botanical origin and physicochemical parameters in the Biobío Region, Chile. Chil. J. Agric. Res. 2020; 80: 675–685. https://doi.org/10.4067/S0718-58392020000400675.

Gupta T, Kataria R. & Sardana SA. Comprehensive Review on Current Perspectives of Flavonoids as Antimicrobial Agents. *Curr. Top. Med. Chem.,* 2022.;*22*(6): 425–434. https://doi.org/10.2174/1568026622666220117104709.

Israili, ZH. Antimicrobial properties of honey. Am. J. Ther. 2014;21(4): 304–323. https://doi.org/10.1097/MJT.0b013e318293b09b.

Karabagias IK, Badeka A, Kontakos S, Karabournioti S. & Kontominas MG. Characterisation and classification of Greek pine honeys according to their geographical origin based on volatiles, physicochemical parameters and chemometrics. Food Chem. 2014; 146: 548–557. https://doi.org/10.1016/j.foodchem.2013.09.105.

Kirkpatrick LA & Feeney BCI, Belmont M. A Simple Guide to IBM SPSS Statistics for Version 20.0, 2013; Wadsworth, Cengage Learning.

Kotz S, Balakrishnan N, Read CB & Vidakovic B. In Encyclopedia of Statistical Sciences (2nd ed.). 2006; Wiley-Interscience.

Kwakman PH & Zaat SA. Antibacterial components of honey. IUBMB Life 2012; 64(1): 48–55. https://doi.org/10.1002/iub.578.

Lehmann DM, Krishnakumar K, Batres MA, Hakola-Parry A, Cokcetin N, Harry E & Carter DA. A cost-effective colourimetric assay for quantifying hydrogen peroxide in honey. Access Microbiology 2019;1(10). https://doi.org/10.1099/acmi.0.000065.

Louveaux J, Maurizio A & Vorwohl G. Methods of Melissopalynology. Bee World 1978; 59: 139-154. http://dx.doi.org/10.1080/0005772X.1978.11097714.

Martinotti S, Laforenza U, Patrone M, Moccia F & Ranzato E. Honey-Mediated Wound Healing: H2O2 Entry through AQP3 Determines Extracellular Ca2+ Influx. Int. J. Mol. Sci. 2019;20(3). https://doi.org/10.3390/ijms20030764.

Ndife J, Abioye L & Dandago M. Quality Assessment of Nigerian Honey Sourced from Different Floral Locations. Niger. Food J. 2013;32(2): 48-55. https://doi.org/10.1016/S0189-7241(15)30117-X.

Ouchemoukh S, Louaileche H & Schweitzer P. Physicochemical characteristics and pollen spectrum of some Algerian honeys. Food Control 2006;18(1): 52-58. https://doi.org/10.1016/j.foodcont.2005.08.007.

Pasias IN, Kiriakou IK & Proestos C. HMF and diastase activity in honeys: A fully validated approach and a chemometric analysis for identification of honey freshness and adulteration. Food Chem. 2017; 229: 425-431. https://doi.org/10.1016/j.foodchem.2017.02.084.

Persano Oddo L, Piana L, Bogdanov S, Bentabol A, Gotsiou P, Kerkvliet J, Martin P, Morlot, M, Ortiz Valbuena A, Ruoff K & Von Der Ohe K. Botanical species giving unifloral honey in Europe. Apidologie 2004; 35: S82-S93. https://doi.org/10.1051/apido:2004045.

Rammali S, Ciobică A, El Aalaoui M, Rahim A, Kamal FZ, Dari K, Khattabi A, Romila L Novac B, Petroaie A & Bencharki B. Exploring the antimicrobial and antioxidant properties of *Lentzea flaviverrucosa* strain E25-2 isolated from Moroccan forest soil. *Front. Microbiol.,* 2024;*15*: 1429035. https://doi.org/10.3389/fmicb.2024.1429035.

Rysha A, Kastrati G, Biber L, Sadiku V, Rysha A & Zogaj F. Evaluating the Physicochemical Properties of Some Kosovo's and Imported Honey Samples. Appl. Sci. 2021;12(2): 629. https://doi.org/10.3390/app12020629.

Saeed MA, Jayashankar M & Tejaswini HK. Evaluation of biochemical and antioxidant properties of Indian and Yemeni honey. J. Apic. Res. 2021; 62(4): 836–849.

https://doi.org/10.1080/00218839.2021.1898835.

Shehata MG, Abd El-Aziz NM, Mehany T & Simal-Gandara J. Taro leaves extract and probiotic lactic acid bacteria: A synergistic approach to improve antioxidant capacity and bioaccessibility in fermented milk beverages. LWT 2023;187: 115280. https://doi.org/10.1016/j.lwt.2023.115280.

Shehata MG, Badr AN, Abdel-Razek AG, Hassanein MM & Hassan A. Oil-bioactive films as antifungal application to save post-harvest food crops. Ann. Res. Rev. Biol. 2017; 16: 1-16.

Siddiqui IR. The sugars of honey. Adv. Carbohydr. Chem. Biochem. 1970; 285-309. https://doi.org/10.1016/s0065-2318(08)60430-8.

Singleton VL & Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Viti c. 1965; 16: 144-158. https://doi.org/10.5344/ajev.1965.16.3.144.

Snowdon JA & Cliver DO. Microorganisms in honey. Int. J. Food Microbiol. 1996; 31, (1-3): 1–26. https://doi.org/10.1016/0168-1605(96)00970-1.

Soares S, Amaral JS, Oliveira MB PP & Mafra I. A comprehensive review on the main honey authentication issues: Production and origin. Compr. Rev. Food Sci. Food Saf. 2017;16(5):1072–1100. https://doi.org/10.1111/1541-4337.12278.

Stefanis C, Stavropoulou E, Giorgi E, Constantinidis TC, Vrioni G & Tsakris A. Honey's Antioxidant and Antimicrobial Properties: A Bibliometric Study. Antioxidants 2023;12(2). https://doi.org/10.3390/antiox12020414.

Tadesse B, Tilahun Y, Woyamo W, Bayu M & Adimasu Z. Factors influencing organic honey production level and marketing: Evidence from southwest Ethiopia. Heliyon 2021;7(9): e07975. https://doi.org/10.1016/j.heliyon.2021.e07975.

Taha EA, Taha RA & AL-Kahtani SN. Nectar and pollen sources for honeybees in Kafrelsheikh province of northern Egypt. Saudi J. Biol. Sci. 2019;26(5): 890-896. https://doi.org/10.1016/j.sjbs.2017.12.010.

White JW. Composition of honey. In: Crane, E. (Ed.), Honey: A Comprehensive Survey. Heinemann, 1975; pp.157-206.

Živkov Baloš M, Popov N, Vidakovic Knezevic S & Jakšić S. Electrical conductivity and acidity of honey. Arch. Vet. Med. 2018;11(1): 91-101. https://doi.org/10.46784/e-avm.v11i1.20.

Citation/Atıf: Karlıdağ S, Solak-Amet O. Türkiye'nin farklı lokasyonlarından temin edilen ticari bal örneklerinin melissopalinolojik ve fiziko-kimyasal analizleri (Melissopalynological and Physicochemical Analyses of Commercial Honey Samples Obtained from Different Locations of Türkiye-Extended Abstract in English). U. Bee J. / U. Arı D. 2025; 25 (1): 89-107. DOI: 10.31467/uluaricilik. 1669569

ARAŞTIRMA MAKALESI / RESEARCH ARTICLE

TÜRKİYE'NİN FARKLI LOKASYONLARINDAN TEMİN EDİLEN TİCARİ BAL ÖRNEKLERİNİN MELİSSOPALİNOLOJİK ve FİZİKO-KİMYASAL ANALİZLERİ

Melissopalynological and Physicochemical Analyses of Commercial Honey Samples Obtained from Different Locations of Türkiye

Semiramis KARLIDAĞ[1*], Omer SOLAK-AMET[2]

[1]Malatya Turgut Özal Üniversitesi, Battalgazi Meslek Yüksekokulu, Bitkisel ve Hayvansal Üretim Bölümü, Malatya, TÜRKİYE. Yazışma yazarı / Corresponding author E-posta: semiramis.karlidag@ozal.edu.tr, ORCID No: 0000-0002-9637-2479

[2]Bursa Uludağ Üniversitesi, Fen-Edebiyat Fakültesi, Biyoloji Bölümü, Bursa, TÜRKİYE. E-posta: omer.solakahmet@gmail.com, ORCID No: 0000-0002-3673-9603

Received / Geliş: 03.04.2025 Accepted / Kabul: 22.04.2025 DOI: 10.31467/uluaricilik. 1669569

ÖZ

Dünya nüfusunun hızla artmasıyla birlikte, gıdaya olan ihtiyaçta artmaktadır. Sentetik katkı maddeleriyle üretilen gıdaların insan sağlığı üzerindeki olumsuz etkilerinden dolayı doğal ürünlere olan talep her geçen gün artmaktadır. Bal, doğal bir madde olup, bal arıları (*Apis mellifera* L.) tarafından bitki nektarlarının, bitkilerin canlı kısımlarının salgılarının veya bitkilerin canlı kısımları üzerinde yaşayan bitki emici böceklerin salgılarının toplanmasıyla olgunlaştırılan tatlı bir maddedir. Bu çalışmada, Türkiye'nin farklı lokasyonlarından temin edilen 9 adet ticari bal örneğinin bazı fiziko-kimyasal özellikleri ve melissopalinolojik analizleri incelenmiştir. Bal örneklerinin ham bal, protein, balda protein ve ham balda δ 13C farkı ve δ 13C değerinden hesaplanan C4 şeker oranı, diastaz, prolin, HMF (5-hidroksimetilfurfural), su muhtevası, iletkenlik, serbest asitlik, fruktoz, glukoz, sakkaroz, maltoz, fruktoz/glukoz, fruktoz+glukoz ortalama değerleri sırasıyla -25.89±1.1‰, -26.12±0.8‰, -0.51±0.3‰, %2.22±2.5, 7.22±0.7, 1003.43±316 mg/kg, 47.18±84.7 mg/kg, %16.62±1.6, 0.55±0.2 mS/cm, 38.56±4.4 mmol/kg, %31.85±6.76, %25.84±6.98, %0.40±0.30, %0.63±0.63, 1.34±0.47 ve %57.69±5.93 olarak bulunmuştur. Elde edilen bulgulara göre, bazı bal örneklerinin Türk Gıda Kodeksi Bal Tebliğine uygun olmadığı tespit edilmiştir.

Anahtar Kelimeler: Fizikokimyasal özellikler, Floral orijin, Kalite parametreleri, Ticari bal

ABSTRACT

With the rapid increase in the world population, the need for food is also increasing. Due to the negative effects of foods produced with synthetic additives on human health, the demand for natural products is increasing day by day. Honey is a natural substance and a sweet substance ripened by honey bees (*Apis mellifera* L.) by collecting plant nectars, secretions of living parts of plants or secretions of plant-sucking insects living on living parts of plants. In this study, some physico-chemical properties and melissopalynological analyses of 9-piece commercial honey samples obtained from different locations in Türkiye were investigated. The mean values of raw honey (δ C13), protein (δ C13), protein in honey (δ C13), C4 sugar ratio, diastase, proline, HMF (5-hydroxymethylfurfural), water content, conductivity, free acidity, fructose, glucose, sucrose, maltose, fructose/glucose, fructose+glucose of honey samples were -25.89±1.1‰, -26.12±0.8‰, -0.51±0.3‰, 2.22±2.5%, 7.22±0.7, 1003.43±316 mg/kg, 47.18±84.7 mg/kg, 16.62±1.6%, 0.55±0.2 mS/cm, 38.56±4.4 mmol/kg, 31.85±6.76%, 25.84±6.98%, 0.40±0.30%, 0.63±0.63%, 1.34±0.47and 57.69±5.93%, respectively.

According to the findings, it was determined that some honey samples did not comply with the Turkish Food Codex Honey Communique.

Keyword: Physicochemical properties, Floral origin, Quality parameters, Commercial honey

EXTENDED ABSTRACT

Objective: The composition, color, aroma and taste of honey vary depending on the source of the nectar, seasonal conditions, climate, bee breed, weather conditions, geographical regions, storage period and conditions. The most important factor affecting the composition of honey is the plant species from which the nectars are collected. The quality of honey is basically evaluated by its plant source and chemical composition. The composition of honey produced in different regions and plant origins varies. According to national and international laws and regulations, no external substance should be added to the honey or one of the components that make up the honey should not be removed. In beekeeping, it is a technically recommended practice to give honey bee colonies supplementary nutrients made with sugar or sugar syrup in early spring to accelerate colony development and prepare the colonies for winter after the honey harvest. Recently, fake honey has been produced by giving sugar syrup to the colonies, especially when the nectar flow period is dry. Of course, adding sugar to honey is an injustice for both consumers and pure honey producers. In this study, the physicochemical properties of commercial honey samples obtained from different locations in Türkiye were examined and their compliance with the Turkish Food Codex-Honey Communique was compared.

Materials and Methods: In this study, 9-piece commercial honey samples were investigated for their raw honey (δ13C), protein (δ13C), protein in honey (δ13C), C4 sugar ratio, diastase, proline, HMF (5-hydroxymethylfurfural), water content, conductivity, free acidity, fructose, glucose, sucrose, maltose, fructose/glucose, fructose+glucose properties and analysed melissopalynologically. Carbon isotope (δ13C) analysis and C4 sugar ratio were determined by IR-MS (Isotope Ratio Mass Spectrometry) using standard analytical methods described in AOAC 998.12 (American Organization of Analytical Chemists International procedures). Free acidity (TS 13360), conductivity (TS 13366), sugar composition (TS 13359), HMF (TS 13356), proline (TS 13357), water content (TS 13365) and diastase number (TS 13364) were analyzed according to the standards described in the Turkish Food Codex Honey Communique. Physico-chemical analyses were performed in the laboratory of Siirt University Science and Technology Application and Research Center.

Results and Discussion: In this study, some physico-chemical properties and melissopalynological analyses of 9-piece commercial honey samples obtained from different locations in Türkiye were investigated. The mean values of raw honey (δ C13) , protein (δ C13), protein in honey (δ C13), C4 sugar ratio, diastase, proline, HMF (5-hydroxymethylfurfural), water content, conductivity, free acidity, fructose, glucose, sucrose, maltose, fructose/glucose, fructose+glucose of honey samples were -25.89±1.1‰,-26.12±0.8‰,-0.51±0.3‰, 2.22±2.5%, 7.22±0.7, 1003.43±316 mg/kg, 47.18±84.7 mg/kg, 16.62±1.6%, 0.55±0.2 mS/cm, 38.56±4.4 mmol/kg, 31.85±6.76%, 25.84±6.98%, 0.40±0.30%, 0.63±0.63%, 1.34±0.47and 57.69±5.93%, respectively.

Conclusion: According to the findings, it was determined that the values of some honey samples did not comply with the Turkish Food Codex Honey Communique standards.

GİRİŞ

Bal, bitki nektarlarının, bitkilerin canlı kısımlarının salgılarının veya bitkilerin canlı kısımları üzerinde yaşayan bitki emici böceklerin salgılarının bal arıları (*Apis mellifera* L.) tarafından toplandıktan sonra kendine özgü maddelerle birleştirerek değişikliğe uğrattığı, su içeriğini düşürdüğü ve petekte depolayarak olgunlaştırdığı, doğası gereği kristallenebilen doğal tatlı maddedir (Bobis vd. 2020, Chen vd. 2019, Keskin vd. 2020, Özcan ve Ölmez 2014, Özenirler vd. 2019, Türk Gıda Kodeksi Bal Tebliği 2020, Xie vd. 2022). Balın bileşimi, rengi, aroması ve tadı nektarın kaynağına, mevsim şartlarına, iklime, arı ırkına, hava koşullarına, coğrafi bölgelere, depolama süresine ve koşullarına bağlı olarak değişiklik gösterir. Balın bileşimini etkileyen en önemli faktör, nektarların toplandığı bitki çeşididir (Bobis vd. 2020, Çiftçi ve Parlat 2018, da Silva vd. 2016, Özcan ve Ölmez 2014, Keskin vd. 2020).

Balın kalitesi temelde bitkisel kaynağı ve kimyasal bileşimi ile değerlendirilmektedir. Farklı bölgelerde ve bitkisel orijinlerde üretilen balların bileşimi farklılık göstermektedir (Çiftçi ve Parlat 2018). Ballar nektar kaynağına göre çiçek veya salgı balı olarak iki genel sınıfa ayrılır. Çiçek balının kökeni nektar, salgı balının kökeni ise bitkilerin canlı kısımlarının salgıları ve/veya bitkilerin canlı kısımları üzerinde yaşayan bitki özsuyu emici böceklerin salgılarıdır (Bobis vd. 2020, Özenirler vd. 2019).

Türkiye zengin bitki örtüsünden dolayı bol miktarda çiçek balı üretme potansiyeline sahiptir. Multifloral balların yanı sıra iki tür salgı balı üretilmektedir. Bunlar çam ağaçlarında beslenen bitki emici böceklerin (*Marchalina hellenica*) salgılarından üretilen çam balı ve meşe ağaçlarının terleme yoluyla yapraklarının salgıladığı tatlı salgılardan üretilen meşe balıdır (Keskin vd. 2020). Ulusal ve uluslararası standartlara göre; bala dışarıdan herhangi bir madde eklenmemeli ya da balı oluşturan bileşenlerden biri çıkarılmamalıdır. Ballar renk, tat ve kompozisyonları bakımından birbirinden farklılık gösterir (Karadal ve Yıldırım 2012).

Bal 200'e yakın bileşen içeren kompleks bir gıda maddesidir (da Silva vd. 2016, Pasias vd. 2022). Balın şeker profili hakkında çok sayıda çalışmalar yapılmıştır. Balda beslenme ve sağlık açısından en önemli bileşenler karbonhidratlardır. Genel olarak bal, kuru ağırlığının yaklaşık %95'ini oluşturan, başlıca şekerler olarak fruktoz (%33-43) ve glukoz (%25-35) ile aşırı doymuş bir şeker çözeltisidir (Bobis vd. 2020, Chen vd. 2019). Esasen, şekerlerin, özellikle fruktoz ve glukozun yoğunlaştırılmış bir karışımıdır (Alghamdi vd. 2020, Keskin vd. 2020).

Bal, temel monosakkaritler olan glukoz ve fruktozla birlikte 25 farklı oligosakkarit içermektedir. Balın bileşiminde genellikle fruktoz, glukoz, sakkaroz, ramnoz, trehaloz, nigerobiyoz, izomaltoz, maltoz, maltotetraoz, maltotrioz, maltuloz, melezitoz, melibiyoz, nigeroz, palatinoz, rafinoz, erlos gibi birçok şeker tespit edilmiştir. Monosakkaritler balda bulunan şekerlerin yaklaşık %75'ini içermektedir. Disakkaritler ve az miktarda diğer şekerler %10-15'ini oluşturmaktadır (da Silva vd. 2016).

Balda bu ana bileşenlere ek olarak yapısında, protein, amino asitler, enzimler, organik asitler, karotenoidler, iz elementler, vitaminler, mineraller, polifenoller ve uçucu bileşikler de bulunur. Bu maddeler bala sadece gıda özelliği kazandırmaz, aynı zamanda antioksidanlar gibi değerli maddeleri de içermesinden dolayı tedavi edici özelliği de bulunmaktadır (Bobis vd. 2020, da Silva vd. 2016, Karadal ve Yıldırım 2012, Özcan ve Ölmez 2014, Pasias vd. 2022, Xie vd. 2022). Nitekim, bal eski çağlardan beri besin maddesi olarak tüketilmesi yanında tıpta da kullanılmaktadır (Alghamdi vd. 2020). Bal, tek bir nektar kaynağından yapılan bal olarak tanımlanan monofloral bal ve farklı nektarların karışımından yapılan bal olarak tanımlanan multifloral bal olmak üzere iki sınıfa ayrılabilir (Bobis vd. 2020, Chen vd. 2019, Terrab vd. 2002).

Piyasada en çok bulunan çiçek balları multifloral ballardır. Bununla birlikte, monofloral ballar, üretildikleri bitkilerin özelliklerini yansıtması nedeniyle tüketicilerin ilgisini çekmiştir (Bobis vd. 2020). Arıcılık faaliyetinde bal arısı kolonilerine, şekerle yapılmış ek besinlerin veya şeker şurubunun erken ilkbaharda koloni gelişimini hızlandırmak ve kolonileri bal hasadından sonra kışa hazırlamak amacıyla verilmesi teknik olarak önerilen bir uygulamadır (Belli 2019).

Son zamanlarda, kolonilere özellikle nektar akımı dönemi kurak geçtiğinde şeker şurubu verilerek tağşiş bal üretilmektedir (Chen vd. 2019, Çiftçi ve Parlat 2018, Simsek vd. 2012). Pirinç şurubu, mısır şurubu, şeker kamışı, şeker pancarı gibi çeşitli şuruplarla kolonilere etik olmayan şekilde besleme yapılması uygun değildir (Brar vd. 2023). Taklit; gıda maddesinin kendisinde olmayan özelliklere sahip gibi gösterilmesidir (Gıdatarım 2025).

Tağşiş; gıda maddelerinin mevzuata veya izin verilen özelliklerine aykırı olarak üretilmesi hali olarak tanımlanmaktadır (Ulusal Gıda Referans Laboratuvar Müdürlüğü 2025). Bal sahteciliği son yıllarda gittikçe önem kazanmaktadır. Çünkü her geçen gün sürekli olarak çok farklı sahtecilik yöntemleri geliştirilmektedir (Zábrodská ve Vorlová 2014). Dünya çapındaki araştırmacılar, bal sahteciliğini tespit etmek ve özgünlüğünü garanti altına almak için ileri teknolojiler geliştirmek ve yenilemek için sürekli çalışmaktadırlar (Brar vd. 2023). Elbette ki, tağşiş bal üretimi hem tüketiciler hem de saf bal üreticileri için bir haksızlık durumudur (Chen vd. 2019, Lozano-Torres vd. 2022).

Yapılan bu çalışmada, Türkiye'nin farklı lokasyonlarından temin edilen bazı ticari bal tiplerinin fiziko-kimyasal özellikleri ve melissopalinolojik analizleri incelenerek, bal örneklerinin Türk Gıda Kodeksi Bal Tebliği standartlarına uygunluğu belirlenmiştir.

GEREÇ VE YÖNTEM

Çalışmada 9 adet ticari bal örneğinin ham bal (δ13C), protein (δ13C), balda protein ve ham balda δ13C farkı ve δ13C değerinden hesaplanan C4 şeker oranı, diastaz, prolin, HMF (5-hidroksimetilfurfural), su muhtevası, iletkenlik, serbest asitlik, fruktoz, glukoz, sükroz, maltoz, fruktoz/glukoz, fruktoz+glukoz özellikleri incelenmiş ve melissopalinolojik analizleri yapılmıştır. 10 g baldaki Toplam Polen Sayısı (TPS10) değeri %45'in üzerinde olan polenlerin ait oldukları bitki taksonları "dominant", %15-45 arasında olanlar "sekonder", %3-15 arasında olanlar "minör", %3'ten daha az oranda rastlanılan polenlerin ait oldukları taksonlar ise "eser" olarak kabul edilmiştir (Louveaux vd. 1978). Karbon izotop(δ13C) analizi ve C4 şeker oranı IR-MS (İzotop Oranı Kütle Spektrometresi) ile AOAC 998.12' e göre (American Organization of Analytical Chemists International prosedürleri) açıklanan standart analitik yöntemler kullanılarak belirlenmiştir. Serbest asitlik (TS 13360), iletkenlik (TS 13366), şeker bileşimi (TS 13359), HMF (TS 13356), prolin (TS 13357), su muhtevası (TS 13365) ve diastaz sayısı (TS 13364) Türk Gıda Kodeksi Bal Tebliği'nde açıklanan standartlara göre analiz edilmiştir. Analizler Siirt Üniversitesi Bilim ve Teknoloji Uygulama ve Araştırma Merkezi laboratuvarında yapılmıştır. Verilere ait istatiksel analizler IBM SPPS (22.0) programının tek yönlü varyans analizi (One Way ANOVA) uygulanarak yapılmıştır. Veriler arasındaki ilişkiler Pearson korelasyon analizi (SPPS 22.0) ile belirlenmiştir.

BULGULAR

TPS değeri %45'in üzerinde olan polenlerin ait oldukları bitki taksonları "dominant", %15-45 arasında olanlar "sekonder", %3-15 arasında olanlar "minör", %3'ten daha az oranda rastlanılan polenlerin ait oldukları taksonlar ise "eser" olarak kabul edilmiştir (Louveaux vd. 1978). Dokuz adet ticari bal örneğinin tanımı ve polen analizi Tablo-1'de verilmiştir. Türkiye'nin farklı lokasyonlarından temin edilen 6 monofloral (devedikeni, meşe, sandal, zahter, çörek otu ve maydanoz balı) ve 3 multifloral (Çelikhan/Adıyaman, Şemdinli/Hakkâri ve Battalgazi/Malatya) etiketli ticari bal örneklerinin yapılan melissopalinolojik analizleri sonucunda; sadece 1 adet bal örneğinin monofloral maydanoz balı, diğer 7 adet bal örneğinin multifloral bal ve 1 adet bal örneğinin ise salgı balı (Bal Çiği Elementi/Toplam Polen Sayısı oranı 3'ün üzerinde bulunmuştur) olduğu tespit edilmiştir. Balın kalitesini belirlemek için bal içerisinde bulunan TPS'nin tespiti önemlidir (Keskin vd. 2020). Ticari bal örneklerinin yapılan melissopalinolojik analizlerinde 11 familyaya ait 17 farklı bitki taksonu belirlenmiştir. Bal örneklerinin botanik orijinleri genel olarak değerlendirildiğinde dominant olarak Apiaceae, sekonder olarak Fabaceae, Asteraceae, Brassicaceae, Amaranthaceae/Chenopodiaceae familyalarına ait polenleri içerdiği, geri kalan familyaların ise minör miktarlarda bulunduğu görülmüştür. Türk Gıda Kodeksi Bal Tebliği (2020/7)'ne göre çiçek balında bulunması gereken standart değerler; nem en fazla %20, sakkaroz en fazla 5g/100g, fruktoz+glukoz en az 60g/100g, fruktoz/glukoz 0.9-1.4 arasında, maltoz en fazla %4, serbest asitlik en fazla 50 meq/kg, elektrik iletkenliği en fazla 0.8 mS/cm, diastaz en az 8, HMF en fazla 40 mg/kg, ham bal (δ 13C) -23 ve daha negatif, protein (δ 13C) -23 ve daha negatif, balda protein ve ham balda δ 13C farkı -1.0 veya daha pozitif, C^{13}/C^{4} şekeri en fazla %7 olmalıdır. Prolin miktarı ise çiçek balı, salgı balı ve çiçek ile salgı balı karışımı ballarda en az 300 mg/kg, kanola, ıhlamur, narenciye, lavanta, ballarında en az 180 mg/kg, biberiye, akasya ballarında en az 120 mg/kg, kestane ballarında en az 500 mg/kg miktarında olmalıdır (Türk Gıda Kodeksi Bal Tebliği 2020) (Tablo-2, 3, 4). Çalışmada, bal örneklerinin δ 13C değerleri -27.97 ile -24.53 arasında değişmekte olup ortalama değer -25.89 olarak tespit edilmiştir (Tablo-2). Türk Gıda Kodeksi Bal Tebliğinde, δ 13C değeri ballar için -23 ve daha negatif olarak belirlenmiştir. Çalışma kapsamında analiz edilen bal örneklerinin ortalama değerinin δ 13C değerinin Türk Gıda Kodeksi Bal Tebliği'nde belirtilen yasal limitlere uygunluk sağladığı görülmektedir.

Tablo-1. Ticari balların tanımı ve ballarda tespit edilen taksonların polen spektrumu (%)

Table-1. Description of commercial honeys and pollen spectrum of taxa identified in commercial honeys (%)

No	Balın ticari satış etiket adı	Balın Tanımı	Türk Gıda Kodeksi Bal Tebliğine Uygunluğu	Dominant Dominant	Sekonder Secondary	Minör Minor
1	Monofloral/ Devedikeni	Multifloral (çiçek balı)	Tebliğe uygun	-	*Helianthus annuus* (%43.61)	Brassicaceae (%10.61), Caryophyllaceae (%8.45), *Cirsium* (%8.25), *Centaurea* (%6.68), Fabaceae (%4.91), *Plantago* (%3.93)
2	Monofloral/ Meşe	Salgı balı	Tebliğe uygun	-	*Laurus nobilis* (%26.74) Asteraceae (%19.38)	Rosaceae (%9.69), *Cistus* (%7.36), Liliaceae (%6.20), *Olea europaea*(%4.26), Boraginaceae (%3.49)
3	Monofloral/ Sandal	Multifloral (çiçek balı)	Tebliğe uygun	-	*Quercus* (%41.65)	Fabaceae (%13.50), Rosaceae (%12.36), Poaceae(%12.36), Ericaceae (%5.26), Brassicaceae (%4.81)
4	Monofloral/ Zahter	Multifloral (çiçek balı)	Tebliğe uygun	-	*Plantago* (%29.69)	Fabaceae (%12.66), Cichorioideae (%9.17), Rosaceae (%7.86), Papaveraceae (%7.42), *Bellis* (%7.42), *Centaurea* (%5.68), Lamiaceae (%4.37), Brassicaceae (%3.93), Betulaceae (%3.43)
5	Monofloral/ Çörekotu	Multifloral (çiçek balı)	Tağşişli bal Tebliğe uygun değil Nişasta var	-	Apiaceae (%17.46) Brassicaceae (%17.01)	Fabaceae (%9.75), *Nigella sativa* (%9.07) Papaveraceae (%7.71), *Castanea sativa* (%4.99), *Olea europaea* (%4.54), *Onobrychis/Hedysarum* (%3.40), *Fraxinus* (%3.17), *Helianthus annuus* (%3.17),
6	Monofloral/ Maydanoz	Monofloral/ Maydanoz	Tebliğe uygun	Apiaceae (%80,72)	-	Fabaceae (%12.50)
7	Multifloral	Multifloral (çiçek balı)	Tebliğe uygun	-	*Trifolium repens* (%29.22) Fabaceae (%20.69)	*Centaurea* (%14.52), Poaceae (%5.81) *Plantago* (%5.44), Papaveraceae (%4.90), Rosaceae (%3.81), *Helianthus annuus* (%3.81), Brassicaceae (%3.09)
8	Multifloral	Multifloral (çiçek balı)	Tebliğe uygun	-	*Helianthus annuus* (%33.99) Amaranthaceae/Chenopodiaceae (%30.03) *Onobrychis/Hedysarum* (%18.48)	Fabaceae (%4.62)
9	Multifloral	Multifloral (çiçek balı)	Tağşişli bal Tebliğe uygun değil Nişasta var	-	*Trifolium repens* (%27.48) *Hypericum* (%24.49)	Papaveraceae (%12.52), *Ranunculus* (%11.96), *Onobrychis/Hedysarum* (%4.11), Poaceae (%3.18)

Bal örneklerinin ortalama protein-26.12, balda protein ve ham balda δ 13C farkı ortalaması -0.51 olarak tespit edilmiş olup Türk Gıda Kodeksi Bal Tebliği'nde bildirilmiş olan yasal limite uygunluk göstermektedir (Tablo-2). Çalışmada δ l3C değerinden hesaplanan C4 şeker oranı ortalama %2.22 olarak tespit edilmiş yasal limitlere (**≤ 7**) uygunluk göstermiştir (Tablo-2). Örneklerin ortalama serbest asitlik değerleri 38.56 mmol/kg olarak bulunmuş, 30.00 mmol/kg ile 44.00 mmol/kg arasında olduğu tespit edilmiştir (Tablo-3). Tüm bal örneklerinde tespit edilen serbest asitlik değerleri yasal standartlara (≤ 50) uygunluk göstermiştir. Elektriksel iletkenlik değeri ortalama 0.55 mS/cm olarak tespit edilmiştir (Tablo-3). Türk Gıda Kodeksi'nin 2020/7 sayılı Bal Tebliği'ne göre; salgı ballarının elektriksel iletkenliği en az 0,8 mS/cm, çiçek ballarının ise en fazla 0,8 mS/cm olarak belirlenmiştir (Türk Gıda Kodeksi Bal Tebliği 2020). Bal örneklerinde diastaz sayısı ortalama 7.22 olup, 5.00 ile 10.90 arasında değişim göstermiş ve 3 adet bal örneğinin diastaz sayısının bal tebliğine uygun olmadığı belirlenmiştir (Tablo-3). Bal örneklerinin prolin değerleri ortalama 640.00 mg/kg ile 1706.60 mg/kg arasında tespit edilmiştir. İncelenen bütün bal örneklerinin prolin değerleri Türk Gıda Kodeksi Bal Tebliğine (2020) uygun olduğu görülmektedir (Tablo-3). Bu çalışmada 3 bal örneğinin HMF değerleri Türk Gıda Kodeksi Bal Tebliğinin (2020) belirlediği yasal limitlere uymadığı tespit edilmiştir. Bal örneklerinin HMF değerleri 1.51 mg/kg ile 267.65 mg/kg arasında değişiklik göstermiştir (Tablo-3).

Tablo-2 Türkiye'nin farklı lokaslarından elde edilen ballarda ve protein fraksiyonlarında ∆13C değerleri

Table-2 ∆13C values of bee-produced honey samples and their proteins from different locations in Türkiye

No	n	Bal Türü Type of Honey	Ham bal (∆13C) (‰)	Protein (∆13C) (‰)	Balda protein ve ham balda ∆13C farkı (‰)	∆13C değerinden hesaplanan C4 şeker oranı (%)
1	1	Multifloral bal	-25.78	-25.99	-0.21	1.30
2	1	Salgı balı	-24.89	-25.45	-0.56	3.55
3	1	Multifloral bal	-27.97	-27.48	-0.49	0.00
4	1	Multifloral bal	-25.11	-25.45	-0.34	2.16
5	1	Multifloral bal	-25.29	-26.64	-1.35	7.99
6	1	Monofloral bal	-24.53	-24.86	-0.33	2.17
7	1	Multifloral bal	-26.46	-26.95	-0.49	2.83
8	1	Multifloral bal	-26.19	-25.89	-0.30	0.00
9	1	Multifloral bal	-26.84	-26.35	-0.49	0.00
			$\bar{x}$ ±S $\bar{x}$	**$\bar{x}$ ±S $\bar{x}$**	**$\bar{x}$ ±S $\bar{x}$**	**$\bar{x}$ ±S $\bar{x}$**
Genel Ortalama	9		-25.89±1.1	-26.12±0.8	-0.51±0.3	2.22±2.5
Min.			-27.97	-27.48	-1.35	0.00
Max.			-24.53	-24.86	-0.21	7.99
Türk Gıda Kodeksi Bal Tebliği			-23 ve daha negatif	-23 ve daha negatif	-1.0 veya daha pozitif	≤ 7
Analiz Metodu			AOAC 998.12	AOAC 998.12	AOAC 998.12	AOAC 998.12

Tablo-3 Bal örneklerinin fiziko-kimyasal özellikleri

Table-3 Physicochemical properties of honey samples

No	n	Bal Türü Type of Honey	Serbest asitlik The level of acidity	Diastaz Sayısı Diastasis	Prolin Proline	HMF	Su muhtevası Moisture	İletkenlik Electrical conductivity
			(mmol/kg)		(mg/kg)	(mg/kg)	(%)	(mS/cm)
1	1	Multifloral bal	36	6.50	711.11	15.65	17.4	0.40
2	1	Salgı balı	43	8.30	1137.70	267.65	18.6	0.65
3	1	Multifloral bal	30	5.00	640.00	42.87	16.8	0.87
4	1	Multifloral bal	40	6.50	924.44	52.19	16.4	0.43
5	1	Multifloral bal	44	10.90	995.56	7.19	15.4	0.67
6	1	Monofloral bal	38	8.30	1706.60	29.00	17.8	0.64
7	1	Multifloral bal	39	6.50	1137.70	2.34	16.6	0.40
8	1	Multifloral bal	42	6.50	782.22	6.24	17.6	0.38
9	1	Multifloral bal	35	6.50	995.56	1.51	13.0	0.51
			$\bar{x}$ ±S $\bar{x}$	**$\bar{x}$ ±S $\bar{x}$**	**$\bar{x}$ ±S $\bar{x}$**	**$\bar{x}$ ±S $\bar{x}$**	**$\bar{x}$ ±S $\bar{x}$**	**$\bar{x}$ ±S $\bar{x}$**
Genel Ortalama	9		38.56±4.4	7.22±1.7	1003.43±316.	47.18±84.7	16.62±1.6	0.55±0.2
Min.			30.00	5.00	640.00	1.51	13.00	0.38
Max.			44.00	10.90	1706.60	267.65	18.60	0.87
Türk Gıda Kodeksi Bal Tebliği			≤ 50	8 ≤	300 ≤	≤ 40	≤ 20	≤ 0.8
Analiz Metodu			TS 13360	TS 13364	TS 13357	TS 13356	TS 13365	TS 13366

Tablo-4 Bal tiplerinin şeker kompozisyonları

Table-4 Sugar compositions of honey samples

No	**n**	Bal Türü Type of Honey	Fruktoz Fructose	Glukoz Glucose	Sakkaroz Sucrose	Maltoz Maltose	Fruktoz/Glukoz Fructose/Glucose	Fruktoz+glukoz Fructose+glucose
			(%)	(%)	(%)	(%)		(%)
1	1	Multifloral bal	36.63	28.55	0.73	1.32	1.28	65.17
2	1	Salgı balı	26.90	29.81	nd	0.94	0.90	56.71
3	1	Multifloral bal	37.63	24.87	0.20	0.70	1.51	62.50
4	1	Multifloral bal	34.64	16.43	0.77	1.25	2.11	51.07
5	1	Multifloral bal	37.05	21.60	0.32	0.00	1.72	58.65
6	1	Monofloral bal	18.94	41.22	nd	1.43	0.46	60.16
7	1	Multifloral bal	24.69	21.77	0.40	0.00	1.13	46.46
8	1	Multifloral bal	37.45	24.91	0.69	0.00	1.50	62.36
9	1	Multifloral bal	32.71	23.40	0.53	0.00	1.40	56.12
			$\bar{x}$ ±S $\bar{x}$	**$\bar{x}$ ±S $\bar{x}$**	**$\bar{x}$ ±S $\bar{x}$**	**$\bar{x}$ ±S $\bar{x}$**	**$\bar{x}$ ±S $\bar{x}$**	**$\bar{x}$ ±S $\bar{x}$**
Genel Ortalama	9		31.85±6.76	25.84±6.98	0.40±0.30	0.63±0.63	1.34±0.47	57.69±5.93
Min			18.94	15.57	0.20	0.00	0.46	46.46
Max			37.63	41.22	4.14	1.79	2.16	65.17
Türk Gıda Kodeksi Bal Tebliği					≤ 5	≤4	0.9-1.4	60 ≤
Analiz metodu			TS 13359	TS 13359	TS 13359	TS 13359		

nd: tespit edilemedi

nd: not detected

Bal örneklerin su muhtevası Türk Gıda Kodeksi'nin 2020/7 sayılı Bal Tebliği'ne göre uygun olduğu tespit edilmiştir (Tablo-3). Bal örneklerinde ortalama fruktoz, glukoz, sukroz, maltoz, fruktoz/glukoz ve fruktoz+glukoz oranı sırasıyla %31.85, %25.84, %0.40, %0.63, %1.34 ve %57.69 olarak bulunmuştur (Tablo-4). İletkenlik ve sukroz arasında negatif bir korelasyon tespit edilmiştir (R^2=-0.737, P≤0.05, Tablo-5).

Tablo-5 Bal örneklerinde Pearson korelasyon matrisi (N: 9)

Table-5 Pearson correlation matrix on the honey samples (N: 9)

Diastaz Sayısı/Diastasis	1	.002	.465	.168	-.146	-.004	-.101	-.370	.184	-.194	.160	.724*	.897**	.764*	-.284	-.674*
Su muhtevası/Moisture		1	.153	.507	-.416	.237	.534	-.308	.471	-.278	.043	.239	.009	-.335	-.392	-.389
Prolin/Proline			1	.144	-.708*	-.289	.244	-.599	.642	-.917**	.063	.288	.276	.029	-.587	-.632
HMF				1	-.282	-.051	.335	-.510	.205	-.256	.297	.300	.156	-.002	-.339	-.356
Fruktoz/Glukoz-Fructose/Glucose					1	-.137	-.266	.691*	-.900**	.810**	-.161	.038	.066	.239	.361	.281
Fruktoz+glukoz/Fructose+glucose						1	.255	-.029	.462	.400	.307	-.256	-.224	-.100	-.069	.094
Maltoz/ Maltose							1	-.141	.478	-.270	.114	-.188	-.132	-.479	-.595	-.468
Sukroz/Sucrose								1	-.621	.616	-.737*	-.005	-.293	-.290	.068	.176
Glukoz/Glucose									1	-.628	.269	-.092	-.104	-.267	-.508	-.365
Fruktoz/Fructose										1	-.008	-.130	-.088	.188	.464	.460
Iletkenlik/ Electrical conductivity											1	-.343	.175	.411	.295	.219
Serbest asitlik/The level of acidity												1	.662	.414	-.445	-.720*
∆13C değerinden hesaplanan C4													1	.846**	-.010	-.529
Balda protein ve ham balda ∆13C farkı														1	.354	-.095
Protein (∆13C)															1	.846**
Ham bal (∆13C)																1

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

*Correlation is significant at the 0.05 level (2-tailed).

TARTIŞMA

İncelenen bal örneklerinin melissopalinolojik analizlerinde monofloral etiketli ticari balların yalnızca 1 adet bal örneğinin monofloral bal (maydanoz balı) olduğu, iki adet bal örneğinin ise tağşişli, nişasta içerdiği ve bal tebliğine uygun olmadığı tespit edilmiştir (Tablo-1). Ballarda nişasta bulunması; kolonilerde pudra şekeri ile varroa tespitinin yapılması, bal üretim döneminde kek ile besleme yapılması veya nişastanın besleme yapılan şekere katılmasından kaynaklanmış olabileceği düşünülmektedir. Ana nektar akımı dönemi bölgelere göre değişmekle birlikte genellikle Mayıs-Temmuz ayları arasındadır ve koloniler bu aylarda yoğun nektar toplamaktadır. Bal örneklerinde Fabaceae familyasına ait polenlerin genel olarak bulunması bu familyaya ait türlerin çiçeklenme dönemleri ve bölgeleri ile ilişkili olabileceği tahmin edilmektedir. Bal sahteciliği için kullanılan maddelerin kaynağı olan bitkiler, karbon metabolizmalarına göre C^3 veya C^4 bitkileri olarak sınıflandırılabilir. Pirinç, buğday, arpa, pamuk, yonca, korunga, elma, üzüm ve şeker pancarı gibi nektarlı bitkilerin çoğu C^3 bitkilerini, mısır, sorgum, darı ve şeker kamışı ise C^4 bitkilerini temsil etmektedirler (Ferek 2016, Zábrodská ve Vorlová 2014).

Fotosentetik döngülerde C^3 ve C^4 bitkilerinin δ 13C (karbon izotop oranları) farklıdır (Ferek 2016, Simsek vd. 2012). Dolayısıyla, arılar nektarlarını dikotiledon çiçekli bitkilerden topladıklarından doğal bir balın C^3 bitkilerinin karakteristik özelliklerini taşıması beklenir. $^{13}C/^{12}C$ izotop oranı, ‰ biriminde δ 13C olarak ifade edilir (Simsek vd. 2012). Bal, en çok tağşişi yapılan gıdalardan birisidir. Bala herhangi bir şeker veya şurup ilavesi olduğunda veya arılar şekerli su veya şurupla beslendiklerinde δ13C sonuçları ile ortaya çıkarılmaktadır. Fruktoz ve glukoz bala bu amaçla eklenen şekerlerin en başında gelir (Belli 2019, Ferek 2016).

Baldaki kamış şekeri veya mısır bazlı şeker katkısının kanıtlanması için en yaygın kullanılan yöntem δ13C analizidir (Chen vd. 2019, Çiftçi ve Parlat 2018). Kocaeli ve İstanbul illerinde piyasadan satın alınan 43 farklı bal örneğinin EA-IRMS ile yapılan $^{13}C/^{12}C$ oranı ölçümlerinde, 10 adet bal örneğinin (%23) sahte olduğu tespit edilmiştir. Çiçek balı örneklerinin $\delta^{13}C$ değerinin -23,91 ile -27,58 ‰ arasında, bal proteinlerinin δ 13C değerinin -24,57 ile -26,76 ‰ arasında olduğu bildirilmiştir (Simsek vd. 2012). Muğla çam balı örneklerinin δ 13C değeri -23,30 ile -24,54 ‰, protein ekstraktlarının ise -24,13 ile -25,41 ‰ arasında olduğu bulunmuştur. Bolu, Kocaeli, Trabzon ve Yalova olmak üzere dört farklı ilden alınan kestane balı örneklerinin δ 13C değeri sırasıyla 25.02, 25.18, 23.98 ve 25.62‰ olduğu ve protein özütlerinin -24.35 ile -25,41 ‰ arasında değiştiği bildirilmiştir. İzmir ili bal örneğinde en yüksek kararlı izotop oranı -27,58 ‰ olarak tespit edilmiştir (Simsek vd. 2012).

Bontempo vd. (2017), İtalya'nın farklı tip ballarının karbon izotop oranıyla ilgili yaptıkları çalışmada δ13C bal değerleri akasya balında ‰ (-24,1), kestane balında ‰ (-25,2), narenciye balında ‰ (-24,3), okaliptüs balında ‰ (-24,4), salgı balında ‰ (-25,3), ormangülü balında ‰ (-24,5), polifloral balda ‰ (-25,3); δ 13Cprotein değerleri akasya balında ‰ (-23,9), kestane balında ‰ (-24,8), narenciye balında ‰ (-24,7), okaliptüs balında ‰ (-24,2), salgı balında ‰ (-24,6), ormangülü balında ‰ (-23,8), polifloral balda ‰ (-24,7) olarak belirlenmiştir. Başka bir çalışmada çeşitli C^3 ve C^4 bitki kaynaklarından elde edilen invert şeker şuruplarıyla beslenen kolonilerden elde edilen 451 adet bal örneklerinin $\delta^{13}C$ oranı -23.0‰ ile -27.3‰ arasında tespit edilmiştir (Elflein ve Raezke 2008).

Bu çalışmada incelenen bal örneklerinde elde edilen $\delta^{13}C$ değerleri -27.97‰ ile -24.53‰ arasında değişmekte ve örnekler Türk Gıda Kodeksi Bal Tebliği'nde bildirilmiş olan yasal limite (-23 ve daha negatif) uygunluk göstermektedir (Tablo-2). Bal protein içeriği bakımından çok zengin değildir. Fakat içerdiği aminoasitler balın elde edildiği kaynağın tespit edilmesinde önemli bir parametredir (Belli 2019). Saf bala mısır ve şeker kamışı karışması durumunda balın $\delta^{13}C$ oranı değişir. Fakat proteinin δ 13C oranı değişmeden kalır. Balın δ 13C oranı ve ekstrakte edilen proteinin δ 13C oranı minimum düzeydeki tağşişin bile kanıtlanmasını sağlar (Belli 2019, Ferek 2016). Yapılan bu çalışmada incelenen balların protein (δ 13C) değerleri -27.48 ile -24.86 arasında değişirken, ortalama protein (δ 13C) değeri -26.12±0.8 olarak tespit edilmiştir (Tablo-2). Çalışmada elde edilen bal örneklerinin ortalama protein (δ 13C) değerleri, Türk Gıda Kodeksi Bal Tebliği'nde belirtilen yasal limitlere uygunluk göstermektedir. Bir çalışmada Tunus'un farklı bölgelerinden sağlanan 6 bal örneğinin (nane, biberiye, okaliptüs, karahindiba, kekik, portakal) incelenen fizikokimyasal özellikleri bakımından Avrupa Mevzuatına (EC Direktifi 2001/110) uygun olduğu ifade edilmiştir. Bal örneklerinin protein

değerinin %0.13 (biberiye) - %0.16 (nane) arasında değiştiği bildirilmiştir (Boussaid vd. 2014).

Başka bir çalışmada çeşitli C^3 ve C^4 bitki kaynaklı invert şeker şuruplarıyla beslenen kolonilerden elde edilen 451 bal örneğinin incelendiği çalışmada, örneklerin δ 13C protein değerleri -22.7‰ ile -26.7‰ arasında bulunmuştur (Elflein ve Raezke 2008). Stabil karbon izotop analizi, karbon izotoplarının miktarını ve doğada daha fazla bulunan (%99) ^{12}C izotopu ile düşük miktarda bulunan (%1) ^{13}C izotopu arasındaki oranın belirlenmesi sağlamaktadır. Bu oran farklı bitkiler tarafından CO_2'in tutulması ve fotosentez sırasında kullanılması ile ilgili döngüyü yansıtmaktadır (Ferek 2016).

Bal ve balın protein fraksiyonu arasında δ 13C farkı, balın saflığının, tağşiş yapılıp yapılmadığının, nitel ve nicel bir göstergesi olarak ifade edilmiştir (Çiftçi ve Parlat 2018, Ferek 2016). Bu tekniği uygulayarak, yapay tatlandırıcı içeren bir bal örneğinin tağşiş yüzdesi belirlenebilir (Simsek vd. 2016). Belli (2019), nektarlı pek çok bitkinin C^3 döngüsü kullandığını ve gerçek balda C^{13}/C^{12} değerinin -25 civarında olması gerektiğini vurgulamıştır. Eğer bala mısır şurubu katılmış ise bu oranın -10'a kadar çıkabileceği belirtilmiştir. İki değer arasındaki farkın -1'den daha negatif ise bala mısır şurubunun katıldığı ifade edilmiştir. Fakat C^3 bitkisi olan pancar şekerinden (çay şekeri) elde edilen şuruplarla beslenen arılardan elde edilen baldaki hileyi tespit etmenin zor olduğunu ifade etmiştir. Hileyi tespit etmede δ 13C analizinin yetersiz kaldığı vurgulanmıştır. Muğla ilinde üretilen ballarda taklit ve tağşiş gibi hileli durumların olup olmadığı araştırmak amacıyla yapılan çalışmada bal örneklerinin δ 13C değerleri farkı (-2,52)-2,51 arasında bulunmuştur (Belli 2019).

Farklı araştırıcılar tarafından incelenen bal örneklerinin, balda protein ve bal δ 13C değerleri arasındaki farkı Elflein ve Raezke (2008) -0.9‰ - 1.5‰, Kambur vd. (2015) -0.39- 0.53 ve Çiftçi ve Parlat (2018) -0.55-1.95 değerleri arasında olduğunu bildirmişlerdir. ∆ 13C değerleri farkı ve C4 şeker oranı balda hilenin olup olmadığı hususunun, taklit ve tağşişin tespit edilmesinde kullanılan kriterlerdendir (Belli 2019, Ferek 2016,). C4 şeker oranı, balda protein ve ham bal δ 13C değerlerinden hesaplanır (Çiftçi ve Parlat 2018). Protein ve balda δ13C değeri arasındaki farkın ‰ 1'den büyük olmaması gerekmemektedir (Ferek 2016). Bir çalışmada çeşitli C3 ve C4 bitki kaynaklarından elde edilen invert şeker şuruplarıyla beslenen kolonilerden elde edilen 451 bal örneğinde bal sahteciliğini tespit etmek amacıyla yapılan çalışmada C4 şeker oranı (%) 0-5.7 arasında tespit edilmiştir (Elflein ve Raezke 2008). Muğla ilinde üretilen ballarda taklit ve tağşiş gibi hileli durumların olup olmadığı araştırmak amacıyla yapılan çalışmada bal örneklerinin C4 şeker oranı (%) 0-16,81 arasında bulunmuştur (Belli 2019). Konya bölgesindeki marketlerde satışa sunulan 5 farklı firmalara ait çiçek ballarının bazı kimyasal özellikleri incelenmiştir. Firmalara ait bal örneklerinin sırasıyla C4 şeker oranı (%); 3.53, 1.93, 0.00, 0.00, 0.00 olarak bulunmuştur. Araştırmada kullanılan firmalara ait bal örnekleri arasında ($P<0.01$) önemli farklar olmasına rağmen Türk Gıda Kodeksi-Bal Tebliğine uygun bulunmuştur (Çiftçi ve Parlat 2018).

Chen vd. (2019) tarafından yapılan çalışmada, bal arısı kolonileri kışın şeker kamışı ile beslendiğinde Mart ayındaki yapılan hasatlarda toplanan bal örneklerinde C4 şekeri "şeker kalıntısı" olarak tespit edilmiştir. Kambur vd. (2015) tarafından yapılan çalışmada ise Yığılca bal örneklerinde C4 oranı %0.43-8.72 arasında bildirilmiştir. ∆ 13C oranı yalnızca C_4 ve C_3 bitkilerinden elde edilen balın kaynağı hakkında bilgi vermektedir. Bu çalışmada incelenen bal örneklerinin balda protein ve ham balda δ 13C farkı değerleri ortalaması -0.51 olarak tespit edilmiş olup, tüm örneklerin değerleri Türk Gıda Kodeksi Bal Tebliği'nde belirtilen yasal limitlere uygun olduğu bulunmuştur. Diğer taraftan, δ 13C değerinden hesaplanan C4 şeker oranı ortalama değeri 2.22 olarak tespit edilmiş, bir adet örneğin Türk Gıda Kodeksi Bal Tebliği'nde belirtilen yasal limitlere uymadığı belirlenmiştir (Tablo-2). Bir bal örneğinin diastaz aktivitesi, HMF içeriği, elektriksel iletkenlik ve nem ölçümleri gibi standart kalite parametreleri, balın genel özelliklerini tanımlamada ve balın kalitesini değerlendirmede önemli bir role sahiptir (Simsek vd. 2012, Baloš vd. 2018).

İncelenen çeşitli bal örneklerinin serbest asitlik değerlerini, Belli (2019) 8.95-27.9 meq/kg, Dadalı (2021) hayıt balında 20.45-27.30 meq/kg, Uçak Koç vd. (2017) hayıt, çiçek ve çam ballarında sırasıyla 26.6±0.3, 36.03±0.534 ve 27.3±0,36 meq/kg, Kambur vd. (2015) 21.0-70.0 meq/kg, Çiftçi ve Parlat (2018) 20.27-34.06 meq/kg, İçli (2022) 5.1-37.7 meq/kg, Yaşar ve Söğütlü (2020) ortalama 10.675 meq/kg, Manolova vd. (2021) 17.70 – 36.00 meq/kg, Yurt ve Çakır (2020) ortalama 22.64 meq/kg, Karatas vd. (2019) 8.96-33.92 meq/kg, Güzel ve Savaş Bahçeci (2020) 21.1-47.8 meq/kg, Gürbüz vd. (2020) ortalama 14.94 meq/kg, Yücel ve Sultanoğlu (2013) 18.06 - 34.88 meq/kg, Bobis vd. (2020)

okaliptus ballarında 15-46 meq/kg, Kilic vd. (2016) 16.0 – 26.1 meq/kg, Boussaid vd. (2014) 7.11 – 27.20 meq/kg, Terrab vd. (2002) 19.5- 88.2 meq/kg, Berhanu vd. (2022) ortalama 23.2 meq/kg ve Baloš vd. (2018) 1.5-30 meq/kg arasında tespit etmişlerdir. Bu çalışmada, bal örneklerinin serbest asitlik değerleri 30 mmol/kg - 44 mmol/kg arasında tespit edilmiş ve incelenen bal örneklerinin yasal limitlere uygun olduğu bulunmuştur (Tablo-3). Balın elektriksel iletkenliği balda kalite kriteri olarak değerlendirilen kimyasal özelliklerden birisidir (Güzel ve Savaş Bahçeci 2020).

Balın elektriksel iletkenliğini mineral içeriği ve asitliği etkiler. Elektriksel iletkenlik değerinin yüksek olması mineral içeriğinin yüksek olmasından veya yüksek asitlikten kaynaklanabilir. Balda kül, proteinler ve bazı kompleks şekerlerin miktarı arttıkça elektriksel iletkenlik de artar. Bu nedenle balın elektriksel iletkenliği iyonların, organik asitlerin ve proteinlerin varlığını ortaya koyar (da Silva vd. 2016, Yücel ve Sultanoğlu, 2013). Elektriksel iletkenlik aynı zamanda balın bitkisel kaynağı ile içerdiği kül miktarını belirlemede de kullanılır. Balın ihtiva ettiği kül miktarı ve asitliği arttıkça elektriksel iletkenliği de artış gösterir (Ferek 2016, Nombre vd. 2010).

Farklı araştırmacılar farklı bal tiplerinin elektriksel iletkenliğini incelemişlerdir. İncelenen bal örneklerinin elektriksel iletkenliği değerlerini Akgün vd. (2021) ortalama 1.13 mS/cm, Baloš vd. (2018) 0.08-1.99 mS / cm, Belli (2019) 0.63-1.67 mS/cm, Berhanu vd. (2022) ortalama 0.41 mS/cm, Manolova vd. (2021) 0.23 – 0.48 mS/cm, Ferek (2016) 0,892-1,838 mS/cm, Gürbüz vd. (2020) ortalama 0.21 mS/cm, Kambur vd. (2015) 0.28-0.80 mS/cm, Malkoç (2019) 0.26-1.25 mS/cm, Uçak Koç vd. (2017) hayıt, çiçek ve çam ballarında sırasıyla 0.42, 0.73 ve 1.17 mS/cm, Chirsanova vd. (2021) ayçiçeği, kolza tohumu, manna ve poliflora ballarında sırasıyla ortalama 371, 161, 775 ve 355µS/cm, Yurt ve Çakır (2020) ortalama 0.17 mS/cm, Boussaid vd. (2014) 0.39 (kekik) – 0.89 mS/cm (portakal), Karatas vd. (2019) 0.15-1.41 µS/cm arasında, Yücel ve Sultanoğlu (2013) 0.17–1.04 $mScm^{1}$, Kilic vd. (2016) 0,18 ms/cm-1 - 0,25 ms/cm-1, Kanbur vd. (2021) 189.66 – 328.05 µS/cm (yayla balı) ile 559.30-714.06 (kestane balı) µS/cm arasında ve Güzel ve Savaş Bahçeci (2020) ortalama 350 µS/cm (205-674) olarak tespit etmişlerdir. Can vd. (2015), en yüksek elektrik iletkenliğini kestane balının gösterdiğini, bunu çam balının takip ettiğini belirtmişlerdir. Akasya (*Robinia pseudoacacia*), lavanta (*Lavandula* sp.) ve geven (*Astragalus* sp.) gibi açık renkli balların daha düşük iletkenlik gösterdiğini vurgulamışlardır. Elektriksel iletkenlik, balın bitki kökeni hakkında bilgi veren bir parametredir (Chirsanova vd. 2021, Çiftci 2014). Nektarın kaynağına ve balın protein miktarına bağlı olarak değişir (Çiftci 2014). Çiçek veya salgı balını birbirinden ayırt etmede kullanılan en önemli kriter balın elektriksel iletkenliğidir (Çiftci 2014, Ferek 2016, Nombre vd. 2010).

Genellikle salgı ballarının elektriksel iletkenliği çiçek ballarından daha yüksektir (Çiftci 2014). Yoğun çam ağaçlarının bulunduğu alanda yapılan bir çalışmada (Karatas vd. 2019), kekik ve funda ballarına salgı karışmasından dolayı iletkenlik seviyesinde artışa neden olduğu vurgulanmıştır. Yapılan bu çalışmada elde edilen sonuçlar ile yukarıda verilen çalışma sonuçlarının farklılık göstermesi farklı bal çeşitleriyle çalışılmasından kaynaklanmaktadır. Bal endüstrisinde, balın kalitesi genel olarak diastaz sayısı ve HMF içeriğine göre de değerlendirilir. Enzimler, balın önemli bileşenlerindendir. Isıya karşı duyarlı oldukları için beslenme yönünden de balın kalitesini belirlemektedirler. Diastaz balda doğal olarak bulunur ve diastaz sayısı balda önemli kalite kriterlerinden birisidir. Isıya daha duyarlı olduğu için bala ısıl işlem uygulanıp uygulanmadığı, diastazın miktarındaki azalmadan tespit edilebilir. Nişastayı parçalayan diastaz enzimi nektara olgunlaşma esnasında bal arısı tarafından ilave edilir. Fakat diastaz enzimin bir kısmının bitki kaynaklı olduğu bilinmektedir (Belli 2019, Ferek 2016).

Bu iki parametre, bal tağşişinin, uygulanan ısıl işlemin yoğunluğunun ve depolama sıcaklığının tahmin edilmesinde kullanılan en kritik parametrelerdir (Ferek 2016). Yapılan bir çalışmada (Flanjak vd. 2022), farklı iki ısıl işlem (45 °C/48 saat ve 65 °C/6 saat) uygulanan adaçayı (*Salvia officinalis* L.) balının iki yıl depolama süreci sonucunda balın içerik değişimi incelenmiştir. Bala kısa süreli yüksek sıcaklık (65 °C/6 saat) ısıl işlem uygulaması enzim aktivitesini azaltmıştır. Balda hem diastaz kaybı hem de yüksek diastaz miktarı istenmeyen bir durumdur. Diastaz sayısının yüksek olması balda asitliği arttıracağı için daha hızlı fermantasyon meydana gelebilmektedir (Ferek 2016).

Türkiye'nin Ordu ili bal örneklerinin diastaz sayısı en yüksek multifloral balda (26.17), en düşük ise Rhododendron balında (11.47) tespit edilmiştir (Akgün vd. 2021). Muğla iline ait bal örneklerinin diastaz sayısı 3.38-13.18 arasında bulunmuştur

(Belli 2019). Dadalı (2021) hayıt balının diastaz sayısını 20.14-27.13 arasında bildirmiştir. Çam balı örneklerinin incelendiği çalışmada diastaz değerleri 9- 21 arasında bulunmuştur (Ferek 2016). Türkiye'nin Bingöl ilinden elde edilen bal örneklerinin diastaz sayısı ortalama 18.39 olarak bildirilmiştir (Bengü ve Kutlu 2018). Yapılan başka çalışmalarda bal örneklerinin diastaz sayısını; Gürbüz vd. (2020) ortalama 10.68, Kilic vd. (2016) 10.0–26.1 mg/100 g, Çiftçi ve Parlat (2018) 12.86-22.45, Uçak Koç vd. (2017) 23.15-27.30, Kambur vd. (2015) 8.45-14.29, Yaşar ve Söğütlü (2020) ortalama 22.5, Simsek vd. (2012) 8.71-31.75, Manolova vd. (2021) 9.00–20.80, Özcan ve Ölmez (2014) %10.9-17.9, Güzel ve Savaş Bahçeci (2020) ortalama 16.4 (0.1-32.2) ve Karatas vd. (2019) 3.44-17.26 g/100g arasında bildirmişlerdir.

Bal örneklerinin incelendiği bu çalışmada 6 adet bal örneğinin diastaz sayısının yasal limitlere uymadığı tespit edilmiştir (Tablo-3). Doğada bulunan 20 farklı aminoasitten 18 tanesi balda bulunmaktadır. Prolin, nektarın bala dönüşmesi esnasında bal arıları tarafından balın yapısına katılan tek aminoasittir. Baldaki aminoasitlerin %50- 85'ini prolin oluşturduğu için balın protein içeriği genelde prolin miktarı olarak belirtilmektedir. Prolin miktarı balda saflığın bir kriteridir ve tağşiş yapılmış ballarda bu değer daha düşük çıkmaktadır (Ferek 2016). Çalışmada prolin ile fruktoz (R^2= -0.917, P≤0.01, Tablo-5) ve prolin ile fruktoz/glukoz oranı (R^2= -0.708, P≤0.05, Tablo-5) arasında güçlü negatif bir korelasyon tespit edilmiştir. Fruktoz/glukoz oranı ballarda kristalleşme eğilimi ve balın orijini hakkında bilgi vermektedir. Fruktoz miktarının fazla olması balın daha tatlı ve lezzetli olmasını sağlamaktadır. Fruktoz oranı diğer bileşenlerle dengeli olmalıdır. Balda prolin düzeyi balın nektar kaynağına, bölgeye, floraya ve balın üretildiği mevsime göre değişiklik göstermektedir (da Silva vd. 2016, Tabay Sümbül 2024).

Bu nedenle, balarısının tarlacılık faaliyetine bağlı olarak kaliteli bir balın doğal olarak yüksek miktarda prolin içermesi ve fruktoz oranının diğer bileşenlerle dengeli olması beklenmektedir. Ferek (2016) Muğla ilinden toplanan çam balı örneklerinin prolin değerlerini 388- 682 mg/kg arasında (ortalama=522,87 mg/kg) bildirmiştir. Bal örneklerinde prolin değerlerini Kilic vd. (2016) 414.2 – 880.8 mg/kg, Akgün vd. (2021) en yüksek 758,56 mg/kg, en düşük ise 377.00 mg/kg (Acacia balında) olarak bildirmişlerdir.

Başka çalışmalarda prolin değerlerini Belli (2019) 158.45-1217.45 mg/kg arasında, Uçak Koç vd. (2017) 980 mg/kg (hayıt balı) ve 922 mg/kg (çiçek balı), Malkoç vd. (2019) ortalama 807,57 mg/kg (karaçalı balı), Manolova vd. (2021) 218.50 – 679.50 mg/kg (ayçiçeği balı) arasında, Çiftçi ve Parlat (2018) 487.81-699.05 mg/kg, Karatas vd. (2019) 204.06-1588.93 mg/kg arasında, Gürbüz vd. (2020) ortalama 420 mg/kg, Terrab vd. (2002) ortalama 227 mg/100 g (salgı balı), Boussaid vd. (2014) 39.62 (biberiye) – 102.60 mg/kg (okaliptüs) arasında ve Łozowicka vd. (2021) 70.8-461.4 mg/kg arasında bildirmişlerdir. Bu çalışmada, incelenen bal örneklerinin HMF değerleri 1.51 mg/kg – 267.65 mg/kg arasında değişmiş, 3 adet bal örneğinin ve bal örneklerinin ortalamasının (47.18 mg/kg) yasal limitlere uymadığı belirlenmiştir (Tablo-3). Türk Gıda Kodeksi'nin 2020/7 sayılı Bal Tebliği'ne göre balda HMF, en fazla 40 mg/kg olmalıdır (Türk Gıda Kodeksi Bal Tebliği 2020).

HMF taze ballarda çok az miktarda bulunur ve balda yüksek miktarlarda bulunmaması gerekmektedir. Balda HMF miktarının artmasına, bala ısıl işlem uygulama, balın depolanma süresi, depo ortamının sıcaklığı ve balın pH'ı etki etmektedir. Balda HMF miktarının artması, diastaz ve invertaz enzimlerinin azalması ve fermantasyon olayının artması gibi istenmeyen durumlar ortaya çıkabilmektedir (Belli 2019, Ferek 2016, Smetanska vd. 2021). HMF düzeyi ve diastaz aktivitesi balın kalitesini belirlenmesinde uzun zamandır kullanılan önemli parametrelerdir (Güzel ve Savaş Bahçeci 2020, Karadal ve Yıldırım 2012). Bu yüzden HMF balın kalitesini belirlemede kullanılan en önemli kriterlerden biridir (Belli 2019, Ferek 2016). Boussaid vd. (2014) inceledikleri tüm bal örneklerinin, incelenen parametreler bakımından Avrupa Mevzuatına (EC Direktifi 2001/110) uygun olduğunu ve örneklerin HMF değerinin 12.07-27.43 mg/kg arasında değiştiğini bildirmişlerdir. Araştırmacılar tarafından incelenen bal örneklerinde HMF içeriğini Belli (2019) 0-93,8 mg/kg, Dadalı (2021) 0.09-1.31 mg/kg, Gürbüz vd. (2020) ortalama 18,5 mg/kg, Özenirler vd. (2018) 1.73 ppm, Bengü ve Kutlu (2018) %36.37, Smetanska vd. (2021) 3.69 - 22.47 arasında, Yaşar ve Söğütlü (2020) 45.148 mg/kg, Yurt ve Çakır (2020) 39.85 mg/kg, Berhanu vd. (2022) ortalama 9.46 mg/kg, Özcan ve Ölmez (2014) 1.34-31.28 mg/kg, Terrab vd. (2002) 3.20-52.60 mg/kg, Bobis vd. (2020) 1.5-40 mg/kg, Ghramh vd. (2020) 0.13 mg/kg, Kambur vd. (2015) 10.50-36.02 mg/kg, Gençay Çelemli (2021) 0,7- 11,31 ppm

değerinde, Güzel ve Savaş Bahçeci (2020) 3.5 (0.3-36.5) mg/kg, Manolova vd. (2021) 0.69 – 12.40mg/kg, Kilic vd. (2016) 6,5 mg/kg - 20,8 mg/kg, Akgün vd. (2021) 11.83 mg/kg ve Ünübol Aypak vd. (2019) ortalama 26.94 mg/kg değerinde bildirmişlerdir. Su muhtevası balın en büyük ikinci bileşenidir (Bobis vd. 2020).

Balın su içeriği, bal arısı tarafından nektarın olgunlaştırılmasından sonraki miktarıdır. Nektarın bitkisel ve coğrafi kökeni, toprak özellikleri, iklim koşulları, hasat sırasındaki koşullar, olgunlaşma derecesi, hasat ve depolamada arıcılar tarafından uygulanan yöntemler balın su muhtevasını etkilemektedir. Su muhtevası, balın depolanması süresince fermentasyonun önlenmesi ve balın dayanıklılığı açısından önem taşımaktadır (Belli 2019, Bobis vd. 2020, Ferek 2016, Smetanska vd. 2021). Ozmofilik mayalar yüksek oranda neme sahip ballarda canlılığını sürdürebilmekte ve sonuç olarak balın bozulmasına sebep olmaktadır. Diğer yandan olgunlaşmış bal nem içeriği düşük olduğu için mikroorganizmaların gelişimine uygun bir ortam oluşturmamaktadır (Ferek 2016).

İncelenen bal örneklerinin su muhtevası yasal limitlere uygun olduğu tespit edilmiştir (Tablo-3). Yapılan çalışmalarda farklı bal tipi örneklerinin su muhtevaları tespit edilmiştir. Bal örneklerinde su muhtevası; Belli (2019) %14.64-20.88, Dadalı (2021) %16.30-17.50, Gürbüz vd. (2020) ortalama %15.91, Akgün vd. (2021) en yüksek Rhododendron balında (%18.89), en düşük ise Akasya balında (%17.99), Uçak Koç vd. (2017) %15.95 (hayıt balı), %16.86 (çiçek balı), %17.11 (çam balı), Manolova vd. (2021) %15.60–19.30, Yurt ve Çakır (2020) ortalama % 15.06, Çiftçi ve Parlat (2018) %15.48-17.63, Boussaid vd. (2014) %17.27 (biberiye) - 19.80 (nane) arasında, Kambur vd. (2015) %16.20-19.40, İçli (2022) %9.8-17.8, Özenirler vd. (2018) ortalama %14.9, Malkoç vd. (2019) %12-16, Karatas vd. (2019) %15.04-19.52, Smetanska vd. (2021) %19.10-19.30, Berhanu vd. (2022) ortalama %18.93, Bengü ve Kutlu (2018) ortalama %15.39, Güzel ve Savaş Bahçeci (2020) %16.9 (14.5-21.7) civarında, Keskin vd. (2020) %15.8-19.5, Özenirler vd. (2019) %13.5-18.3, Özcan ve Ölmez (2014) %17.1-20.0, Gençay Çelemli (2021) %15,8-18,8, Kanbur vd. (2021) %15-22 (çiçek balı) ve %18-20 (kestane), Chirsanova vd. (2021) 18.0 -19.4 g/100g arasında, Kilic vd. (2016) %15.44-17.28 arasında, Bobis vd. (2020) %11- 20 (okaliptüs) ve Ferek (2016) %15.6-18.0 arasında bildirmişlerdir.

Balın kuru maddesinin % 95- 99'unu şekerler oluşturur. Bunun % 85- 95'ini fruktoz ve glukoz şekerleri meydana getirir. Sakkaroz, maltoz, izomaltoz, erloz, kestoz, melezitoz, refinoz, ve dektroz bal içerisinde tespit edilen diğer şekerlerden bazılarıdır (Ferek 2016). Monosakkaritler, balda bulunan en yaygın karbonhidratlardır. Balda en fazla fruktoz (yaklaşık %38,5) ve glukoz (yaklaşık %31,0.) bulunur (da Silva vd. 2016). Bala tadını veren bu iki monosakarit, bitkilerin nektarlarında veya bitkiler üzerinde yaşayan böceklerin salgılarında bulunan sakkarozun invertaz enzimi ile inversiyona uğraması sonucu meydana gelmektedir (Ferek 2016).

Cezayir, Arjantin, Brezilya, Etiyopya ve İspanya'da yapılan çalışmalarda sakkarozun yanı sıra okaliptüs balında çeşitli disakkaritler tanımlanmıştır. Bu balda en yaygın olarak bildirilenler arasında erlos, melezitoz, panoz, maltotrioz ve rafinoz olmak üzere önemli bir trisakkarit grubu da bildirilmiştir (Bobis vd. 2020). Yedi adet bal örneğinin incelendiği çalışmada bakılan parametreler bakımından balların Türk Gıda Kodeksi ve TS 3036 kriterlerine uyduğu bildirilmiştir (Ateş ve Yaşar 2020).

Çeşitli bal örneklerinin incelendiği çalışmalarda örneklerin şeker oranlarını Kilic vd. (2016) %69.09-%75.28 ve İçli (2022) ortalama %67.87 (%46.2-81.6) oranında bildirmişlerdir. Farklı araştırmacılar farklı bal tiplerinin fruktoz oranlarını incelemişlerdir. İncelenen bal örneklerinin fruktoz oranını Güzel ve Savaş Bahçeci (2020) %35.3 (31.5-39.1), İçli (2022) %21.5-43.0, Yurt ve Çakır (2020) % 38.62, Gençay Çelemli (2021) 26.43-35.57g/100g, Ferek (2016) %28.6-35.6, Mahmood ve Abbas (2020) doğal, şekerli ve endüstriyel balın fruktoz oranını sırasıyla %51.86, %36.86 ve %31.27, Ghramh vd. (2020) %33.10-%44.77, Kambur vd. (2015) %30.27-32.73, Kanbur vd. (2021) yayla ve kestane ballarında sırasıyla fruktoz içeriğini 40.91–53.94 g/100 g ve 44.65–53.88 g/100 g, Özenirler vd. (2018) karahindiba monofloral balının fruktoz % 46.02, Malkoç vd. (2019) fruktoz değeri min %30 ve max %38 ve Kilic vd. (2016) 27.9 mg/100 g – 41.3 mg/100 g bildirmiştir.

Özkök vd. (2021) Tunceli/Türkiye bal örneklerinin fruktoz değerlerini 25.97-43.44 g/100g olarak bildirmişlerdir. İçli (2022) incelediği markasız 22 bal örneğinde glukoz oranını %17.5-38.9, Yurt ve Çakır (2020) 30 adet süzme çiçek ballarının incelenmesinde glukoz % 32.46 ± 2.24 olarak, Ferek (2016) 15 adet çam balı örneğinin incelendiği çalışmada, glukoz oranı % 22.1- 28.76, Kanbur vd.

(2021) yayla ballarında ve kestane ballarında glukozu sırasıyla 27.56–40.95 g/ 100 g ve 24.91–30.14 g/100 g olarak, Kambur vd. (2015) Yığılca bal örneklerinde glukoz içeriğini %23.13-26.97, Özenirler vd. (2018) karahindiba monofloral balının glukoz değerini % 35.21, Ozkok vd. (2021) Tunceli bal örneklerinin glukoz değerlerinin; 21.84-45.6 g/100g arasında, Ghramh vd. (2020) glukoz değeri %26.68-%37.91, Güzel ve Savaş Bahçeci (2020) %30.4 (26.0-34.3) ve Gençay Çelemli (2021) Türkiye'nin Rize ili Ayder ilçesinde üretilen 20 adet balın glukoz değerlerini 20.11-30.58 g/100g (ortalama: 26.83 g/100g) olarak tespit etmişlerdir.

Sukroz miktarı balın olgunluğunun belirlenmesinde önemli bir parametre olarak değerlendirilir. Baldaki sukroz analizi, balın sahteciliği konusunda da bilgi verir. Nektar içindeki sukroz bal arısının bal midesinde invertaz enzimiyle glukoz ve fruktoz monosakkaritlerine dönüşür. Balda sukroz düzeyinin yüksek olması bala şeker katkısından olabilir veya erken hasat dolayısıyla, sukrozun, glukoz ve fruktoza tam olarak dönüştürülememiş olmasından da kaynaklanabilir. Diğer taraftan, bal arılarının yoğun şekilde şeker şurubu ile beslenmesi de benzer bir sonuç doğurabilir (Ferek 2016, Güzel ve Savaş Bahçeci 2020). Nitekim, balın içeriğindeki sukroz miktarı olgunlaşma derecesine göre değişmektedir ve zamanından önce hasat edilen ballar fazla miktarda sukroz içerirler.

Ghramh vd. (2020) incelediği 6 bal örneklerinde sakkaroz tespit edememiş, bir unifloral bal örneğinde %0.25 ve multifloral bal örneğinde %3.25 olarak bulmuşlardır. Kambur vd. (2021) yayla ballarının ve kestane ballarının sırasıyla sakkaroz içeriklerini 0.021–0.107 g/100 g ve 0.01–0.05 g/100 g olarak bildirmişlerdir. Muğla ilinde farklı lokasyonlarında bulunan *Pinus brutia* Tenore üzerinde yaşayan *Marchalina hellenica* salgısından üretilen 15 adet çam balı örneğinin incelendiği çalışmada, sakkaroz oranı % 0- 2.1 olarak tespit edilmiştir (Ferek 2016). Bitlis ili Hizan ilçesinde 20 farklı lokasyondan alınan bal örneklerinin çeşitli kimyasal özellikleri incelenmiştir. İki bal örneği dışında hiçbir bal örneğinde sakkaroz bulunmaması, arıların beslenmesinde şeker kullanılmadığını ortaya koyduğu vurgulamışlardır. Sonuçların AB standartlarına ve Türk Gıda Kodeksi Bal Tebliği'ne uygun olduğu ifade edilmiştir (Kilic vd. 2016).

Etiyopya bal örneklerinin incelendiği bir çalışmada balın indirgeyici şeker (%70.46) ve sakkaroz (%2.75) değerlerinin düşük olduğu bulunmuş olup, bu değerin Etiyopya balının kaliteli olduğunu gösterdiği vurgulanmıştır (Berhanu vd. 2022). Başka bir çalışmada, Samarra-Salah al-din-Irak'ta 3 bal tipi (doğal, şekerli ve endüstriyel bal) örneği incelenmiştir. Elde edilen sonuçlara göre sırasıyla doğal, şekerli ve endüstriyel balın sakkaroz oranı %4.07, %34.99 ve %33.88 olarak tespit edilmiştir (Mahmood ve Abbas 2020). Çorum yöresinin 47 bal örneğinde sukroz %0.34 (<0.05-4.64) olarak tespit edilmiştir (Güzel ve Savaş Bahçeci 2020). Dadalı (2021) hayıt balının sükroz içeriğini 1.03-3.27 g/100 g arasında bildirmiştir. Kambur vd (2015) Yığılca dört bal örneğinde sakkaroz oranını %0.16-0.34 arasında olduğunu belirtmiştir. Manolova vd. (2021) Bulgaristan'dan alınan 27 ayçiçeği balı örneğinin sakkaroz içeriğini %0.50 – 3.70 ve indirgeyici şekerleri %72.51 – 80.80 arasında bildirmişlerdir. İçli (2022) incelediği markasız bal örneklerinde sakkaroz oranını ortalama %0.18 olarak bildirmiştir. Sakkaroz içeriğinin hiçbir örnekte %5'lik sınır değerini aşmadığını vurgulamıştır. Başka bir çalışmada (Gürbüz vd. 2020)

Türkiye'nin Güneydoğu Anadolu Bölgesi'nde üretilen 68 adet bal örneğinin balın fizikokimyasal kalite özellikleri araştırılmıştır. Bal örneklerinin ortalama sakkaroz değeri %0,90 olarak bulunmuştur. Tüm örnekler sakkaroz için Türkiye'de belirlenen uluslararası standartları ve yasal limitleri karşılamıştır. Smetanska vd. (2021) inceledikleri bal örneklerinde sakkaroz içeriğini %2.07-6.38 arasında bulmuştur.

Kambur vd. (2015) Yığılca bal örneklerinin maltoz oranını %0.80-1.39 arasında bildirmiştir. Muğla ilinde farklı lokasyonlarında bulunan *Pinus brutia* Tenore üzerinde yaşayan *Marchalina hellenica* salgısından üretilen 15 adet çam balı örneğinin incelendiği çalışmada (Ferek 2016), maltoz oranı %0.02-0.65 arasında bildirilmiştir. Ghramh vd. (2020) incelediği bal örneklerinin maltoz ortalama değerini %0.37-%2.97 arasında açıklamıştır. Kilic vd. (2016) incelediği bal örneklerinin sadece çok az örnekte 2.7 mg/100 g ve 2.1 mg/100 g miktarında maltoz tespit edildiğini bildirmiştir. Gürbüz vd. (2020) bal örneklerinin maltoz ortalama değerini %2.88 olarak bulmuşlardır. Balda bulunan karbonhidratların neredeyse tamamına yakınını (%85'ini) fruktoz ve glukoz oluşturmaktadır. Bazı balların şeker içerikleri ve oranları kaliteyi belirlemede ayırt edici bir özelliktir (Belli 2019, Güzel ve Savaş Bahçeci 2020).

Belli (2019) incelediği bal örneklerinde taklit ve tağşiş gibi hileli durumların olup olmadığını

araştırmak amacıyla yaptığı çalışmasında Fruktoz+Glukoz değerini 41.61-64.85 g/100g arasında tespit etmiştir. Kilic vd. (2016) fruktoz+glukoz miktarını 67.5 g/100 g – 75.9 g/100 g arasında belirlemişlerdir. Gürbüz vd. (2020) inceledikleri bal örneklerinin ortalama fruktoz + glukoz değerlerini %70.97 olarak bildirmişlerdir. Uçak Koç vd. (2017) hayıt, çiçek ve çam ballarında sırasıyla glukoz+fruktoz oranını %73.76, %60.92 ve %60.9 olarak bulmuşlardır. Yurt ve Çakır (2020) inceledikleri 30 adet süzme çiçek ballarının glukoz+fruktoz miktarını % 71.09 civarında tespit etmişlerdir. Kambur vd. (2015) Yığılca bal örneklerinde fruktoz+glukoz içeriğini %53.67-59.61 arasında bildirmişlerdir. İçli (2022) bal örneklerini incelediği çalışmasında bir kalite kriteri olan fruktoz+glukoz içeriğini ortalama %67.69 olarak belirlemiştir. Çiftçi ve Parlat (2018) farklı firmalara ait çiçek ballarının fruktoz+glukoz oranı (%); 70.39, 73.39, 73.52, 65.20 ve 70.30 olarak belirlemiştir. Toplam glukoz+fruktoz oranını Özcan ve Ölmez (2014) %51.31-68.30, Güzel ve Savaş Bahçeci (2020) %65.8, Karatas vd. (2019) %62.02-74.90 olarak bildirmişlerdir. Kristalleşme balın su içeriği ve fruktoz ile glukoz arasındaki oran ile ilgilidir (Çiftçi ve Parlat 2018, Kambur vd. 2021).

Çetin vd. (2011) ve Çiftçi ve Parlat (2018) fruktoz/glukoz oranı arttıkça balın kristalleşme eğiliminin azaldığını belirtmişlerdir. Sakkaroz miktarının balın olgunlaşma derecesine ve nektar içeriğine göre değişiklik gösterdiği, çok erken hasat edilen olgunlaşmamış ballarda sakkaroz değerinin daha yüksek olduğu vurgulanmaktadır (Kambur vd. 2021). Yapılan çeşitli çalışmalarda farklı bal tipi örneklerinin fruktoz/glukoz oranı incelenmiştir. Fruktoz/glukoz oranını çiçek ballarında Yurt ve Çakır (2020) 1.20, Kilic vd. (2016) 1.19 g/100 g – 1.28 g/100 g, İçli (2022) 1.23, Kambur vd. (2015) 1.18-1.32, Ozkok vd. (2021) 0.83-1.13, Uçak Koç vd. (2017) sırasıyla hayıt, çiçek ve çam ballarında %1.16, %1.15 ve %1.36, Özenirler vd. (2018) karahindiba balında 1.3, Güzel ve Savaş Bahçeci (2020) 1.16 (1.03-1.24), Kambur vd. (2021) ortalama 1.43 (yayla balı) ve 1.76 (kestane balı), Gençay Çelemli (2021) 1.03-1.34, Çiftçi ve Parlat (2018) firma ballarında 1.06, 1.09, 1.09, 1.19, 1.09, Çetin vd. (2011) market ballarında 1.01-1.85 ve Keskin vd. (2020) 0.96-1.19 arasında bildirmişlerdir.

Balların şeker içerikleri ve oranları genellikle kaliteyi belirlemede ayırt edici bir özellik olarak kullanılmaktadır. Bal örneklerinin Tablo-4'te verilen şeker kompozisyonları incelendiğinde, fruktoz ortalama değeri %31.85 ve glukoz ortalama değeri %25.84 olarak belirlenmiştir. Örneklerde tespit edilen sakkaroz ve maltoz değerlerinin yasal limitlere uygun olduğu bulunmuştur. Balın orijini, doğal ve gerçekliğini belirlemek amacıyla fruktoz ve glukoz miktarları ile fruktoz /glukoz oranı kullanılmaktadır. Fruktoz/glukoz oranının ortalama değerinin (1.34) yasal limitlere uygun olduğu, ancak 5 adet bal örneğinin yasal limitler dışında olduğu tespit edilmiştir. Bal örneklerinin fruktoz+glukoz ortalama değerinin (%57.69) yasal limitler (%60 ≤) altında kaldığı, sadece 4 bal örneğinin yasal limitlere uygun olduğu belirlenmiştir (Tablo-4). Elde edilen sonuçlara göre fruktoz/glukoz oranı ile fruktoz arasında pozitif bir korelasyon tespit edilmiştir (R^2=0. 810, p≤0.01, Tablo-5). Bala tat veren fruktoz ve glukoz, bitkilerin nektarlarında veya bitkiler üzerinde yaşayan böceklerin salgılarında bulunan sakkarozun invertaz enzimi ile inversiyona uğraması sonucu meydana gelmektedir. Arıların tarlacılık faaliyetleri neticesinde bu şekerler balda belli oranlarda bulunmalıdır. İncelenen bal örneklerinden elde edilen veriler ticari balların bazılarının Türk Gıda Kodeksi Bal Tebliğinin (2020) belirlediği yasal limitlere uymadığı belirlenmiştir.

Sonuç: Çalışmada 6 adet monofloral etiketli ve 3 adet multifloral etiketli ticari balların melissopalinolojik ve fiziko-kimyasal özellikleri incelenmiştir. Melissopalinolojik analiz sonucunda 6 adet monofloral olarak etiketli bal örneklerinin yalnızca 1 adetinin monofloral olduğu, 5 adetinin ise multifloral olduğu tespit edilmiştir. Ayrıca bal örneklerinin 2 adetinin tağşişli bal olduğu ve nişasta içerdiği tespit edilmiştir. Üç adet bal örneğinin HMF ve diastaz sayısının değerlerinin Türk Gıda Kodeksi Bal Tebliğinin (2020) belirlediği yasal limitlere uymadığı, δ l3C, balda protein ve ham balda δ 13C farkı, δ l3C değerinden hesaplanan C4 şeker oranı, serbest asitlik, su muhtevası, fruktoz, glukoz, sukroz, maltoz ve fruktoz/glukoz ortalama değerlerinin yasal limitlere uygun olduğu belirlenmiştir. Balda yapılan taklit, tağşiş veya balın muhafazası sırasında yapılan yanlış uygulamalar balın besin değerini düşürerek tüketicilerin sağlığını olumsuz etkilemektedir. Tüketici sağlığını korumak için Türk Gıda Kodeksi Bal Tebliğine (2020) uygun üretilmiş balların tüketime sunulması son derece önem arz etmektedir.

Çıkar çatışması: Yazarlar arasında herhangi bir çıkar çatışması bulunmamaktadır.

Etik durumu: Çalışmanın yürütülmesi esnasında hayvan veya insan deneyleri yapılmamıştır.

Finansman: Yazarlar herhangi bir fonunun olmadığını beyan eder.

Mali Kaynak: Yazarlar herhangi bir fonunun olmadığını beyan eder.

Veri kullanılabilirliği: Mevcut çalışmada kullanılan veriler talep edilmesi halinde yazarlardan temin edilebilir.

Yazar katkıları: SK: Verilerin elde edilmesi ve yorumlanması, makale yazımı ve editi; OSA: Melissopalynolojik laboratuvar çalışmaları. Yazarlar bu makalenin versiyonunu okumuş ve kabul etmişlerdir.

KAYNAKLAR

Akgün N, Çelik ÖF, Kelebekli L. Physicochemical Properties, Total Phenolic Content, and Antioxidant Activity of Chestnut, Rhododendron, Acacia and Multifloral Honey. Journal of Food Measurement and Characterization. 2021;15: 3501–3508, DOI:10.1007/s11694-021-00937-3

Alghamdi BA, Alshumrani ES, Saeed MSB, Rawas GM, Alharthi NT, Baeshen MN vd. Analysis of Sugar Composition and Pesticides Using HPLC and GC–MS Techniques in Honey Samples Collected from Saudi Arabian Markets. Saudi Journal of Biological Sciences. 2020;27: 3720–3726, DOI:10.1016/j.sjbs.2020.08.018

Ateş Y, Yaşar S. Chemical Investigations on Honey Produced in Bingöl and Surroundings. U. Bee J. 2020; 20(1): 72-80, DOI:10.31467/uluaricilik.713308

Baloš MZ, Popov N, Vidaković S, Pelić DL, PelićM, Mihaljev Z vd. Electrical Conductivity and Acidity of Honey. Arhiv Veterinarske Medicine 2018;11(1): 91–101, DOI:10.46784/e-avm.v11i1.20

Belli T. Muğla ilinde üretilen balların bazı fiziksel ve kimyasal özellikleri. Pamukkale Üniversitesi, Fen Bilimleri Enstitüsü, Gıda Mühendisliği Anabilim Dalı, Yüksek Lisans Tezi, Denizli, 2019, (Erişim tarihi.17.02.2025), https://tez.yok.gov.tr/UlusalTezMerkezi/tezSorguSonucYeni.jsp

Bengü AŞ, Kutlu MA. Identification of the Some Quality Criteria of Honey Produced in Bingol. Tr. J. Nature Sci. 2018;7(1): 7-10, https://dergipark.org.tr/en/download/article-file/495108

Berhanu S, Tadesse DM, Jorge A. Physico-Chemical Properties of Ethiopian *Apis mellifera* Honey: Review. J Agric Sc Food Technol. 2022;8(1): 038-044, DOI: 10.17352/2455-815X.000143

Bobis O, Moise AR, Ballesteros I, Reyes ES, Durán SS, Sánchez-Sánchez J, vd. Lyptus Honey: Quality Parameters, Chemical Composition and Health Promoting Properties. Food Chemistry 2020;325,126870, DOI:10.1016/j.foodchem.2020.126870

Bontempo L, Camin F, Ziller L, Perini M, Nicolini G, Larcher R. Isotopic and Elemental Composition of Selected Types of Italian Honey Measurement 2017;98: 283-289. DOI:10.1016/j.measurement.2015.11.022

Boussaid A, Chouaibi M, Rezig L, Hellal R, Donsí F, Ferrari G vd. Physicochemical and Bioactive Properties of Six Honey Samples from Various Floral Origins from Tunisia. Arabian Journal of Chemistry 2014;11(2): 265-274, DOI:10.1016/j.arabjc.2014.08.011

Brar DS, Pant K, Krishnan R, Kaur S, Rasane P, Nanda V vd. A Comprehensive Review on Unethical Honey: Validation by Emerging Techniques. Food Control 2023;145,109482, DOI:10.1016/j.foodcont.2022.109482

Can Z, Yildiz O, Sahin H, Akyuz E, Silici S, Kolayli S. An Investigation of Turkish Honeys: Their Physico-Chemical Properties, Antioxidant Capacities and Phenolic Profiles. Food Chemistry 2015;180: 133–141, DOI:10.1016/j. foodchem.2015.02.024

Chen CT, Chen BY, Nai YS, Chang YM, Chen KH, Chen YW. Novel Inspection of Sugar Residue and Origin in Honey Based on the $^{13}C/^{12}C$ Isotopic Ratio and Protein Content. Journal of Food and Drug Analysis 2019;27: 175-183, DOI:10.1016/j.jfda.2018.08.004

Chirsanova A, Capcanari T, Boistean A, Siminiuc R. Physico-Chemical Profile of Four Types of Honey from the South of the Republic of Moldova. Food and Nutrition Sciences 2021;12: 874-888,

DOI:10.4236/fns.2021.129065, https://www.scirp.org/journal/fns, ISSN Online: 2157-9458

Çetin K, Alkin E, Ucurum HO. Determination of Quality Parameters of Flower Honey Sold on the Market. Journal of Food and Feed Science – Technology 2011;11: 49–56

Çiftci E. Konya yöresel yayla balı ile püren balının kalite kriterleri yönünden karşılaştırılması. Selçuk Üniversitesi, Sağlık Bilimleri Enstitüsü, Yüksek Lisans tezi, Konya, 2014, (Erişim tarihi: 20.03.2025), https://acikerisim.selcuk.edu.tr/server/api/core/bitstreams/00bf2791-d95e-4996-b190-1eb3af209d34/content

Çiftçi M, Parlat SS. Konya Bölgesindeki Marketlerde Satılan Farklı Ticari Çiçek Ballarının Bazı Kimyasal Özelliklerinin Türk Gıda Kodeksi Bal Tebliğine Uygunluğunun Araştırılması. Selcuk J Agr Food Sci. 2018;32(1): 38-42, DOI: 10.15316/SJAFS.2018.61

da Silva PM, Gauche C, Gonzaga LV, Costa ACO, Fett R. Honey: Chemical Composition, Stability and Authenticity. Food Chemistry 2016;196: 309–323, DOI: 10.1016/j.foodchem.2015.09.051

Dadalı C. Characterization of Volatile Compounds and Sensory Properties of Chaste (*Vitex agnus-castus* L.) Honeys. Turkish Journal of Agriculture Food Science and Technology 2021;9(3): 621-631, DOI:10.24925/turjaf.v9i3.621-631.4165

Elflein L, Raezke KP. Improved Detection of Honey Adulteration by Measuring Differences Between 13C/12C Stable Carbon Isotope Ratios of Protein and Sugar Compounds with a Combination of Elemental Analyzer - Isotope Ratio Mass Spectrometry and Liquid Chromatography - Isotope Ratio Mass Spectrometry (δ13C-EA/LC-IRMS). Apidologie 2008;39: 574–587, DOI:10.1051/apido:2008042

Ferek Ö. Muğla ili çam ballarının bazı kalite özelliklerinin belirlenmesi. Namık Kemal Üniversitesi, Fen Bilimleri Enstitüsü, Gıda Mühendisliği Anabilim Dalı, Yüksek Lisans Tezi, Tekirdağ, 2016 (Erişim tarihi:17.02.2025), https://tez.yok.gov.tr/UlusalTezMerkezi/tezSorguSonucYeni.jsp

Flanjak I, Kenjerić D, Strelec I, Rajs BB, Primorac L. Effect of Processıng and Storage on Sage (*Salvia officinali*s L.) Honey Quality, J Microbiol Biotech Food Sci. 2022;11(6) e3375, DOI: 10.55251/jmbfs.3375

Gençay Çelemli Ö, Özkök A, Özenirler Ç, Mayda N, Zare G, Sorkun K. Highlighting the Melissopalynological and Physicochemical Characteristics of Ayder-Rize (Turkey). Commun. Fac. Sci. Univ. Ank. Ser. 2021;30(2): 119-133, ISSN 1303-6025 E-ISSN 2651-3749, DOI:10.53447/communc.906788, https://communications.science.ankara.edu.tr

Ghramh HA, Khan KA, Zubair A, Ansari MJ. Quality Evaluation of Saudi Honey Harvested from the Asir Province by Using High-Performance Liquid Chromatography (HPLC). Saudi Journal of Biological Sciences 2020;27: 2097–2105, DOI: 10.1016/j.sjbs.2020.04.009

Gıdatarım. 2025. https://gidatarim.com/bakanlik-tek-tek-ifsa-ettitaklit-ve-tagsis-listesine-1-gunde-11-bal-markasi-eklendi/, (Erişim tarihi: 13.03.2025)

Gürbüz S, Çakıcı N, Mehmetoğlu S, Atmaca H, Demir T, Apan MA vd. Physicochemical Quality Characteristics of Southeastern Anatolia Honey, Turkey. Hindawi International Journal of Analytical Chemistry 2020; ID 8810029, DOI: 10.1155/2020/8810029

Güzel N, Savaş Bahçeci K. Çorum Yöresi Ballarının Bazı Kimyasal Kalite Parametrelerinin Değerlendirilmesi. Gıda The Journal of Food 2020;45 (2): 230-241, E-ISSN 1309-6273, DOI: 10.15237/gida.GD19129

İçli N. Evaluation of HMF Levels in Unbranded Flower Honeys in Terms of Food Safety. Ankara Univ Vet Fak Derg. 2022;69: 431-436, DOI: 10.33988/auvfd. 886000

Kambur M, Kekeçoğlu M, Yıldız İ. Assesment of the Honey Samples Produced in Yığılca District of Düzce City by Using Chemical and Palynological Analysis, U. Arı D.- U. Bee J. 2015;15(2): 67-79

Kanbur ED, Yuksek T, Atamov V, Ozcelik AE. A Comparison of the Physicochemical

Properties of Chestnut and Highland Honey: The Case of Senoz Valley in the Rize Province of Turkey. Food Chemistry 2021;345, 128864, DOI: 10.1016/j.foodchem.2020.128864

Karadal F, Yıldırım Y. The Quality Parameters and Nutritional and Health Effect of Honey. J FacVetMedUniv Erciyes 2012;9(3): 197-209

Karatas S, Aktumsek A, Duru ME. Investigation of Physicochemical Properties of Some Monofloral Honeys in South Western Anatolia. International Journal of Secondary Metabolite, 2019;6(3): 251–262, ISSN-e: 2148-6905 /© IJSM 2019, DOI: 10.21448/ijsm.623262

Keskin Ş, Mayda N, Keskin M, Özkök A. Bilecik Ballarının Melissopalinolojik ve Kimyasal Özellikleri Açısından Değerlendirilmesi. Gıda The Journal of Food 2020;45(2): 275-289, DOI: 10.15237/gida.GD19107

Kilic O, Kutlu MA, Ozdemir FA. Examination of the Various Chemical Properties of the Hizan Region (Bitlis-Turkey) Honey Types. Progress in Nutrition 2016;18(4): 411-420

Louveaux J, Maurizio A, Vorwohl G. Methods of Melissopalynology. Bee World 1978; 59: 139–157

Lozano-Torres B, Martínez-Bisbal MC, Soto J, Borr´as MJ, Martínez-M´a˜nez R, Escriche I. Monofloral Honey Authentication by Voltammetric Electronic Tongue: A Comparison with 1H NMR Spectroscopy. Food Chemistry 2022;383,132460, DOI: 10.1016/j.foodchem.2022.132460

Łozowicka B, Kaczy´nski P, Iwaniuk P. Analysis of 22 Free Amino Acids in Honey from Eastern Europe and Central Asia Using LC-MS/MS Technique Without Derivatization Step. Journal of Food Composition and Analysis 2021;98,103837, DOI: 10.1016/j.jfca.2021.103837

Mahmood QH, Abbas KhM. Comparative Of Glucose, Fructose and Sucrose Ratios in Honey Fed Honey Bees on Citrus Flowers (Citrus Singensis), Honey Fed Honey Bees on Sucrose with Artificial Honey in HPLC. Eurasia J Biosci 2020;14(2): 7197-7204

Malkoç M, Kara Y, Özkök A, Ertürk Ö, Kolayli S. Characteristic Properties of Jerusalem Thorn (*Paliurus spina-christi* Mill.) Honey U. Arı D. - U. Bee J. 2019;19(1): 69-81, DOI: 10.31467/uluaricilik.535658

Manolova V, Parvına I, Yankovska–Stefanova T, Balkanska R. Physicochemical Analysis of Sunflower Honey From Bulgaria U. Arı D. - U. Bee J. 2021;21(2): 168-176, DOI: 10.31467/uluaricilik.960751

Nombre I, Schweitzer P, Boussim JI, Rasolodimby JM. Impacts of Storage Conditions on Physicochemical Characteristics of Honey Samples from Burkina Faso. Afr J FoodSci. 2010;4(7): 458–463, https://academicjournals.org/journal/AJFS/article-full-text-pdf/885C8A625138.pdf

Ozkok A, Gencay Celemli O, Zare G, Ozenirler Ç, Mayda N, Sorkun K. Geographical Indication Study of Tunceli Honeys. U. Arı D. - U. Bee J. 2021;21(1): 39-53, DOI: 10.31467/uluaricilik.896300

Özcan M, Ölmez Ç. Some Qualitative Properties of Different Monofloral Honeys. Food Chem. 2014;163: 212–218, DOI: 10.1016/j.foodchem.2014.04.072

Özenirler Ç, Mayda M, Gençay Çelemli Ö, Özkök A., Sorkun K. Paketli Balların Botanik Orijin Tespiti ve Mikroskobik Analizleri: Etiket Bilgileri ile İçerik Uyumluluğunun Karşılaştırılması. Gıda 2019;44(5): 861-865, DOI:10.15237/gida.GD19058

Özenirler Ç, Mayda N, Gençay Çelemli Ö, Özkök A, Sorkun K. Dandelion Honey: A New Monofloral Honey Record for Turkey U. Arı D. - U. Bee J. 2018;18(2): 87-93, DOI: 10.31467/uluaricilik.485024

Pasias IN, Raptopoulou KG, Makrigennis G, Ntakoulas DD, Lembessis D, Dimakis V vd. Finding the Optimum Treatment Procedure to Delay Honey Crystallization Without Reducing its Quality. Food Chemistry 2022;381,132301, DOI: 10.1016/j.foodchem.2022.132301

Simsek A, Bilsel, Goren AC. $^{13}C/^{12}C$ Pattern of Honey from Turkey and Determination of Adulteration in Commercially Available Honey Samples Using EA-IRMS. Food Chemistry 2012;130: 1115–1121, DOI: 10.1016/j.foodchem.2011.08.017

Smetanska I, Alharthi SS, Selim KA. Physicochemical, Antioxidant Capacity and Color Analysis of Six Honeys from Different Origin. Journal of King Saud University – Science 2021;33,101447, DOI: 10.1016/j.jksus.2021.101447

Tabay Sümbül D. Chemical Quality of Geven Honey from Ağrı Region: A Study on Proline Content and Sugar Profile. GUFBD / GUJS 2024;14(4): 1195-1206, DOI: 10.17714/gumusfenbil.1394347

Terrab A, Diez MJ, Heredia FJ. Characterisation of Moroccan Unifloral Honeys by their Physicochemical Characteristics. Food Chemistry 2002;79: 373–379, DOI: 10.1016/S0308-8146(02)00189-9

Türk Gıda Kodeksi Bal Tebliği (Tebliğ No: 2020/7, Sayı 31107). 2020. https://www.resmigazete.gov.tr/eskiler/2020/04/20200422-13.htm, (Erişim tarihi:16.02.2025)

Uçak Koç A, Karacaoğlu M, Doğan M. Hayıt (*Vitex agnus-castus*), Çam ve Karışım Çiçek Balının Bazı Kalite Kriterleri Açısından Karşılaştırılması. Adü Ziraat Derg. 2017; 14(1): 17-21, DOI: 10.25308/aduziraat.294889

Ulusal Gıda Referans Laboratuvar Müdürlüğü. 2025. https://gidalab.tarimorman.gov.tr/gidareferans/Menu/73/Katki-Orijin-Tespiti-Taklit-Ve-Tagsis, (Erişim tarihi: 13.03.2025)

Ünübol Aypak S, İnci A, Bakırcı S, Dereli Fidan E, Soysal M. Comparision of the Antioxidant Activity and Hydroxymethylfurfural (HMF) Levels in Honey Taken from Hives and Markets. Gida The Journal of Food 2019;44(1): 86-92, E-ISSN 1309-6273, ISSN 1300-3070, DOI: 10.15237/gida.GD18056

Xie Q, Huang X, Ji X, Jia G, Sang Y, Cui Z vd. Comprehensive Investigation of Psicose in Chinese Honeys and the Assessment of Its Potential As A New Marker for Honey Adulteration Detection. Journal of Food Composition and Analysis 2022;108,104444, http://www.elsevier.com/locate/jfca, DOI: 10.1016/j.jfca.2022.104444

Yaşar S, Söğütlü İ. Investigation of Acidity, Diastase Number, HMF, Insoluble Dry Matter and Ash Percentage Values of Some Honey Samples Produced in Bingöl and Districts Van Vet J. 2020;31(1): 42-45, ISSN: 2149-3359, e-ISSN: 2149-8644, DOI: 10.36483/vanvetj.631565

Yurt B, Çakır S. Some Physicochemical Properties of Honey Produced in Iğdır and Region. Tr. J. Nature Sci., 2020;9:51-57 Special Issue, DOI: 10.46810/tdfd.761654

Yücel Y, Sultanoğlu P. Characterization of Honeys from Hatay Region by Their Physicochemical Properties Combined with Chemometrics. Food Bioscience 2013;1: 16–25, www.elsevier.com/locate/fbio, DOI: 10.1016/j.fbio.2013.02.001

Zábrodská B, Vorlová L. Adulteration of Honey and Available Methods for Detection – A Review. Acta Vet. BRNO, 2014;83: S85–S102; DOI:10.2754/avb201483S10S85

Citation/Atıf: Fişne A, Pehlivan S. Pollen analysis of honeys from Trabzon (Türkiye). U. Bee J. / U. Arı D. 2025; 25 (1): 108-130. DOI: 10.31467/uluaricilik.1641493

ARAŞTIRMA MAKALESI / RESEARCH ARTICLE

POLLEN ANALYSIS OF HONEYS FROM TRABZON (TÜRKİYE)

Trabzon (Türkiye) Ballarında Polen Analizi

Ahter FİŞNE[1*], Sevil PEHLİVAN[1]

[1]Department of Biology, Faculty of Science, Gazi University, Ankara, TÜRKİYE, *Corresponding author/ Yazışma Yazarı: E-mail: ahter@gazi.edu.tr, ORCID No: 0000-0001-6895-8870, E-mail: sevil.pehlivan.1948@gmail.com, ORCID No: 0000-0002-7002-2331

Received / Geliş: 17.02.2025 Accepted / Kabul: 29.04.2025 DOI: 10.31467/uluaricilik.1641493

ABSTRACT

This study presents the results of pollen analysis on honey samples collected from 85 different locations across all districts of Trabzon, Türkiye, during the months of June to October between 2009 and 2012. A total of 50 pollen taxa were identified, including 23 families, 25 genera, and 2 species. The most dominant pollen type was *Castanea sativa*, found in 65 samples, reflecting the regional floral characteristics. Lamiaceae was dominant in only one sample. Secondary pollen types commonly observed included Apiaceae, Asteraceae, Brassicaceae, *Carduus*, *Cistus*, *Cynoglossum*, Fabaceae, *Hedysarum*, *Laurus nobilis*, *Rhododendron*, and Rosaceae. Four samples were classified as monofloral honeys, all identified as *Castanea sativa* honey. *Rhododendron*, known for its toxic effects when present above a certain threshold in honey, was detected in 48 samples, indicating the necessity of evaluating these honeys in terms of consumer health. TPN-10 ranged from 2,845 to 1,525,683 per 10 g of honey. Correlation analysis showed that floral diversity increased with altitude, while cluster analysis indicated that total pollen count was the most influential factor in sample classification. These findings underline both the rich botanical diversity of Trabzon honeys and the impact of ecological variables on honey composition.

Keywords: Botanical origin, *Castanea sativa*, Melissopalynology, *Rhododendron,* Total pollen number

ÖZ

Bu çalışmada, 2009-2012 yılları arasında Trabzon ilinin tüm ilçelerinde, Haziran-Ekim aylarında 85 farklı bölgeden toplanan bal örnekleri üzerinde polen analizi gerçekleştirilmiştir. Yapılan analizler sonucunda, 23 familya, 25 cins ve 2 tür düzeyinde olmak üzere toplam 50 taksona ait polen varlığı tespit edilmiştir. Bölgenin karakteristik türlerinden *Castanea sativa*, 65 örnekte dominant polen olarak en yüksek orana ulaşmıştır. Lamiaceae familyası ise yalnızca bir örnekte dominant olarak saptanmıştır. Sekonder polen grubunda ise Apiaceae, Asteraceae, Brassicaceae, *Carduus*, *Castanea sativa*, *Cistus*, *Cynoglossum*, Fabaceae, *Hedysarum*, Lamiaceae, *Laurus nobilis*, *Rhododendron* ve Rosaceae taksonları öne çıkmıştır. İncelenen örneklerin 4'ü monofloral bal olarak sınıflandırılmış ve tamamı *Castanea sativa* balı olarak tanımlanmıştır. Balda belli bir miktarın üzerinde bulunduğunda zehirleme etkileriyle bilinen *Rhododendron* cinsine ait polenler 48 örnekte belirlenmiş, bu durum balın tüketici sağlığı açısından değerlendirilmesi gerektiğini göstermiştir. TPS-10 g değerine göre polen sayıları 2.845 ile 1.525.683 arasında değişmiştir. Korelasyon analizleri, rakım yükseldikçe balın floristik çeşitliliğinin arttığını ortaya koyarken; kümeleme analizinde örneklerin sınıflandırılmasında en belirleyici unsur toplam polen sayısı olmuştur. Bu bulgular, Trabzon balının floristik zenginliğini ortaya koyarken, aynı zamanda ekolojik değişkenlerin bal kompozisyonu üzerindeki etkilerini de net bir şekilde gözler önüne sermektedir.

Anahtar kelimeler: Botanik köken, *Castanea sativa*, Melissopalinoloji, *Rhododendron,* Toplam polen sayısı

GENİŞLETİLMİŞ ÖZET

Giriş: Balın polen içeriği, üretim alanının floristik kompozisyonuyla doğrudan ilişkilidir. Türkiye, iklimsel çeşitliliği ve 12.000'i aşkın bitki türüyle arıcılık açısından son derece yüksek bir potansiyele sahip bir ülkedir. Ancak, bitki çeşitliliğinin bilinmesine rağmen, bu bitkilerden hangilerinin bal üretimine ne ölçüde katkı sağladığı konusunda yeterli düzeyde detaylı ve bölgesel temelli bilimsel çalışmalara ihtiyaç duyulmaktadır. Bitki çeşitliliğinin bal üretimindeki rolünü değerlendirmede en etkili yöntemlerden biri melissopalinolojik analizlerdir. Son yıllarda Türkiye'de üretilen ballar üzerine yapılan palinolojik araştırmaların sayısında artış gözlemlenmekte; bu çalışmalar sayesinde, nektar kaynağı bitki türlerinin belirlenmesi ve üretilen balların kalite standartlarının yükseltilmesi amaçlanmaktadır. Bu çalışmanın temel amacı, Trabzon il merkezi ve bağlı ilçelerden toplanan 85 adet bal örneğinin polen içeriklerini analiz ederek, bölge ballarının botanik kökenini ve kalite düzeyini ortaya koymaktır.

Gereç ve Yöntem: Bal örnekleri, Trabzon il merkezinden ve tüm ilçelerinden 2009-2012 yılları arasında Haziran-Ekim ayları arasında üreticilerden temin edilmiştir. Toplanan bal örneklerinden kalitatif ve kantitatif melissopalinolojik analizler için preparatlar hazırlanmıştır (Louveaux et al. 1978). Homojenize edilmiş stok baldan alınan 10 gram alınarak steril tüplere konulup üzerine 20 mL distile su eklenmiş ve balın suda çözünmesi için 45°C'de su banyosunda bekletilmiştir. Numuneler 3500 rpm'de 45 dakika santrifüj edilerek tüplerin dibinde oluşan polen tortusu, kalıcı bir preparat hazırlamak amacıyla bazik fuksinli gliserin jelatin kullanılarak hazırlanmıştır. Hazırlanan preparatlarda polenlerin ait olduğu bitki taksonları belirlenmiş ve yüzde oranları hesaplanmıştır. İncelenen bal örnekleri, eser (3%'ten az), minör (3-15%), sekonder (16-44%) ve dominant (>45%) olmak üzere 4 grupta incelenmiştir. Bal örneklerindeki toplam polen sayısı (TPS-10 g), *Lycopodium* spor tabletleri kullanılarak hesaplanmıştır (Moar 1985). Polenler, TPS'ye göre kategori I (< 20 000), kategori II (20 000-100 000), kategori III (100 000-500 000), kategori IV (500 000-1 000 000), kategori V (>1 000 000) olmak üzere 5 kategoriye sınıflandırılmıştır (Jose et al. 1989). Elde edilen parametreler arasındaki ilişkiyi belirlemek amacıyla bulgulara korelasyon analizi, polen spektrumu analizi ve hiyerarşik kümeleme analizi uygulanmıştır.

Bulgular: Yapılan analizler sonucunda, 36 familyaya ait 50 takson tespit edilmiştir. 65 örnekte *Castanea sativa*'ya, 1 örnekte ise Lamiaceae familyasına ait polenlerin dominant miktarda bulunduğu belirlenmiştir. Sekonder miktarda polen bulunan taksonlar ise 10 örnekte *Castanea sativa*, 9 örnekte Fabaceae, 8 örnekte *Cistus*, 7 örnekte *Rhododendron*, 5 örnekte Lamiaceae ve *Hedysarum* L., 3 örnekte Apiaceae ve Brassicaceae, 2 örnekte Rosaceae ve 1 örnekte ise Asteraceae, *Cynoglossum* L., *Carduus* L. ve *Laurus nobilis* L. olmuştur. İncelenen bal örneklerinde belirlenen bitki taksonlarının sayısının 2 ile 24 arasında değiştiği gözlemlenmiştir. Ayrıca yapılan analizler sonucunda belli bir miktarın üzerinde tüketildiğinde zehirlenmeye neden olduğu bilinen *Rhododendron* (Ericaceae) polenlerine de 48 adet bal örneğinde çeşitli miktarlarda rastlanmıştır. Bal örnekleri, toplam polen sayısı (TPS-10 g) miktarına göre sınıflandırıldığında; 19 tanesi kategori I (% 22.3), 39 tanesi kategori II (% 45.9), 23 tanesi kategori III (% 27), 2'şer tanesi ise kategori IV (% 2.4) ve kategori V (% 2.4) olarak belirlenmiştir. 10 gram baldaki TPS-10 değerleri 2845 ile 1 525 683 arasında hesaplanmıştır**.**

Tartışma-Sonuç: Yapılan palinolojik analizler, Trabzon yöresine ait bal örneklerinin botanik kökenine ışık tutarak bölgenin arıcılık potansiyelini ortaya koymuştur. Çalışma sonucunda, Fagaceae familyasına ait *Castanea sativa* yöre balları için başlıca nektar ve polen kaynağı olarak belirlenmiştir. Bal örneklerinde en fazla tespit edilen ikinci polen taksonu Lamiaceae, üçüncü ise Rosaceae familyası olmuştur. Toplamda analiz edilen 85 bal örneğinin 4'ü, unifloral (tek çiçek türüne dayalı) bal olarak sınıflandırılmıştır. Bu unifloral balların tamamı kestane balıdır. Geriye kalan 81 örnek ise çok sayıda bitki türünden polen içermesi nedeniyle multifloral (çok çiçekli) bal olarak tanımlanmıştır. Analizler sonucunda, Trabzon ballarında 23'ü familya, 25'i cins ve 2'si tür düzeyinde olmak üzere toplam 50 farklı taksonun polenine rastlanmıştır. Bu polenlerin büyük çoğunluğu Apiaceae, Asteraceae, Brassicaceae, Cistaceae (*Cistus*), Ericaceae (*Rhododendron*), Fabaceae, Fagaceae, Lamiaceae, Poaceae ve Rosaceae familyalarına aittir. İstatistiksel değerlendirmeler, bal kalitesinin belirlenmesinde en önemli faktörün toplam polen sayısı olduğunu ortaya koymuştur. Bu araştırma, yalnızca Trabzon bölgesinin nektar bitkileri potansiyelini belirlemekle kalmayıp, aynı zamanda

Türkiye nektarlı bitkileri listesine de katkı sunmayı hedeflemektedir. Bölgesel bitki çeşitliliğini yansıtan bu veriler, arıcılık faaliyetlerinin yönlendirilmesinde, coğrafi işaretleme çalışmalarında ve bal kalite kontrol süreçlerinde önemli bir referans niteliği taşımaktadır.

INTRODUCTION

The floristic composition of the production area determines the pollen content of honey. With its climatic features and 11 707 plant species (Güner et al. 2012), Türkiye has a very high potential in beekeeping. However, Türkiye's rich plant diversity is known, so detailed studies on which plant contributes to honey production are needed. For this reason, the most important method to help determine the contribution of plant diversity to honey production is pollen analysis in honey. Therefore, with the increasing number of studies on the palynological examination of honey produced in Türkiye in recent years, the aim is to determine the nectar-producing plant species and to increase product quality.

Honey has been a valuable nutrient for humans since ancient times. However, the quality of honey varies depending on the geographical structure and herbal characteristics of the place where it is produced. Honey is a natural product that can be produced anywhere in the world without any preparation and can be used in human nutrition. The increasing understanding of honey's composition has made it a more preferred food product among consumers. The market value of honey varies depending on its floral source; in some Northern European countries, honeydew honey is preferred and valued more highly than blossom honey, while in other countries, monofloral blossom honeys may be preferred (Bogdanov et al. 2004, Kenjeric et al. 2008, Prodolliet and Hischenhuber 1998). Monofloral honeys are considered more valuable because they are easier to market and consumers can more easily find the type of honey they want; therefore, they are commercially important and are generally sold at higher prices than polyfloral honeys (Atanassova et al. 2012, Deodikar 1965, Oddo et al. 1988, Oddo and Bogdanov 2004,). This price variability based on consumer preference is likely one of the main reasons honey is a frequent target for food fraud, particularly concerning its botanical origin. Melissopalynological analyses are conducted to determine the botanical origin of honey. Thus, it is accepted that honey is sourced from pollen-producing plants in proportion to the pollen ratio (Sorkun 1985). To identify the plant source and geographical indication of honey, pollen analysis must be conducted on honey from that region (Güzel 2014). No honey produced is the same as the other since the herbal resources used by the bees are very diverse and obtained in different climatic conditions. Therefore, honeys show significant differences, especially in taste and aroma (Crane 1990).

Pollen analysis in honey was first performed by Pfister in 1845. Pollen analysis in Turkish honey was first studied by Quistani in 1976 (Sorkun et al. 1989). Sorkun and İnceoğlu (1984), one of the Turkish researchers, conducted pollen analysis in honey for the first time between 1979-1981. In recent years, melissopalynological studies have gained importance worldwide (Barth and Luz 2022, Giorgi et al. 2011, Makhloufi et al. 2010, Matthew et al. 2018, Samrat et al. 2023, Sanz et al. 2005) and in Türkiye (Bağcı and Tunç 2006, Bayram 2019, Özler 2015, Silici and Gökceoglu 2007). In the Eastern Black Sea Region, where our study area is located, pollen analysis studies on honey are carried out, albeit in small numbers (Bayram et al. 2019, Sorkun et al. 1989, Sorkun and Yuluğ 1985, Tosunoğlu et al. 2023, Uzunca et al. 2023). These studies positively affect honey's quality and increase honey's value in marketing.

Beekeeping has been done in Trabzon province for many years. In order to clarify the current state of beekeeping in Trabzon province, official data regarding the number of enterprises and colonies, as well as production figures, were taken into consideration. According to the most recent statistics, there are 2,629 registered beekeeping enterprises in the province, with a total of 155,829 colonies. Annual honey production is recorded at 781 tons, corresponding to an average yield of 5.01 kg per colony (Tarım ve Orman Bakanlığı 2025). In line with the "Development of Modern Beekeeping" project studies by the Provincial Directorate of Food, Agriculture and Livestock, many beekeeping courses, technical meetings and symposiums were organized and technical information about modern beekeeping was given to people engaged in beekeeping. Trabzon region has important beekeeping potential, unique climate and vegetation, and different nectar plants. Botanical origin analyzes have been carried out on the honeys of the neighboring provinces (Gümüşhane, Rize, Bayburt) of the region and this research conducted

in the Trabzon region is complementary (Bayram et al. 2019, Sorkun et al. 1989, Sorkun and Yuluğ 1985, Tosunoğlu et al. 2023). For this reason, Trabzon was chosen as the study area.

The aims of the research are to identify the botanical origins of honeys and to explore the diversity of pollen types present, to provide information on the relationship between the flora of the region and the characteristics of local honeys, and to understand the relationships between the number of plant taxa in honey, altitude, pollen spectrum, and total pollen count through correlation analysis, pollen spectrum analysis, and hierarchical clustering analysis. Additionally, this study contributes to the authenticity and quality of honey, which are of critical importance for consumers and producers in the honey industry.

MATERIALS AND METHODS

Study area

Trabzon province; in the Eastern Black Sea Region, it covers 0.6% of the country's territory with a surface area of 4685 km², between 40° 30' and 41° 07' north latitudes and 39° 07' and 40° 30' east longitudes. Starting from the sea level and increasing towards the south, the altitude reaches 3000 meters in the region (Haldizen Mountain 3325 m).

In the province of Trabzon, the annual precipitation amount is 820.60 mm, the maximum temperature average of the hottest month is 26.80 °C, the minimum temperature of the coldest month is 4.30 °C, the summer precipitation is 133.50 mm, the precipitation-temperature equivalent (Q) is 126.39, and the Emberger drought index is 4.98, and there is a Transitional climate, which is considered as the Submediterranean bioclimate type, between the Ocean-Mediterranean climates (Kurt 2014).

Trabzon province, is in the Europe-Siberian phytogeographic region (Davis 1965-2000). A few flora studies of vascular plants were carried out in Trabzon (Palabaş-Uzun and Anşin 2006, Palabaş-Uzun and Terzioğlu 2019, Uzun and Terzioğlu 2008). Uzun and Terzioğlu (2008) investigated the vascular flora of forest vegetation in Altındere Valley (Maçka-Trabzon) in 2001-2002. 383 vascular taxa belonging to 246 genera and 84 families were identified. The richest family was Asteraceae 35 taxa (9.1%), followed by Lamiaceae 27 taxa (7.0%) and Fabaceae 23 taxa (6.0%). Additionally, the richest genus was *Campanula* L. 7 taxa (1.82%), followed by *Trifolium* L. 6 taxa (1.56%) and *Acer* L. 6 taxa (1.56%). Palabaş-Uzun and Terzioğlu (2019) studied Flora of Sisdağı (Şalpazarı/Trabzon) and Environs, and 472 vascular plant taxa belonging to 84 families and 254 genera were determined. Depending on determined vascular taxa, the richest plant families are as follows; Asteraceae 52 taxa (11.02%), Rosaceae 38 taxa (8.05%), Poaceae 29 taxa (6.14%), Fabaceae 27 taxa (5.72%), Lamiaceae 26 taxa (5.51%), Apiaceae 18 taxa (3.81%), Ranunculaceae 14 taxa (2.97%), Plantaginaceae 13 taxa (2.75%), Brassicaceae 12 taxa (2.54%), Orchidaceae 10 taxa (2.12%), Polygonaceae 10 taxa (2.12%).

Honey sampling

85 honey samples, for which pollen analyses were made, were collected from Trabzon city center and all its districts in June-October between 2009 and 2012 with the help of the Trabzon Beekeepers Association (Figure 1). While collecting honey samples, the distance between the villages and their altitudes was considered, and special attention was paid to the fact that the hives were settled (fixed) in the province of Trabzon, where migratory beekeeping is quite common. Honey samples taken by opening the hive with bee producers were placed in transparent and sterile jars and labeled. Different honey samples taken from the same village or neighborhood of the district are numbered 1, 2, 3 etc. to avoid confusion. In the region where the hives are located, the plants that the bees can go to were collected and identified, and reference pollen preparations were prepared from these plants according to the Wodehouse method (Wodehouse 1935).

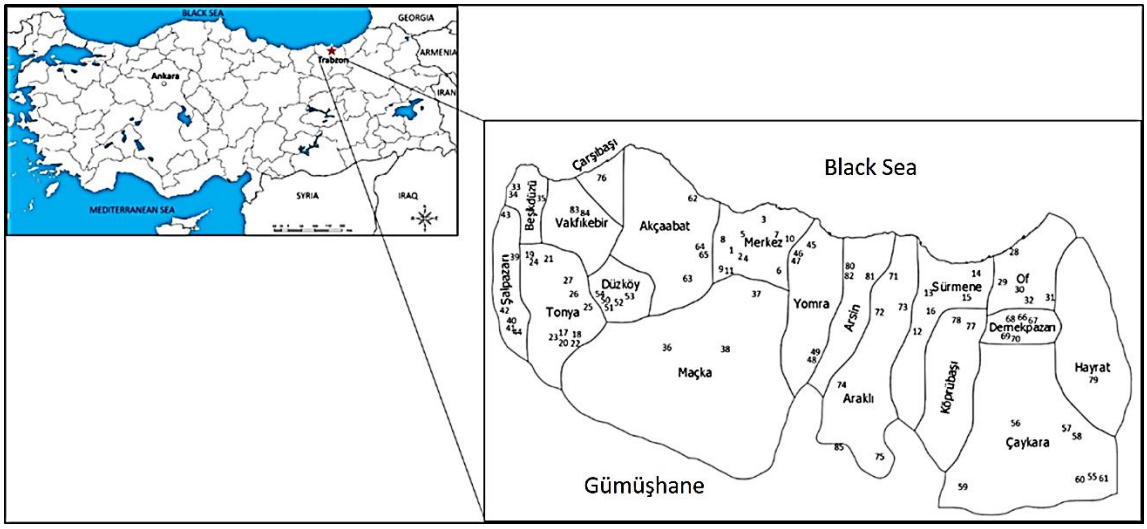


Figure 1. Locations of the collected honey samples

Melissopalynological analyses

Qualitative analysis: The method Louveaux et al. (1978) suggested for the qualitative analysis was followed. To prepare pollen slides, 10 grams of honey taken from the homogenized stock honey into sterile tubes was added to 20 mL of distilled water and kept in a water bath at 45 ˚C to dissolve the honey in water. After the samples were centrifuged at 3500 rpm for 45 minutes, the pollen precipitate formed at the bottom of the tubes was smeared with some basic fuchsin glycerin gelatin to prepare a permanent slide. In the prepared slides, the plant taxa to which the pollen belonged and the number of pollen were calculated. The investigated honey samples were examined in 4 groups as trace (<3%), minor (3-15%), secondary (16-44%), and dominant (>45%) (Louveaux et al. 1978).

Quantitative analysis: The total pollen number (TPN in 10 g honey) in honey samples was calculated according to Moar (1985) by using tablets of *Lycopodium* spores (Stockmarr 1971). Based on the TPN-10 g value, the pollen grains were classified into 5 categories; Category I (<20 000 pollen grains per 10 g honey), Category II (20 000-100 000 pollen grains), Category III (100 000-500 000 pollen grains), Category IV (500 000-1 000 000 pollen grains), and Category V (>1 000 000 pollen grains) (Maurizio 1979).

Pollen slides were examined with a Leica ICC50 HD imaging system connected to a Leica DM 750 microscope, and pollen counts and identifications were made. An immersion lens (×100) was used to identify pollen and take microphotographs. The entire lamella area of 22×22 mm was scanned in the examinations, and the pollen in this area was identified. Two slides were prepared from each sample for microscopic analysis of pollen taxa of honey samples. Pollen averages and pollen % of taxa were determined. While identifying the pollens, those that could be identified at the species and genus levels were diagnosed accordingly, while those that could not be classified at these levels were identified at the family level. The occurrence rates and percentages of pollens identified at the genus level within the same family were calculated separately from those at the family level. The contribution of pollen belonging to these taxa in the honey studied was determined. When describing pollen types, palynological literature (Aytuğ 1971, Blackmore and Ferguson 1986, Erdtman 1969, Faegri and Iversen 1989, Kapp 1971, Pehlivan 1995, Punt 1976, Punt and Clarke 1980, Sawyer 1988, Sorkun 2008) and reference pollen slides prepared from plants collected from the research area, and the collection of pollen preparations in the Palynology Laboratories of Gazi and Hacettepe University Biology Department were used.

Statistical analyses

For statistical data analysis, Python's Pandas and NumPy libraries were used, while Matplotlib & Seaborn and Scikit-learn libraries were used for visualization (Harris et al. 2020, Hunter 2007, McKinney 2010, Pedregosa et al. 2011, Wascom

2021). The analysis process consists of four main parts: data preparation, correlation, pollen spectrum, and hierarchical clustering analysis. The correlation matrix is a fundamental statistical tool used to examine linear relationships between variables. Correlation coefficients (ranging from -1 to +1) indicate the direction and strength of the relationship between variables (Pearson 1896). Based on the data distribution, a linear relationship was found between the variables, so Pearson Correlation was calculated and visualized in the heatmap. Hierarchical Clustering analysis is a clustering method that groups data based on their similarities or differences. Each data point initially belongs to its own cluster, and then clusters are merged based on the distance between similar data (Everitt 2011).

RESULTS

As a result of pollen analysis of Trabzon honey, a total of 50 taxa were identified, 23 at the family, 25 at the genus, and 2 at the species level (Figure2-4, Table 1,). Percentage rates of the most abundant taxa in the samples; *Castanea sativa* (95.2%), Lamiaceae (87%), Rosaceae (83.5%), Apiaceae (74.1%), Fabaceae (74.1%), *Cistus* L. (70.5%), *Rhododendron* L. (56%), Poaceae (54.1%), Brassicaceae (40%) and Asteraceae (36.4%). The taxon whose pollen is most frequently found in honey samples is *Castanea sativa*, one of the region's natural plants. As a result of the pollen analysis, it was observed that the highest number of taxa was found in the trace group, followed by the minor, secondary, and dominant groups (Table 1). 4 of the honey samples (40, 59, 78, 81) were defined as unifloral and *Castanea sativa* pollen was determined to be dominant in these honeys, and they were named as chestnut honey. The remaining 81 honey samples were named multifloral honey. Although the honey collected from the Trabzon region is generally seen to be of multi-plant origin, the majority of honeys called multifloral contain not only dominant and trace amounts of pollen, but also low amounts of minor pollen. For example, the presence of *Castanea sativa* pollen in 24 honey samples is over 80%. However, low amounts of minor pollen from other taxa were detected in these honey samples, these honeys were named multifloral honey.

As a result of melissopalynological analysis, it was determined that *Castanea sativa* pollen was dominant in 65 samples, and Lamiaceae pollen was found in dominant amounts in one sample. Taxa with secondary quantities of pollen were *Castanea sativa* in 10, Fabaceae in 9, *Cistus* in 8, *Rhododendron* in 7, Lamiaceae and *Hedysarum* L. in 5, Apiaceae and Brassicaceae in 3, Rosaceae in 2 samples, and Asteraceae, *Cynoglossum* L., *Carduus* L., and *Laurus nobilis* L. in 1 sample. It was observed that the total number of plant taxa whose pollen was encountered in the honey samples examined varied between 2 and 24 (Table 1).

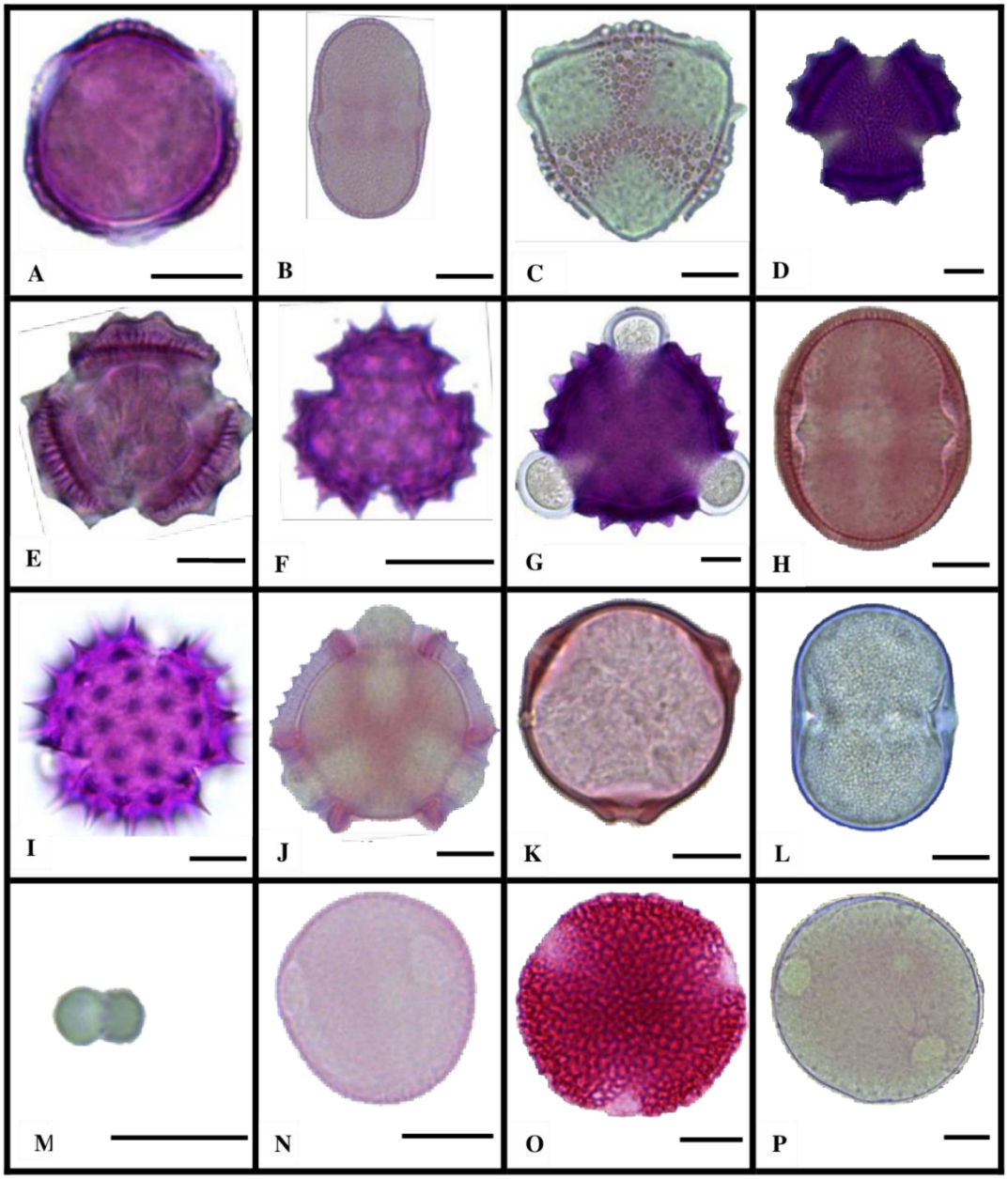


Figure 2. A- Adoxaceae-*Sambucus* sp. B- Apiaceae C- Aquifoliaceae-*Ilex* sp. D- Asteraceae E- Asteraceae-*Anthemis* sp. F- Asteraceae-*Bellis* sp. G- Asteraceae-*Carduus* sp. H- Asteraceae-*Centaurea* sp. I- Asteraceae-*Helianthus* sp. J- Asteraceae-*Taraxacum* sp. K- Betulaceae L- Boraginaceae M- Boraginaceae-*Cynoglossum* sp. N- Boraginaceae-*Echium* sp. O- Brassicaceae P- Campanulaceae Scale bars-10 µm.

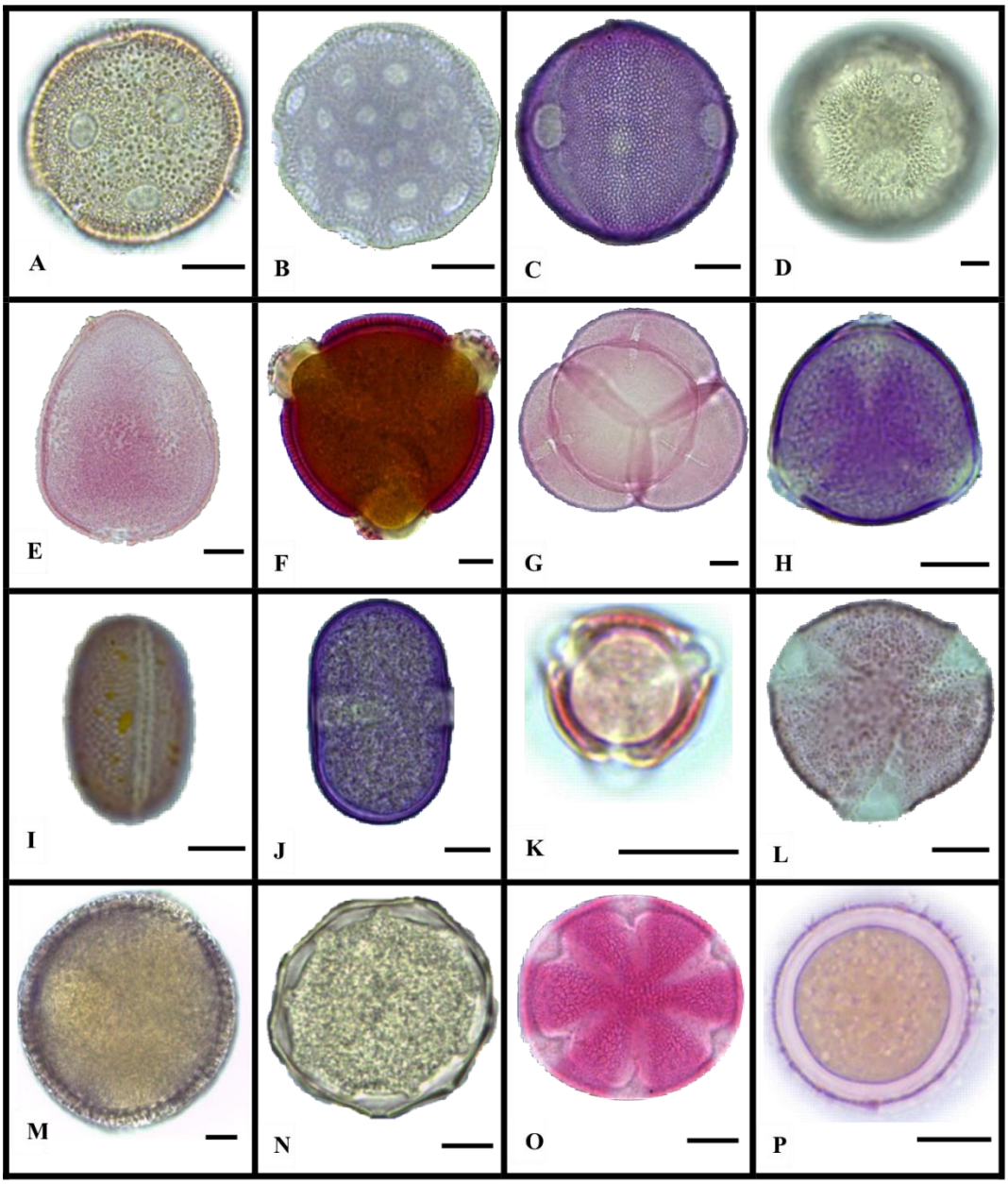


Figure 3. A- Caryophyllaceae B- Amaranthaceae (=Chenopodiaceae) C- Cistaceae-*Cistus* sp. D- Convolvulaceae E- Cyperaceae-*Carex* sp. F- Dipsacaceae G- Ericaceae-*Rhododendron* sp. H- Fabaceae I- Fabaceae-*Hedysarum* sp. J- Fabaceae-*Vicia* sp. K- Fagaceae-*Castanea sativa* L- Fagaceae-*Quercus* sp. M- Geraniaceae N- Juglandaceae O- Lamiaceae P- Lauraceae- *Laurus nobilis* Scale bars-10 µm

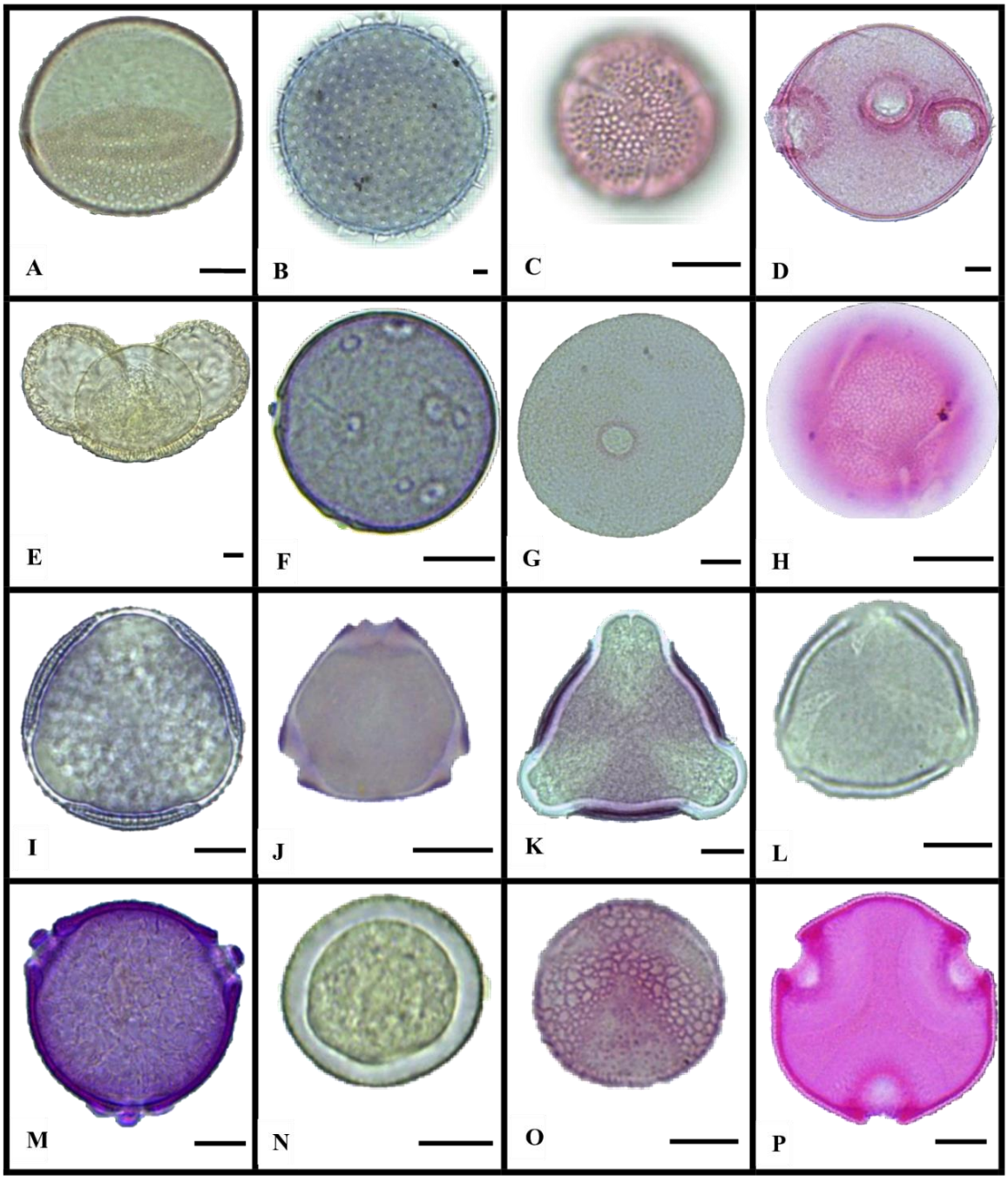


Figure 4. A- Liliaceae B- Malvaceae C- Oleaceae D- Onagraceae-*Epilobium* sp. E- Pinaceae F- Plantaginaceae-*Plantago* sp. G- Poaceae H- Polygonaceae-*Rumex* sp. I- Ranunculaceae J- Rhamnaceae K- Rosaceae L-Rosaceae-*Rubus* sp. M-Rosaceae-*Sanguisorba* sp. N- Salicaceae–*Populus* sp. O- Salicaceae-*Salix* sp. P- Tiliaceae-*Tilia* sp. Scale bars-10 µm

Table 1. Number of samples, location and altitudes, TPN-10 g values, number of taxa in each sample, pollen spectrum, and percentages for the Trabzon honey samples (* Dominant pollen, ** secondary pollen, *** minor pollen, ****trace pollen)

Sample number	Locations/ Altitude (m)	TPN-10 g /Pollen status	Number of taxa	Pollen spectrum and percentage (%)
1	Ortahisar - Akkaya 550	16.853 Category I	8	* *Castanea sativa* (81.5) *** Fabaceae (6), Rosaceae (5) ****Apiaceae (2.5), Lamiaceae (1), Poaceae (1.5), *Rumex* (1), Ranunculaceae(1.5)
2	Ortahisar - Geçit (1) 500	95.156 Category II	13	* *Castanea sativa* (63) ** Apiaceae (19) *** Fabaceae (8.5) **** Brassicaceae (1), Lamiaceae (2.5), Rhamnaceae (0.5), Rosaceae (1.5), *Rubus* (0.5), *Rumex* (0.5), *Sambucus* (1.5), *Taraxacum* (0.5), *Tilia* (0.5), *Vicia* (0.5)
3	Ortahisar - Başkurt 600	112.316 Category III	23	* *Castanea sativa* (44) ** Brassicaceae (26.5) *** *Cistus* (4), Juglandaceae (4), *Rhododendron* (5), Rosaceae (3.5) **** Apiaceae (1), *Carduus* (0.5), *Carex* (0.5), Caryophyllaceae (0.5), *Echium* (0.5), Fabaceae (2.5), *Hedysarum* (1.5), Lamiaceae (1.5), *Laurus nobilis* (0.5), Malvaceae (0.5), Oleaceae (0.5), Pinaceae (0.5), *Plantago* (0.5), Poaceae (0.5), *Rubus* (0.5), *Rumex* (0.5), *Sanguisorba* (0.5)
4	Ortahisar - Geçit (2) 600	147.610 Category III	12	* *Castanea sativa* (65) ***Cistus* (8), Fabaceae (10.5), Rosaceae (9) ****Apiaceae (0.5), *Taraxacum* (0.5), *Rhododendron* (1.5), *Hedysarum* (1), Poaceae (1), *Rubus* (0.5), *Rumex* (2), *Vicia* (0.5)
5	Ortahisar - Bengisu 50	102.164 Category III	9	* *Castanea sativa* (65.5) *** Apiaceae (7.5), Fabaceae (11.5), Rosaceae (3), *Tilia* (8) **** Campanulaceae (0.5), *Cistus* (0.5), Lamiaceae (2), Rhamnaceae (1.5)
6	Ortahisar - Yeşilyurt 250	42.112 Category II	7	* *Castanea sativa* (84.5) ***Apiaceae (6.5), Lamiaceae (4) ****Cistus* (2), Fabaceae (2), Oleaceae (0.5), Rosaceae (0.5)
7	Ortahisar- Yanyamaç 500	61.485 Category II	12	* *Castanea sativa* (73.5) *** *Cistus* (5.5), Fabaceae (5), *Rhododendron* (3), Rosaceae (5) **** Apiaceae (2.5), Geraniaceae (0.5), Juglandaceae (0.5), Lamiaceae (1), Oleaceae (2), Poaceae (0.5), *Tilia* (1)
8	Ortahisar- Karakaya 150	7.506 Category I	8	* *Castanea sativa* (88) *** Apiaceae (5), Lamiaceae (3) **** Pinaceae (0.5), Poaceae (0.5), Ranunculaceae (0.5), Rhamnaceae (1), Rosaceae (1.5)
9	Ortahisar- Ağıllı (2) 400	200.257 Category III	7	* *Castanea sativa* (83) *** Apiaceae (7.5), Lamiaceae (4.5) **** Asteraceae (0.5), *Cistus* (0.5), Fabaceae (2), Rosaceae (2)
10	Ortahisar- Kendirli 450	24.129 Category II	8	* *Castanea sativa* (63.5) ** *Cistus* (24) *** Fabaceae (3), Rosaceae (5) ****Amaranthaceae (1), *Hedysarum* (1), *Plantago* (2), Scrophulariaceae (0.5)
11	Ortahisar- Ağıllı (2) 500	109.757 Category III	17	* *Castanea sativa* (69.5) *** Apiaceae (3), *Cistus* (6), Fabaceae (5.5), *Rhododendron* (3.5), Rosaceae (3) ****Ailanthus* (0.5), Brassicaceae (2), Campanulaceae (0.5), *Centaurea* (0.5), *Hedysarum* (0.5), Lamiaceae (2), *Laurus nobilis* (1), Malvaceae (0.5), Oleaceae (0.5), Poaceae (0.5), *Rumex* (1)
12	Sürmene- Yeşilköy 930	48.407 Category II	6	* *Castanea sativa* (65) ** Lamiaceae (22) *** Apiaceae (5), *Rhododendron* (6) **** *Cistus* (0.5), Poaceae (1.5)

Table 1. Continued

Sample number	Locations/ Altitude (m)	TPN-10 g /Pollen status	Number of taxon	Pollen spectrum and percentage (%)
13	Sürmene-Gültepe 350	301.616 Category III	14	* *Castanea sativa* (58.5) *** Apiaceae (10.5), Asteraceae (3.5), Oleaceae (5.5), Poaceae (3), Rosaceae (4), *Taraxacum* (3), *Vicia* (3.5) **** *Bellis* (1.5), Brassicaceae (2.5), *Centaurea* (2), Fabaceae (1), Lamiaceae (1), *Rhododendron* (0.5)
14	Sürmene-Yeniay 500	25.732 Category II	7	* *Castanea sativa* (63) ** *Rhododendron* (17), Rosaceae (16) ***** Brassicaceae (0.5), *Ilex* (0.5), *Laurus nobilis* (2.5), Rhamnaceae (0.5)
15	Sürmene-Ormanseven 410	118.522 Category III	7	* *Castanea sativa* (84) *** Fabaceae (5), *Rhododendron* (7.5) **** Boraginaceae (1), *Helianthus* (0.5), Lamiaceae (1), *Salix* (1)
16	Sürmene-Aşağıovalı 430	33.298 Category II	4	* *Castanea sativa* (93) *** Apiaceae (4) **** Fabaceae (1), Rosaceae (2)
17	Tonya-Kalınçam (1) 1.100	369.710 Category III	13	* *Castanea sativa* (70.5) *** *Cistus* (4), Fabaceae (4), *Hedysarum* (7), Poaceae (4), Rosaceae (3) **** Apiaceae (0.5), Boraginaceae (1.5), Amaranthaceae (0.5), Geraniaceae (0.5), Lamiaceae (2), *Rhododendron* (2), *Sanguisorba* (0.5)
18	Tonya-Kalınçam (2) 1.100	26.459 Category II	10	* *Castanea sativa* (84.5) ****Rhododendron* (8) **** Asteraceae (1), Boraginaceae (1), *Cistus* (2), Caprifoliaceae (1), Fabaceae (1), Pinaceae (0.5), Poaceae (0.5), Rosaceae (0.5)
19	Tonya-İskenderli (1) 750	72 164 Category II	12	* *Castanea sativa* (67) ** *Hedysarum* (16) *** Apiaceae (3), Fabaceae (5.5), Poaceae (3) **** Boraginaceae (0.5), *Centaurea* (0.5), Amaranthaceae (0.5), Lamiaceae (1.5), *Populus* (1), Rosaceae (1), *Rumex* (0.5)
20	Tonya-Kalınçam (3) 1.100	46.425 Category II	11	* *Castanea sativa* (79.5) *** Apiaceae (3), *Rhododendron* (5), Rosaceae (5) **** Betulaceae (0.5), Brasicaceae (0.5), *Cistus* (0.5), Fabaceae (2), *Hedysarum* (0.5), *Plantago* (1.5), Poaceae (2)
21	Tonya-Hoşarlı 755	65.210 Category II	11	** *Castanea sativa* (25), *Hedysarum* (30) *** Apiaceae (7.5), *Centaurea* (4), *Cistus* (5), Fabaceae (13.5), Lamiaceae (9), Rosaceae (3) ****Amaranthaceae (0.5), Geraniaceae (1), *Vicia* (1.5)
22	Tonya-Erikbeli 1.500	10.855 Category I	11	** *Carduus* (19.5), *Cistus* (22.5), Fabaceae (18) *** Apiaceae (3), *Castanea sativa* (14.5), *Hedysarum* (4.5), Lamiaceae (6), *Plantago* (3), Rosaceae (6) ****Poaceae (1.5), *Salix* (1.5)
23	Tonya-Zevon 1.200	52.489 Category II	15	* *Castanea sativa* (68.5) *** Fabaceae (5), Poaceae (3.5), *Rhododendron* (12.5) **** Asteraceae (1), *Centaurea* (0.5), *Cistus* (1), *Cynoglossum* (0.5), Caprifoliaceae (2), *Ilex* (0.5), Lamiaceae (2.5), Pinaceae (0.5), *Plantago* (0.5), *Populus* (0.5), Rosaceae (1)
24	Tonya-İskenderli (2) 750	2.845 Category I	12	** *Castanea sativa* (16.5), Fabaceae (30), Lamiaceae (32.5) *** Asteraceae (6), Brassicaceae (4) **** *Anthemis* (1), Apiaceae (2), Boraginaceae (2), *Centaurea* (1), Poaceae (2), Rosaceae (2), *Taraxacum* (1)
25	Tonya-Biçinlik 1.100	6.408 Category I	9	** *Castanea sativa* (16), Fabaceae (31), *Hedysarum* (20), Lamiaceae (18) *** Rosaceae (6.5) **** Brassicaceae (2.5), Campanulaceae (2,5), *Rhododendron* (2.5), Scrophulariaceae (1)
26	Tonya-Kadıralak 1.800	21.926 Category II	14	*** *Cistus* (25), Lamiaceae (26) *** Asteraceae (3.5), *Castanea sativa* (7.5), Fabaceae (10.5), *Hedysarum* (10), Rosaceae (5), Scrophulariaceae (3.5) **** Apiaceae (2.5), Campanulaceae (0.5), Convolvulaceae (0.5), *Cynoglossum* (0.5), Geraniaceae (2.5), Poaceae (2.5)

Table 1. Continued

Sample number	Locations/ Altitude (m)	TPN-10 g / Pollen status	Number of taxon	Pollen spectrum and percentage (%)
27	Tonya-Zere 1.850	26.494 Category II	16	** *Cistus* (20), *Hedysarum* (22.5) *** Asteraceae (12), Fabaceae (8.5), Geraniaceae (5), Lamiaceae (6), Rosaceae (12.5), *Sanguisorba* (4) **** Apiaceae (0.5), Brassicaceae (2), *Castanea sativa* (2.5), Caryophyllaceae (0.5), Poaceae (0.5), Rhamnaceae (2.5), *Taraxacum* (0.5), *Vicia* (0.5)
28	Of- Sulaklı 500	77.014 Category II	9	* *Castanea sativa* (73) *** Boraginaceae (6), Lamiaceae (12), *Tilia* (3) **** Apiaceae (1), *Cistus* (2), Pinaceae (1), Poaceae (1), Rhamnaceae (1)
29	Of-Yazlık 350	46.576 Category II	12	** Apiaceae (26.5), *Castanea sativa* (21.5), *Rhododendron* (26.5) *** *Cistus* (7.5), Fabaceae (4.5), *Hedysarum* (5) **** Campanulaceae (2.5), Caprifoliaceae (0.5), *Ilex* (0.5), Lamiaceae (2), *Laurus nobilis* (0.5), Rosaceae (2.5)
30	Of-Ballıca 400	38.032 Category II	10	* *Castanea sativa* (79) *** *Cistus* (3), Lamiaceae (3.5), *Rhododendron* (10) **** Campanulaceae (0.5), Fabaceae (0.5), Poaceae (1), Rosaceae (1.5), *Rumex* (0.5), *Vicia* (0.5)
31	Of-Ağaçseven 500	159.218 Category III	6	* *Castanea sativa* (92.5) *** *Rhododendron* (3) **** Boraginaceae (0.5), Fabaceae (1), Lamiaceae (1), Rosaceae (2)
32	Of-Uğurlu	94 179 Category II	2	* Castanea sativa (93) *** Lamiaceae (7)
33	Beşikdüzü-Oğuz (1) 302	274.863 Category III	12	* *Castanea sativa* (72.5) *** Apiaceae (7.5), Lamiaceae (4.5), Poaceae (4) ****Amaranthaceae (0.5), *Cistus* (1), Fabaceae (2), *Rhododendron* (2.5), Rosaceae (2), *Rumex* (2), Scrophulariaceae (0.5), *Vicia* (1)
34	Beşikdüzü-Oğuz (2) 470	439.580 Category III	9	* *Castanea sativa* (81) *** Apiaceae (3), *Cistus* (9) **** Brassicaceae (0.5), Fabaceae (1), Lamiaceae (0.5), Poaceae (1), *Rhododendron* (2), Rosaceae (2)
35	Beşikdüzü-Ağaçlı 300	96.960 Category II	11	* *Castanea sativa* (68) *** Boraginaceae (3.5), Amaranthaceae (8), *Cistus* (4.5), Lamiaceae (6.5), Poaceae (4) **** Apiaceae (0.5), *Epilobium* (1.5), *Hedysarum* (1), *Plantago* (1), Rosaceae (1.5)
36	Maçka-Ormanüstü 1.000	7.956 Category I	5	* *Castanea sativa* (55) *** Campanulaceae (7.5), *Cistus* (15), *Echium* (7.5), *Rhododendron* (15)
37	Maçka-Temelli 650	15.985 Category I	6	* *Castanea sativa* (62.5) ** *Rhododendron* (32.5) **** Apiaceae (1), Boraginaceae (1), Malvaceae (2), Poaceae (1)
38	Maçka-Yazlık 800	16.217 Category I	11	** *Laurus nobilis* (16), *Rhododendron* (43) *** Brassicaceae (6), *Cistus* (13), Geraniaceae (5), Lamiaceae (5), Pinaceae (3), Poaceae (3), Rosaceae (4) **** *Ilex* (1), Liliaceae (1)
39	Şalpazarı-Kasımağzı 800	75.873 Category II	7	* *Castanea sativa* (82.5) *** Apiaceae (6), Boraginaceae (4.5) **** *Cistus* (1.5), Lamiaceae (2.5), *Rhododendron* (2.5), *Tilia* (0.5)
40	Şalpazarı-Gökçeköy 1 900	38.611 Category II	9	* *Castanea sativa* (92.5) **** Apiaceae (1), Boraginaceae (2.5), *Cistus* (0.5), Lamiaceae (0.5), Oleaceae (0.5), Pinaceae (1), *Rhododendron* (1), Rosaceae (0.5)
41	Şalpazarı-Gökçeköy 2 900	345.648 Category III	11	* *Castanea sativa* (56) ** *Rhododendron* (29.5) *** Rosaceae (7) **** Apiaceae (1), Brassicaceae (0.5), *Cistus* (1), Lamiaceae (0.5), Oleaceae (1), Pinaceae (1), *Plantago* (2), Poaceae (0.5)

Table 1. Continued

Sample number	Locations/ Altitude (m)	TPN-10 g / Pollen status	Number of taxon	Pollen spectrum and percentage (%)
42	Şalpazarı-Yeşilyurt 900	10.147 Category I	15	* *Castanea sativa* (56) ** *Rhododendron* (27.5) *** Rosaceae (4) **** Apiaceae (2.5), Asteraceae (0.5), Boraginaceae (0.5), Brassicaceae (1), Fabaceae (2), Lamiaceae (0.5), Malvaceae (0.5), Oleaceae (0.5), *Plantago* (2.5), Poaceae (0.5), *Rumex* (1), *Salix* (0.5)
43	Şalpazarı-Akkiriş 700	10.092 Category I	9	*** *Castanea sativa* (21.5), Fabaceae (37.5) *** *Ailanthus* (4), Asteraceae (5), Lamiaceae (11.5), Pinaceae (7.5), *Plantago* (4), Rosaceae (5), Scrophulariaceae (4)
44	Şalpazarı-Gökçeköy-3 900	95.575 Category II	21	** Brassicaceae (33) *** Apiaceae (3), Asteraceae (6), *Cistus* (15), Fabaceae (6.5), *Hedysarum* (6), Lamiaceae (8.5), *Quercus* (3), Rosaceae (5) **** *Castanea sativa* (1.5), *Centaurea* (0.5), Amaranthaceae (0.5), *Echium* (1.5), *Laurus nobilis* (1.5), Malvaceae (0.5), Oleaceae (1.5), Poaceae (0.5), Rhamnaceae (1.5), *Rumex* (0.5), *Salix* (2.5), *Vicia* (1.5)
45	Yomra-Çınarlı 20	214.760 Category III	14	* *Castanea sativa* (69.5) *** Apiaceae (6), *Cistus* (5), *Rhododendron* (10.5) **** Asteraceae (1.5), Brassicaceae (0.5), Fabaceae (2.5), Lamiaceae (0.5), *Laurus nobilis* (0.5), Oleaceae (0.5), *Plantago* (0.5), Poaceae (1), Rhamnaceae (0.5), Rosaceae (1)
46	Yomra-İkisu (1) 350	114.159 Category III	5	* *Castanea sativa* (88) *** Apiaceae (4), *Cistus* (3), *Rhododendron* (4) **** Lamiaceae (1)
47	Yomra-İkisu (2) 350	194.237 Category III	21	* *Castanea sativa* (52) *** Apiaceae (6), Brassicaceae (3.5), Fabaceae (7), Lamiaceae (5.5), Poaceae (3.5), Rosaceae (7.5) **** Asteraceae (1.5), *Cistus* (2.5), Caprifoliaceae (0.5), *Echium* (1), Geraniaceae (0.5), *Hedysarum* (0.5), Malvaceae (1.5), Oleaceae (1.5), Rhamnaceae (1), *Salix* (0.5), *Sanguisorba* (0.5), Scrophulariaceae (2), *Taraxacum* (0.5), *Vicia* (1)
48	Yomra-Çamlıyurt 1.200	10.398 Category I	9	* *Castanea sativa* (70) *** Asteraceae (4), Campanulaceae (5), Lamiaceae (6), *Rhododendron* (10) **** Brassicaceae (2), *Cistus* (1), Fabaceae (1), Malvaceae (1)
49	Yomra-Çamlıyurt -2 750	27.512 Category II	8	* *Castanea sativa* (66) *** Apiaceae (7.5), Fabaceae (7.5), *Rhododendron* (12.5), Rosaceae (3.5) **** Lamiaceae (1.5), *Plantago* (0.5), Rhamnaceae (1)
50	Düzköy-Çayırbağı (1) 1.150	20.932 Category II	18	** *Castanea sativa* (41), *Rhododendron* (16.5) *** Campanulaceae (3), Caryophyllaceae (10), Lamiaceae (4.5), Malvaceae (3), Rhamnaceae (3.5), Rosaceae (11) **** Apiaceae (0.5), Asteraceae (0.5), *Cistus* (0.5), *Epilobium* (1.5), Fabaceae (0.5), Geraniaceae (1.5), *İlex* (0.5), Pinaceae (0.5), Scrophulariaceae (1), *Taraxacum* (0.5)
51	Düzköy-Çayırbağı (2) 1.100	64.416 Category II	11	* *Castanea sativa* (65) *** Apiaceae (7), *Cistus* (3), Lamiaceae (5), *Rhododendron* (13.5) **** Asteraceae (1.5), Fabaceae (1), Liliaceae (0.5), Malvaceae (1), Rhamnaceae (0.5), Rosaceae (2)
52	Düzköy-Çayırbağı (3) 1.100	535.995 Category IV	13	* *Castanea sativa* (70.5) *** Lamiaceae (15) **** Apiaceae (1), Asteraceae (2), Brassicaceae (0.5), *Cistus* (2), Fabaceae (0.5), Geraniaceae (0.5), Malvaceae (2.5), Rhamnaceae (1.5), *Rhododendron* (2), Rosaceae (1.5), *Tilia* (0.5)
53	Düzköy-Mezere 2.000	94.848 Category II	15	* *Castanea sativa* (49.5) *** Apiaceae (13), Brassicaceae (3.5), Fabaceae (7), Lamiaceae (12.5), Rosaceae (4.5) **** Asteraceae (1), Boraginaceae (1.5), *Cistus* (1.5), Convolvulaceae (0.5), Geraniaceae (2.5), Oleaceae (0.5), *Plantago* (0.5), Poaceae (1.5), Ranunculaceae (0.5)

Table 1. Continued

Sample number	Locations/ Altitude (m)	TPN-10 g /Pollen status	Number of taxon	Pollen spectrum and percentage (%)
54	Düzköy-Çayırbağı (4) 1.100	75.371 Category II	7	* *Castanea sativa* (78) *** Apiaceae (12), Rhamnaceae (4) **** Asteraceae (1), Fabaceae (1), Malvaceae (2), *Rhododendron* (2)
55	Çaykara-Demirkapı (1) 1.150	12.341 Category I	11	** Brassicaceae (24.5), *Cistus* (22) *** Apiaceae (5), *Cynoglossum* (14.5), Fabaceae (5.5), Lamiaceae (12), Poaceae (12.5) **** Asteraceae (0.5), *Hedysarum* (0.5), Pinaceae (0.5), Rosaceae (2.5)
56	Çaykara-Taşkıran 1.000	26.583 Category II	5	* *Castanea sativa* (93) *** *Rhododendron* (3) **** Apiaceae (2), Asteraceae (1), *Cistus* (1)
57	Çaykara-Çayıroba (1) 2.200	4.629 Category I	11	* *Castanea sativa* (62.5) *** Campanulaceae (3), *Cistus* (7.5), Fabaceae (6), Lamiaceae (7.5), Rosaceae (6) **** Apiaceae (1.5), Caryophyllaceae (1.5), Geraniaceae (1.5), Oleaceae (1.5), *Rhododendron* (1.5)
58	Çaykara-Çayıroba (2) 2.000	10.504 Category I	12	* *Castanea sativa* (72) *** Brassicaceae (7), *Cistus* (5), *Hedysarum* (3), Lamiaceae (3), *Rhododendron* (3) **** *Cynoglossum* (1), Campanulaceae (2), Fabaceae (1), Liliaceae (1), Rosaceae (1), *Taraxacum* (1)
59	Çaykara-Merkez 300	6.083 Category II	11	* *Castanea sativa* (91.5) **** Apiaceae (2), Brassicaceae (0.5), Fabaceae (1), Lamiaceae (1), Malvaceae (0.5), Pinaceae (0.5), Poaceae (0.5), *Rhododendron* (1), Rosaceae (1), *Tilia* (0.5)
60	Çaykara-Demirkapı (2) 1.150	20.708 Category II	12	** Asteraceae (16), Fabaceae (39.5) *** Apiaceae (12.5), Brassicaceae (3.5), *Cistus* (3), Oleaceae (8), Rhamnaceae (6.5), Rosaceae (5) **** *Echium* (1.5), Geraniaceae (1.5), *Plantago* (1.5), *Vicia* (1.5)
61	Çaykara-Demirkapı (3) 1.150	3.485 Category I	14	****** Fabaceae (27) ******* Apiaceae (4), Boraginaceae (4), *Castanea sativa* (8.5), *Centaurea* (6.5), *Echium* (8), *Hedysarum* (12), Lamiaceae (9), *Plantago* (4), Rosaceae (9) ******** Brassicaceae (2), Malvaceae (2), Oleaceae (2), Poaceae (2)
62	Akçaabat-Yıldızlı 50	63.404 Category II	13	***** *Castanea sativa* (45) ****** *Cynoglossum* (23.5) ******* Apiaceae (14), Lamiaceae (3), Rosaceae (4) ******** Boraginaceae (1), *Cistus* (1.5), Fabaceae (1), *Hedysarum* (2), Poaceae (1.5), Rhamnaceae (1), *Rumex* (2), Scrophulariaceae (0.5)
63	Akçaabat-Uçarsu 250	45.110 Category II	8	***** *Castanea sativa* (60.5) ******* Apiaceae (7.5), *Cistus* (6.5), Lamiaceae (7.5), Poaceae (4.5), Rhamnaceae (3), *Rhododendron* (6), Rosaceae (4.5)
64	Akçaabat-Akçaköy (1) 200	1.525.683 Category V	9	***** *Castanea sativa* (67) ****** Lamiaceae (18) ******* *Cistus* (5), Rosaceae (4.5) ******** Apiaceae (1), Betulaceae (0.5), Fabaceae (2), Geraniaceae (0.5), Poaceae (1.5)
65	Akçaabat-Akçaköy (2) 200	5.564 Category I	12	****** Fabaceae (30), *Hedysarum* (17.5) ******* Brassicaceae (9), *Castanea sativa* (7.5), *Echium* (11), Lamiaceae (9), Rosaceae (8.5) ******** Asteraceae (1.5), *Centaurea* (1.5), *Cistus* (1.5), *Cynoglossum* (1.5), *Salix* (1.5)
66	D.pazarı-Kondu (1) 650	120.583 Category III	6	***** *Castanea sativa* (94) ******* Lamiaceae (3.5) ******** *Plantago* (0.5), Poaceae (0.5), *Rhododendron* (1), Scrophulariaceae (0.5)
67	D.pazarı-Kondu (2) 550	178.166 Category III	9	***** *Castanea sativa* (81) ******* Lamiaceae (4.5), *Rhododendron* (5) ******** Apiaceae (1), *Carduus* (0.5), *Cistus* (2.5), *Plantago* (1), Poaceae (2.5), Rosaceae (2)
68	D.pazarı-Kondu (3) 600	107.508 Category III	5	***** *Castanea sativa* (78) ****** *Cistus* (18) ******** Fabaceae (0.5), Lamiaceae (1), *Rhododendron* (2.5)

Table 1. Continued

Sample number	Locations/ Altitude (m)	TPN-10 g /Pollen status	Number of taxon	Pollen spectrum and percentage (%)
69	D.pazarı-Yenice (1) 2.000	50.190 Category II	5	***** Lamiaceae (82) ******* Asteraceae (6), Brassicaceae (5), *Cistus* (6) ********Rosaceae (1)
70	D.pazarı-Yenice (2) 2.000	38.571 Category II	18	****** *Castanea sativa* (28.5), Rosaceae (36) ******* Apiaceae (4.5), Fabaceae (7.5), Lamiaceae (8), *Rhododendron* (3.5) ******** Asteraceae (1), Brassicaceae (0.5), Campanulaceae (1), Caryophyllaceae (0.5), *Cistus* (2), *Cynoglossum* (0.5), *Hedysarum* (0.5), *İlex* (1.5), Pinaceae (0.5), Poaceae (2), Scrophulariaceae (1.5), *Vicia* (0.5)
71	Araklı-Yeşilce 220	61.633 Category II	11	***** *Castanea sativa* (64.5) ******* Apiaceae (5), Fabaceae (8.5), Lamiaceae (12.5), Poaceae (3.5) ******** Asteraceae (2), Brassicaceae (0.5), Rhamnaceae (0.5), *Rhododendron* (0.5), Rosaceae (2), *Salix* (0.5)
72	Araklı-Değirmencik 1.200	136.103 Category III	9	***** *Castanea sativa* (89.5) ******* *Cistus* (3.5) ******** Apiaceae (1.5), Asteraceae (0.5), Lamiaceae (1.5), Poaceae (1), *Rhododendron* (1.5), Rosaceae (0.5), *Vicia* (0.5)
73	Araklı-Yoncalı 350	1.096.031 Category V	12	* *Castanea sativa* (50) ** Apiaceae (30) *** Lamiaceae (3), Rosaceae (4.5), *Rhododendron* (6.5) **** Asteraceae (1), Brassicaceae (1), *Centaurea* (0.5), *Cistus* (2), Fabaceae (0.5), Geraniaceae (0.5), Rhamnaceae (0.5)
74	Araklı-Merkez 100	85.392 Category II	7	* *Castanea sativa* (58) ** Fabaceae (18) *** *Cistus* (10), Rosaceae (9) **** Apiaceae (2), Lamiaceae (2), Scrophulariaceae (1)
75	Araklı-Bahçecik 1.550	6.266 Category I	11	** *Castanea sativa* (27.5), *Cistus* (37) *** Fabaceae (10), *Hedysarum* (4.5), Lamiaceae (9), Rosaceae (4) **** Apiaceae (2.5), Brassicaceae (1.5), Geraniaceae (1.5), Oleaceae (2), Scrophulariaceae (0.5)
76	Çarşıbaşı-Kavaklı 211	27.342 Category II	8	* *Castanea sativa* (80) *** Lamiaceae (6), Rosaceae (4) **** Asteraceae (2), Brassicaceae (2), *Cistus* (2), Fabaceae (2), Poaceae (2)
77	Köprübaşı-Çifteköprü 530	102.708 Category III	4	* *Castanea sativa* (93.5) *** *Rhododendron* (5) **** Lamiaceae (0.5), *Plantago* (1)
78	Köprübaşı-Gündoğan 350	145.933 Category III	6	* *Castanea sativa* (93) **** Apiaceae (2), Fabaceae (1), Lamiaceae (1), Poaceae (1), Rosaceae (2)
79	Hayrat-Sarmaşık 2.500	6.057 Category I	9	** *Castanea sativa* (30), *Cistus* (20) *** Asteraceae (5), Brassicaceae (4), *Cynoglossum* (3), Fabaceae (10), Lamiaceae (12), Rosaceae (7), Scrophulariaceae (9)
80	Arsin-Harmanlı -1 150	49.039 Category II	8	* *Castanea sativa* (80) *** Apiaceae (4), *Tilia* (6) **** *Cistus* (2), Lamiaceae (2), Oleaceae (2), Poaceae (2), Rosaceae (2)
81	Arsin-Yolüstü 200	245.498 Category III	11	* *Castanea sativa* (90.5) **** Apiaceae (1), Brassicaceae (0.5), *Cistus* (0.5), Convolvulaceae (0.5), Fabaceae (1.5), Lamiaceae (1), Poaceae (0.5), *Rhododendron* (2.5), Rosaceae (1), *Taraxacum* (0.5)
82	Arsin-Harmanlı (2) 150	278.474 Category III	8	* *Castanea sativa* (78) *** Apiaceae (4), Fabaceae (9), Rosaceae (5) **** Asteraceae (0.5), *Cistus* (2), *Quercus* (0.5), Lamiaceae (1)
83	Vakfıkebir-Deregözü (1) 600	832.896 Category IV	7	* *Castanea sativa* (68) *** Apiaceae (12.5), Fabaceae (5.5), *Rhododendron* (9.5) **** Asteraceae (1.5), Lamiaceae (1.5), Rosaceae (1.5)
84	Vakfıkebir-Deregözü (2) 700	43.099 Category II	4	* *Castanea sativa* (92) *** Rosaceae (5) **** Apiaceae (1), Lamiaceae (2)
85	Gümüşhane Yağmurdere 2.000	11.175 Category I	11	** *Castanea sativa* (20), Fabaceae (24), Lamiaceae (32) *** Brassicaceae (4), *Cistus* (8), *Hedysarum* (6), Rosaceae (4) **** Geraniaceae (0.5), Oleaceae (0.5), Poaceae (0.5), Scrophulariaceae (0.5)

Honey samples according to total pollen count (TPN-10 g) amounts are grouped; 22.3% Category I (19 samples), 45.9% Category II (39 samples), 27% Category III (23 samples), 2.4% Category IV (2 samples) and 2.4% Category V (2 samples) (Figure 5, Table 1). The total number of pollen in 10 grams

of honey (TPN-10 g) has been determined to be at least 2845 (sample 24) and at most 1 525 683 (sample 64) (Table 1). The graph showing the grouping of honey samples according to the amount of TPN-10 g is given below (Figure 5).

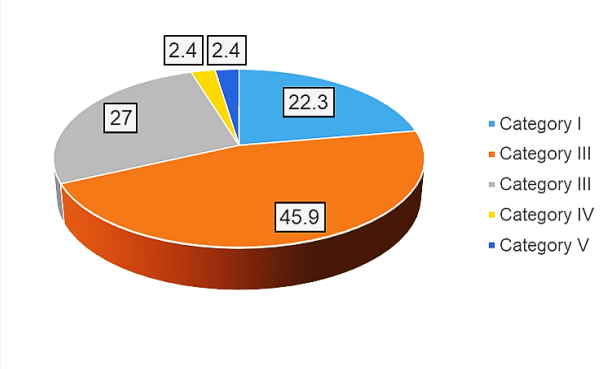


Figure 5. Percentage distribution of honey samples according to total pollen number (TPN-10 g)

Correlation analysis was performed to examine linear relationships between variables. This analysis used altitude, TPN-10 g, number of taxa, and pollen status variables. The correlation matrix was calculated, and the heat map was visualized. The map expresses correlation coefficients with color tones; dark colors indicate high correlation, and light colors indicate low correlation.

When the correlation between altitude and both TPN-10 g and pollen status was calculated, it was determined that there was a negative relationship between them. As the altitude increases, there is a decrease in TPN-10 g value and pollen status. However, there is a positive relationship between altitude and the number of taxa. In other words, as the altitude increased, the number of taxa contained in honey also increased. Similarly, it was determined that there was a positive relationship between the total pollen count and the pollen status. On the other hand, when the relationship between the number of taxa and both the total pollen number and the pollen status was examined, almost no connection was found between these characters. It was determined that the results of the correlation analysis were compatible with the results of the research. Pearson correlation coefficients of the analyses are given in Figure 6.

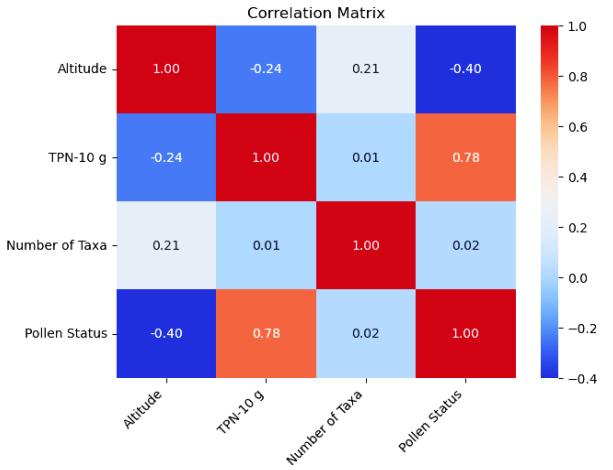


Figure 6. Correlation matrix heat map made with altitude, TPN-10 g, number of taxa, and pollen status characters

Pollen spectrum analysis examined the percentage distribution of plant taxa found in honey. By defining the relevant columns, the percentages of occurrence in honey samples were calculated for each plant taxon, and these data were visualized using a polar plot. Color intensity indicates the abundance of taxon types in honey (Figure 7).

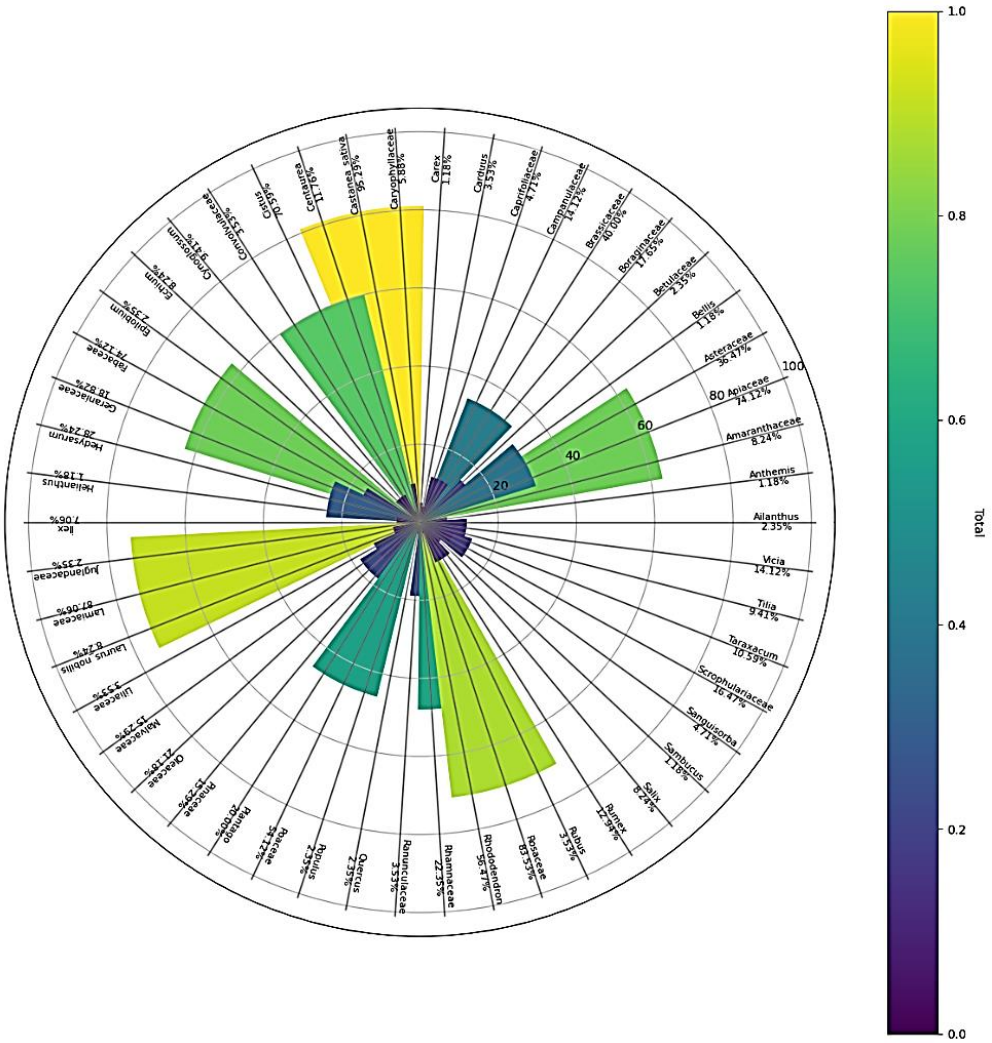


Figure 7. Polar graph resulting from pollen spectrum analysis

A cluster analysis was performed on all the data (altitude, TPN-10 g, pollen spectrum, and pollen status) characteristics of the 85 honey samples produced in Trabzon (Türkiye). When the hierarchical clustering method was applied, the samples were grouped in a tree structure, and a dendrogram was drawn. While each node represents an example, the distances between nodes show the similarity or distance between these examples. There are 2 main groups, each with 2 subgroups of honey samples according to the hierarchical clustering dendrogram. Samples in the green and orange subsets have similar characteristics and converge at small distances. The same situation is observed in the red and purple clusters. When the subclusters are examined, the closest similarity is the red subcluster, while the examples in the green subcluster converge at larger distances, and these clusters show more significant differences (Figure 8).

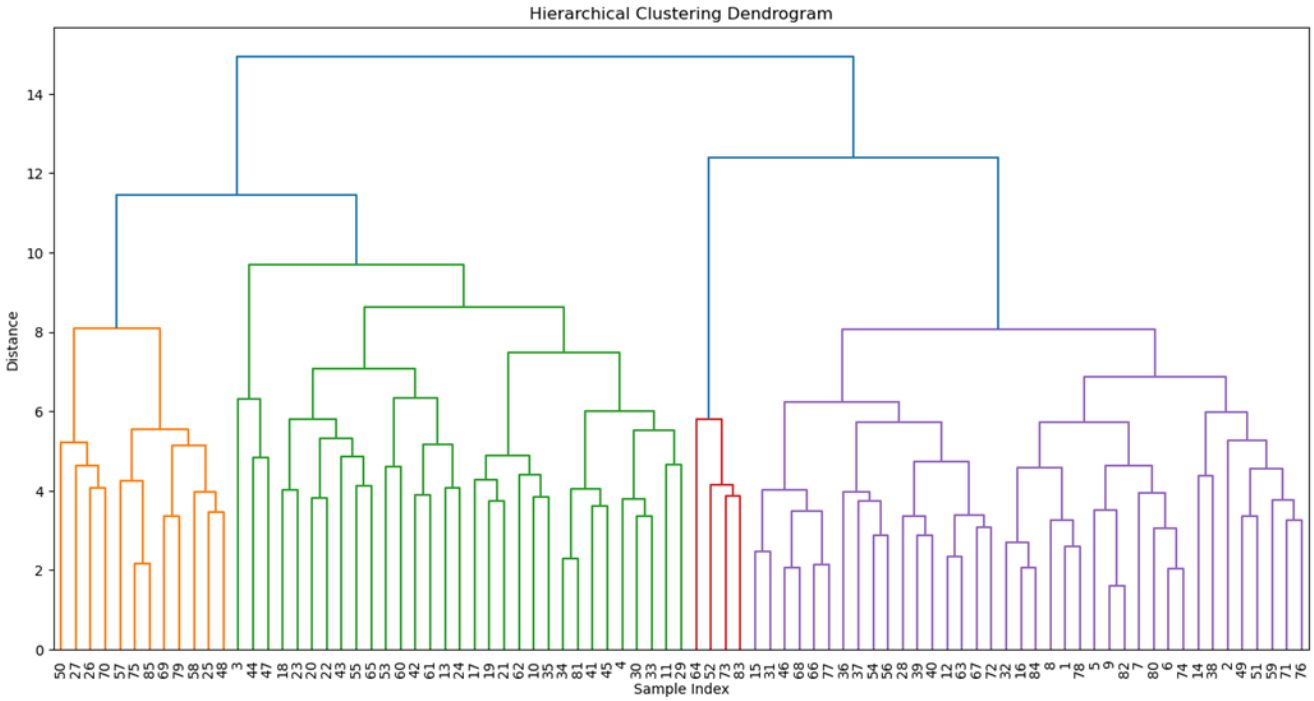


Figure 8. Cluster dendrogram of the honey samples investigated using all data (altitude, TPN-10 g, pollen spectrum, and pollen status)

DISCUSSION

To develop beekeeping in a region and obtain high efficiency from beekeeping, along with the colony strength, nectar and pollen must be diverse and abundant (Bijev 1958). Therefore, knowing what honey plants are necessary to make beekeeping activities more efficient.

As a result of pollen analysis of Trabzon honey, 50 taxa belonging to 36 families were identified. Most of these taxa belong to Apiaceae, Asteraceae, Brassicaceae, Cistaceae, Ericaceae, Fabaceae, Fagaceae, Lamiaceae, Poaceae and Rosaceae. Similarly, it is known that the plant taxa that the source of flower honey produced in Türkiye are Asteraceae, Brassicaceae, Fabaceae, Fagaceae, Lamiaceae, Malvaceae, Myrtaceae, Oleaceae, and Scrophulariaceae families (Sorkun et al. 1999). Sorkun and Doğan (1995), reported in their study that the diversity of taxa in the dominant group is always less, and the diversity of taxa in the trace group is always greater. In this study, as a result of pollen analysis, it was observed that the trace group had the highest number of taxa, followed by the minor, secondary, and dominant groups (Table 1).

The dominance of *Castanea sativa* pollen in the honey samples clearly indicates that this species is one of the most important nectar and pollen sources in the region. The presence of *Castanea sativa* pollen in 81 out of 85 honey samples (Figure 9), and its classification as dominant in 65 of them, secondary in 10, minor in 4, and trace in 2 samples (Table 1), underlines its pivotal role in local honey production. This dominance is closely related to Trabzon's unique geography and vegetation. Located along the Black Sea coast, the province has a humid and mild climate that supports the growth of broad leaved moist forests where *Castanea sativa* is widespread. Naturally growing at elevations of 300-1000 meters, chestnut trees bloom in June, providing abundant nectar and pollen for honeybees (Dalgıç 1994, Sorkun and Yuluğ 1985).

Accordingly, four honey samples were identified as unifloral and labeled as chestnut honey. However, in many multifloral honey samples, *Castanea sativa* remained the dominant pollen source. In our research, several samples with over 80% *Castanea sativa* pollen were still classified as multifloral due to the presence of minor amounts of other taxa. This

reflects both the foraging behavior of bees and the botanical richness of the region.

From both floristic and apicultural perspectives, chestnut has a high value. In our study, 65 samples with dominant *Castanea sativa* pollen also had high total pollen counts (TPN-10 g), confirming that this species is dominant not only floristically but also in terms of pollen production. Particularly in the red cluster subgroup, samples had notably high TPN values, supporting their chestnut origin. The widespread presence of chestnut pollen is attributed not only to the tree's abundance but also to its preference by bees. Its small sized pollen grains are easily collected, its flowering is intense, and its fragrance is highly attractive to bees (Dalgıç 1994, Sorkun and Doğan 1995).

Samples 64 and 73 had the highest total pollen counts (1.525.683 and 1.096.031), placing them in Category V, which is typically associated with additive honey. In both samples, *Castanea sativa* was the dominant taxon. According to the study by Sorkun and Doğan (2002), total pollen count (TPN-10 g) is an important parameter for distinguishing natural from artificial honey. In line with their findings, these two samples could be interpreted as not being natural. However, considering the high prevalence of *Castanea sativa* pollen in our samples and the fact that the chestnut tree is a highly attractive, nectar and pollen rich source for bees, these high pollen count samples are likely to be natural as well. It is also important to note that TPN-10 g alone is not sufficient to determine the authenticity of honey; chemical and physical analyses are also required. Supporting findings from neighboring provinces with similar ecological characteristics such as Rize, Sinop, Adapazarı and Kastamonu have also reported dominant levels of *Castanea sativa* pollen in honey samples (Erdoğan et al. 2006; 2009, Özler 2015, Sorkun et al. 1989, Sorkun and Yuluğ 1985, Uzunca et al. 2023). These consistent results reinforce the significant potential of the Black Sea region in chestnut honey production.

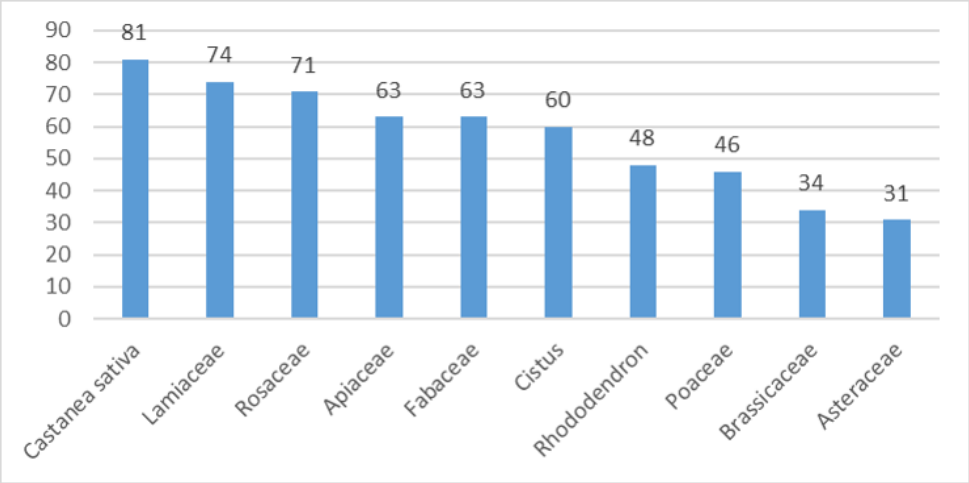


Figure 9. The most common taxa in honey samples

Lamiaceae and Rosaceae were identified as the second and third most frequently encountered pollen families in Trabzon honey, respectively. Lamiaceae pollen was detected in 74 out of 85 samples, being dominant in 1, secondary in 6, minor in 35, and present in trace amounts in 33 samples. Due to their long flowering periods, aromatic nature, and rich nectar content, members of the Lamiaceae family constitute an important source for beekeeping (Sorkun 1986). Rosaceae pollen was identified in 71 samples, mostly in minor (39 samples) and trace (30 samples) amounts. The prevalence of this family's pollen is likely due to its early spring blooming, high pollen production, and the abundance of fruit orchards in the region. The presence of pollen from both families has been frequently reported in studies conducted in various regions of Türkiye (Dalgıç 1994, Erdoğan et al. 2006, 2009, Sabuncu et al. 2002, Sorkun et al. 1999, Sorkun ve İnceoğlu 1984).

The detection of pollen from the *Rhododendron* genus, belonging to the Ericaceae family, in honey holds particular significance. It has been reported that honey produced by bees collecting nectar from *R. ponticum* L. and *R. luteum* Sweet, both species of this taxon, may cause poisoning when consumed in excess due to its high grayanotoxin content. These types of honey have also been shown to affect the cardiovascular system (Ergun et al. 2005). *Rhododendron* pollen contains an alkaloid called andromedotoxin (Grayanotoxin I), and excessive exposure to such honey has been associated with symptoms of hypotension, including vomiting, dizziness, visual disturbances, and tinnitus (Tutkun 2000). *Rhododendron* pollen was detected in 48 out of 85 honey samples. Among these, it was found as a secondary pollen type in 7 samples, as a minor pollen in 24 samples, and in trace amounts in 17 samples. Pollen of this taxon has also been identified in studies conducted in provinces neighboring Trabzon (Erdoğan et al. 2006, 2009, Sorkun et al. 1989). In the present study, *Rhododendron* pollen was detected in 16 of Trabzon's 18 districts. Notably, the amount of pollen increased in honey samples collected from districts such as Düzköy, Maçka, Of, Sürmene, and Şalpazarı, where this taxon is known to be densely distributed.

In this study, statistical analyses conducted on 85 honey samples produced in Trabzon provide significant insights into the relationship between the pollen content of honey, its floristic diversity, and environmental factors. The results of the Pearson correlation analysis revealed meaningful relationships between altitude and certain variables. A positive correlation was found between altitude and the number of taxa, indicating that a greater diversity of plant taxa was observed at higher elevations, and these taxa were reflected in the honey samples. This suggests that certain plant species may be more dominant at higher altitudes or that bees in these regions may utilize a broader range of floral sources. However, the lack of a significant correlation between the number of taxa and both the total pollen count and pollen status indicates that diversity does not necessarily correlate with abundance. In other words, a honey sample may contain pollen from many different plant taxa, but each taxon may be represented in small quantities.

In the pollen spectrum analysis, the percentage distributions of plant taxa present in the honey samples were examined. This analysis enabled the identification of the botanical origins of the honey and their relative abundances. The polar plot served as an effective tool for understanding the floristic composition, with taxa shown in more intense colors representing dominant plant sources in the honey.

The hierarchical clustering analysis grouped the honey samples based on their similarities and identified two main clusters, each consisting of two subgroups. These subgroups were differentiated based on the floristic composition, total pollen count, and environmental characteristics of the samples. The red subgroup, which includes the most similar samples, is noteworthy for containing honey samples with the highest total pollen counts. This finding highlights the total pollen count (TPN-10 g) as the most influential variable in the clustering process. In contrast, the green subgroup, which showed greater distances between samples, reflects higher variability among its samples, indicating a more heterogeneous set of characteristics.

Conclusion: The findings of this study reveal the significant potential of the Black Sea Region particularly Trabzon province for the production of high-quality chestnut honey. This highlights the importance of regional branding, improvement of quality control processes, and the development of sustainable beekeeping practices. In this context, expanding floristic mapping studies and supporting them with chemical analyses will facilitate science-based progress in the apiculture sector.

Acknowledgements: The authors would like to thank PhD. Cahit Doğan who passed away in June 2023 for support in diagnosis. This study has been produced from a doctoral thesis.

Author Contribution: The authors have contributed equally.

Data Availability: Data are available in the manuscript.

Declaration of Interest: The authors declare they have no conflict of interest.

Ethics: Not applicable.

Financial Support: This study has been supported by the Scientific Research Projects (BAP) unit of Gazi University with the project number 05/2009-02.

REFERENCES

Atanassova J, Yurukova L, Lazarova M. Pollen and inorganic characteristics of Bulgarian unifloral honeys. Czech J Food Sci. 2012; 30(6): 520-526, doi.org/10.17221/44/2012-CJFS

Aytuğ B. İstanbul Çevresi Bitkilerinin Polen Atlası. İstanbul Üniversitesi Orman Fakültesi Yayınları, İstanbul, Türkiye, 1971, p. 2-325.

Bağcı Y, Tunç B. Pollen analysis of honeys from Hadim-Taşkent (Konya) and Sarıveliler (Karaman) region. Selçuk Üniversitesi Fen Fakültesi Fen Dergisi. 2006; 2: 73-82.

Barth OM, Luz CFP. Melissopalynology in Brazilian restinga areas, a mini review. Grana. 2022; 61: 355-365, doi.org/10.1080/00173134.2022.2117570

Bayram EN. Quality evaluation and pollen profile of honey samples from different locations. Progress in Nutrition. 2019; 21: 928-934, doi.org/10.23751/pn.v21i4.8862

Bayram NE, Yüzer MO, Bayram S. Melissopalynology analysis, physicochemical properties, multi-element content, and antimicrobial activity of honey samples collected from Bayburt, Turkey. U. Bee J. 2019;19(2): 161-176, doi.org/10.31467/uluaricilik.618289

Bijev B. Rıkovodstvo Za Uprajneniya Po Pçelarstvo. Sofya,1958, p. 27-34.

Blackmore S, Ferguson IK. Pollen and spores: Form and function. Academic Press, London, United Kingdom, 1986, p.1-443.

Bogdanov S, Ruoff K, Persano Oddo L. Physico-chemical methods for the characterisation of unifloral honeys: A review. Apidologie. 2004; 35: 4-17, doi.org/10.1051/apido:2004047

Crane, E. Bees and Beekeeping: Science, Practice and World Resources. Heinemann Professional Publishing, London, United Kingdom, 1990, p.1-614.

Dalgıç R. Biochemical and palynological investigation of Turkish Aegean region honey. Ege University, Institute of Science, PhD Thesis, İzmir, 1994, (erişim tarihi. 21.08.2015) https://tez.yok.gov.tr/UlusalTezMerkezi/tezSorguSonucYeni.jsp

Davis PH. Flora of Turkey and the East Aegean Islands: Vol 1-10. Edinburg University Press, Edinburg, United Kingdom, 1965-2000.

Deodikar G.B. Melittopalynology. Indian Bee Journal. 1965; 27:59-69.

Erdoğan N, Pehlivan S, Doğan C. Pollen analysis of honeys from Hendek, Akyazı, and Kocaali districts of Adapazarı province (Turkey). Mellifera. 2006; 6(10-12): 20-27.

Erdoğan N, Pehlivan S, Doğan C. Pollen analysis of honeys from Sapanca, Karapürçek, Geyve and Taraklı districts of Adapazarı province (Turkey). Mellifera. 2009; 9(17): 9-18.

Erdtman G. Handbook of palynology, morphology, taxonomy and ecology. Munksgaard International Publishers, Copenhagen, Denmark, 1969, p. 8-496.

Ergun K, Tüfekçioğlu O, Aras D, Korkmaz Ş, Pehlivan S. A rare cause of atrioventricular block: Mad Honey intoxication. International Journal of Cardiology. 2005; 99(2): 347-348, doi.org/ 10.1016/j.ijcard.2003.11.041

Everitt BS, Landau S, Leese M, Stahl D. Cluster Analysis (5th ed.). John Wiley & Sons Ltd, Chichester, United Kingdom, 2011, p. 20.

Faegri K, Iversen J. Textbook of pollen analysis. Wiley and Sons Publishers, Chichester, United Kingdom,1989, p.1-328.

Giorgi A, Madeo M, Baumgartner J, Lozzia GC. The relationships between phenolic content, pollen diversity, physicochemical information and radical scavenging activity in honey. Molecules. 2011;16: 336-347, doi.org/10.3390/molecules16010336

Güner A, Aslan S, Ekim T, Vural M, Babaç MT. (edlr.). Türkiye Bitkileri Listesi (Damarlı bitkiler), Nezahat Gökyiğit Botanik Bahçesi ve Flora Araştırmaları Derneği, İstanbul, 2012.

Güzel F. Melitopalynological, physical and chemical analysis of Ardahan province honey. Hacettepe University, Institute of Science, MSc Thesis, Ankara, 2014, (erişim tarihi 10.09.2015), https://tez.yok.gov.tr/UlusalTezMerkezi/tezSorguSonucYeni.jsp

Harris CR, Millman KJ, Walt SJ, van der Gommers R, Virtane P, Cournapeau D, Wieser, E, Taylor J, Berg S, Smith NJ, Kern R, Picu M,

Hoyer S, Kerkwijk MH, van Brett M, Haldane A, R'ıo JF, del Wiebe M, Peterson P, Oliphant TE. Array programming with NumPy. Nature. 2020; 585(7825): 357-362, doi.org/10.1038/s41586-020-2649-2

Hunter JD. Matplotlib: A 2D Graphics Environment. Computing in Science & Engineering. 2007; 9(3): 90-95.

Jose M, Demalsy F, Parent J, Alexander AS. Microscopic analysis of honey from Manitoba, Canada. Journal of Apicultural Research. 1989; 2(1): 41-49.

Kapp RO. Illinoian and Sangamon vegetation in Southwestern Kansas and Adjacent. University of Michigan Museum of Paleontology. 1971; 45(3): 494-501.

Kenjeric D, Mandić ML, Primorac L, Čačić F. Flavonoid pattern of sage (*Salvia officinalis* L.) unifloral honey. Food Chemistry. 2008; 110(1): 187-192, doi.org/10.1016/j.foodchem.2008.01.031

Kurt L. Biyoiklim, Ed. Güner A, Ekim T, "Resimli Türkiye Florası, Vol 1", Ali Nihat Gökyiğit Vakfi, Flora Araştırmaları Derneği ve Türkiye İş Bankası Kültür Yayınları, İstanbul, 2014, p. 1:117-137.

Louveaux J, Maurizio A, Vorwohl G. Methods of melissopalynology. Bee World. 1978; 59(4):139-157, doi.org/10.1080/0005772X.1978.11097714

Makhloufi C, Kerkvliet JD, D'albore GR, Choukri A, Samar R. Characterization of Algerian honeys by palynological and physico-chemical methods. Apidologie. 2010;41: 509-521, doi.org/10.1051/apido/2010002

Matthew P, Alice D, Jessica M, Jessica P. Melissopalynology of honey from Ponteland, UK, shows the role of *Brassica napus* in supporting honey production in a suburban to rural setting. Palynology. 2018; 42(3): 400-405, doi.org/10.1080/01916122.2017.1362485

Maurizio A. Microscopy of honey, Ed. Crane E, "Honey. A comprehensive survey" Heinemann, London, United

Kingdom, 1979. p. 1-608.

McKinney W. Data Structures for Statistical Computing in Python. 9th Python in Science Conference, Book of proceedings, Austin, 28 June-3 July 2010, p. 56-61, doi.org/10.25080/Majora-92bf1922-00a

Moar NT. Pollen analysis of New Zealand honey. Journal of Agricultural Research. 1985; 28: 38-70.

Oddo LP, Stefanini R, Piazza MG, Accorti M. Diagnosis of Unifloral Honeys, III. Application of a Statistical Approach to Honey Classification. Apicoltura. 1988; 4: 27-38.

Oddo LP, Piazza MG, Sabatini AG, Accorti M. Characterization of unifloral honeys. Apidologie. 1995; 26 (6): 453-465, https://hal.science/hal-00891311/document

Oddo PL, Bogdanov S. Determination of honey botanical origin: problems and issues. Apidologie. 2004; 35: S2-S3, doi.org/ 10.1051/apido:2004044

Özler H. Melissopalynological analysis of honey samples belonging to different districts of Sinop, Turkey. Mellifera. 2015;15 (1): 1-11.

Palabaş-Uzun S, Anşin R. Subalpine and Alpine Flora of Altındere Valley (Maçka, Trabzon). Turkish Journal of Botany. 2006; 30(5): 381-398.

Palabaş-Uzun S, Terzioğlu S. Sisdağı (Şalpazarı/Trabzon) ve Yöresinin Florası. Düzce Üniversitesi Bilim ve Teknoloji Dergisi. 2019; 7: 1523-1573, doi.org/10.29130/dubited.538068

Pearson K. Mathematical contributions to the theory of evolution. III. Regression, heredity, and panmixia. Philosophical Transactions of the Royal Society of London. Series A, Containing Papers of a Mathematical or Physical Character. 1896;187: 253-318.

Pedregosa F, Varoquaux G, Gramfort A, Michel V, Thirion B, Grisel O; Blondel M, Prettenhofer P, Weiss R, Dubourg V, Vanderplas J, Passos A, Cournapeau D, Brucher M, Perrot M, Duchesnay E. Scikit-Learn: Machine Learning in Python. Journal of Machine Learning Research. 2011;12(85):2825-2830, doi.org/10.48550/arXiv.1201.0490

Pehlivan S. Türkiye'nin Alerjen Polenleri Atlası. Ünal Offset Matbaacılık, Ankara, Türkiye, 1995, s. 6-165.

Prodolliet J, Hischenhuber C. Food authentication by carbohydrate chromatography. Zeitschrift für Lebensmittel Untersuchung und Forschung.

1998; *A* 207(1): 1-12, doi.org/10.1007/S002170050286x

Punt W. The Northwest European Pollen Flora I. Elsevier Scientific Publishing Company, Amsterdam, 1976, p.1-145.

Punt W, Clarke GCS. The Northwest European Pollen Flora II. Elsevier Scientific Publishing Company, Amsterdam, 1980, p. 1-30.

Sabuncu İ, Bıçakçı A, Tatlıdil S, Malyer H. Bursa piyasasında satılan ve Uludağ ile Karacabey yörelerine ait olduğu belirtilen polenlerin mikroskobik analizi. Uludağ Arıcılık Dergisi. 2002; 2(3): 3-9.

Samrat S, Riju N, Adrish D, Nripendra L, Avijit K. Melissopalynological analysis of Apis honey from northern districts of West Bengal. Grana. 2023; 62(5-6): 352-368, doi.org/ 10.1080/00173134.2023.2273542

Sanz ML, Gonzalez M, Lorenzo C, Sanz J, Castro I. A contribution to the differentiation between nectar honey and honeydew honey. Food Chemistry. 2005; 91: 313-317, doi.org/10.1016/j. foodchem.2004.06.013

Sawyer R. Honey identification. Cardiff Academic Press, United Kingdom, 1988, p. 1-115.

Silici S, Gökceoglu M. Pollen analysis of honeys fromMediterranean region of Anatolia. Grana. 2007; 46: 57-64. doi.org/10. 1080/00173130601138783

Sorkun K. Balda polen analizi. Teknik Arıcılık Dergisi, 1985; 1:28-30.

Sorkun K. Ballı bitkiler. Teknik Arıcılık Dergisi. 1986; 4: 7-10.

Sorkun K. Türkiye'nin Nektarlı Bitkileri, Polenleri ve Balları. Palme Yayıncılık, Ankara, Türkiye, 2008, p.1-352.

Sorkun K, İnceoğlu Ö. İç Anadolu Bölgesi ballarında polen analizi. Doğa Bilim Dergisi. 1984; 8(2): 222-228.

Sorkun K, Yuluğ N. Rize-İkizdere yöresi ballarında polen analizi ve antimikrobik özellikleri. Doğa Bilim Dergisi. 1985; A2 9(1):118-123.

Sorkun K, Güner A, Vural M, Rize ballarında polen analizi. Doğa Türk Botanik Dergisi. 1989;13(3): 547-554.

Sorkun K, Doğan C. Türkiye'nin çeşitli yörelerinden toplanan ballarda polen analizi. Hacettepe Fen ve Mühendislik Bilimleri Dergisi. 1995; 24(A-C): 15-24.

Sorkun K, Doğan C, Gümüş Y, Başoğlu N, Bulakeri N, Ergün K. "Türkiye'de üretilen doğal ve yapay kaynaklı balların ayırt edilmesine esas olacak fiziksel, kimyasal ve palinolojik kriterlerin belirlenmesine yönelik araştırmalar", Tübitak Tarım, Orman ve Gıda Teknolojileri Araştırma Grubu, Proje No: TOGTAG-1270, 1999.

Sorkun K, Doğan C. The importance of the total number of pollen types in 10 g of honey in distinguishing between natural honey and artificial honey produced in Turkey. Mellifera. 2002; 2(3): 34-38.

Stockmarr J. Tablets with spores used in absolute pollen analysis. Pollen spores. 1971; 13: 615-621.

Tarım ve Orman Bakanlığı. Arıcılık İstatistikleri. https://arastirma.tarimorman.gov.tr/aricilik/Link/2/Aricilik-Istatistikleri, (Erişim tarihi: 19.04.2025).

Tosunoğlu H, Tosunoğlu A, Ergün N, Bıcakcı A. Botanical characterization of natural honey samples from a high altitudinal region, Gümüshane, east-Türkiye. Grana. 2023; 62(1): 59-69. doi.org/10.1080/00173134.2022.2138532

Tutkun E. Teknik arıcılık el kitabı. Türkiye Kalkınma Vakfı Yayınları, Ankara, 2000, p.20-35.

Uzun A, Terzioğlu S. Vascular Flora of Forest Vegetation in Altındere Valley (Maçka-Trabzon). Turk J Bot. 2008; 32:135-153.

Uzunca H, Çelemli ÖG, Biyiklioğlu O, Karabicak S, Çeter T. Characterisation of Kastamonu honeys by palynological and physicochemical methods. Grana. 2023; 62(3), 192-205. doi.org/10.1080/00173134.2023.2210578

Waskom ML. Seaborn: statistical data visualization. Journal of Open Source Software, 2021; 6(60): 3021, doi.org/10.21105/joss.03021

Wodehouse RP. Pollen Grains. Their Structure, Identification, and Significance in Science and Medicine. Mc. Graw-Hill Book Company, New York, 1935, p.13-400.

Citation/Atıf: Birinci C, Kolaylı S. A comparatiive study of phenolic and antioxidant properties of propolis and sumac (Rhus coriaria L.). U. Bee J. / U. Arı D. 2025; 25 (1): 131-139. DOI: 10.31467/uluaricilik.1624649

ARAŞTIRMA MAKALESI / RESEARCH ARTICLE

A COMPARATIVE STUDY OF PHENOLIC AND ANTIOXIDANT PROPERTIES OF PROPOLIS AND SUMAC (*RHUS CORIARIA* L.)

Propolis ve Sumak'ın (*Rhus coriaria* L.) Fenolik ve Antioksidan Özelliklerinin Karşılaştırmalı Bir Çalışması

Ceren BİRİNCİ[1]*, Sevgi KOLAYLI[1]

[1*]Department of Chemistry, Faculty of Sciences, Karadeniz Technique University, Trabzon, TÜRKİYE, Corresponding author/ Yazışma Yazarı: E-mail E-mail: cerendidar.birinci@gmail.com, ORCID No: 0000-0002-0167-6809

[1]Department of Chemistry, Faculty of Sciences, Karadeniz Technique University, Trabzon, TÜRKİYE, E-mail: skolayli61@yahoo.com, ORCID No:0000-0003-0437-6139

Received / Geliş: 21.01.2025 Accepted / Kabul: 05.05.2025 DOI: 10.31467/uluaricilik.1624649

ABSTRACT

Sumac (*Rhus coriaria* L.) is a sour spice widely used in Türkiye and especially in the Southeastern Anatolia region to give a distinctive color and sharp taste to dishes. Propolis is a resinous natural product collected from beehives, and due to its wide range of biologically active properties, it is an important dietary supplement. In this study, the phenolic and antioxidant properties of sumac and propolis from Malatya region were examined and compared. Ethanolic extracts of sumac and propolis were analyzed for their phenolic properties, including total phenolic content (TPC), total flavonoid content (TFC), and phenolic composition. The phenolic profile was determined using HPLC-PDA based on 26 phenolic standards. Antioxidant activities were evaluated using the ferric reducing/antioxidant power (FRAP) assay and ABTS radical scavenging activity. The total phenolic content was measured at 49.12 mg GAE/g in sumac and 159.30 mg GAE/g in propolis. Sumac was found to be particularly rich in phenolic acids, including gallic acid, protocatechuic acid, and syringic acid. In contrast, propolis exhibited a higher content of flavonoids such as pinocembrin, hesperetin, caffeic acid, and CAPE. Chrysin was identified as a common flavonoid present in both natural products. The findings indicate that sumac contains a significant concentration of biologically active compounds, similar to propolis, and therefore has the potential to be utilized not only as a spice but also as a dietary supplement.

Keywords: Sumac, Propolis, Phenolics, Antioxidant activity

ÖZ

Sumak (*Rhus coriaria* L.), Türkiye'de ve özellikle Güneydoğu Anadolu Bölgesi'nde yemeklere belirgin bir renk ve keskin bir tat vermek için yaygın olarak kullanılan ekşi bir baharattır. Propolis, arı kovanlarından toplanan reçineli bir doğal üründür ve çok çeşitli biyolojik olarak aktif özellikleri nedeniyle önemli bir besin takviyesidir. Bu çalışmada, Malatya bölgesinden elde edilen sumak ve propolisin fenolik ve antioksidan özellikleri incelenmiş ve karşılaştırılmıştır. Sumak ve propolisin etanol ekstraktları, toplam fenolik içerik (TPC), toplam flavonoid içerik (TFC) ve fenolik bileşim dahil olmak üzere fenolik özellikleri açısından analiz edilmiştir. Fenolik profil, 26 fenolik standarda dayalı HPLC-PDA kullanılarak belirlenmiştir. Antioksidan aktiviteler, ferrik indirgeyici/antioksidan güç (FRAP) testi ve ABTS· radikal süpürücü aktivitesi kullanılarak değerlendirilmiştir. Toplam fenolik içerik, sumağın içinde 49,12 mg GAE/g ve propolisin içinde 159,30 mg GAE/g olarak ölçülmüştür. Sumak, gallik asit, protokatekuik asit ve şiringik asit dahil olmak üzere fenolik asitler açısından özellikle zengin bulundu. Buna karşılık, propolis, pinocembrin, hesperetin, kafeik asit ve CAPE gibi daha yüksek bir flavonoid içeriği sergiledi. Krisin, her iki doğal üründe de bulunan yaygın bir flavonoid olarak

tanımlandı. Bulgular, sumağın propolise benzer şekilde önemli miktarda biyolojik olarak aktif bileşik içerdiğini ve bu nedenle yalnızca bir baharat olarak değil aynı zamanda bir diyet takviyesi olarak da kullanılma potansiyeline sahip olduğunu göstermektedir.

Anahtar kelimeler: Sumak, Propolis, Fenolik, Antioksidan aktivite

GENİŞLETİLMİŞ ÖZET

Amaç: Sumak (*Rhus coriaria* L.), Türkiye'de ve özellikle Güneydoğu Anadolu Bölgesi'nde yemeklere kendine özgü bir renk ve keskin bir tat vermek amacıyla yaygın olarak kullanılan ekşi bir baharattır. Propolis ise arı kovanlarından toplanan reçinemsi bir doğal ürün olup, geniş biyolojik aktif özellikleri sayesinde önemli bir besin takviyesidir. Sumak ve propolis her ikisi de koyu renkli doğal ürünlerden olup, yüksek fenolik madde miktarlarına sahip doğal bitkisel karışımlardır. Bu çalışmanın amacı Malatya bölgesinde üretilen sumak ve propolisin örneklerinin fenolik ve antioksidan özelliklerinin incelenmesi ve karşılaştırılmasıdır.

Gereç-Yöntem: Malatya Doğanşehir'den toplanan Sumak ve propolis örneklerinden elde edilen etanolik özütlerin fenolik madde miktarları toplam fenolik madde miktarı (TFM), toplam flavonoid madde miktarı (TFM) cinsinden ölçüldü. Fenolik kompozisyonları ise 26 fenolik bileşene göre valide edilmiş HPLC-PDA ile analiz edildi Antioksidan özellikleri ise, demir-III-indirgeme /antioksidan test (FRAP) ve ABTS radikali temizleme aktivitesine göre belirlendi.

Bulgular: Etanolik örneklerin toplam fenolik madde miktarları, sumakta 49,12 mg GAE/g, propoliste ise 159,30 mg GAE/g olarak ölçüldü. Sumak bitkisinin, gallik asit, protokatekuik asit ve şiringik asit fenolik asitlerce zengin açısından zengin bulundu. Buna karşılık, propolisin flavonoidlerce zengin içerikli pinosembrin, hesperetin, kafeik asit ve CAPE (kafeik asit fenetil esteri) bulundu. Her iki doğal ürünün krisince zengin olması dikkat çekti.

Sonuç: Bu bulgular, sumak ve propolisin her ikisinin de biyolojik olarak aktif bileşenler açısından zengin olduğunu ve sumak bitkisinin sadece bir baharat olarak değil, aynı zamanda bir besin takviyesi olarak da değerlendirilebileceğini ortaya koymaktadır. Her ikisinin birlikte oluşturacağı karışımların, sinerjik olarak etkin potansiyel oluşturması düşünülmektedir. Bunun için daha ileri çalışmalara ihtiyaç vardır.

INTRODUCTION

Sumac (*Rhus coriaria* L.), a member of the Anacardiaceae family, is native to the Mediterranean region and typically grows as a shrub reaching heights of 1 to 3 meters. During the summer, it produces small, greenish flowers, followed by reddish-brown fruits in the autumn (Alsamri et al., 2021). Sumac is a popular spice in culinary applications and is widely used in Middle Eastern, Mediterranean and Turkish cuisines. It is often sprinkled on salads, meat dishes, and marinades to enhance flavor. Additionally, it is used in sauces, yogurt-based dips, and even some beverages to impart a tangy taste. Sumac serves as both a natural source of acidity and a rich reservoir of antioxidants, making it a valuable ingredient in the kitchen (Alsamri et al., 2021; Shabbir, 2012). Sumac fruits are rich in tannins, giving them a characteristic sour taste. They are known for their antioxidants, antimicrobial, and anti-inflammatory properties. Traditionally, sumac has been used in herbal medicine to treat digestive disorders and as an anti-inflammatory agent. Additionally, its fruits are widely used as a spice, especially in Middle Eastern and Mediterranean cuisines (Alsamri et al., 2021).

Sumac plants bloom in spring and summer, and fruit ripening occurs in late summer and early autumn. The harvest period generally takes place in August and September, when the fruits reach full maturity and develop their characteristic reddish-brown color. After harvesting, the fruits are dried and processed for use as a spice. Dried sumac flowers, which are dark red or burgundy in color, are commonly used in cooking either directly or by soaking in water to prepare sumac juice. The resulting liquid, after straining, is utilized as a flavoring agent in various dishes (Rayne and Mazza, 2007; Ünver and Özcan, 2006). The planted area of sumac (Rhus *coriaria* L.) varies significantly depending on the region, with a higher concentration found in the Mediterranean and Middle Eastern regions, where the plant is native and widely cultivated. In Turkey, particularly in the southeastern Anatolia region, sumac is extensively grown both for culinary and medicinal purposes. The cultivation of sumac remains significant due to its

economic and cultural value, especially in local markets where it is used as a spice, as well as its potential as a source of bioactive compounds for the pharmaceutical and nutraceutical industries.

Propolis is a resinous substance collected by honeybees from tree buds and other botanical sources. It contains a diverse range of biologically active compounds such as flavonoids and phenolic acids, which contribute to its antioxidant, antimicrobial, and anti-inflammatory properties. Historically used in traditional medicine, propolis has gained renewed scientific interest for its potential roles in immunomodulation, wound healing, and cancer prevention (Kasote et al., 2022; Kolaylı et al., 2023).

Numerous studies have demonstrated that *Rhus coriaria* possesses a wide range of biologically active properties, including antioxidant (Alsamri et al., 2021; Bursal & Köksal, 2011), antimicrobial (Ashoori et al., 2020), antidiabetic (Tohma et al., 2019), antiinflammation (Momeni et al., 2019), neuroprotective (Khalilpour et al., 2019) and antitumor (Athamneh et al., 2017) activities. This study was aimed, the phenolic and antioxidant properties of propolis were compared with sumac (*Rhus coriaria*) plant grown in Doğanşehir district of Malatya province. Considering the growing demand for plant-based antioxidants with functional health benefits, sumac presents itself as a promising candidate for comparison with propolis, not only due to its rich phenolic content and traditional use, but also because it remains underrepresented in antioxidant research despite exhibiting comparable bioactive potential.

MATERIALS AND METHODS

Samples

Fresh sumac plant was collected from Erkenek/Karanlıkdere neighborhood of Doğanşehir district of Malatya province, Turkey in September 2023. Raw propolis sample was obtained from Malatya region in 2023 and used by pulverizing (Figure1). Approximately 10 g of dried sumac and powdered propolis samples were extracted in 100 mL of 70% ethanol. The extraction process was carried out in two stages: first, the sample was subjected to ultrasonic bath extraction for 2 h, then shaken in a shaker for 24 h. Filtration was carried out in two stages: first, coarse filtration was performed, then fine filtration was performed using high-quality, fine-pore filter paper. The obtained clear sumac and propolis extract was stored in a closed container in the refrigerator to be used in the study (Kara et al., 2022; Kolaylı and Birinci, 2024)

A) Propolis

B) Sumac

Figure 1. Powdered sumac and propolis samples

The selection of 70% ethanol as the extraction solvent was based on both literature precedence and its proven efficiency in recovering a broad spectrum of phenolic and flavonoid compounds from plant-based matrices. Ethanol–water mixtures, particularly at concentrations between 50–80%, have been widely reported to offer an optimal polarity for extracting both hydrophilic and moderately lipophilic compounds (Dai and Mumper, 2010; Do et al., 2014). In this context, 70% ethanol was chosen as a balanced solvent system capable of efficiently solubilizing a wide range of antioxidant and phenolic constituents from both sumac and propolis.

Total Phenolic Content (TPC)

TPC was evaluated spectrophotometrically using the Folin-Ciocalteu method (Slinkard and Singleton, 1977). In this procedure, a reaction mixture was

prepared by combining 20 µL of the extract with 400 µL of 0.2 N Folin-Ciocalteu reagent and then diluted with 680 µL of distilled water. The mixture was incubated at room temperature for 3 min, then 400 µL of 10% Na_2CO_3 solution was added and the mixture was incubated at 25 °C for 2 h. The absorbance of the solution was then measured at 760 nm using a UV-Vis spectrophotometer (Thermo Scientific Evolution TM 201, Madison, USA). In the preparation of the standard graph, different concentrations of gallic acid (1; 0.5; 0.25; 0.125; 0.0625 and 0.03125 mg/mL) were used. The graph was drawn with the absorbance values found against concentration and the amount of phenolic substance as gallic acid equivalent was found according to the graph.

Total Flavonoid Content (TFC)

The total flavonoid content in the ethanolic extract was measured using a modified procedure based on the method of Fukumoto and Mazza (Fukumoto and Mazza, 2000) In this modification, Al $(NO_3)_3$ was used instead of $AlCl_3$ due to its lower water solubility. To initiate the assay, 50 µL of the ethanolic extract was mixed with 100 µL of 10% Al $(NO_3)_3$ and 100 µL of 1.0 M NH_4CH_3COO. The resulting solution was diluted to a final volume of 3.0 mL using 99% methanol and incubated at 25 °C for 45 min. After incubation, the absorbance was measured at 415 nm. A calibration curve was established using six quercetin standards with concentrations ranging from 0.031 to 0.50 mg QUE/mL. Total flavonoid content was calculated and expressed as milligrams of quercetin equivalents (QUE) per 100 grams of extract according to the standard curve.

Ferric Reducing/Antioxidant Power (FRAP)

The total antioxidant capacity of the ethanolic extract was evaluated using the Ferric Reducing Antioxidant Power (FRAP) test according to method Benzie (Benzie and Strain, 1996). For the preparation of FRAP reagent (Fe-III-TPTZ), a mixture of TPTZ dissolved in 40 mM HCl, acetate buffer and 20 mM $FeCl_3.6H_2O$ solution was made in a test tube. Then, 3 mL of the prepared FRAP reagent was combined with 100 µL of the extract and incubated at 37 °C for 4 min. The absorbance was measured at 595 nm. A calibration curve was established using varying $FeSO_4.7H_2O$ concentrations (1000 to 31.25 µmol/mL). The results were expressed as µmol $FeSO_4 .7H_2O$ equivalent per grams of weight.

ABTS• Radical Scavenging Capacity

The radical scavenging activity of ethanolic extracts was evaluated by a spectrophotometric method using 2,20-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt ($ABTS^{\cdot}$). $ABTS^{\cdot}$ was dissolved with deionized water to a concentration of 7 mM and potassium persulfate was added to 2.45 mM. The mixture was incubated at room temperature and in the dark for 12 h. Then, the mixture was diluted with 0.01 M PBS (phosphate buffered saline), pH 7.4, to obtain an absorbance value of 0.70 at 734 nm. Trolox was used as a standard and the results obtained were expressed as SC_{50} value as the concentration of samples that scavenged 50% of $ABTS^{\cdot}$ radicals (Kolayli et al., 2016).

Phenolic Compounds in RP-HPLC-PDA

Before the analysis of phenolic components of the ethanolic extracts by RP-HPLC-PDA, liquid-liquid extraction was performed to enrich the sample (Kolaylı and Birinci, 2024). A 10 mL portion of the extract was evaporated using a rotary evaporator at 40 °C. The resulting residue was redissolved in 10 mL of distilled water and the pH was adjusted to 2 with concentrated HCl. The organic phases were collected after three consecutive extractions with diethyl ether and ethyl acetate. After evaporation of the solvent, the residue was dissolved in 2 mL of methanol, filtered through a 0.45 µm RC membrane and analyzed for phenolic content. The phenolic composition of the extract was determined using an RP-HPLC-PDA system equipped with a photodiode array (PDA) detector (Shimadzu Liquid Corporation LC 20AT) and a C18 column (250 mm × 4.6 mm, 5 µm; GL Sciences) (Kara and Birinci, 2024). A calibration curve was established with 26 phenolic standards. The mobile phase was (A) 2% acetic acid in water and (B) 70:30 mixture of acetonitrile and water, the injection volume was optimized at 20 µL, the column temperature was 30 °C, and the flow rate was 1.0 mL/min.

RESULTS

The total phenolic and flavonoid content of ethanolic sumac and propolis extracts are presented in Table 1. The average total phenolic content (TPC) was 49 mg GAE/g in the sumac extract, while it was approximately three times higher in the propolis extract. The total flavonoid content was approximately 31.7 mg QUE/g in the propolis

extract, compared to 3.36 mg QUE/g in the sumac extract. A comparative analysis of the two extracts reveals that propolis exhibits a higher flavonoid content. Specifically, flavonoids account for approximately 20% of the total phenolic content in propolis, whereas they represent only about 6.8% of the total phenolic content in sumac.

Table 1. Total phenolic and flavonoid contents of the samples

	Total Phenolic Content (mg GAE/g)	Total Flavonoid Content (mg QUE/g)	%TFC/TPC
Sumac	49.11 ± 1.32	3.36 ±0.15	%6.84
Propolis	156.20±2.78	31.70±1.21	%20.29

In this study, the antioxidant capacity of propolis and sumac extracts was compared as a measure of their biological activity. The ferric reducing antioxidant power (FRAP) method, a straightforward and reliable assay for determining total antioxidant capacity, was used. Higher FRAP values correspond to greater antioxidant potential, with the FRAP value of propolis found to be approximately three times higher than that of sumac extracts (Table 2). The antioxidant capacity of both sumac and propolis is primarily attributed to their polyphenolic content. However, unlike propolis, sumac is a natural product that also contains significant amounts of ascorbic acid, which contributes to its antioxidant capacity. Indeed, the ascorbic acid content in sumac has been reported to range from 10 to 44 mg/kg, indicating that polyphenols are not the sole determinant of its antioxidant activity (Fereidoonfar et al., 2019).

Table 2. Total antioxidant capacities of the samples

	Total antioxidant capacity FRAP (µM $FeSO_4.7H_2O$/g)	ABTS radical scavenging capacity SC_{50}(mg/mL)
Sumac	463.17 ± 2.26	0.93±0.01 *(Std.Troloks0.20±0.01)*
Propolis	1662.30±68.20	0.021±0.00

Free radicals are atoms or molecules that contain unpaired electrons, making them highly reactive. In this study, ABTS molecule was used as a model radical to evaluate antioxidant activity (Kolayli et al., 2016). ABTS radical is a radical commonly used to evaluate antioxidant activity, especially to evaluate radical scavenging capacity. The SC_{50} value calculated in this experiment represents the concentration of an antioxidant required to neutralize 50% of radicals in the experimental medium. Consequently, a lower SC_{50} value corresponds to a higher antioxidant capacity, which reflects the effectiveness of the substance in radical scavenging. In our study, it is seen that the radical scavenging activity of propolis is much higher than that of sumac.

In our study, the phenolic profiles of both ethanolic extracts were analyzed using RP-HPLC-PDA. Samples enriched through liquid-liquid extraction were evaluated against 26 phenolic standards. The results are summarized in Table 3. The identified phenolic compounds were categorized into two main groups, phenolic acids and flavonoids, and their contents were compared within the table. Gallic acid was identified as the major component of the sumac plant, followed by protocatechuic acid and syringic acid. Chrysin and pinocembrin were determined as the flavonoids present in sumac. Similar to our study, it has been reported that gallic acid and protocatechuic acid are the major phenolic compounds in sumac (Elagbar et al., 2020; Kosar et al., 2007). In propolis, gallic acid was not detected, while hydroxycinnamic acids were found to be more abundant, with flavonoids being higher in concentration. Among the phenolic acids, caffeic acid and its ester derivative, caffeic acid phenethyl ester (CAPE), are among the most important markers of propolis, while chrysin, pinocembrin, and rhamnetin were also identified as flavonoids present in propolis.

Table 3. The phenolic compounds of the samples using HPLC-PDA

	Phenolic Standards (µg/g)	Sumac	Propolis
Phenolic acids	***Hydroxybenzoic acids***		
	p-OH Benzoic acid	-	23.10
	Vanillic acid	-	
	Protocathehuic acid	143.11	-
	Gallic acid	1693.58	-
	Chlorogenic acid	-	-
	Syringic acid	68.98	-
	Ellagic acid	-	-
	Hydroxycinnamic acids		
	t-cinnamic acid	-	-
	Ferulic acid	-	960.80
	p-Coumaric acid	-	1210.20
	Caffeic acid	-	1430.22
	Caffeic acid phenethyl ester (CAPE)	-	1750.09
Flavonoid	***Flavonol***		
	Rhamnetin	-	-
	Quercetin	-	760.20
	Rutin	-	-
	Myricetin	-	-
	Galangin	-	-
	Flavan-3-ols		
	Epicatechin	-	-
	Catechin hydrate	-	-
	Flavones		
	Chrysin	8.14	1820.33
	Daidzein	-	-
	Apigenin	-	-
	Luteolin	-	-
	Flavanones		
	Pinocembrin	8.58	2055.06
	Hesperetin	-	806.45
	Naringenin	-	-

(-): not detected

DISCUSSION

Propolis is a resinous substance collected from the hives of honeybees, renowned for its high biologically active value due to its rich polyphenol content. The most prominent feature of this complex natural mixture, which is poorly soluble in water but highly soluble in ethanol, is its substantial polyphenolic composition (Kumova, 2002).

Similarly, sumac (*Rhus spp*.) is a plant belonging to the Anacardiaceous family (gum tree family), usually growing in the Mediterranean, and Middle East regions. The red-colored fruits of this shrub-shaped plant are used as a spice after being dried and ground. Sumac spice adds flavor to dishes with its sour and aromatic taste and is frequently preferred in salad dressings, meat dishes and appetizers

(Batiha et al., 2022). Apart from its culinary uses, sumac water extracts are valued for their health benefits, including antioxidant, antimicrobial, and anti-inflammatory properties. Traditionally, sumac has been utilized in the treatment of sore throat, digestive disorders, and inflammation, and it is also a rich source of vitamin C (Zannou et al., 2025).

This study compares the phenolic composition and antioxidant properties of propolis and sumac extracts. For the first time, the phenolic content of these two natural extracts was directly compared, revealing that both are polyphenol-rich, with propolis exhibiting approximately three times the polyphenol content of sumac. Additionally, propolis demonstrated a higher concentration of flavonoids. Polyphenols, secondary metabolites in plants, play a critical role in their biological activities. These compounds are primarily categorized into two major subclasses: phenolic acids and flavonoids, with flavonoids being the largest subclass. They are particularly significant due to their potent anti-inflammatory effects, contributing to their overall biological efficacy (Abbas et al., 2017).

A study conducted on 136 sumac trees in Iran reported that the total phenolic content (TPC) ranged from 77.54 to 389.30 mgGAE/g DW (Fereidoonfar et al., 2019). These values are notably higher than those observed in our study, suggesting significant methodological differences in the calculation or measurement techniques. Conversely, the total flavonoid content (TFC) in the same study was reported to range between 2.19 and 7.15 mg, which aligns closely with our findings. A study conducted using LC/MS-MS identified the presence of 25 phenolic compounds in sumac. Additionally, the study reported that the sumac plant is characterized by its high tannin and anthocyanins content (Tohma et al., 2019). In this study, the amount of TPC in aqueous sumac extracts was reported as 55 mg GAE/g. However, ethanolic extracts have been reported to contain higher TPC.

In a study, it was reported that sumac fruits contain Delphinidin-3-glucoside, Cyanidin-3-glucoside, Cyanidin-3-rutinoside, and Cyanidin chloride from anthocyanins. In the same study, it was reported that the sour taste of sumac fruits is caused by organic acids such as citric acid, malic acid, oxalic acid, tartaric acid (Zannou et al., 2025).

The total phenolic content of propolis extracts varies significantly depending on regional, national, and floral characteristics. Studies focusing on Anatolian propolis have demonstrated a wide range of total phenolic content, typically between 45 mgGAE/g and 274 mgGAE/g. This variation highlights the influence of geographical and botanical factors on the phenolic composition of propolis (Can et al., 2024; Kolaylı et al., 2023). Our results showed that the antioxidant capacities of both extracts were found to be related to the total amount of phenolic substances.

In this study, the FRAP method reflects total antioxidant capacity, while ABTS method indicates radical scavenging activity. Although there is a positive correlation between both methods, there are differences between their antioxidant mechanisms of action. In this study, antiradical activity of the extracts was analyzed according to ABTS methods. ABTS radical (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) is a chemical substance widely used in antioxidant capacity measurements. In particular, the radical form of ABTS ($ABTS^{-+}$) is blue-green in color and its color decreases or disappears when it reacts with an antioxidant substance. This color change is measured spectrophotometrically to determine the antioxidant capacity of a substance (Kut et al., 2022). The ABTS radical is commonly used in what is known as the Trolox Equivalent Antioxidant Capacity (TEAC) test and the results are expressed in Trolox (vitamin E analog) equivalents. The ABTS method is widely preferred in the food, pharmaceutical and biochemistry fields as it is effective in measuring hydrophilic and lipophilic antioxidants (Cano et al., 2023).

According to the results from our HPLC analysis based on 26 phenolic standards, it is evident that propolis has a richer polyphenolic profile compared to sumac. Our findings indicated that sumac extract was predominantly rich in gallic acid, with smaller amounts of protocatechuic acid, chrysin, and pinocembrin. Consistent with our results, previous studies have reported sumac extracts as being particularly high in gallic acid (Zannou et al., 2025). Propolis was characterized by the presence of hydroxycinnamic acids and flavanones, which are typical of Anatolian propolis (Can et al., 2024). Moreover, key active constituents in propolis, including caffeic acid, rutin, quercetin, pinocembrin, and hesperetin, were identified, although their concentration and presence vary according to the regional floral characteristics. The quality and biological value of Turkish (Anatolian) propolis demonstrate regional variations, with distinct types such as pine propolis in areas rich in oak forests and

chestnut propolis in regions abundant with chestnut trees (Cora et al., 2023).

Conclusıon: In conclusion, both propolis and sumac are natural botanical products characterized by high polyphenolic content and significant antioxidant properties, with notable similarities and differences between them.

REFERENCES

Abbas M, Saeed F, Anjum FM, Afzaal M, Tufail T, Bashir MS, Ishtiaq A, Hussain S, Suleria HAR. Natural polyphenol: An overview. International Journal of Food Properties. 2017; 20(8):1689–1699, doi.org/10.1080/10942912.2016.1220393

Alsamri H, Athamneh K, Pintus G, Eid AH, Iratni R. Pharmacological and Antioxidant Activities of *Rhus coriaria* L. (Sumac). Antioxidants. 2021;10(1):1–28, doi.org/10.3390/Antiox10010073

Ashoori F, Fakhar, Goli HR, Mirzaee F, Faridnia R, Kalani H, Shahani S. Antileishmanial and antibacterial activities of the hydroalcoholic extract of *Rhus coriaria* L. Annals of Parasitology. 2020;62(2):157–163, doi.org/10.17420/AP6602.250

Athamneh K, Hasasna H El, Samri H Al, Attoub S, Arafat K, Benhalilou N, Rashedi A Al, Dhaheri Y Al, Abuqamar S, Eid A, Iratni R. Rhus coriaria increases protein ubiquitination, proteasomal degradation and triggers non-canonical Beclin-1-independent autophagy and apoptotic cell death in colon cancer cells. Scientific Reports. 2017; 7(1), doi.org/10.1038/S41598-017-11202-3

Batiha GES, Ogunyemi OM, Shaheen HM, Kutu FR, Olaiya CO, Sabatier JM, De Waard M. *Rhus coriaria* L. (Sumac), a Versatile and Resourceful Food Spice with Cornucopia of Polyphenols. Molecules. 2022; 27(16): 5179, doi. org/10.3390/molecules27165179

Benzie IFF, Strain JJ. The Ferric Reducing Ability of Plasma (FRAP) as a Measure of "Antioxidant Power": The FRAP Assay. Anal Biochem 1996; 239:70–6, doi.org/10.1006/ABIO.1996.0292

Bursal E, Köksal E. Evaluation of reducing power and radical scavenging activities of water and ethanol extracts from sumac (Rhus coriaria L.). Food Research International 2011; 44:2217–21, doi.org/10.1016/j.foodres.2010.11.001

Can Z, Kara Y, Kolayli S, Çakmak I. Antioxidant activity and phenolic composition of propolis from Marmara region, Turkey. J Apic Res 2024; 63:542–8, doi.org/10.1080/00218839.2022.2157582

Cano A, Maestre AB, Hernández-Ruiz J, Arnao MB, Cano A, Maestre AB. ABTS/TAC Methodology: Main Milestones and Recent Applications. Processes. 2023; 11:185, doi.org/10.3390/pr11010185

Cora M, Buruk CK, Ünsal S, Kaklikkaya N, Kolayli S. Chemical Analysis and in Vitro Antiviral Effects of Northeast Türkiye Propolis Samples against HSV-1. Chem Biodivers. 2023;20, doi.org/10.1002/cbdv.202300669

Dai J, Mumper R. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. *Molecules* 2010; *15*(10), 7313-7352, doi.org/10.3390/molecules15107313

Do QD, Angkawijaya AE, Tran-Nguyen PL, Huynh LH, Soetaredjo FE, Ismadji S, Ju YH. Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. Journal of food and drug analysis 2014; 22(3), 296-302, doi.org/10.1016/j.jfda.2013.11.001

Elagbar ZA, Shakya AK, Barhoumi LM, Al-Jaber HI. Phytochemical Diversity and Pharmacological Properties of *Rhus coriaria*. Chem Biodivers. 2020;17, doi.org/10.1002/cbdv.201900561

Fereidoonfar H, Salehi-Arjmand H, Khadivi A, Akramian M, Safdari L. Chemical variation and antioxidant capacity of sumac (*Rhus coriaria* L.). Ind Crops Prod. 2019;139, doi.org/10.1016/j.indcrop.2019.111518

Fukumoto LR, Mazza G. Assessing antioxidant and prooxidant activities of phenolic compounds. J Agric Food Chem. 2000; 48:3597–604, doi.org/10.1021/jf000220w

Kara Y, Birinci C. Usability of the phenolic profile analysis method developed in RP-HPLC-PDA in natural products. Journal of Apitherapy and Nature. 2024; 7:14–27, doi.org/10.35206/jan.1430767

Kara Y, Can Z, Kolaylı S. What Should Be the Ideal Solvent Percentage and Solvent-Propolis ratio in the Preparation of Ethanolic Propolis Extract? Food Anal Methods. 2022; 15:1707–1719, doi.org/10.1007/s12161-022-02244-z/tables/6

Kasote D, Bankova V, Viljoen AM. Propolis: chemical diversity and challenges in quality control. Phytochem Rev. 2022; 21:1887–1911, doi.org/10.1007/s11101-022-09816-1

Khalilpour S, Sangiovanni E, Piazza S, Fumagalli M, Beretta G, Dell'Agli M. In vitro evidences of the traditional use of *Rhus coriaria* L. fruits against skin inflammatory conditions. J Ethnopharmacol. 2019;238, doi.org/10.1016/j.jep.2019.111829

Kolayli S, Can Z, Yildiz O, Sahin H, Karaoglu SA. A comparative study of the antihyaluronidase, antiurease, antioxidant, antimicrobial and physicochemical properties of different unifloral degrees of chestnut (*Castanea sativa* Mill.) honeys. J Enzyme Inhib Med Chem. 2016;31(sup3):96-104, doi: 10.1080/14756366.2016.1209494

Kolaylı S, Birinci C. A comparative study of solvent effect on propolis extraction by ultrasound-assisted extraction. Turkish Journal of Analytical Chemistry. 2024; 6:11–17, doi.org/10.51435/turkjac.1445121

Kolaylı S, Birinci C, Kara Y, Ozkok A, Samancı AET, Sahin H. A melissopalynological and chemical characterization of Anatolian propolis and an assessment of its antioxidant potential. European Food Research and Technology. 2023; 249:1213–1233, doi.org/10.1007/s00217-023-04208-x

Kosar M, Bozan B, Temelli F, Baser KHC. Antioxidant activity and phenolic composition of sumac (*Rhus coriaria* L.) extracts. Food Chem. 2007; 103:952–959, doi.org/10.1016/j.foodchem.2006.09.049

Kumova U. Önemli Bir Arı Ürünü: Propolis. U. Arı D.-U. Bee J. 2002;02(2):10-24

Kut K, Cieniek B, Stefaniuk I, Bartosz G, Sadowska-Bartosz I. A Modification of the ABTS• Decolorization Method and an Insight into Its Mechanism. Processes. 2022; 10:1288, doi.org/10.3390/pr10071288.

Momeni A, Maghsoodi H, Rezapour S, Shiravand M, Mardani M. Reduction of expression of IL-18, IL-1β genes in the articular joint by sumac fruit extract (Rhus coriaria L.). Mol Genet Genomic Med 2019;7, doi.org/10.1002/mgg3.664

Rayne S, Mazza G. Biological activities of extracts from sumac (*Rhus spp*.): a review. Plant Foods Hum Nutr. 2007; 62:165–75, doi.org/10.1007/S11130-007-0058-4

Shabbir A. Rhus Coriaria Linn, A Plant of Medicinal, Nutritional And Industrial Importance: A Review. Journal of Animal and Plant Sciences. 2012; 22:505-512

Slinkard K, Singleton V L. Total Phenol Analysis: Automation and Comparison with Manual Methods. American Journal of Enology and Viticulture. 1977; 28(1): 49–55, doi.org/10.5344/ajev.1974.28.1.49

Tohma H, Altay A, Köksal E, Gören AC, Gülçin İ. Measurement of anticancer, antidiabetic and anticholinergic properties of sumac (*Rhus coriaria*): analysis of its phenolic compounds by LC–MS/MS. Journal of Food Measurement and Characterization 2019;13:1607–19, doi.org/10.1007/S11694-019-00077-9

Ünver A, Özcan MM. Determination Of Physical and Chemical Properties Of Some Sumach (*Rhus coriaria* L.) Fruits Growing Wild In Turkey. Selcuk Journal of Agriculture and Food Sciences. 2006; 40(20):111–116

Zannou O, Oussou KF, Chabi I B, Alamou F, Awad NMH, Miassi YE, Loké FCV, Abdoulaye A, Pashazadeh H, Redha A A, Kpoclou YE, Guclu G, Koca I, Selli S, Ibrahim SA. Phytochemical and nutritional properties of sumac (*Rhus coriaria*): a potential ingredient for developing functional foods. Journal of Future Foods. 2025; 5(1): 21–35, doi.org/10.1016/j.jfutfo.2024.01.002

Citation/Atıf: Sakr SA, El-Basiony MN, Omar MS, Mahfouz HM. Assessment Efficiency of Chinese, and Local Propolis Against the Greater, and Lesser Wax Moths in Honeybee (*Apis mellifera,* L.) Colonies. U. Bee J. / U. Arı D. 2025; 25 (1): 140-157 DOI:10.31467/uluaricilik.1638254

ARAŞTIRMA MAKALESI / RESEARCH ARTICLE

ASSESSMENT EFFICIENCY OF CHINESE, AND LOCAL PROPOLIS AGAINST THE GREATER, AND LESSER WAX MOTHS IN HONEYBEE (*APIS MELLIFERA,* L.) COLONIES

Bal Arısı (*Apis mellifera*, L.) Kolonilerinde Büyük ve Küçük Balmumu Güvelerine Karşi Çin ve Yerel Propolisin Etkinliğinin Değerlendirilmesi

Samy H. SAKR[1*]; Mohammed N. El-BASIONY[2]; Mahmoud S. OMAR[1], Hatem M. MAHFOUZ[2]

[1]Bee Research Department, Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, 12619, Agriculture ministry, EGYPT, * Corresponding author / Yazışma yazarı E-mail: samy0sakr777@gmail.com, ORCID No: 0009-0005-9588-106X; E-mail: Profomarms72717@yahoo.com, ORCID No: 0009-0007-1733-2318

[2]Department of Plant Production, Faculty of Environmental Agriculture Sciences, Arish University, 45511, EGYPT, E-mail: mnaguib@agri.aru.edu.eg, ORCID No: 0009-0005-2070-5993, E-mail: hmahfouz@agri.aru.edu.eg, ORCID No: 0000-0001-6858-2316

Received / Geliş: 12.02.2025 Accepted / Kabul: 22.05.2025 DOI: 10.31467/uluaricilik.1638254

ABSTRACT

This study analyzed the biochemical composition and effectiveness of Chinese and local propolis extracts against greater (*Galleria mellonella* L.) and lesser (*Achroia Grisella*, L.) wax moth larvae, while also assessing their impact on adult honeybee workers. Propolis extracts were prepared at 10% concentration using ethanol, acetone, olive oil, water, thyme, and sage at 90%, 50%, and 25% concentrations. Biochemical analysis revealed Chinese propolis contained higher total flavonoids (2.47%), amino acids (13.69%), and lipids (31.36 mg/g) than local propolis (0.95%, 6.21%, and 26.96 mg/g, respectively). Laboratory experiments showed that increasing extract concentration (90%>50%>25%) generally increased mortality in wax moth larvae and adult workers for both propolis types. Chinese propolis extracts were more effective against both wax moth species than local extracts across all concentrations. However, Chinese propolis also caused higher mortality in adult workers, especially at higher concentrations. Ethanolic and olive oil extracts were most effective against wax moth larvae but also negatively affected adult worker survival compared to other extracts.

Key words: Propolis, Biochemical analysis, Extracts, Wax moths, Alternative control

ÖZ

Bu çalışmada, Çin ve yerel propolis ekstraktlarının biyokimyasal bileşimi ve büyük (*Galleria mellonella* L.) ve küçük (*Achroia Grisella*, L.) balmumu güvesi larvalarına karşı etkinliği analiz edilirken, yetişkin bal arısı işçileri üzerindeki etkileri de değerlendirilmiştir. Propolis ekstraktları %90, %50 ve %25 konsantrasyonlarda etanol, aseton, zeytinyağı, su, kekik ve adaçayı kullanılarak %10 konsantrasyonda hazırlanmıştır. Biyokimyasal analizler Çin propolisinin yerel propolise göre (sırasıyla %0,95, %6,21 ve 26,96 mg/g) daha yüksek toplam flavonoid (%2,47), amino asit (%13,69) ve lipid (31,36 mg/g) içerdiğini ortaya koymuştur. Laboratuvar deneyleri, artan ekstrakt konsantrasyonunun (%90>%50>%25) her iki propolis türü için de balmumu güvesi larvalarında ve yetişkin işçilerde ölüm oranını genel olarak artırdığını göstermiştir. Çin propolisi ekstraktları her iki mum güvesi türüne karşı da tüm konsantrasyonlarda yerel ekstraktlardan daha etkili olmuştur. Bununla birlikte, Çin propolisi özellikle yüksek konsantrasyonlarda yetişkin işçilerde daha yüksek ölüm oranına neden olmuştur. Etanolik ve

zeytinyağı özütleri balmumu güvesi larvalarına karşı en etkili özütler olmakla birlikte, diğer özütlere kıyasla yetişkin işçilerin hayatta kalmasını olumsuz yönde etkilemiştir.

Anahtar kelimeler: Propolis, Biyokimyasal analiz, Ekstraktlar, Balmumu güveleri, Alternatif kontrol

GENİŞLETİLMİŞ ÖZET

Çalışmanın amacı: Propolis, farklı bitki parçaları ve arıların oluşturduğu moleküllerin karışımından elde edilen reçinemsi bir maddedir. Bu çalışma, iki farklı arı sakızının biyokimyasal bileşim analizini gerçekleştirmiş ve iki tür propolisin (Çin ve Yerel), Laboratuvar koşullarında, bal arısında büyük ve küçük balmumu güvesi larvalarını kontrol etmedeki etkinliğini belirlemiştir.

Materyal ve yöntem: Laboratuvar deneyi, altı propolis özütünün (etanolik, aseton, zeytinyağı, su, kekik ve adaçayı) hem büyük (*G. mellonella*) hem de küçük (*A. grisella*) balmumu güvesi larvaları üzerindeki etkisinin yanı sıra bu özütlerin yetişkin işçiler (*Apis mellifera*, L.) üzerindeki etkisini incelemek üzere tasarlanmıştır. Bal arısı kolonisindeki yetişkin işçileri olumsuz etkilemeden balmumu güvelerini kontrol etmede Çin ve yerel propolis özütlerinin kullanımının uygunluğunu değerlendirmek için etanol, aseton, zeytinyağı, su, kekik ve adaçayının her biri için %90, 50 ve %25'lik konsantrasyonlarda çözünen %10'luk propolis (w/w) konsantrasyonunun kullanıldığı bir laboratuvar deneyi gerçekleştirerek bal arısının yetişkin işçileri (Apis mellifera, L) üzerindeki etkisini araştırmıştır**.**

Bulgular: Biyokimyasal analiz sonuçları, Çin propolisinin toplam flavonoidler (%2,47), toplam amino asitler (%13,69) ve toplam lipitler (31,36 mg/g. vücut ağırlığı) bakımından yerel propolisteki muadillerine (sırasıyla %0,95, %6,21 ve 26,96 mg/g) göre daha üstün olduğunu göstermiştir. Genel olarak, Çin ve yerel propolis ekstraktlarının tüm konsantrasyonları altında büyük, küçük balmumu güvesi larvaları ve yetişkin işçilerin toplam ölüm oranı artmıştır ve bu artış sırasına göre aşağıdaki gibi sınıflandırılabilir: 90% > 50% > 25%. Çin propolisi ekstraktlarının tüm konsantrasyonlarda (%90, 50 ve 25) hem büyük hem de küçük balmumu güvesi larvalarıyla mücadelede Yerel'e üstünlüğü görülmüştür. Öte yandan, Çin propolisinin artan konsantrasyonu ile yetişkin işçi ölüm oranları üzerinde Yerel propolis ile karşılaştırıldığında olumsuz bir etki vardı. Etanolik ve zeytinyağı ekstraktlarının tüm konsantrasyonlarda (%90, 50 ve 25) hem büyük hem de küçük balmumu güvesi larvalarıyla mücadelede tüm ekstraktlara göre üstünlüğü tespit edilmiştir. Öte yandan, etanolik ve zeytinyağı ekstraktlarının artan konsantrasyonu ile diğer ekstraktlara kıyasla yetişkin işçi ölüm oranları üzerinde olumsuz bir etki vardı.

Sonuç: Propolis ekstraktları balmumu güvesi larvalarını öldürmede etkilidir. Daha yüksek konsantrasyonlar ve daha uzun maruz kalma süreleri larva ölümünün artmasıyla sonuçlanmıştır. Zeytinyağı bazlı ekstraktlar en etkili olanlardır. Çin propolisi özütleri yerel propolis özütlerinden daha iyi performans göstermiştir.

Çalışma, her bir propolis ekstraktının test edilen tüm konsantrasyonlarının 4. instar büyük ve küçük balmumu güvesi larvalarını etkili bir şekilde öldürdüğünü ve ölüm oranlarının daha yüksek konsantrasyonlar ve daha uzun maruz kalma süreleri ile arttığı önemli ölçüde daha yüksek ölüm oranları ile sonuçlandığını göstermiştir. Spesifik olarak, her bir ekstraktta %90 oranında çözünmüş %10 Propolis konsantrasyonu, 24 saat sonra büyük ve küçük balmumu güvesi için sırasıyla %81,8 ve %95,79 ölümle sonuçlanmıştır. Ayrıca, zeytinyağı diğer tüm ekstraktlara göre daha üstün olup, büyük ve küçük mum güvesi için sırasıyla %93.33 ve %82.8 ölüm oranı kaydetmiştir ve tüm bu sonuçlar Çin propolisi ile Yerel propolise kıyasla daha belirgindir.

INTRODUCTION

Propolis is known as bee glue. Bees need propolis in the formation and preservation of their hives because of its waxy nature and mechanical properties for sealing gaps, smoothing out the internal walls, and as a protective barrier against external strangers such as snakes, lizards, wind, and rain (Abdallah et al., 2023; Wagh, 2013). Propolis has attracted public interest since it is a natural product with many biological properties. It has been used to possess anti-bacterial, anti-viral, and pests (Omar and Fathy, 2016). Propolis also serves as a protective shield for the bee colony due to its antibacterial and antifungal properties, which contribute to shielding the colony from diseases (Bogdanov, 2017; Özdemir et al*.,* 2022).

The increasing resistance of microorganisms to conventional chemicals and drugs has prompted scientists to search for novel sources of biocides with broad-spectrum activities (Abad et al., 2007). The chemical composition of propolis varies according to its botanical source but mainly it is composed of 30% wax, 50% resin, 10% essential and aromatic oils, 5% pollen and 5% other substances including organic debris (NMKL, 2013). Propolis color ranges from transparent or yellow to dark brown according to the source of resin. Ethanol (ethyl alcohol) ether, glycol, and water are commonly used to extract the propolis (Abdulkhani et al., 2017; Anjum et al*.,* 2019). Propolis has more than 300 compounds with various compositions and isomers. Among these compounds, vitamins (C, B, B1, B2, A, and E), acids (organic acid, gallique acid, isoferulic acid, ferulic acid, and phenolic acids), flavonoids (flavones, flavonol, flavonones, and flavononol), cafeique, pectolinarigenine, and chrysine, are the most common bioactive chemical agents present in all kinds of propolis (Anjum et al*.,* 2019; Bogdanov, 2017; and Özdemir et al*.,* 2022). The best solvent for propolis extraction is ethanol but it is also a limiting factor for the usage of propolis in certain areas (Muz et al*.,* 2024).

Researchers evaluated an ethanolic propolis extract against young wax moth larvae in the laboratory using propolis extracted from 70% ethanol, dissolved in 55% ethanol at concentrations of 2%, 4%, 6%, 8%, and 10% (w/v), and distilled water and 55% ethanol as control samples. The results showed significant values after 24 and 48 hours, with mortality rates accounting for 90% and 80% of wax moth larval mortality, respectively. On the other hand, treatment with a 10% (w/v) propolis extract resulted in 100% moth mortality regardless of treatment time also, the most effect was in sixth- and seventh-instar larvae which, resulting 100% mortality (Ararso and Legesse, 2016). The direct treatment of the larvae of the major wax moth and the food provided to them indicated that the ethanolic propolis extract showed effectiveness in destroying the third larval age the major wax moth (Hussein et al., 2022 a). The propolis ethanolic extracts (15%) have insecticidal action against the 3rd larval instar of *G. mellonella*. in field conditions (Hussien et al., 2022 b). The ethanolic extract of Propolis was significantly toxic on against the third instar larvae of GWM more than *Nigella sativa*, and *Carum carvi* with high percentage of mortality by increasing the concentration and elapse of time. (Algalil et al., 2022). The larger wax moth larvae *Galleria mellonella* responded differently to different plant extracts. *Rosmarinus officinalis*, *Eucalyptus* spp and *Cinnamomum verum* extracts triggered the greatest larval mortality after application %15 and %20 after 48,72hr respectively (Omer et al., 2023).

MATERIALS AND METHODS

This investigation aims to study the biochemical composition of two different bee gum (Chinese, and Local propolis) then using some propolis extracts in controlling greater (*G. mellonella*), and lesser (*A. Grisella*) wax moth larvae.

Propolis samples

1) Chinese propolis which imported from China and purchased commercially in Egyptian market.
2) Local propolis which collected by different traps (normal, glass slide, plastic mesh sheet, and fiber mesh sheet) from honey bee colonies located in the apiary of Agricultural Research Station, El-Arish, North Sinai, Egypt (31.06"46.1"N3349"37.1"E) through two years (2022- 2023).

Propolis Biochemical Analysis

Biochemical analysis of Chinese and local propolis samples were conducted at Plant Protection Research Institute, Agricultural Research Center, Giza, Egypt to estimate the total lipids, amino acids, and flavonoids, with noting that the local propolis sample was estimated at 3 stages (fresh, 6 months and one year). Table 1 is shown total biochemical analysis of local, and Chinese propolis.

Preparation of homogenate samples

Samples were homogenized in distilled water and then centrifuged at 6000 rpm for 10 min at 5°C using (BECKMAN GS-6R Centrifuge). After centrifugation, the supernatant fluid was divided into small aliquots (0.5 ml) and stored at -20 °C until analysis of main components. Three replicates were carried out for each biochemical determination.

Table 1. Total Biochemical Analysis of Local, and Chinese Propolis

Total Analysis	Propolis Samples			
	Local			Chinese
	Fresh	6 months	Year	
Flavonoids (%)	0.24	1.65	0.95	2.47
Amino acid (%)	7.75	8.82	6.21	13.69
Lipid (mg/g. B. W.*)	20.03	28.34	26.96	31.36

*B.W.: Body weight

Total Lipid

Determination of total lipid content

Total lipid content in homogenate was estimated according to Knight et al. (1972) using phosphovanillin reagent and standard curve.

A. Preparation of Phosphovanillin reagent

Pure vanillin (0.6 g) was dissolved in 10 ml absolute ethanol and completed to 100 ml with distilled water. Concentrated phosphoric acid (400 ml) was added, and the solution was stored in a dark glass bottle at room temperature.

B. Procedure

A sample solution of homogenate (250 µl) was added to concentrated sulfuric acid (5 ml) in a test tube and heated in a boiling water bath for 10 min. After cooling to room temperature, 500 µl of the digest was added to the phosphovanillin reagent (6.0 ml). After 45 min incubation in dark, the developed color was measured at 525 nm against a reagent blank prepared from 500 µl distilled water and 6.0 ml phosphovanillin reagent. The content of lipid was expressed as mg/gram of weight.

C. Preparation of standard curve of lipid

For the standard curve, serial concentrations of oleic acid and palmitic acid mixture (7:3) from 0.5 to 5 mg/ml were prepared in absolute ethanol were used and treated in the same manner as the unknown. The standard curve was blotted by O.D. (Optical Density) against concentration.

Total Amino Acids

Determination of total amino acids content

Total amino acids content in homogenate was estimated according to Lee and Takahashi (1966) using colorimetric determination of amino acids with Ninhydrine.

Preparing of Solution

Citrate Buffer = 0.5Molar (Sodium Citrate 129.03g in one liter of Distilled Water) and adjust pH by 0.1N HCI 5.5. Ninhydrine = 1% in (1g in 100ml Citrate Buffer) . Glycerol (pure).

$$Mixture\ of\ solution = 0.5ml\ Ninhydrine + 1.2ml\ Glycerol + 0.2ml\ Citrate\ buffer$$

Preparing standard: Prepare 100 ppm Glycine (0.01g of Glycine in 100ml Distilled water) . Prepare different concentrations of standard in tubes (20 ppm-40 ppm - 60 ppm - 80 ppm-100 ppm) .

Digestion

- Take 0.2g of sample + put 10 ml HCI (6 Molar) in Sealed tube and leave it in sand path in oven at 105°C overnight.
- After that take the digested sample and put it in beaker and add some distilled water and evaporate in water path to get rid of excess of HCI.
- Filtrate in volumetric 25ml using Citrate Buffer.
- Take 1 ml of sample and add 1.9 ml of the mixture in glass tube.
- Do blank and the St. Concentration as the same sample.
- Put them all in water path from 12 min to 20 min and notice formation of reaction color in tubes.
- Leave for cooling and shake vigorously and read on spectrophotometer at 570nm.
- Calculate the Total Amino Acids using St. Curve.

Total Flavonoids

Determination of total flavonoids content

Total flavonoids content in homogenate was estimated according to Zhishen et al. (1999).

Extraction

- Take 0.5g of fresh plant sample and extract with 10ml of ethanol absolute in mortar.
- Filtrate the extract in dark bottle to be ready for analysis.

Preparation

Prepare the standard form Catechin standard 1000 ppm (0.1g/100ml Ethanol) (stock Solution) and 100ppm (take 0.1ml from the stock solution / 100 100ml Ethanol) (working solution). Prepare freshly standard curve using 5 point of Catechin St. (20, 40, 80, 160,320 and 640ppm.(Prepare NaNO2 (5%) solution. Prepare Alcl (10%) solution. Prepare NaOH (1Molar) solution.

Assay

Take 125 ul form the extract in tube and add 75ul from NaNO2 (5%) solution and wait for 6min. Then add 150 ul Alcl (10%) solution and wait for 5 min. Add 750 ul NaOH (1Molar) solutions. Adjust the final volume of solution to 2500 ul with distilled water.

$$Total\ Flavonoide\ (Catechin)\% = (concentration\ from\ St.curve\ ber\ Wt.of\ sample) \times dilution\ factor\ \times 10000$$

Laboratory Experimental Design

Laboratory experiment was designed to examine the possibility of controlling some pests and external parasites using different of propolis extracts. Then the effect of using six propolis extracts (ethanolic, acetone, olive oil, water, thyme, and sage) on both greater (*G. mellonella*), and lesser (*A. grisella*) wax moth larvae were evaluated through conducting a laboratory experiment in which a concentration of 10% propolis (w/w) dissolved in concentrations of 90, 50, and 25% for each of ethanol, acetone, olive oil, water, thyme, and sage to judge the suitability of using Chinese and local propolis extracts in controlling wax moths.

Raring of wax moth larvae

The larvae of greater wax moth were collected from naturally infested honeybee hives. Wood boxes of 40 x 30 x 30cm were used for rearing under laboratory conditions with 25 ± 5°C and 70 ± 5% relative humidity. Collected larvae were introduced into the boxes with infested wax combs and left to feed and grow (Mohamed and Amro, 2022). Boxes were covered with polyethylene plastic. Wax combs were added as needed until pupation, then after the emergence of moths that laid eggs, hatched into larvae. For toxicological tests, fourth instar larvae were used (Elkhiat, 2012).

Wax moth larvae assay

The assay was performed on fourth instar larvae, 20 of which were used (replicated). Three replicates were used for each propolis extracts. The larvae were transferred into Jars with circular holes (1 mm diameter = 625 hole/ inch^2) provided with 20 gm wax. Samples of wax moths were sprayed with previously prepared propolis extracts at different concentrations. Dead larvae were counted and recorded for 24 hours, and mortality rates were evaluated. Larval mortality rate = number of dead larvae / number of tested larvae × 100.

Experimental Design

The experimental design was a factorial randomized complete block involving 3 factors: Factor A: Two propolis samples (Chinese, and Local). Factor B: Six propolis extracts (ethanolic, acetone, olive oil, water, thyme, and sage). Factor C: Four timing (6, 12, 18, and 24 hours). Thus there were 48 treatment combinations in 3 replicates overall total 144 sample with each concentration (90, 50, and 25%). Each sample of the laboratory experimental contains 20 wax moths to estimate the death rate of it as a percentage according to the equation:

$$Death\ rate\ (\%) = \frac{Death\ No.of\ wax\ moths}{Total\ No.of\ wax\ moths} \times 100$$

Preparation of raw propolis extract

Propolis samples were extracted by maceration at room temperature, with occasional shaking, in the proportion of 10 g of (Chinese and Local) propolis to 100 ml of solvent (ethanol 80% w/v), extracts were obtained after 7 days of maceration and the ethanolic extracts were then filtered by Whatman (No.1) filter paper and incubated at room temperature until ethanol evaporated and the product obtained a honey-like consistence are referred to as ethanolic extract propolis, this method was reported by Ildenize et al. (2004).

Statistical Analysis

A completely randomized experimental design was tested. Data were analyzed using SAS program (SAS Institute, 1989). The general linear models were carried out to test differences (alpha = 0.05) and the least significant difference (LSD) mean separation tests were determined.

RESULTS

Positive effect of some propolis extracts on greater wax moth larvae

Generally, increased total mortality rate of greater wax moth larvae (*G. mellonella*) under all concentrations of Chinese, and local propolis extracts (ethanolic, acetone, olive oil, water, thyme, and sage), which can be classified in order of increasing as follows: 90% > 50% > 25% with rates 81.8, 76.51, and 50% respectively, addition to superiority of Chinese over Local propolis extracts with rates 81.29, and 64.33% under all concentrations of dissolved solutions (90, 50, 25%). Effect of some propolis extracts on greater wax moth larvae with 10% concentration (w/w) dissolved 90, 50, 25% in each extract are shown in Table 2(a, b, and c).

The positive effect under dissolved in 90% for each extract

Concerning the effect of some propolis extracts on greater wax moth larvae with 10% concentration (w/w) dissolved 90% in each extract, data reveal that all factors gave high significant values on mean mortality rate of greater wax moth larvae except interaction effect with factors (A×C), and (A×B×C).

Main effects

The effect of propolis kind showed that, Superiority Chinese propolis over Local propolis in total death number. Results recorded 107 while, the Local propolis recorded 89.33 under dissolved 90% in each extract with increasing rate 19.78%. Regarding the effect of extracts kind data revealed that, Superiority Ethanolic, and Olive oil extract over all other extracts with total death rate 100% while, the lowest rate was water extract with death rate 50%. As well as, the highest positive effect was after the first 6 hours where, recorded 77.67 with increasing rate 126.25% comparing with the lowest value (34.33) after 24 hours.

Interaction effects

Concerning the effect between propolis and extracts the results indicated that, The greater effect of Ethanolic, and Olive oil extract over all extract was particularly marked where Chinese, and Local propolis was present; and the higher rate 100% caused by Ethanolic, and Olive oil extract comparing with lower rate of water extract (43.35%) was particularly evident where Local propolis was present. While, the effect of extracts with timing showed that, The highest positive effect was after the first 6 hours under conditions of all extracts comparing with the death number of greater wax moth after 24 hours; and the positive effect was more marked with Ethanolic, and Olive oil extract. On the other hand, there were no interaction effects between propolis (A) and timing (C). Also, there were no interaction effects between the three factors as well as kind of propolis (A), extracts (B), and timing (C).

Table 2.a. Effect of propolis extracts on Greater Wax Moth Larvae (*Galleria mellonella L*.) with 10% concentration (w/w) dissolved in 90% for each extract.

Propolis (A)	Extract (B)	Timing (C)				Mean	Total
		1	2	3	4		
Chinese	Ethanolic	10.00	10.00	0.00	0.00	5.00	20.00
	Acetone	3.33	5.33	3.00	3.00	3.67	14.66
	Olive oil	18.67	0.67	0.33	0.33	5.00	20.00
	Water	5.00	3.33	3.00	3.00	3.58	14.33
	Thyme	1.33	4.67	6.67	6.67	4.83	19.34
	Sage	2.67	6.00	5.00	5.00	4.67	18.67
	Total	41.00	30.00	18.00	18.00	26.75	107.00
Local	Ethanolic	10.00	10.00	0.00	0.00	5.00	20.00
	Acetone	3.33	1.33	1.67	1.67	2.00	8.00
	Olive oil	18.67	1.33	0.00	0.00	5.00	20.00
	Water	4.67	0.00	2.00	2.00	2.17	8.67
	Thyme	0.00	2.67	6.33	6.33	3.83	15.33
	Sage	0.00	4.67	6.33	6.33	4.33	17.33
	Total	36.67	20.00	16.33	16.33	22.33	89.33
	G. Total	77.67	50.00	34.33	34.33	49.08	196.33
		Mean of Extract solution					
	Ethanolic	10.00	10.00	0.00	0.00	5.00	20.00
	Acetone	3.33	3.33	2.33	2.33	2.83	11.32
	Olive oil	18.67	1.00	0.17	0.17	5.00	20.00
	Water	3.33	1.67	2.50	2.50	2.50	10.00
	Thyme	0.67	3.67	6.50	6.50	4.33	17.34
	Sage	1.33	5.33	5.67	5.67	4.50	18.00
L.S.D. 0.05%		A=0.32	B=0.50	C=0.66	A×B=0.71	A×C=N.S	
		B×C=1.63	A×B×C=N.S				

- A, B, and C means factors of propolis, extract, and timing.
- 1, 2, 3, and 4= 6, 12, 18, and 24 hours.

The positive effect under dissolved in 50% for each extract

Interaction effects

Concerning the effect between propolis and extracts the results indicated that, there were no interaction effects between which kind of propolis (A) and extracts (B). But in general, the effect of Olive oil extract over all extract was particularly marked where Chinese, and Local propolis was present; and the higher rate 100% caused by Olive oil extract comparing with lower rate of Thyme extract (50%) was particulary evident where Local propolis was present. While, the effect of propolis with timing showed that, the highest positive effect was after 12 hours under conditions of two propolis kinds comparing with the death number of greater wax moth after 24 hours; and the positive effect was more marked with Chinese propolis. Also, the highest positive effect was after 12 hours under conditions of all extracts comparing with the death number of greater wax moth after 24 hours; and the positive effect was more marked with Olive oil extract.

Table 2.b. Effect of propolis extracts on Greater Wax Moth Larvae (*Galleria mellonella L.*) with 10% concentration (w/w) dissolved in 50% for each extract.

Propolis (A)	Extract (B)	Timing (C)				Mean	Total
		1	2	3	4		
Chinese	Ethanolic	9.33	9.33	0.33	0.33	4.83	19.32
	Acetone	4.00	8.00	1.33	1.33	3.67	14.66
	Olive oil	7.33	12.67	0.00	0.00	5.00	20.00
	Water	5.00	2.67	3.00	3.00	3.41	13.67
	Thyme	1.33	8.67	2.33	2.33	3.67	14.66
	Sage	7.33	8.67	1.33	1.33	4.67	18.66
	Total	34.32	50.01	8.32	8.32	25.25	100.97
Local	Ethanolic	5.00	5.00	0.33	0.33	2.67	10.66
	Acetone	8.67	3.33	0.33	0.33	3.17	12.66
	Olive oil	13.33	6.67	0.00	0.00	5.00	20.00
	Water	5.33	4.00	1.33	1.33	3.00	11.99
	Thyme	1.33	5.33	1.67	1.67	2.50	10.00
	Sage	4.00	8.00	2.67	2.67	4.33	17.34
	Total	37.66	32.33	6.33	6.33	20.67	82.65
	G. Total	71.98	82.34	14.65	14.66	45.92	183.63
		Mean of Extract solution					
	Ethanolic	7.17	7.17	0.33	0.33	3.75	15.00
	Acetone	6.33	5.67	0.83	0.83	3.41	13.66
	Olive oil	10.33	9.67	0.00	0.00	5.00	20.00
	Water	5.67	3.33	2.17	2.17	3.33	13.34
	Thyme	1.33	7.00	2.00	2.00	3.08	12.33
	Sage	5.67	8.33	2.00	2.00	4.50	18.00
L.S.D. 0.05%		A=N.S	B=0.80	C=1.25	A×B=N.S	A×C=1.77	
		B×C=3.06	A×B×C=N.S				

- A, B, and C means factors of propolis, extract, and timing.
- 1, 2, 3, and 4= 6, 12, 18, and 24 hours.

The positive effect under dissolved in 25% for each extract

Concerning the effect of some propolis extracts on greater wax moth larvae with 10% concentration (w/w) dissolved 25% in each extract, data reveal that all factors gave high significant values on mean mortality rate of greater wax moth larvae except interaction effect with factors (A×B), and (A×B×C).

Main effects

The effect of propolis kind showed that, Superiority Chinese propolis over Local propolis in total death number. Results recorded 84.66 while, the Local propolis recorded 59.62 under dissolved 50% in each extract with increasing rate 42%. Regarding the effect of extracts kind data revealed that, Superiority Olive oil extract over all other extracts with total death rate 80% while, the lowest rate was Water extract with death rate 48.35%. As well as, the highest positive effect was after the first 6 hours where, recorded 63.98 with increasing rate 220.1% comparing with the lowest value (19.99) after 24 hours.

Table 2.c. Effect of propolis extracts on Greater Wax Moth Larvae (*Galleria mellonella L*.) with 10% concentration (w/w) dissolved in 25% for each extract.

Propolis (A)	Extract (B)	Timing (C)				Mean	Total
		1	2	3	4		
Chinese	Ethanolic	10.00	2.00	1.33	1.33	3.67	14.66
	Acetone	6.67	6.00	1.00	1.00	3.67	14.67
	Olive oil	10.00	4.00	1.67	1.67	4.33	17.34
	Water	7.33	1.33	1.00	1.00	2.67	10.66
	Thyme	3.33	4.67	2.33	1.67	3.00	12.00
	Sage	2.67	8.00	2.33	2.33	3.83	15.33
	Total	40.00	26.00	9.66	9.00	21.17	84.66
Local	Ethanolic	5.33	3.33	1.67	2.00	3.08	12.33
	Acetone	3.33	1.33	1.00	1.00	1.67	6.66
	Olive oil	7.33	1.33	3.00	3.00	3.67	14.66
	Water	3.33	2.67	1.33	1.33	2.17	8.66
	Thyme	1.33	4.00	2.33	2.33	2.50	9.99
	Sage	3.33	1.33	1.33	1.33	1.83	7.32
	Total	23.98	13.99	10.66	10.99	14.92	59.62
	G. Total	63.98	16.59	20.32	19.99	36.09	120.88
		Mean of Extract solution					
	Ethanolic	7.67	2.67	1.50	1.67	3.38	13.51
	Acetone	5.00	3.67	1.00	1.00	2.67	10.67
	Olive oil	8.67	2.67	2.33	2.33	4.00	16.00
	Water	5.33	2.00	1.17	1.17	2.42	9.67
	Thyme	2.33	4.33	2.33	2.00	2.75	10.99
	Sage	3.00	4.67	1.83	1.83	2.83	11.45
L.S.D. 0.05%		A=0.47	B=0.88	C=1.03	A×B=N.S	A×C=1.45	
		B×C=2.52	A×B×C=N.S				

- A, B, and C means factors of propolis, extract, and timing.
- 1, 2, 3, and 4= 6, 12, 18, and 24 hours.

Interaction effects

Concerning the effect between propolis and extracts the results indicated that, there were no interaction effects between which kind of propolis (A) and extracts (B). But in general, the effect of Olive oil

extract over all extract was particularly marked where Chinese, and Local propolis was present; and the higher rate 86.7% caused by Olive oil extract comparing with lower rate of Acetone extract (33.3%) was particulary evident where Local propolis was present. While, the effect of propolis with timing showed that, the highest positive effect was after 12 hours under conditions of two propolis kinds comparing with the death number of greater wax moth after 24 hours; and the positive effect was more marked with Chinese propolis. Also, the highest positive effect was after the first 6 hours under conditions of all extracts comparing with the death number of greater wax moth after 24 hours; and the positive effect was more marked with Olive oil extract.

Positive effect of some propolis extracts on lesser wax moth larvae

Generally, increased total mortality rate of lesser wax moth larvae (*A. Grisella*) under all concentrations of Chinese, and local propolis extracts (ethanolic, acetone, olive oil, water, thyme, and sage), which can be classified in order of increasing as follows: 90% > 50% > 25% with rates 95.79, 71.39, and 51.79% respectively, addition to superiority of Chinese over Local propolis extracts with rates 80.56, and 69.59% under all concentrations of dissolved solutions (90, 50, 25%). Effect of some propolis extracts on lesser wax moth larvae with 10% concentration (w/w) dissolved 90, 50, 25% in each extract are shown in Table 3(a, b, and c).

The positive effect under dissolved in 90% for each extract

Concerning the effect of some propolis extracts on lesser wax moth larvae with 10% concentration (w/w) dissolved 90% in each extract, data reveal that all factors gave high significant values on mean mortality rate of lesser wax moth larvae except main and interaction effect with factors (A), (A×B), and (A×B×C).

Main effects

The effect of propolis kind showed that, The results were no significant although, superiority Chinese propolis slightly over Local propolis in total death number. Results recorded 118.01 while, the Local propolis recorded 111.88 under dissolved 90% in each extract with increasing rate 5.48%. Regarding the effect of extracts kind data revealed that, All extracts recorded high total death rate with 100% while, sage extract was slightly lower total death rate with 90%. As well as, the highest positive effect was after the first 6 hours where, recorded 123.88 with increasing rate 317.53% comparing with the lowest value (29.67) after 24 hours.

Interaction effects

Concerning the effect between propolis and extracts the results indicated that, there were no interaction effects between which kind of propolis (A) and extracts (B). But in general, the higher rate 100% caused by all extracts was particularly marked where Chinese, and Local propolis was present. While, the effect of propolis with timing showed that, the highest positive effect was after the first 6 hours under conditions of two propolis kinds comparing with the death number of lesser wax moth after 24 hours; and the positive effect was more marked with Chines propolis. Also, the highest positive effect was after the first 6 hours under conditions of all extracts comparing with the death number of lesser wax moth after 24 hours; and the positive effect was more marked with all extracts while, sage extract was slightly lower total death rate under the same conditions. On the other hand, there were no interaction effects between the three factors as well as kind of propolis (A), extracts (B), and timing (C).

Table 3.a. Effect of propolis extracts on Lesser Wax Moth Larvae (*Achroia Grisella L.*) with 10% concentration (w/w) dissolved in 90% for each extract.

Propolis (A)	Extract (B)	Timing (C)				Mean	Total
		1	2	3	4		
Chinese	Ethanolic	15.33	4.67	0	0	5	20
	Acetone	16	2.67	0.67	0.67	5	20
	Olive oil	19.33	0.67	0	0	5	20
	Water	14	4.67	0.67	0.67	5	20
	Thyme	2.67	6	5	5	4.67	18.67
	Sage	1.33	4.67	6.67	6.67	4.83	19.34
	Total	68.66	23.35	13.01	13.01	29.5	118.01
Local	Ethanolic	17.22	2.67	0	0	5	19.89
	Acetone	8.67	6	2.67	2.67	5	20
	Olive oil	19.33	0.66	0	0	5	19.99
	Water	10	7.33	1.33	1.33	5	19.99
	Thyme	0	4.67	6.33	6.33	4.33	17.33
	Sage	0	2.67	6.33	6.33	3.83	15.33
	Total	55.22	23.34	16.66	16.66	28.16	111.88
	G. Total	123.88	46.69	29.67	29.67	57.66	229.9
		Mean of Extract solution					
	Ethanolic	16.33	3.67	0	0	5	20
	Acetone	12.33	4.33	1.67	1.67	5	20
	Olive oil	19.33	0.67	0	0	5	20
	Water	12	6	1	1	5	20
	Thyme	1.33	5.33	5.67	5.67	4.5	18
	Sage	0.67	3.67	6.5	6.5	4.33	17.34
L.S.D. 0.05%		A=N.S	B=0.4	C=1.13	A×B=N.S	A×C=1.6	
		B×C=2.77	A×B×C=N.S				

- A, B, and C means factors of propolis, extract, and timing.
- 1, 2, 3, and 4= 6, 12, 18, and 24 hours.

The positive effect under dissolved in 50% for each extract

Concerning the effect of some propolis extracts on lesser wax moth larvae with 10% concentration (w/w) dissolved 50% in each extract, data reveal that all factors gave high significant values on mean mortality rate of lesser wax moth larvae.

Main effects

The effect of propolis kind showed that, Superiority Chinese propolis over Local propolis in total death number. Results recorded 94.67 while, the Local propolis recorded 76.67 under dissolved 50% in each extract with increasing rate 23.48%. Regarding the effect of extracts kind data revealed that, Superiority Olive oil, and Thyme extract over all other extracts with total death rate 91.7, and 90% respectively while, the lowest rate was water extract with death rate 53.35%. As well as, the highest positive effect was after 12 hours where, recorded 89.34 with increasing rate 346.7% comparing with the lowest value (20) after 24 hours.

Interaction effects

Concerning the effect between propolis and extracts the results indicated that, the greater effect of Olive oil, and Thyme extract over all extract was

particularly marked where Chinese, and Local propolis was present; and the higher rate 100, and 93.3% caused by Olive oil, and Thyme extract respectively, comparing with lower rate of water extract (43.35%) was particularly evident where Local propolis was present. While, the effect of propolis with timing showed that, the highest positive effect was after the first 6 hours under conditions of two propolis kinds comparing with the death number of lesser wax moth after 24 hours; and the positive effect was more marked with Chinese propolis. Also, the highest positive effect was after 12 hours under conditions of all extracts comparing with the death number of lesser wax moth after 24 hours; and the positive effect was more marked with Olive oil, and Thyme extract. As well as, there were a significant, and interaction effects between the three factors as well as kind of propolis (A), extracts (B), and timing (C).

Table 3.b. Effect of propolis extracts on Lesser Wax Moth Larvae (*Achroia Grisella L*.) with 10% concentration (w/w) dissolved in 50% for each extract.

Propolis (A)	Extract (B)	Timing (C)				Mean	Total
		1	2	3	4		
Chinese	Ethanolic	0	9.33	2	2	3.33	13.33
	Acetone	2.67	8.67	2	2	3.83	15.34
	Olive oil	10.67	9.33	0	0	5	20
	Water	0.67	4.67	3.67	3.67	3.17	12.68
	Thyme	7.33	8.67	1.33	1.33	4.67	18.66
	Sage	1.33	8.67	2.33	2.33	3.67	14.66
	Total	22.67	49.34	11.33	11.33	23.67	94.67
Local	Ethanolic	4.67	4.67	1.33	1.33	3	12
	Acetone	0.67	6	2	2	2.67	10.67
	Olive oil	5.33	12	0.33	0.33	4.5	17.99
	Water	3.33	4	0.67	0.67	2.17	8.67
	Thyme	4	8	2.67	2.67	4.33	17.34
	Sage	1.33	5.33	1.67	1.67	2.5	10
	Total	19.33	40	8.67	8.67	19.17	76.67
	G. Total	42	89.34	20	20	42.84	171.34
		Mean of Extract solution					
	Ethanolic	2.33	7	1.67	1.67	3.17	12.67
	Acetone	1.67	7.33	2	2	3.25	13
	Olive oil	8	10	0.17	0.17	4.75	18.34
	Water	2	4.33	2.17	2.17	2.67	10.67
	Thyme	5.67	8.33	2	2	4.5	18
	Sage	1.33	7	2	2	3.08	12.33
L.S.D. 0.05%		A=0.21	B=0.74	C=1.19	A×B=1.04	A×C=1.69	
		B×C=2.92	A×B×C=4.13				

- A, B, and C means factors of propolis, extract, and timing.
- 1, 2, 3, and 4= 6, 12, 18, and 24 hours.

The positive effect under dissolved in 25% for each extract: Concerning the effect of some propolis extracts on lesser wax moth larvae with 10% concentration (w/w) dissolved 25% in each extract, data reveal that all factors gave high significant values on mean mortality rate of lesser wax moth larvae except main effect with factor (A).

Main effects

The effect of propolis kind showed that, The results were no significant although, superiority Chinese propolis slightly over Local propolis in total death number. Results recorded 77.35 while, the Local propolis recorded 61.96 under dissolved 25% in each extract with increasing rate 24.84%. Regarding the effect of extracts kind data revealed that,

Superiority Ethanolic, and Olive oil extract over all other extracts with total death rate 73.35, and 61.7% respectively while, the lowest rate was Acetone extract with death rate 45%. As well as, the highest positive effect was after 12 hours where, recorded 56 with increasing rate 236.13% comparing with the lowest value (16.66) after 24 hours.

Table 3.c. Effect of propolis extracts on Lesser Wax Moth Larvae (*Achroia Grisella L*.) with 10% concentration (w/w) dissolved in 25% for each extract.

Propolis (A)	Extract (B)	Timing (C)				Mean	Total
		1	2	3	4		
Chinese	Ethanolic	10.67	4.67	1	1	4.33	17.34
	Acetone	2.67	2.67	1.67	1.67	2.17	8.68
	Olive oil	2	8	1.33	1.33	3.17	12.66
	Water	6	4	0.67	0.67	2.83	11.34
	Thyme	2.67	8	2.33	2.33	3.83	15.33
	Sage	3.33	4.67	2.33	1.67	3	12
	Total	27.34	32.01	9.33	8.67	19.33	77.35
Local	Ethanolic	6.67	3.33	1	1	3	12
	Acetone	2	4.67	1.33	1.33	2.33	9.33
	Olive oil	5.33	5.33	0.67	0.67	3	12
	Water	3.33	5.33	1.33	1.33	2.83	11.32
	Thyme	3.33	1.33	1.33	1.33	1.83	7.32
	Sage	1.33	4	2.33	2.33	2.5	9.99
	Total	21.99	23.99	7.99	7.99	13.66	61.96
	G. Total	49.33	56	17.32	16.66	32.99	124.31
		Mean of Extract solution					
	Ethanolic	8.67	4	1	1	3.67	14.67
	Acetone	2.33	3.67	1.5	1.5	2.25	9
	Olive oil	3.67	6.67	1	1	3.08	12.34
	Water	4.67	4.67	1	1	2.83	11.34
	Thyme	3	4.67	1.83	1.83	2.83	11.33
	Sage	2.33	4.33	2.33	2	2.75	10.99
L.S.D. 0.05%		A=N.S	B=0.64	C=0.92	A×B=0.89	A×C=1.30	
		B×C=2.26	A×B×C=3.20				

- A, B, and C means factors of propolis, extract, and timing.
- 1, 2, 3, and 4= 6, 12, 18, and 24 hours.

Interaction effects

Concerning the effect between propolis and extracts the results indicated that, The greater effect of Ethanolic extract over all extract was particularly marked where Chinese, and Local propolis was present; and the higher rate 86.7% caused by Ethanolic extract comparing with lower rate of Thyme extract (36.6%) was particularly evident where Local propolis was present. While, the effect of propolis with timing showed that, Highest positive effect was after 12 hours under conditions of two propolis kinds comparing with the death number of lesser wax moth after 24 hours; and the positive effect was more marked with Chinese propolis. Also,

Highest positive effect was after 12 hours under conditions of all extracts comparing with the death number of lesser wax moth after 24 hours; and the positive effect was more marked with Ethanolic extract.As well as, there were a significant, and interaction effects between the three factors as well as kind of propolis (A), extracts (B), and timing (C).

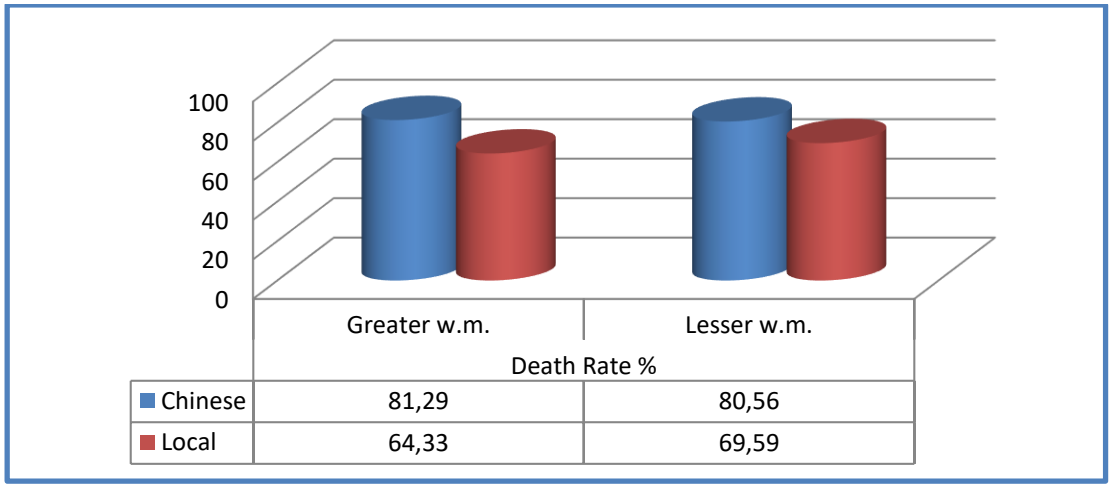


Figure 1. Effect of Chinese and Local propolis on death rate (%) of greater, and lesser wax moth.

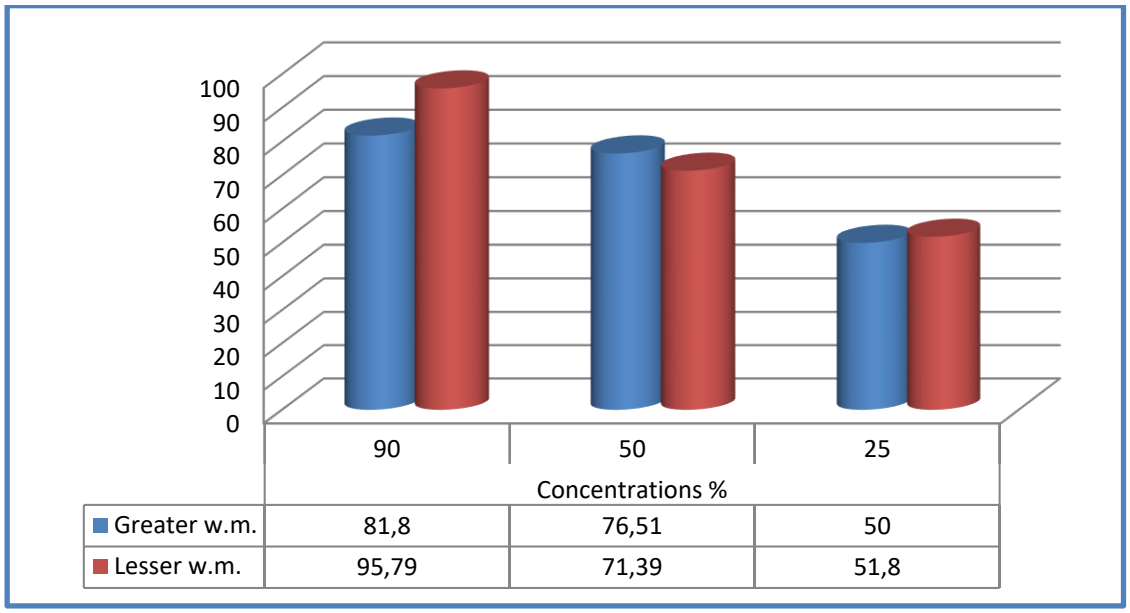
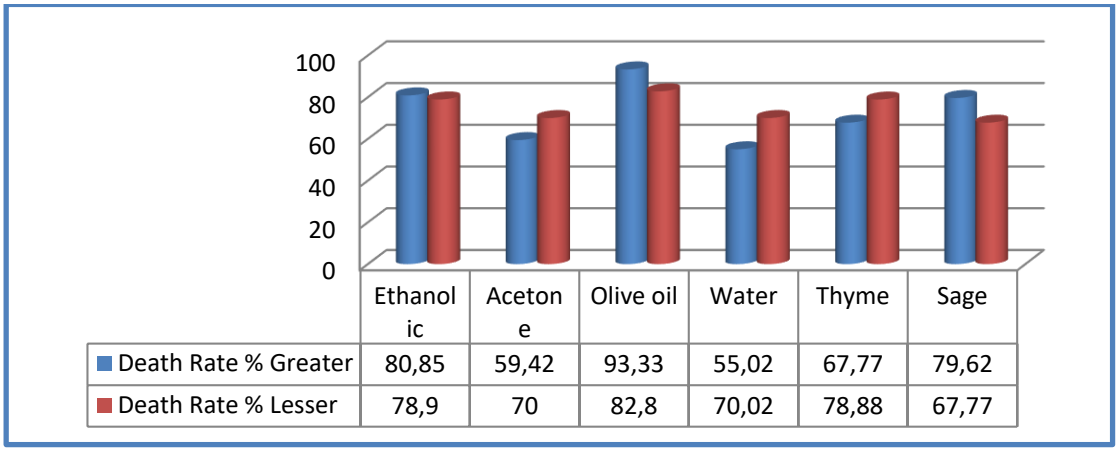


Figure 2. Effect of extracts in solvent concentration (90, 50 and 25%) on death rate (%) of greater, and lesser wax moth.



	Ethanolic	Acetone	Olive oil	Water	Thyme	Sage
Death Rate % Greater	80,85	59,42	93,33	55,02	67,77	79,62
Death Rate % Lesser	78,9	70	82,8	70,02	78,88	67,77

Figure 3. Effect of different extracts on death rate (%) of greater, and lesser wax moth.

DISCUSSION

Propolis Biochemical Analysis

In general, the color of the propolis used in this experiment was dark brown with a pungent odor and was extracted using ethanol ether because it is more effective, and this is consistent with the results of Abdulkhani et al., 2017 and Anjum et al., 2019 who found that proplis color ranges from transparent or yellow to dark brown according to the source of resin. Bogdanov, 2017; and Özdemir et al., 2022 reported that propolis serves as a protective shield for the bee colony due to its antibacterial and antifungal properties, which contribute to shielding the colony from diseases. Our results of the biochemical analysis showed that Chinese propolis is superior in total flavonoids (2.47%), total amino acids (13.69%), and total lipids (31.36 mg/g. body weight) over its counterparts in local propolis (0.95%, 6.21%, and 26.96 mg/g), respectively. These results were consistent with Anjum et al., 2019; Bogdanov, 2017; and Özdemir et al., 2022 who reached that Propolis has more than 300 compounds with various compositions and isomers. Among these compounds, vitamins (C, B, B1, B2, A, and E), acids (organic acid, gallique acid, isoferulic acid, ferulic acid, and phenolic acids), flavonoids (flavones, flavonol, flavonones, and flavononol), cafeique, pectolinarigenine, and chrysine, are the most common bioactive chemical agents present in all kinds of propolis.

Positive effect of some propolis extracts on greater wax moth larvae

Generally, increased total mortality rate of greater wax moth larvae (*G. mellonella*) under all concentrations of Chinese, and local propolis extracts (ethanolic, acetone, olive oil, water, thyme, and sage), which can be classified in order of increasing as follows: 90% > 50% > 25% with rates 81.8, 76.51, and 50% respectively, addition to superiority of Chinese over Local propolis extracts with rates 81.29, and 64.33% under all concentrations of dissolved solutions (90, 50, 25%).

Assessment in this respect, the results align with Fawzy et al. (2017), who found that a 55% ethanolic propolis extract at higher concentrations significantly increased mortality in wax moth larvae after 24 hours compared to lower concentrations and controls. Then the larvicidal action of propolis improved with the increasing concentration. However, the larvae of wax moth responded similarly to all concentrations after 48 hrs. from treatment, but significantly a greater number of larvae which reached 90% were killed in propolis treatment than the control. Propolis extracts were evaluated for their insecticidal effectiveness against GWM by Sturm and Ulrih (2020) who proved that the extracts were found to have high levels of alkaloids, saponins, tannins, and resins. Terpenoids, which contribute to the distinct scent of propolis, also enhance its biological properties, including antibacterial and anti-inflammatory effects. Lee et al. (2008) demonstrated that natural products, like propolis and their

constituents, have been shown to interfere with this symbiosis.

Also, Fathy and Elettreby (2024) found that the highest concentration of propolis (1.4 ppm) extract recorded 99.9 % of mortality percentage in larvae *G. mellonella* after 24 hours. However, the larvae of wax moth responded similarly to all concentrations after 48 hrs. Significantly a greater number of larvae which reached 90% were killed in propolis treatment than the control. Muslem (2012) reported that using Egyptian ethanolic propolis extract (EP) as dietary supplement combined with sugar syrup in honeybee colonies aims to enhance disease resistance and address issues related to harmful effects on beneficial microorganisms and potential residue problems in honeybee products highlighting the need for alternative treatments or control methods in beekeeping.

Positive effect of some propolis extracts on lesser wax moth larvae

Generally, increased total mortality rate of lesser wax moth larvae (*A. Grisella*) under all concentrations of Chinese, and local propolis extracts (ethanolic, acetone, olive oil, water, thyme, and sage), which can be classified in order of increasing as follows: 90% > 50% > 25% with rates 95.79, 71.39, and 51.79% respectively, addition to superiority of Chinese over Local propolis extracts with rates 80.56, and 69.59% under all concentrations of dissolved solutions (90, 50, 25%).

Assessment in this respect, the results align with Ararso and Legesse (2016) who evaluated the ethanol propolis extract against larvae of lesser wax moth in the laboratory using 70% ethanol extracted propolis that dissolved in 55% ethanol at the concentrations of 2%, 4%, 6%, 8% and 10% (w/v), and distilled water and 55% ethanol as controls. The results showed a significant value after 24hrs and 48 hrs at percent mortality caused 90% and 80% mortality of wax moth larvae, respectively. Garedew et al. (2004) found that propolis extract dissolved in 55% ethanol at higher concentrations caused significantly ($p<0.05$) higher mortality to wax moth larvae than the lower concentrations and untreated controls 24 hrs after treatment. On the other hand, adult emergence was observed in treatments of higher concentrations. This may suggest propolis extract at higher concentration accelerated larva and pupa development stages. The abnormally higher rate of development may lead to malformed and immature individuals. Ararso and Legesse (2016) and Garedew et al. (2004); reported that treatment of 10% (w/v) propolis extract resulted in 100% of mite mortality regardless of a treatment time. The sixth and seventh larval instars were reported to be more sensitive to treatments with propolis concentrations of 10% propolis that was resulted in 100% mortality of seventh larval instars. On the other hand, the abnormally higher rate of development may lead to malformed and immature individuals.

In general, using propolis as an insecticide may decrease environmental damage caused by synthetic insecticides in pest management. This diversity can prevent pests from developing resistance as quickly. Because propolis is a complex natural substance, having many components with diverse modes of action is implausible or very slow (Imdorf et al., 1999).

Conclusion: The study demonstrated that all tested concentrations of each propolis extracts effectively killed 4th instar greater, and lesser wax moth's larvae, resulting in significantly higher mortality rates where, mortality rates increased with higher concentrations and longer exposure times. Specifically, a 10% concentration of Propolis dissolved 90% in each extracts resulted in 81.8, and 95.79% mortality for greater, and lesser wax moth respectively, after 24 hours. Additionally, olive oil superior over all other extracts at death rate where, recorded 93.33, and 82.8% for greater, and lesser wax moth respectively, and all this results were more marked with Chinese propolis comparing with Local propolis.

Source of Finance: Not applicable because there is no funding source for this study.

Conflict of interest: Authors declare that they have no any conflict of interests to be reported.

Authors' contributions: The authors contributed equally in the study. They designed, performed, analyzed the data, wrote and revised the manuscript.

Ethical issue: Not applicable because this study on insect and not animals or humans.

Data availability: All data and materials used and/or analyzed during the current study are available in this manuscript.

REFERENCES

Abad MJ, Ansuategui M, Bermejo P. Active antifungal substances from natural sources. Arkivoc. 2007; 7(11): 6-145. 2: 116-145. doi.org/10.3998/ark.5550190.0008.711

Abdallah, SA, El-shemy AA, El Moghazy GM, Fahmy HN, Abd Allah AE. Studying the quality of local propolis and evaluation of its effect as antimicrobial food additive Egypt. J. Chem. 2023; 66(3): 381-389.

Abdulkhani A, Hosseinzadeh J, Ashori A, Esmaeeli H. Evaluation of the antibacterial activity of cellulose nanofibers/polylactic acid composites coated with ethanolic extract of propolis. Polym. Compos. 2017; 38(1): 13-19. doi.org/10.1002/pc.23554

Algalil WAHN, Khaeir SM, Ali AE, Mahmoud MEE. Bioactivity and prospects of using ethanolic extracts of some plants and bee glue (propolis) to control the greater wax moth *Galleria mellonella* (L) (Lepidoptera: Pyralidae). Global Journal of Agricultural Innovation, Research & Development, 2022; (9): 100-109. doi.org/10.15377/2409-9813.2022.09.8

Anjum SI, Ullah A, Khan KA, Attaullah M, Khan H, Ali H, Bashir MA, Tahir M, Ansari MJ, Ghramh HA. Composition and functional properties of propolis (bee glue): A review. Saudi J. Biol. Sci. 2019; 26(7): 1695-1703. doi.org/10.1016/j.sjbs.2018.08.013

Ararso Z, Legesse G. Insecticidal action of honeybees propolis extract against larvae of lesser wax moth. Agric. Biol. J. N. Am. 2016; 7(6): 302-306. doi.org/10.5251/abjna.2016.7.6.302.306

Bogdanov S. Propolis: Biological properties and medical applications. The propolis book. 2017; Ch. 2: 1-41. doi.org/10.1055/s-0035-1545982

Elkhiat BAA. Effect of some plant formulation on greater wax moth *Galleria mellonella*, L. (Lepidoptera: Pyralidae). Ph. D. Agricultural Sciences (Economic Entomology and Pest Control) Menoufiya University. 2012. doi.org/10.21608/SVUIJAS.2019.67090

Fathy DM, Elettreby ShF. Effectiveness of propolis propylene glycol extract on controlling the greater wax moth (*Galleria mellonella*, L.) and colony activation. J. Plant Prot. Pathol., Mansoura Univ. 2024; 15 (10): 329-333. doi.org/10.21608/jppp.2024.317650.1261

Fawzy AM, Al-Ahmadi SS, Al-Hazmi HM. Influence of some natural substances for control the greater wax moth *Galleria mellonella*, L. (Lepidoptera: Pyralidae). J. Plant Prot. Pathol. 2017; 8(8): 407-413. doi.org/10.21608/jppp.2017.46358

Garedew AA, Schmolz E, Lamprecht I. Effect of the bee glue (propolis) on the calorimetrically measured metabolic rate and metamorphosis of the greater wax moth *Galleria mellonella*, L. Thermochim. Acta. 2004; (413):63-72. doi.org/10.1016/j.tca.2003.10.014

Hussein HM, Hadi MH,Hassoni AA.(a) Evaluation of the efficiency of alcoholic propolis extract and *Bacillus thuringiensis* in the mortality rate of the third larval age of the great wax Moth *Galleria melonella* (L.). International Journal of Health Sciences, 2022; 6(S2), 11413–11419. doi.org/10.53730/ijhs.v6nS2.827

Hussien WA, Adam AHM, Khaeir SM, Ali AEE.(b) Effect of biocontrolling methods (proplis and bacteria) on the 3rd larval instar of *Galleria mellonella* (Lepidoptera: Pyralidae). J. Agron. Res. 2022; 4(4): 24-29. doi.org/10.14302/issn.2639-3166.jar-22-4389

Ildenize BS, Cunha I, Alexandra CHF, SawayaI FMC, Mario TS, Maria CM, Flavia TD, Giovanna SP, Patricia DOC. Factors that influence the yield and composition of Brazilian propolis extracts. J. Braz. Chem. Soc. 2004; 15(6): 964-970. doi.org/10.1590/S0103-50532004000600026

Imdorf A, Bogdanov S, Ochoa RI, Calderone NW. Use of essential oils for control of *Varroa jacobsoni* Oud. in honeybee colonies. Apidologie. 1999; (30): 209-228. doi.org/10.1051/apido:19990210

Knight JA, Anderson S, Rawle JM. Chemical basis of the sulfophospho-vanillin reaction for estimating total serum lipids. Clin. Chem. 1972; (18): 199-202. doi.org/10.1093/CLINCHEM/18.3.199

Lee KW, Kang NJ, Kim JH, Lee KM, Lee DE, Hur HJ, Lee HJ. Caffeic acid phenethyl ester inhibits invasion and expression of matrix

metalloproteinase in SK-Hep1 human hepatocellular carcinoma cells by targeting nuclear factor kappa B. Gen. & Nutr. 2008; (2): 319-322. doi.org/10.1007/s12263-007-0067-9

Lee YP, Takashi T. An improved colorimetric determination of amino acids with the use of ninhydrin. Biochem.1966; (14): 71-77. doi.org/10.1016/0003-2697(66)90057-1

Mohamed HO, Amro A. Impact of different diets' nutrition on the fitness and hemocytic responses of the greater wax moth larvae, *Galleria mellonella* (Linnaeus) (Lepidoptera: Pyralidae). J. Basic Appl. Zool. 2022; 83(1): p.10. doi.org/10.1186/s41936-022-00274-x

Muslem AA. The influence phenolic proplis extract and some insects growth regulators on *Galleria mellonella* (Lepidoptera: Pyralidae). Kufa J. Agri. Sci. 2012; 4(1): 159-166. https://www.iraqoaj.net/iasj/article/49933

Muz ÖE, Keskin S, Kara Y, Alpay Karaoğlu S, Keskin M. Isolation and characterization of water soluble fraction of propolis and its antibacterial potential on bacteria causing conjunctivitis. U. Bee J. / U. Arı D. 2024; 24 (2): 167-176. doi.org/10.31467/uluaricilik.1465376

NMKL. Aerobic microorganisms. Determination in foods at 37 °C, 30 °C, 25 °C, 20 °C, 17/7 °C or 6.5 °C by the colony count method. Nordic committee on food analysis. Newsletter. 2013; 86, 5th Ed: 1-8.

Omar SA, Fathy DM. Evaluation of controlling silkworm bacterial diseases using propolis extract and cinnamon oil. J. Agric. Chem. Biotechn. Mansoura Univ.2016;(7): 213-218. doi.org/10.21608/jacb.2016.40892

Omer ShA, Faraj IM, Faraj NM. Insecticidal Activity of Some Plant Extract Against Greater Wax Moth Larvae (*Galleria mellonella* L) Journal of Agricultural Science and Agriculture Engineering, Faculty of Agriculture, Merdeka University Surabaya,Indonesia.2023;6(2).doi.org/10.55173/agriscience.v6i2.88

Özdemir V, Yanar M, Koçyiğit R. General properties of propolis and its usage in ruminants. J. Hellenic Vet. Med. Soc. 2022; 73(2): 3905-3912. doi.org/10.12681/jhvms.26334

SAS Institute Inc. SAS/STAT User's Guide. Cary, NC. 1989; 2(6). doi.org/10.4236/ojs.2015.55045

Sturm L, Ulrih NP. Advances in the propolis chemical composition between 2013 and 2018: A review. *EFood*. 2020; 1(1): 24-37. doi.org/10.2991/efood.k.191029.001

Wagh VD. Propolis: a wonder bees product and its pharmacological potentials. Adv. Pharmacol. Sci. 2013; (1). p.308249. doi.org/10.1155/2013/308249

Zhishen J, Mengcheng, T, Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food chem.1999; (64): 555-559. doi.org/10.1016/S0308-8146(98)00102-2

Citation/Atıf: Abou-Shaara HF. Strategies to temporarily repel honey bees from pesticide-treated areas, U. Bee J. / U. Arı D. 2025; 25 (1): 158-170. DOI: 10.31467/uluaricilik.1609659

DERLEME /REVIEW

STRATEGIES TO TEMPORARILY REPEL HONEY BEES FROM PESTICIDE-TREATED AREAS

Pestisit Uygulanan Alanlardan Bal Arılarını Geçici Olarak Uzaklaştırma Stratejileri

Hossam F. ABOU-SHAARA

Department of Plant Protection, Faculty of Agriculture, Damanhour University, Damanhour, 22516, EGYPT, E-posta: hossam.farag@agr.dmu.edu.eg, ORCID No: 0000-0001-7208-6526

Received / Geliş: 30.12.2024 Accepted / Kabul: 04.02.2025 DOI: 10.31467/uluaricilik.1609659

ABSTRACT

The exposure of forager honey bees, *Apis mellifera*, to pesticides during the treatment period or shortly after can result in instant death or direct impairment of their behaviors. Beekeepers are often faced with limited choices when pesticides are applied near their colonies. One proposed method is the use of repellent materials shortly before pesticide spraying or synchronizing the application time. This article aims to highlight key trends that can be employed to temporarily repel honey bees and emphasizes areas where further studies are needed. The significance of this article lies in the destructive impact of pesticides on honey bees, which are crucial global plant pollinators. The decline of honey bee colonies due to pesticide exposure is a growing concern. The article specifically identifies five strategies that can be employed to repel honey bees: 1) plant-based materials, 2) chemicals, 3) simulation, 4) technology-based repellents, and 5) physical barriers. However, some strategies have been overlooked in previous studies, leading to noticeable gaps in knowledge that should be addressed in further research. The article also presents some perspectives on the beneficial utilization of these specified strategies, paving the way for more innovative methods to mitigate the negative effects of pesticides on honey bees.

Keywords: Pesticides, Honey bees, Stressors, Colonies, Repelling

ÖZ

Toplayıcı bal arılarının, *Apis mellifera*, tedavi süresince veya hemen sonrasında pestisitlere maruz kalması anında ölüme veya davranışlarının doğrudan bozulmasına neden olmaktadır. Arıcılar, kolonilerinin yakınına pestisit uygulandığında genellikle sınırlı seçeneklerle karşı karşıya kalmaktadır. Önerilen yöntemlerden biri, pestisit püskürtmeden hemen önce kovucu malzemelerin kullanılması veya uygulama zamanının senkronize edilmesidir. Bu makale, bal arılarını geçici olarak kovmak için kullanılabilecek temel eğilimleri işaret etmeyi ve daha fazla çalışmaya ihtiyaç duyulan alanları vurgulamak amacındadır. Bu makalenin önemi, küresel bitki polinatörleri olan bal arıları üzerindeki pestisitlerin yıkıcı etkisinde yatmaktadır. Bal arısı kolonilerinin pestisit maruziyeti nedeniyle azalması giderek artan bir endişe kaynağıdır. Makale, bal arılarını kovmak için kullanılabilecek beş eğilimi özel olarak tanımlamaktadır: 1) bitki bazlı malzemeler, 2) kimyasallar, 3) simülasyon, 4) teknoloji tabanlı kovucular ve 5) fiziksel bariyerler. Bunlarla birlikte, önceki çalışmalarda bazı eğilimler göz ardı edilmiş ve bu da daha ileri araştırmalarda ele alınması gereken dikkat çekici bilgi boşluklarına yol açmıştır. Makale ayrıca, bu belirtilen eğilimlerin faydalı kullanımıyla ilgili bazı bakış açıları sunarak, pestisitlerin bal arıları üzerindeki olumsuz etkilerini azaltmak için daha yenilikçi yöntemlerin önünü açmaktadır.

Anahtar Kelimeler: Pestisitler, Bal arıları, Stres faktörleri, Koloniler, Uzaklaştırma

GENİŞLETİLMİŞ ÖZET

Amaç: Bu makale, bal arılarını geçici olarak kovmak için kullanılabilecek temel eğilimleri işaret etmeyi ve daha fazla çalışmaya ihtiyaç duyulan alanları vurgulamak amacındadır. Bu makalenin önemi, küresel bitki polinatörleri olan bal arıları üzerindeki pestisitlerin yıkıcı etkisinde yatmaktadır.

Giriş:Toplayıcı bal arılarının, *Apis mellifera*, tedavi süresince veya hemen sonrasında pestisitlere maruz kalması anında ölüme veya davranışlarının doğrudan bozulmasına neden olmaktadır. Arıcılar, kolonilerinin yakınına pestisit uygulandığında genellikle sınırlı seçeneklerle karşı karşıya kalmaktadır. Önerilen yöntemlerden biri, pestisit püskürtmeden hemen önce kovucu malzemelerin kullanılması veya uygulama zamanının senkronize edilmesidir.. Bal arısı kolonilerinin pestisit maruziyeti nedeniyle azalması giderek artan bir endişe kaynağıdır. Makale, bal arılarını kovmak için kullanılabilecek beş eğilimi özel olarak tanımlamaktadır: 1) bitki bazlı malzemeler. Örneğin, citronella yağı bal arıları için en umut verici kovuculardan biridir. Sarımsak, maydanoz, limon otu, tütün, acı bakla ve asır bitkisi özleri gibi bazı özler bal arıları üzerinde kovucu etki göstermiştir ve sarımsak en güçlü kovucu etkiyi göstermiştir. 2) kimyasallar. Örneğin, bal arısı alarm feromonları, 2-heptanon ve izopentil asetat. Yağlı tohumlu kolza tarlalarına, tarla fasulyelerine ve ayçiçeği başlarına uygulandığında, toplayıcılarda kovucu etki yaratmıştır. 3) simülasyon. Örneğin, bal arıları belirli böceklerin bulunduğu çiçekleri ziyaret etmekten kaçınırlar. Polen böceği *Meligethes aeneus*, Arjantin karıncaları veya *Plecia nearctica*'nın bulunduğu çiçekler. Bu kovuculuk eğiliminde, bu tür böceklere benzeyen yapay nesnelerin kullanılması, bal arılarının belirli çiçeklerde yiyecek aramasını görsel olarak bozabilir ve önleyebilir. 4) teknoloji tabanlı kovucular. Örneğin, insan duyma aralığının üzerinde frekanslara sahip ultrasonik dalgalar yayan ultrasonik cihazların kullanımı. Bu frekanslar böcekleri kovmak için yeterlidir. Başka bir yaklaşım, bal arılarının karanlığa maruz kaldıklarında anormal yiyecek arama davranışı sergilemeleri nedeniyle ışığa ve 5) fiziksel bariyerlere dayanmaktadır. Örneğin, ince ağ veya file gibi bariyerler kurmak, bal arılarının belirli yerlere erişmesini önleyebilir. Dışlama çitleri, bal arılarını kovmak için öldürücü olmayan ve fiziksel olarak göze çarpmayan bir yol sağlar.

Tartışma ve sonuç: Bu makale, bal arılarının pestisit uygulanmış alanlara erişimini sınırlandırmayı amaçlayan beş strateji sunmaktadır. Ancak, literatürde bal arılarının gerçekçi saha koşulları altında çeşitli pestisit türleriyle işlenmiş alanlardan başarılı bir şekilde uzaklaştırıldığını belgeleyen kapsamlı bir veriye rastlanmamıştır. Özellikle pestisitlerin bal arısı kolonileri için önemli bir tehlike oluşturmaya devam ettiği göz önünde bulundurulduğunda, bal arıları için kovucu kullanımının bu yönüyle ilgili belirgin bilgi boşlukları bulunmaktadır.

Bununla birlikte önceki çalışmalarda bazı eğilimler göz ardı edilmiş ve bu da daha ileri araştırmalarda ele alınması gereken dikkat çekici bilgi boşluklarına yol açmıştır. Makale ayrıca, bu belirtilen eğilimlerin faydalı kullanımıyla ilgili bazı perspektifler sunarak, pestisitlerin bal arıları üzerindeki olumsuz etkilerini azaltmak için daha yenilikçi yöntemlerin önünü açmaktadır. Pestisit uygulaması sırasında arı kolonilerini korumak için fiziksel bariyerlerin kullanılması en pratik yaklaşım gibi görünmektedir. Genel olarak, uygulama sırasında bal arıları ile pestisitler arasındaki etkileşimi azaltmak için yöntemler mevcuttur, ancak bunlar ticari ölçekte etkili bir şekilde uygulanmamaktadır.

INTRODUCTION

The honey bee, *Apis mellifera*, assumes a pivotal role in agriculture and plant pollination, contributing significantly to the global economy with annual earnings amounting to billions of US dollars (Morse and Calderone 2000, Paudel et al., 2015; Sillman et al., 2021). Estimates suggest that honey bees play a role in pollinating around 35% of global crop production (Aizen et al. 2009, Klein et al., 2007), with their pollination services enhancing crop quality, leading to amplified yields and improved fruit set (Abou-Shaara 2014). The foraging behavior of honey bees facilitates the crucial transfer of pollen from male to female flower parts, a vital process for the propagation of numerous plant species, encompassing fruits, vegetables, and wildflowers (Abou-Shaara 2014, Bänsch et al. 2021, Halder et al. 2019, Kendall et al. 2021, Rader et al. 2024). This mutualistic relationship between honey bees and agriculture underscores the indispensable role these insects play in sustaining food production

(Etxegarai-Legarreta and Sanchez-Famoso 2022, Patel et al. 2021).

The decline in honey bee populations, as evidenced by prior studies like Hristov et al. (2021) and Panziera et al. (2022), poses a significant threat to global food security and biodiversity. This decline can lead to diminished crop yields, compromised crop quality, and reduced genetic diversity, underscoring the importance of maintaining robust honey bee populations and implementing conservation strategies to uphold agricultural productivity and preserve plant species diversity (Halvorson et al., 2021). Concerns regarding the impact of pesticides on honey bees have escalated due to their potential adverse effects on bee health and colony viability (Abati et al. 2021, Johnson et al. 2010). Recent research has illuminated the harmful repercussions of pesticide exposure on honey bees. For instance, neonicotinoids, a prevalent class of insecticides, have been associated with hindered colony growth, compromised bee health, heightened vulnerability to pathogens, and negative effects on foraging behaviour (Dirilgen et al. 2023, Li et al. 2023, Woodcock et al. 2017). Numerous studies have delved into the immediate effects of pesticide exposure on honey bees, revealing the detrimental outcomes they experience (Johnson et al. 2010, Koch and Weisser 1997, Okubo et al. 2021). These investigations underscore the concerning impact of pesticides on honey bees and emphasize the urgency of adopting sustainable practices to mitigate risks to honey bee colonies.

Safeguarding honey bee colonies from the deleterious effects of pesticides is imperative for their survival and the continuity of pollination services. Various strategies have been proposed to mitigate the risks posed by pesticides to honey bees (Zhang et al. 2023). Integrated pest management approaches, involving pest level monitoring, alternative pest control methods, and reduced pesticide application during active bee foraging periods, offer a viable solution (Lundin et al. 2021, Pecenka et al. 2023). Additionally, modern pest control technologies like gene editing and silencing can aid in diminishing reliance on chemical pesticides (Gossen and McDonald 2020). Encouraging diverse floral resources and establishing pollinator-friendly habitats can furnish bees with alternative foraging options, thereby reducing exposure to pesticide-contaminated crops (Obregon et al. 2021, Zhang et al. 2023). When employed collectively, these methods can shield honey bee colonies from the adverse impacts of pesticides, ensuring their well-being and preserving the invaluable pollination services they provide.

Deterring honey bees from pesticide-treated areas can mitigate their exposure to harmful chemicals and diminish health risks. Various methods, encompassing natural plant extracts and synthetic compounds, have been explored to achieve this objective (Deshpande and Naik 2016, Malerbo-Souza and Nogueira-Couto 2004, Sidhu and Wilson Rankin 2016). This review article aims to underscore the significance of protecting honey bees by repelling them from pesticide-treated regions, focusing on strategies that merit exclusive scrutiny to enhance awareness and furnish updates on this critical subject.

Plant-based materials

Plant extracts and essential oils have been used to control *Varroa destructor* mites and other bee pathogens, in addition to improving honey bee health (Abou-Shaara 2017, Abou-Shaara et al. 2017, Abou-Shaara et al. 2023, Bava et al. 2023, Garrido et al. 2024, Jack and Ellis 2021). Using repellent materials as additives to pesticides is not a novel idea, but it has been commercially used for a long time. For example, QCymbush, a commercial formulation, has been found to repel honey bees for about 2 days after treatment, but the repellency is due to the added ingredients and not the insecticide cypermethrin itself (Delabie et al. 1985). Similarly, plant-derived materials have long been suggested as repellents to honey bees in pesticide-treated areas (e.g., Atkins et al. 1975, Jones 1952, Woodrow et al. 1965). This topic has been covered in detail in a chapter by Deshpande and Naik (2016). As quick snippets, citronella oil is one of the most promising repellents to honey bees (Kumar et al. 1986). Some extracts, such as garlic, parsley, citronella, tobacco, rue, and century plant extracts have shown repellent effects on honey bees, with garlic exhibiting the strongest repellent action lasting for 2.5 hours in the yellow passion-fruit crop and 6 hours in confined beef cattle feeders (Nicodemo and Nogueira Couto 2004).

The leaf extract and essential oil of *Ocimum sanctum* have demonstrated repellent activity against honey bees under semi-field conditions, with the essential oil showing the highest efficacy (Gill et al. 2002). Certain materials have shown repellent effects on the dwarf bees *Apis florea*, such as the essential oil of *Terminalia chebula* (Naik et al. 2010), as well as

linalool and α-terpeniol, which are components of the essential oil derived from the flower of *Swertia densifolia* (Naik et al. 2015). Additionally, additives like ethylene glycol and glycerol have been found to enhance repellence against honey bees (Mishra and Sihag 2010), but further testing with other plant-based materials or pesticides is necessary. Consistent with that, the repellency of citronellal to honey bees from basil (*Ocimum sellowii*) crops has been increased when diluted in water and glycerine compared to water alone (Malerbo-Souza and Nogueira-Couto 2004).

It is worth noting that the ingestion of plant extracts, such as those from *Matricaria chamomilla*, *Origanum majorana*, *Punica granatum*, and *Echinodorus grandiflorus*, can have harmful effects on honey bees, leading to a reduction in their survival (Potrich et al. 2020). Therefore, when utilizing repellency strategies, it is crucial to select materials that are highly repellent to honey bees without causing subsequent harm to them. Furthermore, considering the target insect group is important when using plant-based products. For instance, certain plant-based insecticides like oil-free neem seed extract containing azadirachtin as the main active ingredient have shown no deterrent effects on honey bees (Naumann et al. 1994), indicating no negative impact on bee foraging. However, their toxicity to honey bees still remains a concern. Nicotine-based insecticides can also pose problems for honey bees, as even the highest nicotine concentrations did not completely repel them (Köhler et al. 2012). Similarly, some repellent oils, including eucalyptus, neem, citronella, garlic extract, and rotenone, have been found to have toxic effects on honey bees (Xavier et al. 2015). Therefore, it is essential to search for repellent materials that are safe for honey bees, even in cases of occasional ignition or topical exposure. Additionally, this highlights the importance of conducting selectivity tests when using plant-based pesticides to ensure minimal impact on honey bees, which are a non-target group (Cunha Pereira et al. 2020, Da Silva et al. 2020).

Repellent chemicals

There are certain compounds that can be extracted from honey bees or other insects, or artificially synthesized, which have deterrent effects. An example of this is the honey bee alarm pheromones, 2-heptanone and isopentyl acetate. When applied to oil-seed rape plots, field beans, and sunflower heads, they caused a repellent action to foragers (Free et al. 1985). 2-heptanone, which is secreted from honey bee mandibles, can cause temporary local anesthesia when honey bees bite their enemies (Papachristoforou et al. 2012) and has gained much attention as a repellent. It has shown a 2.5-hour repellent action in the yellow passion-fruit crop (Nicodemo and Nogueira Couto 2004). This chemical has also been found to be repellent to *A. florea* (Naik et al. 2002). However, when used as additives to insecticides in an agricultural setting, 2-heptanone has not shown significant repellent effects on honey bees (Rieth et al. 1986). The repellency of n.octyl.acetate and 2-heptanone to honey bees from basil crops has been increased when diluted in water and glycerine compared to water alone (Malerbo-Souza and Nogueira-Couto 2004). This indicates that the repellence of 2-heptanone is case-dependent, affected by crop type, application method, and additives.

Other compounds containing nitrogen, short side-chain substituted phenyl acetates, and/or tolyl compounds have shown promise as honey bee repellents (Atkins et al. 1975). Compounds such as diethyl-meta-toluamide, 2-ethyl-1,3-hexanediol, dimethyl phthalate, benzaldehyde, and menthol have caused a significant reduction in the number of bees around treated areas (Collins et al. 1996). Additionally, ketones, aldehydes, and phenols have exhibited approximately 80% repellency to honey bees under semi-field conditions, particularly p-ethoxyacetophenone, m-bromoacetophenone, 3,4,5-trimethoxyacetophenone, phenylacetaldehyde, 4-nitrobenzaldehyde, p-bromophenol, and p-cresol (Mishra and Sihag 2009).

Some pollinators exhibit a rejection behavior towards revisiting flowers that have been previously visited by conspecifics or heterospecifics, particularly when the nectar has been depleted. This behavior has been well-documented in honey bees (e.g., Giurfa 1993). Similar repellence has also been observed in various species of bumble bees from the genus *Bombus* spp., which utilize repellent forage-marking scents on flowers of *Symphytum officinale* to temporarily deter subsequent foragers for about 20 minutes (Stout et al. 1998). Chemicals from other insects can induce such rejection behavior in pollinators. For example, Argentine ants (*Linepithema humile*) employ chemical marking using iridomyrmecin from their pygidial glands, which affects certain bee species but not honey bees

(*A. mellifera*) (Wilson Rankin et al. 2020). However, honey bees tend to avoid flowers occupied by Argentine ants or treated with their pheromone (Sidhu and Wilson Rankin 2016). The possibility of developing effective repellent chemicals based on compounds extracted from insects deserves more attention, as it appears to have been overlooked in previous studies.

Simulation

In certain instances, honey bees exhibit avoidance behavior towards flowers already inhabited by specific insects. For instance, flowers hosting the pollen beetle *Meligethes aeneus* (Kirk et al. 1995), Argentine ants *Linepithema humile* (Sidhu and Wilson Rankin 2016), or the lovebug *Plecia nearctica* (Abou-Shaara et al. 2022) are examples of such cases. This phenomenon of repulsion can be leveraged through the use of artificial objects mimicking these insects, visually interfering with and deterring honey bee foraging activities on particular flowers. For instance, the implementation of artificial black spots on flower petals, resembling pollen beetles, demonstrated a dissuasive impact on honey bee foragers, effectively deterring them without necessitating landing on these flowers (Kirk et al. 1995). Despite the promising implications of such experimental paradigms, research in this domain remains relatively scarce. This strategy may be most applicable to limited areas such as small plots or gardens.

An alternative approach involves the simulation of certain bird vocalizations. Observations have indicated that bee-eaters, such as *Merops* spp., known predators of honey bees, can disrupt honey bee foraging patterns (Goras et al. 2023), with their vocalizations potentially impeding flying activities from bee colonies (Bota et al. 2022). Devices emitting simulated bee-eater vocalizations could prove effective in deterring bees from entering pesticide-treated zones. Nevertheless, empirical field data on the efficacy of this method remains scarce and warrants further investigation. Subsequent studies should be undertaken to evaluate the practicality of these devices, encompassing their deployment within apiaries or in proximity to treated areas.

Technology-based repellents

Various technologies exist that can effectively repel insects within designated areas. One such technology involves the utilization of ultrasonic devices that emit ultrasonic waves at frequencies beyond the human auditory threshold. These frequencies have demonstrated the capacity to repel insects (Kaila et al. 2015, Yturralde and Hofstetter 2012). An ultrasonic system underwent testing against varroa mites within colonies, yielding results that showcased its efficacy in combating varroa mites while leaving honey bees unaffected (Barry et al. 2018). Despite these promising outcomes, this particular avenue of research remains relatively unexplored, warranting further investigations into honey bee responses under diverse experimental settings. Noteworthy attributes of this technology include its cost-effectiveness, minimal environmental residue, and ease of application.

An alternative strategy revolves around light manipulation, spurred by observations of honey bees displaying aberrant foraging behavior during solar eclipses. Instances of interrupted foraging trips have been documented during partial solar eclipses (Hains and Gamper 2017). Total solar eclipses, inducing complete darkness, have been shown to impede flying activities (Galen et al. 2019), albeit not entirely halting them (Waiker et al. 2019), and reducing the diversity of bee species visiting floral resources (Sinu et al. 2024). These findings suggest that diminished sunlight prompts the cessation of foraging endeavors, prompting bees to return to their hives. Therefore, strategies that manipulate light within pesticide-treated zones can effectively dissuade honey bees from frequenting these areas. For instance, employing dark covers over treated regions until the conclusion of the spraying period or immediately prior to its commencement. Research highlights that particular wavelengths of LED lights, like ultraviolet, can deter honey bees by disrupting their visual perception (Kevan et al. 2001). Indeed, artificial lighting can adversely affect insect activities (Juddin et al. 2023). By deploying LED lights around apiaries in conjunction with other deterrent measures, it becomes feasible to establish visual barriers that discourage bees from departing their hives. Furthermore, outfitting unmanned aerial vehicles (UAVs) or drones with sound or light-emitting apparatuses could facilitate the development of dynamic and mobile deterrent systems, thereby presenting a worthwhile avenue for exploration under field conditions.

Physical barriers

The implementation of barriers, such as fine mesh or netting, has been shown to effectively deter honey

bees from accessing specific sites (Sonter et al. 2024). Exclusion fencing offers a non-lethal physical method for repelling honey bees. Notably, greenhouses exemplify environments where exclusion fencing can be particularly beneficial, even though honey bees might not represent the most suitable pollinators for greenhouse crops (Nicodemo et al. 2018, Kiatoko et al. 2023). During pesticide applications, greenhouses can be sealed off to prevent incidental honey bee entry.

Similarly, beekeepers have the option to shield their colonies or apiaries with mesh or netting prior to pesticide treatments, allowing for adequate preparation time. This protective measure can be upheld for up to two days, ensuring adequate ventilation while securing food and water supplies for the colonies. Despite considerations regarding cost-effectiveness and labor, there is currently a dearth of empirical data regarding the merits and drawbacks of this approach.

On small farms, temporary covers can be placed over plants without disrupting pesticide application procedures. These covers can be installed before or shortly after spraying, particularly following evening treatments. In larger expanses, tall mesh barriers can be temporarily erected around farms. Leveraging technology, these barriers can be engineered for easy installation and removal through remote control functionalities.

DISCUSSION

Although repellents to deter honey bees from pesticide-treated areas have been suggested and studied for a long time, their impact is not significantly evident. One piece of evidence is the persistent and considerable impact of pesticides on honey bees, causing numerous direct and indirect effects on them. Even during the dominance of research studies on honey bees focusing on colony collapse disorder (CCD), the contribution of pesticide hazards to this issue has been suggested (Frazier et al. 2008, Frazier et al. 2011, Gross 2008). However, no studies have comprehensively discussed the effectiveness of repellents to protect honey bees from pesticide-treated areas in the context of CCD occurrence. Pesticide manufacturers, bee researchers, and other related organizations have not given adequate attention to this research area, and the absence of clear reasons for this oversight is notable. Perhaps the development of suitable repellent materials for field application is progressing slower than the development of pesticides. Specifically, such repellents should be able to restrict honey bee access to certain areas for at least 72 hours after application, rather than just a few minutes. Likewise, the limited longevity of repellents, particularly volatile substances, to maintain their effectiveness for an extended period after application in the field has been recognized as one of the challenges associated with their utilization (Zhang et al. 2023).

Without a doubt, the direct effects of pesticides on honey bee colonies when they are exposed to pesticides during application and in the few days following application are more harmful than the pesticide residues subsequently available in the environment (e.g. Johnson et al. 2010, Okubo et al. 2021, Tosi and Nieh 2019, Yao et al. 2018). Therefore, it is essential to develop effective formulations using plant extracts, synthetic chemicals, insect extracts, or their mixtures for practical application, while considering their repellent duration to be as long as 72 hours. In addition to using repellent materials to keep bees away from specific areas, attractants can be applied to untreated areas to draw honey bees towards them. For example, the attraction of honey bees towards untreated yards for a few days can be achieved by using attractants such as liquid paraffin as an additive to the leaf extract of *Swertia densifolia* (Naik et al. 2007). Research should investigate appropriate concentrations, carriers, and application methods to optimize the repellent effects while minimizing any negative impacts on the environment or non-target organisms. When considering application, it is crucial to apply these materials before pesticide application or develop slow-release devices that can be fixed around treated areas, instead of mixing them with pesticides. The latter approach is not ideal for protecting the bees, as they can still come into contact with pesticides and transfer the toxins to their colonies, thereby negatively affecting them.

The use of technology-based repellents, simulation approaches, and physical barriers shows great promise for repelling bees from certain areas for a relatively extended period, but further studies are still required to validate their effectiveness. These strategies require the involvement of technology companies to develop appropriate methods, as highlighted in the article. Applying pesticides during periods when honey bees are less active, such as

early morning or late evening, allows for ample time to apply repellent methods that facilitate the bees' return to their hives before the application, thus avoiding immediate exposure. Careful studies and evaluations are necessary to determine effective methods to repel honey bees while preserving their vital role as pollinators and minimizing any impact on non-target organisms (Figure 1). Additionally, careful planning and coordination with beekeepers are essential to ensure the safety and well-being of the colonies during the implementation of any repellent method.

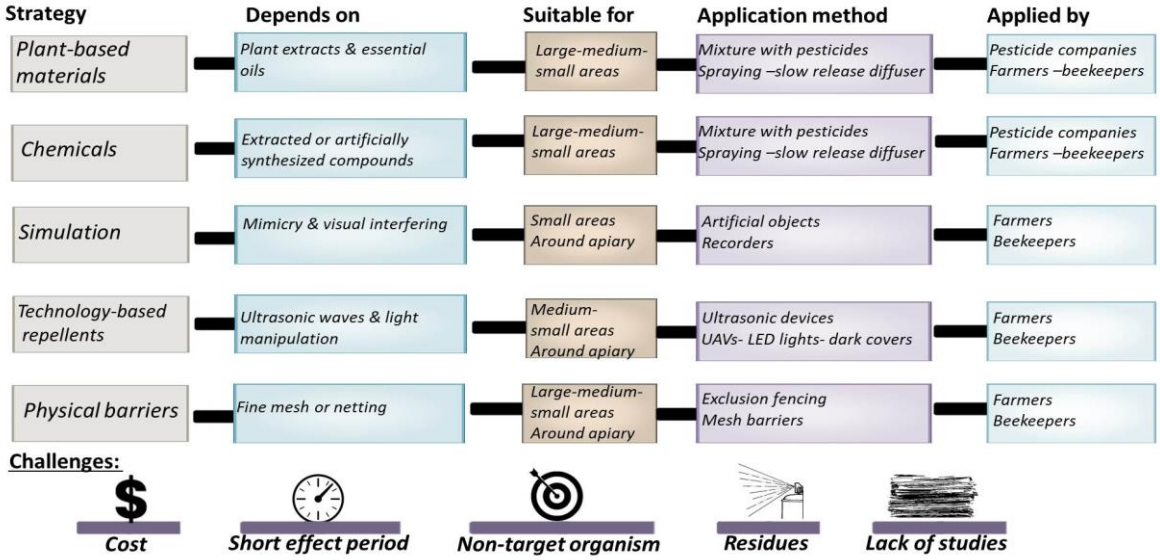


Figure 1. A summary of the strategies presented in the article and key points related to their application.

Conclusion: This article presents five strategies aimed at limiting the access of honey bees to pesticide-treated areas. However, no comprehensive data documenting successful repelling of honey bees from areas treated with various types of pesticides under realistic field conditions have been found in the literature. There are noticeable knowledge gaps in this aspect of using repellents for honey bees, particularly considering that pesticides continue to pose a significant hazard to honey bee colonies. The use of physical barriers to safeguard bee colonies during pesticide application appears to be the most practical approach. In general, methods to mitigate the interaction between honey bees and pesticides during application are available, but they are not effectively implemented on a commercial scale.

Author contribution: The author designed, wrote, and revised the manuscript.

Conflict of Interest: No conflict of interests to be reported.

Ethical issue: Not applicable because this article is a review on honey bees.

Source of Finance: Not applicable.

REFERENCES

Abati R, Sampaio AR, Maciel RMA, Colombo FC, Libardoni G, Battisti L, Lozano ER, Ghisi NC, Costa-maia FM, Potrich M.. Bees and pesticides: The research impact and scientometrics relations. Environmental Science and Pollution Research. 2021;28:32282-32298. https://doi.org/10.1007/s11356-021-14224-7.

Abou-Shaara HF. The foraging behaviour of honey bees, *Apis mellifera*: a review. Veterinarni Medicina. 2014;59:1-10.

Abou-Shaara HF. Using safe materials to control *Varroa* mites with studying grooming behavior of honey bees and morphology of *Varroa* over winter. Annals of Agricultural Sciences. 2017;62:205-210.

https://doi.org/10.1016/j.aoas.2017.12.002.

Abou-Shaara HF, Amiri E, Parys KA. Tracking the effects of climate change on the distribution of *Plecia nearctica* (Diptera, Bibionidae) in the USA using MaxEnt and GIS. Diversity. 2022;14:690. https://doi.org/10.3390/d14080690.

Abou-Shaara H, Jack C, Ellis JD. The impact of smoke and fogged/vaporized thyme oil on honey bee (*Apis mellifera*) survival and behavior in vitro. Journal of Apicultural Research. 2023;62:643-645. https://doi.org/10.1080/00218839.2022.2135809.

Abou-Shaara H, Staron M, Cermakova T. Impacts of oxalic acid, thymol, and potassium citrate as *Varroa* control materials on some parameters of honey bees. Turkish Journal of Veterinary & Animal Sciences. 2017;41:238-247. https://doi.org/10.3906/vet-1606-92.

Aizen MA, Garibaldi LA, Cunningham SA, Klein AM. How much does agriculture depend on pollinators? Lessons from long-term trends in crop production. Annals of Botany. 2009;103:1579-1588. https://doi.org/10.1093/aob/mcp076.

Atkins EL, Macdonald RL, Mcgovern TP, Beroza M, Greywood-Hale EA. Repellent additives to reduce pesticide hazards to honeybees: laboratory testing. Journal of Apicultural Research. 1975; 14: 85-97. https://doi.org/10.1080/00218839.1975.11099808.

Bänsch S, Tscharntke T, Gabriel D, Westphal C. Crop pollination services: Complementary resource use by social vs solitary bees facing crops with contrasting flower supply. Journal of Applied Ecology. 2021;58:476-485. https://doi.org/10.1111/1365-2664.13777.

Barry BC, Verstraten L, Butler FT, Whelan PM, Wright WM. The use of airborne ultrasound for *Varroa destructor* mite control in beehives. In *2018* IEEE International Ultrasonics Symposium (IUS) pp. 1-9.

Bava R, Castagna F, Palma E, Marrelli M, Conforti F, Musolino V, Musella V. Essential oils for a sustainable control of honeybee varroosis. Veterinary Sciences. 2023;10:308. https://doi.org/10.3390/vetsci10050308.

Bota G, Traba J, Sarda-palomera F, Giralt D, Pérez-Granados C.Passive acoustic monitoring for estimating human-wildlife conflicts: The case of bee-eaters and apiculture. Ecological Indicators. 2022;142:109158. https://doi.org/10.1016/j.ecolind.2022.109158.

Collins AM, Rubink WL, Cuadriello Aguilar JI, Hellmich RL. Use of insect repellents for dispersing defending honey bees (Hymenoptera: Apidae). Journal of Economic Entomology. 1996;89(3):608-613. https://doi.org/10.1093/jee/89.3.608.

Cunha Pereira R, Faria Barbosa W, Pereira Lima MA, Vieira JOL, Carvalho Guedes RN, Rodrigues Da Silva BK, Lemes Fernandes F. Toxicity of botanical extracts and their main constituents on the bees *Partamona helleri* and *Apis mellifera*. Ecotoxicology. 2020;29:246-257. https://doi.org/10.1007/s10646-020-02167-7.

Da Silva IM, Zanuncio JC, Brügger BP, Soares MA, ZAnuncio AJV, Wilcken CF, Sediyama CS. Selectivity of the botanical compounds to the pollinators *Apis mellifera* and *Trigona hyalinata* (Hymenoptera: Apidae). Scientific Reports. 2020;10:4820. https://doi.org/10.1038/s41598-020-61469-2.

Delabie J, Bos C, Fonta C, Masson C. Toxic and repellent effects of cypermethrin on the honeybee: Laboratory, glasshouse and field experiments. Pesticide Science. 1985;16:409-415. https://doi.org/10.1002/ps.2780160417.

Deshpande PV, Naik DG. Repellents for honey bees. Mutualistic interactions between flowering plants and animals. 2016;148-173.

Dirilgen T, Herbertsson L, O'reilly AD, Mahon N, Stanley DA. Moving past neonicotinoids and honeybees: A systematic review of existing research on other insecticides and bees. Environmental Research. 2023:235:116612. https://doi.org/10.1016/j.envres.2023.116612.

Etxegarai-Legarreta O, Sanchez-Famoso V. The role of beekeeping in the generation of goods and services: The interrelation between environmental, socioeconomic, and sociocultural utilities. Agriculture. 2022;12:551. https://doi.org/10.3390/agriculture12040551.

Frazier J, Mullin C, Frazier M, Ashcraft S. Pesticides and their involvement in colony collapse disorder. American Bee Journal. 2011;151:779-781.

Frazier M, Mullin C, Frazier J, Ashcraft S. What have pesticides got to do with it? American Bee Journal. 2008;148:521-524.

Free JB, Pickett JA, Ferguson AW, Simpkins JR, Smith MC. Repelling foraging honeybees with alarm pheromones. Journal of Agricultural Science. 1985;105,:255-260.

Galen C, Miller Z, Lynn A, Axe M, Holden S, Storks L, Kephart J. Pollination on the dark side: Acoustic monitoring reveals impacts of a total solar eclipse on flight behavior and activity schedule of foraging bees. Annals of the Entomological Society of America. 2019.;12:20-26. https://doi.org/10.1093/aesa/say035

Garrido PM, Porrini MP, AlBeroni D, Baffoni L, Scott D, Mifsud D, Di Gioia D. Beneficial bacteria and plant extracts promote honey bee health and reduce nosema ceranae infection. Probiotics and Antimicrobial Proteins. 2024;16:259-274. https://doi.org/10.1007/s12602-022-10025-7

Gill K, Malik OP, Kalidhar SB, Mishra RC, Malik MS. Honey bee repellent activity of leaf extract and essential oil of *Ocimum sanctum* and isolation of a new compound. Journal of Medicinal and Aromatic Plant Sciences. 2002;24:951-956.

Giurfa M. The repellent scent-mark of the honeybee *Apis mellifera tigustica* and its role as communication cue during foraging. Insectes Sociaux. 1993;40:59-67. https://doi.org/10.1007/BF01338832

Goras G, Tananaki C, Liolios V, Kanelis D, Tofaris C, Giannouris E, Thrasyvoulou A. The conflict between avian predators and domestic honey bees: a case study of European bee-eater (*Merops apiaster* L.) preying on the honey bee (*Apis mellifera* L.) in Cyprus. Journal of Apicultural Research. 2023;62:1115-1122. https://doi.org/10.1080/00218839.2022.2069642

Gossen BD, Mcdonald MR. New technologies could enhance natural biological control and disease management and reduce reliance on synthetic pesticides. Canadian Journal of Plant Pathology. 2020;42:30-40. https://doi.org/10.1080/07060661.2019.1697370

Gross M. Pesticides linked to bee deaths. Current Biology. 2008;18, R684.

Hains BC, Gamper H. Disruption in honey bee (*Apis mellifera*) foraging flight activity during a partial solar eclipse shown by individual flight path tracking. Bulletin of Insectology. 2017;70:315-320.

Halder S, Ghosh S, Khan R, Khan AA, Perween T, Hasan MA. Role of pollination in fruit crops: A review. Pharma Innovation Journal. 2019;8,:695-702.

Halvorson K, Baumung R, Leroy G, Chen C, Boettcher P. Protection of honeybees and other pollinators: one global study. Apidologie. 2021;52:535-547. https://doi.org/10.1007/s13592-021-00841-1.

Hristov P, Shumkova R, Palova N, Neov B. Honey bee colony losses: Why are honey bees disappearing? Sociobiology. 2021; 68:e5851-e5851.

Jack CJ, Ellis JD. Integrated pest management control of *Varroa destructor* (Acari: Varroidae), the most damaging pest of (*Apis mellifera* L.(Hymenoptera: Apidae)) colonies. Journal of Insect Science.

2021;21:6. https://doi.org/10.1093/jisesa/ieab058

Johnson, R. M., Ellis, M. D., Mullin, C. A., & Frazier, M. 2010. Pesticides and honey bee toxicity–USA. Apidologie, 41, 312-331. https://doi.org/10.1051/apido/2010018

Jones, G. G. 1952. The responses of the honey-bee to repellent chemicals. Journal of Experimental Biology, 29, 372-386. https://doi.org/10.1038/s41598-017-15066-5

Juddin, A. S. S., Ngah, N., Umar, R., Asante, K., & Abdullahi, M. G. 2023. A review influence of light on insect activity and behavior: sustainable lighting and light pollution. J Sustainabil Sci Manag 18: 231-247.

Kaila, K., Bodkepranali, S., & Namdev, K. K. 2015. Development performance and evaluation of ultrasonic pest and insect repelling system. International Journal of Engineering Research and General Science, 3,19-25.

Kendall, L. K., Evans, L. J., Gee, M., Smith, T. J., Gagic, V., Lobaton, J. D. & Rader, R. 2021. The effect of protective covers on pollinator health and pollination service delivery. Agriculture, Ecosystems & Environment, 319, 107556. https://doi.org/10.1016/j.agee.2021.107556.

Kevan, P. G., Chittka, L., & Dyer, A. G. 2001. Limits to the salience of ultraviolet: lessons from colour vision in bees and birds. Journal of Experimental Biology, 204, 2571-2580. https://doi.org/10.1242/jeb.204.14.2571

Kiatoko, N., Pozo, M. I., Van Oystaeyen, A., Musonye, M., Klka, J., Wäckers, F. & Jaramillo, J. 2023. African endemic stingless bees as an efficient alternative pollinator to honey bees in greenhouse cucumber (*Cucumis sativus* L). Journal of Apicultural Research, 62, 1017-1029. https://doi.org/10.1080/00218839.2021.2013421.

Kirk, W. D., Ali, M., & Breadmore, K. N. 1995. The effects of pollen beetles on the foraging behaviour of honey bees. Journal of Apicultural Research, 34, 15-22.

Klein, A. M., Vaissière, B. E., Cane, J. H., Steffan-Dewenter, I., Cunningham, S. A., Kremen, C., & Tscharntke, T. 2007. Importance of pollinators in changing landscapes for world crops. Proceedings of the royal society B: biological sciences, 274, 303-313.

Koch, H., & Weisser, P. 1997. Exposure of honey bees during pesticide application under field conditions. Apidologie, 28, 439-447. https://doi.org/10.1051/apido:19970610.

Köhler, A., Pirk, C. W., & Nicolson, S. W. 2012. Honeybees and nectar nicotine: deterrence and reduced survival versus potential health benefits. Journal of Insect Physiology, 58, 286-292. https://doi.org/10.1016/j.jinsphys.2011.12.002.

Kumar, J., Patyal, S. K., & Mishra, R. C. 1986. Evaluation of some essential oils as repellents to the Indian honeybee *Apis cerana indica* 1. Gustatory repellency and toxicity. Journal of Apicultural Research, 25, 256-261. https://doi.org/10.1080/00218839.1986.11100726.

Li, W., Lv, L., Wang, Y., & Zhu, Y. C. 2023. Mixture effects of thiamethoxam and seven pesticides with different modes of action on honey bees (*Aplis mellifera*). Scientific Reports, 13, 2679. https://doi.org/10.1038/s41598-023-29837-w.

Lundin, O., Rundlöf, M., Jonsson, M., Bommarco, R., & Williams, N. M. 2021. Integrated pest and pollinator management–expanding the concept. Frontiers in Ecology and the Environment, 19, 283-291.

Malerbo-souza, D. T., & Nogueira-Couto, R. H. 2004. Efficiency of n-octyl-acetate, 2-heptanone and citronellal in repelling bees from basil (*Ocimum sellowii*-Labiatae). Brazilian Archives of Biology and Technology, 47, 121-125.

Mishra, S., & Sihag, R. C. 2009. Efficacy of some chemicals as repellents against two honey bee species, *Apis mellifera* L. and *Apis*

florea F. in semi-field trials. Journal of Apicultural Science, 53, 53-66.

Mishra S, Sihag RC. Efficacy of some chemicals and additives as bee repellents against two honeybee species, *Apis mellifera* L. and *Apis florea* F. in semi-field trials. Journal of Apicultural Science. 2010;54:21-34.

Morse RA, Calderone NW. The value of honey bees as pollinators of US crops in 2000. Bee culture. 2000;128:1-15.

Naik DG, Banhatti P, Chadawa SS, Thomas D. 2-Heptanone as a repellent for *Apis florea*. Journal of Apicultural Research. 2002;4: 59-61. https://doi.org/10.1080/00218839.2002.11101069.

Naik DG, Dandge C, Puntambekar H. *Swertia densifolia* leaf extract as a dose-dependant attractant or repellent for *Apis florea*. Journal of Apicultural Research. 2007;46:15-18. https://doi.org/10.1080/00218839.2007.11101361.

Naik DG, Puntambekar H, Anantpure P. Essential oil of *Terminalia chebula* fruits as a repellent for the Indian honeybee *Apis florea*. Chemistry & Biodiversity. 2010;7:1303-1310. https://doi.org/10.1002/cbdv.200900274.

Naik DG, Vaidya-Kannur H, Deshpande PV, Dandge CN, Reddy GV. Potential use of an essential oil from the flower of *Swertia densifolia* as a repellent for *Apis florea* (Hymenoptera: Apidae). Annals of the Entomological Society of America. 2015;108:18-25. https://doi.org/10.1093/aesa/sau005.

Naumann K, Currie RW, Isman MB. Evaluation of the repellent effects of a neem insecticide on foraging honey bees and other pollinators. The Canadian Entomologist. 1994;126:225-230.

Nicodemo D, Nogueira Couto RH. Use of repellents for honeybees (*Apis mellifera* L.) in vitro in the yellow passion-fruit (*Passiflora edulis* Deg) crop and in confined beef cattle feeders. Journal of Venomous Animals and Toxins including Tropical Diseases. 2004;10:77-85.

Nicodemo D, Malheiros EB, De Jong D, Couto RHN. Improved pollination efficiency and reduced honey bee colony decline in greenhouses by allowing access to the outside during part of the day. Sociobiology. 2018;65:714-721. https://doi.org/10.13102/sociobiology.v65i4.3455.

Obregon D, Guerrero OR, Stashenko E, Poveda K. Natural habitat partially mitigates negative pesticide effects on tropical pollinator communities. Global Ecology and Conservation. 202;28:e01668. https://doi.org/10.1016/j.gecco.2021.e01668.

Okubo S, Shoji A, Kimura K, Morimoto N, Yoshiyama M. Effectiveness of floral enhancement in reducing honeybee exposure to insecticides. Applied Entomology and Zoology. 2021;56:207-215. https://doi.org/10.1007/s13355-021-00727-9.

Panziera D, Requier F, Chantawannakul P, Pirk CW, Blacquière T. The diversity decline in wild and managed honey bee populations urges for an integrated conservation approach. Frontiers in Ecology and Evolution. 2022;10:767950. https://doi.org/10.3389/fevo.2022.767950.

Papachristoforou A, Kagiava A, Papaefthimiou C, Termentzi A, Fokialakis N, Skaltsounis AL, Theophilidis G. The bite of the honeybee: 2-heptanone secreted from honeybee mandibles during a bite acts as a local anaesthetic in insects and mammals. PLoS ONE. 2012;7:e47432. https://doi.org/10.1371/journal.pone.0047432.

Patel V, Pauli N, Biggs E, Barbour L, Boruff B. Why bees are critical for achieving sustainable development. Ambio. 2021;50:49-59.

Paudel YP, Mackereth R, Hanley R, Qin W. Honey bees (*Apis mellifera* L.) and pollination issues: Current status, impacts, and potential drivers of decline. Journal of Agricultural Science. 2015;7:93-109.

Pecenka JR, Ingwell LL, Krupke, CH, Kaplan I. Implementing IPM in crop management simultaneously improves the health of managed bees and enhances the diversity of wild pollinator communities. Scientific Reports. 2023;13:11033. https://doi.org/10.1038/s41598-023-38053-5.

Potrich M, Silva RTLD, Maciel RM, Costa-Maia FM, Lozano ER, Rossi RM, Dallacort S. Are plant extracts safe for honey bees (*Apis mellifera*)? Journal of Apicultural Research. 2020;59:844-851. https://doi.org/10.1080/00218839.2020.1735733.

Rader R, Schmidt L, Rocchetti M, Wright D, Bezerra Da Silva Santos K, Dingley A, Preradovic J, Jones J, Dawso, B, Devetter LW, Perović DJ, Davis AE. Future-proofing the pollination services to insect-pollinated crop production. Acta Horticulturae. 2024;388:17-36.

Rieth JP, Wilson WT, Levin MD. Repelling honeybees from insecticide-treated flowers with 2-heptanone. Journal of Apicultural Research. 1986;25:78-84. https://doi.org/10.1080/00218839.1986.11100697.

Sidhu CS, Wilson Rankin EE. Honey bees avoiding ant harassment at flowers using scent cues. Environmental Entomology. 2016;45:420-426. https://doi.org/10.1093/ee/nvv230.

Sillman J, Uusitalo V, Tapanen T, Salonen A, Soukka R, Kahiluoto H. Contribution of honeybees towards the net environmental benefits of food. Science of the Total Environment. 2021;756:143880. https://doi.org/10.1016/j.scitotenv.2020.143880.

Sinu PA, Jose A, Varma S. Impact of the annular solar eclipse on December 26, 2019 on the foraging visits of bees. Scientific Reports. 2024;14:17458 (2024). https://doi.org/10.1038/s41598-024-67708-0.

Sonter CA, Jones J, Dawson BM, Reid JN, Santos KC, Fuller S, Tighe M, Wilson C, Rader R. Protective covers impact honey bee colony performance and access to outside resources. Agriculture, Ecosystems & Environment. 2024;368:109028. https://doi.org/10.1016/j.agee.2021.107556.

Stout JC, Goulson D, Allen JA. Repellent scent-marking of flowers by a guild of foraging bumblebees (*Bombus* spp.). Behavioral Ecology and Sociobiology. 1998;43:317-326. https://doi.org/10.1007/s002650050497.

Tosi S, Nieh JC. Lethal and sublethal synergistic effects of a new systemic pesticide, flupyradifurone (Sivanto®), on honeybees. Proceedings of the Royal Society B. 2019;286:20190433. https://doi.org/10.1098/rspb.2019.0433.

Waiker P, Baral, S, Kennedy A, Bhatia S, Rueppell A, Le K, Rueppell O. Foraging and homing behavior of honey bees (*Apis mellifera*) during a total solar eclipse. The Science of Nature. 2019;106:1-10. https://doi.org/10.1007/s00114-018-1597-2.

Wilson Rankin EE, Cecala JM, Hernandez Pineda N, Lu, QY, Pelayo E, Choe DH. Differential feeding responses of several bee species to sugar sources containing iridomyrmecin, an Argentine ant trail pheromone component. Journal of Insect Behavior. 2020;33:83-90. https://doi.org/10.1007/s10905-020-09748-8.

Woodcock BA, Bullock JM, Shore RF, Heard MS, Pereira MG, Redhead J, Pywell RF. Country-specific effects of neonicotinoid pesticides on honey bees and wild bees. Science. 2017;356:1393-1395. https://doi.org/10.1126/science.aaa1190.

Woodrow AW, Green N, Tucker H, Schonhorst MH, Hamilton C. Evaluation of chemicals as honey bee attractants and repellents. Journal of Economic Entomology. 1965;58:1094-1102. https://doi.org/10.1093/jee/58.6.1094.

Xavier VM, Message D, Picanço MC, Chediak M, Júnior PAS, Ramos RS, Martins, JC. Acute toxicity and sublethal effects of

botanical insecticides to honey bees. Journal of Insect Science. 2015;15:137. https://doi.org/10.1093/jisesa/iev110.

Yao J, Zhu YC, Adamczyk J. Responses of honey bees to lethal and sublethal doses of formulated clothianidin alone and mixtures. Journal of Economic Entomology. 2018;111:1517-1525. https://doi.org/10.1093/jee/toy140.

Yturralde KM, Hofstetter RW. Efficacy of commercially available ultrasonic pest repellent devices to affect behavior of bed bugs (Hemiptera: Cimicidae). Journal of Economic Entomology. 2012;105:2107-2114. https://doi.org/10.1603/ec12166

Zhang G, Olsson RL, Hopkins BK. Strategies and techniques to mitigate the negative impacts of pesticide exposure to honey bees. Environmental Pollution. 2023;318:120915. https://doi.org/10.1016/j.envpol.2022.120915.

Citation/Atıf: Ilyasov RA, Ilyasova AY, Danilenko VN, Meral Kekeçoğlu M, Rašić SB, Thai PH et al. Strategies and mechanisms of plant-microbiome-pollinator coadaptation**. U. Bee J. / U. Arı D. 2025; 25 (1): 171-196. DOI: 10.31467/uluaricilik.1675598**

DERLEME /REVIEW

STRATEGIES AND MECHANISMS OF PLANT-MICROBIOME-POLLINATOR COADAPTATION

Bitki-Mikrobiyom-Polinatör Ko-adaptasyon Stratejileri ve Mekanizmaları

Rustem A. ILYASOV [1, *], Alla Yu. ILYASOVA [1], Valery N. DANILENKO [2], Meral KEKEÇOĞLU [3], Slađan B. RAŠIĆ [4], Pham Hong THAI [5], Svetlana N. KHRAPOVA [6], Alfir G. MANNAPOV [6],Sofia S. PROKUDINA [1], Vener N. SATTAROV [7], Dmitry V. BOGUSLAVSKY [1]

[1]Koltsov Institute of Developmental Biology of the Russian Academy of Sciences, Moscow, RUSSIA, [*]Corresponding author / Yazışma yazarı E-mail: ilyasov_ra@idbras.ru, ORCID No: 0000-0003-2445-4739; E-mail: ilyasova_ay@idbras.ru, ORCID No: 0000-0002-7505-6805; E-mail: sofia2y2@gmail.com, ORCID No: 0009-0003-2594-7663; E-mail: boguslavsky@rambler.ru, ORCID No: 0000-0001-9601-640X

[2]Vavilov Institute of General Genetics of the Russian Academy of Sciences, Moscow, RUSSIA, E-mail: valerid@vigg.ru, ORCID No: 0000-0001-5780-0621

[3]Duzce University, Faculty of Arts and Sciences, Department of Biology, Center for Apiculture Research, Development and Application, Duzce, TURKEY, E-mail: meralkekecoglu@gmail.com, ORCID No: 0000-0002-2564-8343

[4]Educons University, Faculty of Ecological Agriculture, Sremska Kamenica, SERBIA, E-mail: rasic.sladjan@gmail.com, ORCID No: 0000-0001-6859-2784

[5]Vietnam National University of Agriculture, Research Center for Tropical Bees and Beekeeping, Hanoi, VIETNAM, E-mail: phthai@vnua.edu.vn, ORCID No: 0000-0002-6441-1402

[6]Russian State Agrarian University named after K.A. Timiryazev, Moscow, RUSSIA, E-mail: 54alfir@mail.ru, ORCID No: 0000-0002-5093-9740

[7]Bashkir State Pedagogical University named after M. Akmulla, Ufa, RUSSIA, E-mail: wener5791@yandex.ru, ORCID No: 0000-0001-6331-4398

Received / Geliş: 14.04.2025 Accepted / Kabul: 13.05.2025 DOI: 10.31467/uluaricilik.1675598

ABSTRACT

Plant-pollinator interactions showcase mutualistic coevolution, but the role of microorganisms in these relationships is often overlooked. Nectar-dwelling microorganisms, mainly yeasts and bacteria, significantly influence floral chemistry, pollinator behavior, and plant reproduction. These microorganisms alter nectar's sugar content, amino acid profiles, pH, and scent emissions, shaping pollinator preferences. For example, the yeast *Metschnikowia reukaufii* produces fruity esters that attract bumble bees, while some bacteria lower pH, repelling honey bees. Pollinators spread these microorganisms between flowers, creating a feedback loop that shapes microbial communities and drives coevolution. Beyond nectar, microorganisms' impact on thermal regulation through metabolic heat, pollen health, and pollinator gut microbiomes. Specialized bacteria like *Rosenbergiella nectarea* and *Acinetobacter spp.* thrive in nectar's high-sugar environment, while pollinator microorganisms, such as *Lactobacillus kunkeei,* protect honey bees from pathogens. Microbial diversity varies by region, with tropical flowers hosting richer communities than temperate ones. This review highlights how microorganisms act as key players in plant-pollinator networks, boosting pollinator nutrition, immunity, and foraging efficiency. It explores microbial spread, competition, and chemical influence, calling for studies that blend microbiology, ecology, and evolution. Understanding these interactions is vital for predicting how climate change and habitat loss threaten pollination, affecting agriculture and biodiversity.

Keywords: Honey bee, Pollinators, Microbiome, Coadaptation, Coevolution

ÖZ

Bitki-polinatör etkileşimleri, karşılıklı faydaya dayalı ko-evrimi sergilemektedir; ancak mikroorganizmaların bu ilişkilerdeki rolü genellikle göz ardı edilmektedir. Başlıca mayalar ve bakterilerden oluşan nektarda yaşayan mikroorganizmalar, çiçek kimyasını, polinatör davranışını ve bitki üremesini önemli ölçüde etkilemektedir. Bu mikroorganizmalar, nektarın şeker içeriğini, amino asit profillerini, pH'ını ve koku emisyonlarını değiştirerek polinatör tercihlerini şekillendirmektedir. Örneğin, *Metschnikowia reukaufii* mayası, *bombus* arılarını cezbeden meyvemsi esterler üretirken, bazı bakteriler pH'ı düşürerek bal arılarını uzaklaştırmaktadır. Polinatörler, bu mikroorganizmaları çiçekler arasında yayarak, mikrobiyal toplulukları şekillendiren ve ko-evrimi yönlendiren bir geri bildirim döngüsü oluşturmaktadır. Nektarın ötesinde, mikroorganizmalar metabolik ısı yoluyla termal düzenlemeyi, polen sağlığını ve polinatör bağırsak mikrobiyomlarını etkilemektedir. *Rosenbergiella nectarea* ve *Acinetobacter spp.* gibi özelleşmiş bakteriler, nektarın yüksek şekerli ortamında gelişirken, *Lactobacillus kunkeei* gibi polinatör mikroorganizmaları, bal arılarını patojenlerden korumaktadır. Mikrobiyal çeşitlilik bölgeye göre değişmekte olup, tropikal çiçekler ılıman iklim çiçeklerine göre daha zengin topluluklara ev sahipliği yapmaktadır. Bu derleme, mikroorganizmaların polinatör beslenmesini, bağışıklığını ve besin arama verimliliğini artırarak bitki-polinatör ağlarında nasıl kilit oyuncular olarak rol oynadığını vurgulamaktadır. Mikrobiyal yayılımı, rekabeti ve kimyasal etkileri inceleyerek, mikrobiyoloji, ekoloji ve evrimi harmanlayan çalışmalara çağrı yapmaktadır. Bu etkileşimleri anlamak, iklim değişikliğinin ve habitat kaybının tozlaşmayı nasıl tehdit ettiğini tahmin etmek, tarımı ve biyoçeşitliliği etkilemek için hayati öneme sahiptir.

Anahtar Kelimeler: Bal arısı, Polinatörler, Mikrobiyom, Ko-adaptasyon, Ko-evrim

GENİŞLETİLMİŞ ÖZET

Giriş: Nektar, 1884 yılında Boutroux tarafından ilk kez belgelendiği gibi zengin ve az keşfedilmiş bir mikrobiyal habitattır. Çalışmalar, çiçek nektarının *Metschnikowia* gibi mayalar ve *Acinetobacter* ve *Pseudomonas* gibi bakteriler de dahil olmak üzere, öncelikle tozlayıcılar tarafından getirilen çeşitli mikrobiyal toplulukları desteklediğini ortaya koymuştur. Bu mikroorganizmalar nektar ekolojisinde merkezi bir rol oynamakta, bitki ve böceklerin davranış ve uygunluğunun yanı sıra nektarın tadı, aroması, şeker içeriği ve amino asit bileşimini de etkilemektedir.

Böcekler, özellikle de bal arıları, kovanla ilişkili türleri nektara bırakarak çiçekler arasında mikropların aktarılmasında önemli vektörler olarak hareket ederler. Bu mikrobiyal değişim nektarın mikrobiyomunu şekillendirir ve tozlayıcıların tercihlerini ve bitkilerin üreme başarısını etkileyebilir. Mikroorganizmalar nektara rüzgar veya yağmur gibi abiyotik yollarla girebilse de, tozlayıcı aktivitesi bu habitattaki mikrobiyal çeşitliliğin birincil itici gücü olmaya devam etmektedir. Bal arıları, çiçeklere yaptıkları tekrarlı ziyaretler ve kovandaki sabit mikrobiyotaları sayesinde, nektarı tozlaşma dinamiklerini etkileyecek şekilde değiştirerek ekosistem mühendisleri olarak hareket ederler. Çiçek mikrobiyal toplulukları genellikle tozlayıcı ziyaret modellerini yansıtır ve iklim ve coğrafya topluluk kompozisyonunu daha da etkiler. Bu derleme, nektarda yaşayan mikropların nektar kimyasını dönüştürerek tozlayıcı davranışını ve bitki-polinatör etkileşimlerini nasıl değiştirdiğine dair mevcut bilgileri özetlemektedir.

Bitki-polinatör birlikte evriminde çiçek adaptasyon stratejileri: Bitkiler, tozlayıcıların davranışlarını manipüle etmek için karmaşık çiçek adaptasyonları geliştirmiştir. Bunu esas olarak nektar salgılayarak yaparlar. Böceklerin ziyaretleri, genellikle mikroorganizmaların katılımı yoluyla nektarın kimyasını önemli ölçüde değiştirebilir. Örneğin, bal arıları bez salgıları ve polen biriktirme yoluyla nektardaki amino asit seviyelerini artırır. Bombus arıları ise muhtemelen ağız parçalarından maya transferi nedeniyle şeker konsantrasyonlarını azaltır. Yalnız yaşayan arıların nektar üzerinde çok az etkisi vardır. Tozlayıcılar tarafından getirilen mikroorganizmalar, özellikle de *Metschnikowia* gibi mayalar, şeker bileşimini, pH'ını ve uçucu emisyonlarını değiştirerek nektarı modifiye eder. Bu değişiklikler tozlayıcıların çekiciliğini ve yiyecek arama davranışını etkiler. Maya metabolizması ısı üreterek nektarın sıcaklığını 6 santigrat dereceye kadar yükseltir, çiçek kokusu salınımını artırır ve daha soğuk iklimlerde böcekleri çeker. *Nelumbo*

nucifera gibi ısı üreten türler, tozlayıcı enerji maliyetlerini azaltarak ve ziyaret süresini artırarak fayda sağlar. Koku taklidi ve renk değişiklikleri gibi çiçek ipuçları, bitki çekim stratejilerini daha da geliştirir. Örneğin orkideler ve Ceropegia türleri, belirli tozlayıcıları çekmek için böcek ipuçlarını kimyasal olarak taklit eder. UV işaretleri ve anthesis ile bağlantılı renk değişimleri, yiyecek arama davranışını yönlendirir. Bununla birlikte, son derece uzmanlaşmış stratejiler, tozlayıcıların azalması karşısında üreme başarısını riske atabilir. Mikrobiyal uçucu organik bileşikler (VOC'ler) hem tozlayıcıları çekebilir hem de rakip mikroorganizmaları bastırarak ekolojik dinamikleri şekillendirebilir. Bu nedenle, mikroplar sadece nektar modülatörleri olarak değil, aynı zamanda bitki-polinatör iletişimini rafine eden ekolojik aracılar olarak da hareket eder.

Çiçek nişlerinde mikroorganizmaların uzmanlaşması ve adaptasyonu: Çiçeklerde yaşayan mikroorganizmalar hava, toprak, su ve bitki dokularından türetilen dinamik topluluklar oluşturur. Bakteriler ve mantarlar çiçek dokularını erken dönemde, hatta tomurcuklar açılmadan önce kolonize eder, ancak bollukları genellikle düşük kalır ve bu da geçici kolonizasyona işaret eder. Bu mikroorganizmaların kalıcılığı, UV radyasyonu, kuruma, rekabet ve sınırlı dağılma gibi çevresel stres faktörlerinin üstesinden gelme yeteneklerine bağlıdır. Tozlayıcılar gibi böcekler mikroorganizmaların yayılmasında önemli bir rol oynar, ancak yalnızca ozmotik strese toleranslı olanlar ve diğerleriyle rekabet edebilenler nektara yerleşebilir. Diğer yayılma şekilleri arasında rüzgar, yağmur sıçraması ve bazı mikroorganizmaların çiçek dokularına göç etmesiyle bitkiler içinde iç taşınma yer alır. Çiçeklerin taç yaprakları ve yapraklarındaki mikrobiyal topluluklar sıklıkla örtüşse de, çiçek mikrobiyomu tipik olarak daha az çeşitlidir ve eşit olmayan bir şekilde dağılmıştır. Bazı mikroorganizmaların çiçek ortamında oldukça uzmanlaştığı, çiçeklerde bol miktarda bulunurken diğer habitatlarda nadiren görüldüğü görülmektedir. Bu uzmanlar genellikle hızlı büyüme, nektar ozmolaritesine tolerans ve çiçeğe özgü bileşikleri metabolize etme yeteneği gibi özellikler sergiler. Çiçekler yaşlandıkça, özellikle nektarda ve pistillerde mikrobiyal bolluk artar. Tozlayıcılar, temas, dışkı veya tımarlama yoluyla mikroorganizmalar getirerek çiçek kısımlarındaki mikrobiyal çeşitliliği önemli ölçüde etkiler. Metodolojik zorluklara rağmen, mikrobiyal yaşam döngülerini izlemek ve konakçıya adapte olmuş türler arasında ayrım yapmak, çiçek nişlerindeki mikroorganizmaların uzmanlaşmasını ve evrimini anlamak için kritik öneme sahiptir.

Nektar ve bal mikrobiyal topluluklarının polinatör aracılı şekillendirilmesi: Nektar, özelleşmiş mikrobiyal toplulukları destekleyen dinamik bir mikrohabitattır. Bu topluluklar öncelikle tozlayıcılardan etkilenen bakteri ve mayalardan oluşur. Tozlayıcılar hem polen vektörleri hem de mikrobiyal dağıtıcılar olarak hareket ederek farklı çiçek türleri arasında nektar mikrobiyomunun bileşimini şekillendirir. Nektardaki mikrobiyal yoğunluklar, bir çiçeğin ömrü boyunca mikrolitre başına 105 maya hücresine ve 107 bakteri hücresine ulaşabilir. Nektardaki koşullar, yüksek ozmotik basınç ve düşük nitrojen mevcudiyeti ile sert olsa da, *Metschnikowia*, *Wickerhamiella*, *Acinetobacter* ve *Rosenbergiella* gibi ozmotoleranslı türler gelişebilir. Ilıman bölgelerde, nektar topluluğu sınırlı çeşitlilik nedeniyle genellikle tek bir tür tarafından domine edilir. Bununla birlikte, çiçeklenme mevsiminin daha uzun sürdüğü ve bitki çeşitliliğinin daha fazla olduğu tropikal bölgelerde, nektar daha çeşitli mikrobiyal topluluklar sağlar. Tozlayıcılar nektarın mikrobiyal bileşiminin şekillenmesinde önemli bir rol oynar. Böcekler tarafından ziyaret edilen çiçekler ascomycetous maya topluluklarına sahip olma eğilimindeyken, tozlayıcılar tarafından ziyaret edilmeyen çiçekler daha fazla basidiomycete türüne sahiptir. Bazı nektar mikropları bal arılarının bağırsaklarında da yaşar ve patojenleri engelleyen antibiyotikler ve organik asitler üreterek koloni sağlığını etkiler. Nektar ve balın yanı sıra arı bağırsaklarında da bol miktarda bulunan *Lactobacillus* ve *Bifidobacterium* laktik ve asetik asitler üreterek pH'ı düşürür ve patojenlerin büyümesini sınırlar. Bu etkileşimler nektarın kimyasını ve tozlayıcıların sağlığını geliştirerek, karşılıklı fayda sağlayan ve antagonistik etkileşimler yoluyla çiçekleri, mikropları ve tozlayıcıları birbirine bağlayan birlikte evrimleşmiş bir mikrobiyal ekolojiye işaret etmektedir.

Nektar mikrobiyom dinamikleri ve tozlaşma için işlevsel sonuçları: Çiçek nektarında yaşayan mikroorganizmalar, nektarın kimyasal bileşimini değiştirerek bitki-polinatör etkileşimleri üzerinde önemli bir etkiye sahiptir. Neokomagataea ve Rosenbergiella nectarea gibi özelleşmiş bakteriler ve *Metschnikowia reukaufii* gibi mayalar, metabolizmaları yoluyla sükroz seviyelerini düşürüp nektardaki glikoz ve fruktoz konsantrasyonlarını artırmanın yanı sıra amino asit konsantrasyonlarını ve asitliği modüle eder. Bu değişiklikler nektarın

lezzetini ve besin değerini etkileyerek tozlayıcı tercihlerini ve yiyecek arama davranışını doğrudan etkiler. Bazı mikroorganizmalar, tozlayıcıları çekebilen veya itebilen uçucu organik bileşikler yayar; *M*. *reukaufii* meyvemsi aroması nedeniyle özellikle çekicidir. Ayrıca, bu mikroplar nektar hacmini, şeker oranlarını ve çiçek kokusunu etkileyerek tozlaşma başarısını ve üreme verimini etkileyebilir. Bazı mikroplar nektar kalitesini veya polen canlılığını azaltabilirken, diğer mikroplar tozlayıcı sağlığını iyileştirir, bağırsak mikrobiyotasını modüle eder veya Crithidia bombi gibi patojenleri inhibe eder. Bu mikrobiyal ajanlar, çekici meyvemsi esterlerden itici asitlere kadar bir dizi bileşik üretir ve bu bileşikler böcek tozlayıcıların yiyecek arama tercihlerini doğrudan etkiler. Buna ek olarak, tozlayıcıların çiçekler arasında belirli mikrobiyal toplulukları dağıtmak için vektör görevi görmesi ve bağışıklıklarını, besin asimilasyonunu ve patojenlere karşı dirençlerini artıran bağırsak simbiyotik mikroorganizmalardan faydalanması ile ilişki çift yönlüdür. Tropikal ve ılıman ekosistemler farklı mikrobiyal bileşimler sergilediğinden, coğrafi ve ekolojik faktörler bu karmaşıklığa daha da katkıda bulunur. Bitkiler, mikrobiyal aktiviteye yanıt olarak kimyasal savunmalar geliştirmiştir. Bu savunmalar, nektarlarındaki mikropları kullanabilir ya da bastırabilir. Mikrobiyal metabolizma çiçek sıcaklığının düzenlenmesine bile yardımcı olabilir, bu da daha soğuk koşullarda tozlayıcı ziyaretini artırabilir. Son zamanlarda elde edilen bilgilere rağmen, özellikle pestisitler ve habitat parçalanması gibi insan etkilerine ilişkin hala önemli bilgi boşlukları bulunmaktadır. Polinatör popülasyonları azalmaya devam ederken, bitki-polinatör etkileşimlerinin mikrobiyal yönünü anlamak çok önemlidir. Bu, mikroorganizmaların bu hassas biyolojik ağlarda temel ekolojik oyuncular olarak kabul edilmesiyle, biyoçeşitliliği ve tarımsal üretkenliği desteklemek için hedeflenen probiyotiklerin geliştirilmesine yol açabilir.

Sonuç: Mikroorganizmalar, bitki-polinatör ilişkilerinin karmaşık ağında çok önemli bir rol oynar ve ekolojik sonuçları incelikli ancak derinden etkiler. Nektarda yaşayan mayalar ve bakteriler çiçek nektarında sadece pasif olarak bulunmaz, aynı zamanda kimyasal ve fiziksel özelliklerini aktif olarak yeniden şekillendirir. Bu durum tozlayıcı davranışını değiştirir ve nihayetinde bitkinin üreme başarısını etkiler. Bu mikrobiyal ajanlar, çekici meyvemsi esterlerden itici asitlere kadar, böcek tozlayıcılarının besin arama tercihlerini doğrudan etkileyen bir dizi bileşik üretir. Buna ek olarak, tozlayıcıların çiçekler arasında belirli mikrobiyal toplulukları dağıtmak için vektör görevi görmesi ve bağışıklıklarını, besin asimilasyonunu ve patojenlere karşı direncini artıran bağırsak simbiyotik mikroorganizmalardan faydalanması ile ilişki çift yönlüdür. Bu savunmalar, nektarlarındaki mikropları kullanabilir ya da bastırabilir. Mikrobiyal metabolizma çiçek sıcaklığının düzenlenmesine bile yardımcı olabilir, bu da daha soğuk koşullarda tozlayıcı ziyaretini artırabilir. Son zamanlarda elde edilen bilgilere rağmen, özellikle pestisitler ve habitat parçalanması gibi insan etkilerine ilişkin hala önemli bilgi boşlukları bulunmaktadır. Tozlayıcı popülasyonları azalmaya devam ederken, bitki-polinatör etkileşimlerinin mikrobiyal yönünü anlamak çok önemlidir. Bu, mikroorganizmaların bu hassas biyolojik ağlarda temel ekolojik oyuncular olarak kabul edilmesiyle, biyoçeşitliliği ve tarımsal üretkenliği desteklemek için hedeflenen probiyotiklerin geliştirilmesine yol açabilir.

INTRODUCTION

Nectar remains one of the least studied microbial habitats, despite over a century of research. As early as 1884, Boutroux documented yeasts in flowers, fruits, and insects, using morphological and physiological methods to assess their diversity. Subsequent studies have revealed that nectar from diverse plant species worldwide hosts bacterial and yeast communities, with *Metschnikowia* (Ascomycota, Fungi) being one of the most common genera (Boutroux 1884, Álvarez-Pérez and Herrera 2013, Aleklett et al. 2014).

Nectar, rich in carbohydrates and amino acids, attracts insect pollinators and supports dynamic microbial communities, including yeast and bacteria (Willmer 1980, Nicolson and Thornburg 2007). These microorganisms colonize floral surfaces, pollen, and nectar (Álvarez-Pérez et al. 2012, Aleklett et al. 2014, Manirajan et al. 2016) and are dispersed by both biotic and abiotic vectors. Although microorganisms exist in nectar before pollination, animals - particularly pollinators - play a key role in introducing and spreading microorganisms among flowers, enriching floral microbiome diversity (Boutroux 1884,de Vega and Herrera 2012).

Pollinating insects play a significant role in structuring the microbiome of nectar, pollen, and

floral surfaces, with their visitation patterns directly shaping microbial composition (Aizenberg-Gershtein et al. 2013, Morris et al. 2020, Vannette 2020, de Vega et al. 2021, Hietaranta et al. 2022). Honey bees, for instance, act as microbial vectors, introducing nest-associated bacteria and fungi into floral resources (Keller et al. 2021), which can subsequently influence plant fitness and pollinator behavior.

Honey bees harbor a distinct in-hive microbiome that differs from environmental microbial communities (Santorelli et al. 2023). Their social structure reinforces microbiome stability, enabling intergenerational transmission (Canto et al. 2008). When deposited into nectar, these microorganisms can modify floral conditions, effectively acting as ecosystem engineers that mediate plant-pollinator interactions. Thus, nectar functions as a dynamic micro-ecosystem, linking macro- and microorganisms (Vannette et al. 2013).

Microbial communities in nectar can reduce sugar concentration (Herrera et al. 2008, de Vega et al. 2009,de Vega and Herrera 2012), alter carbohydrate and amino acid composition (Herrera et al. 2008, de Vega and Herrera 2012, Lenaerts et al. 2017, Vannette and Fukami 2018), induce floral heating (Herrera and Pozo 2010), and release volatile organic compounds (VOCs) (Rering et al. 2017, Schaeffer et al. 2019). These changes influence pollinator behavior and pollination success (Vannette et al. 2013, Herrera et al. 2013, Junker et al. 2014, Schaeffer and Irwin 2014, Vannette and Fukami 2017, Schaeffer et al. 2017).

Flowers are known to harbour a diverse array of microorganisms, which have the capacity to be passively dispersed by wind, rain, or soil (Zarraonaindia et al. 2015, Sharaby et al. 2020). However, microbial colonization of nectar also depends on animal vectors (Peay et al. 2011, Tucker and Fukami 2014, Álvarez-Pérez et al. 2019). Yeasts and bacteria in nectar are often introduced by birds, bats, beetles, butterflies, ants, flies, and honey bees (Sandhu and Waraich 1985, Lachance et al. 2001, de Vega and Herrera 2012, Canto and Herrera 2012, Vannette 2020, Keller et al. 2021, de Vega et al. 2021). Early work by Boutroux (1884) showed that honey bee-visited flowers contain more enzymatic yeasts than those not visited by honey bees (Boutroux 1884, de Vega and Herrera 2012).

Nectar hosts specific yeast and bacterial species commonly found in this ecological niche across plants worldwide (Brysch-Herzberg 2004, Pozo et al. 2011, Belisle et al. 2012, Fridman et al. 2012, Álvarez-Pérez and Herrera 2013, Jacquemyn et al. 2013, Canto et al. 2017). The bacterial community is often dominated by Proteobacteria, particularly *Acinetobacter*, *Rosenbergiella*, and *Pseudomonas* (Aizenberg-Gershtein et al. 2013, Álvarez-Pérez and Herrera 2013, Álvarez-Pérez et al. 2013, Morris et al. 2020, Sharaby et al. 2020), while the yeast community is frequently represented by *Metschnikowia* species (Brysch-Herzberg 2004, Belisle et al. 2012, Pozo et al. 2012, Schaeffer et al. 2019). Less common nectar-associated yeasts include *Cryptococcus*, *Papiliotrema*, *Rhodotorula*, and *Sporobolomyces* (Basidiomycetes), as well as *Aureobasidium*, *Clavispora, Debaryomyces*, *Hanseniaspora*, *Kodamaea*, *Starmerella*, and *Wickerhamiella* (Ascomycetes) (Klaps, 2019). Ongoing research continues to expand the known diversity of floral yeasts (Klaps et al. 2020), with over a dozen new species described in the past two decades (de Vega et al. 2017, Klaps 2019).

Pollinators act as biological vectors, facilitating the exchange of microorganisms between flowers and insects through the different microbial communities they carry (Brysch-Herzberg 2004, Keller et al.). It has been demonstrated that flowers visited by the same pollinator species possess compositionally similar nectar microbiomes (de Vega et al. 2017). Each pollinator species leaves a unique microbial signature on the flower it visits (Lachance et al. 2001, Ushio et al. 2015, Morris et al. 2020, de Vega et al. 2021), suggesting that the observed differences in floral microbiota are shaped not only by environmental but also pollinator-specific effects. Additionally, nectar microbial diversity varies with climate, with tropical plants typically supporting greater species richness than those in temperate regions (Álvarez-Pérez et al. 2012, de Vega et al. 2017, Canto et al. 2017).

This review explores how microorganisms mediate plant-insect pollinator interactions, focusing on their role in shaping pollination dynamics. Specifically, it examines how microorganisms alter the properties and chemical composition of nectar and honey, influencing the behavior and health of honey bees and other insect pollinators. The discussion highlights the mechanisms by which microorganisms modify nectar's nutritional and chemical profiles, ultimately affecting pollinator activity.

Floral adaptation strategies in plant-pollinator coevolution

Plants have evolved mechanisms to interact with insect pollinators, primarily through nectar secretion, which functions to manipulate pollinator behavior (Pyke 2016). Insect pollinators can directly alter nectar chemistry, partly due to microorganisms they introduce (Aizenberg-Gershtein et al. 2013). For example, honey bee visits increase amino acid concentrations in nectar through mandibular gland secretions, cell wall damage, and pollen transfer (Corbet et al. 1979, Willmer 1980).

The impact on nectar composition varies by pollinator species. While solitary bees (*Andrena* and *Lasioglossum*) do not affect sugar content, bumble bees (*Bombus terrestris* and *B. pratorum*) reduce it (Canto et al. 2008). Honey bees and bumble bees actively modify nectar chemistry, altering acidity and fructose-to-sucrose ratios, likely due to yeast inoculation from their mouthparts (Aizenberg-Gershtein et al. 2013). Pollinator sociality may influence yeast transfer frequency, as bumble bee visits correlate with higher floral yeast densities, while solitary bee visits show an inverse relationship (Herrera et al. 2009).

Nectar-inhabiting yeasts can influence floral thermal microclimates by generating heat through sugar catabolism, elevating both nectar and internal floral temperatures. In nectaries with high yeast densities, this temperature gradient can reach up to 6°C (Herrera and Pozo 2010). For early-flowering plants such as *Helleborus foetidus*, this warming effect has significant ecological consequences, including enhanced pollinator attraction through increased volatile organic compound emissions and improved floral visibility to insects.

Thermogenic flowers, such as *Nelumbo nucifera*, use microbe-mediated heat production to maintain temperatures above ambient levels, attracting pollinators in cooler conditions (Seymour and Schultze-Motel 1998). This thermogenesis not only boosts scent dispersion but also reduces pollinators' thermoregulatory energy costs, increasing their foraging activity (Rands and Whitney 2008). Some species, like *Cistus ladanifer*, combine thermal cues with structural adaptations - such as sticky surfaces that deter predators - though larger flowers face a trade-off, as their size increases both pollinator attraction and susceptibility to herbivory (Teixido et al. 2016).

Chemical signals play an important role in plant-pollinator communication. Yeasts, as part of these mutualistic interactions, can have both beneficial and detrimental effects. While their metabolism of nectar sugars may seem parasitic - exploiting resources meant to attract pollinators (Herrera et al. 2008) - yeasts can also enhance pollination success. For example, they may raise nectar temperature or produce compounds like ethanol, which attract certain pollinators (Wiens et al. 2008, Herrera et al. 2010).

Floral volatile organic compounds (VOCs) act as long-range insect attractants. Some plants employ deceptive strategies, such as *Philodendron solimoesense*, which mimics the scent of decaying matter to lure dung beetles (Seymour et al. 2003). Similarly, *Ophrys* orchids imitate female honey bee pheromones, triggering pseudocopulation in males (Peakall et al. 2010). While these tactics reduce nectar production costs, they may also lower repeat pollinator visits (Jersáková et al. 2006). Nocturnal flowers often emit fungal or carrion-like scents, adapting to the preferences of night-active pollinators (Stökl et al. 2010).

Floral adaptations often involve color variations linked to geographic range and pollinator preferences. For example, *Gentiana lutea* displays flower colors ranging from yellow to greenish hues, corresponding to local honey bee activity (Sobral et al. 2015). Some Asteraceae species feature ultraviolet markings - invisible to humans but visible to insects - that guide pollinators to nectar (Miller et al. 2011). Additionally, color changes during the anthesis period, as seen in *Quisqualis indica*, indicate flower maturity. White blooms attract nocturnal moths, while pink ones attract diurnal honeybees and butterflies (Yan et al. 2016).

Some plants employ highly specialized pollination strategies. The South African *Ceropegia gerrardii* lures kleptoparasitic flies (*Desmometopa spp.*) by mimicking the scent of injured honey bees and offering nectar resembling honey bee hemolymph in protein and sugar content (Heiduk et al. 2023). While such adaptations demonstrate evolutionary plasticity, they also make plants vulnerable to declines in their specialized pollinator populations (Zariman et al. 2022).

Nectar microorganisms, such as yeasts and bacteria, play a significant role in plant-pollinator interactions. By colonizing nectar and changing its chemistry, they modify its nutritional value and

attractiveness, ultimately influencing insect foraging behavior (Vannette et al. 2013, Martin et al. 2022). For instance, yeasts metabolize sucrose into fructose and produce volatile organic compounds (VOCs), such as alcohols and ketones, which pollinators can detect (Herrera et al. 2013, Rering et al. 2017). Although these changes reduce nectar's caloric content, they often enhance its appeal to bumble bees and honey bees (Schaeffer et al. 2017).

Critically, microbial VOCs not only attract pollinators but may also suppress competing microorganisms, creating a dynamic interplay between nectar microorganisms and plant chemistry (Good et al. 2014, Cullen et al. 2021). These volatiles can either increase or decrease nectar attractiveness, fine-tuning pollinator preferences (Rering et al. 2017). Thus, microorganisms act as both biochemical modifiers and ecological mediators, bridging plant and pollinator interactions (Table 1).

Table 1. Plant-pollinator-microbe interaction mechanisms and examples

Strategy	Mechanism	Specific Microbial Taxa and Metabolite Involved	Effects on Nectar/Pollinators	Plant Examples	Citations
Nectar Chemistry Modification	Pollinators introduce microorganisms that alter sugar composition and pH	*Metschnikowia reukaufii* (yeast), *Acinetobacter spp.* (bacteria)	Converts sucrose to fructose+glucose, reduces sugar concentration by 15-30%, lowers pH by 1-2 units	General across angiosperms	Herrera et al. 2008, Canto et al. 2008, Álvarez-Pérez and Herrera 2013
Thermal Microclimate Regulation	Microbial metabolism generates heat	*Metschnikowia gruessii* (yeast)	Increases nectar temp by 2-6°C, enhances VOC emission by 20-40%	*Helleborus foetidus*, *Nelumbo nucifera*	Herrera and Pozo, 2010, Seymour and Schultze-Motel, 1998
Volatile Organic Compound (VOC) Production	Microorganisms synthesize attractant/deterrent compounds	*M. reukaufii* (fruity esters), *Asaia astilbes* and *Neokomagataea sp.* (2,5-dimethylfuran)	Increases bumble bee visits by 25-50%, repulses honey bees at high concentrations	*Mimulus aurantiacus*	Rering et al. 2017, Schaeffer et al. 2017, Good et al. 2014
Nutritional Quality Modulation	Microbial consumption/transformation of nutrients	*Rosenbergiella nectarea* (reduces amino acids), *Neokomagataea sp.* (increases amino acids)	Alters pollinator foraging duration by 30-60%, affects colony growth rates	*Epilobium canum*	Lenaerts et al. 2017, Pozo et al. 2021
Antimicrobial Defense	Plant secondary metabolites regulate microorganisms	Callunene in heather inhibits the bumble bee parasite *Crithidia bombi*	Reduces *Crithidia bombi* infections by 70-90% in bumble bees	*Calluna vulgaris*	Koch et al. 2019, Carter and Thornburg,, 2004
Pollen-Nectar Microbiome Linkage	Gut microbiota acquisition from flowers	*Lactobacillus kunkeei, Bifidobacterium spp.*	Improves honey bee pathogen resistance (30-50% survival increase)	General across bee-pollinated plants	Arredondo et al. 2018, Vásquez et al. 2012
Specialized Pollinator Attraction	Co-evolved scent/chemical mimicry	*Saccharomyces spp.* (ethanol production)	Attracts kleptoparasitic flies (*Desmometopa spp.*)	*Ceropegia gerrardii*	Wiens et al. 2008, Heiduk et al. 2023

Specialization and adaptation of microorganisms in floral niches

The microorganisms inhabiting flowers form complex, dynamic communities derived from diverse environmental sources, including air, soil, and water. Both bacteria and fungi colonize flowers early in development, appearing on buds and petals as soon as they form (Shade et al. 2013, Morris et al. 2020). Notwithstanding their early presence, microbial abundance remains low, thus indicating transient colonization (Brysch-Herzberg 2004, Morris et al. 2020).

An important factor shaping nectar microbial communities is limited dispersal. Insects, such as pollinators, introduce yeasts and bacteria into nectar, but only those capable of tolerating high osmotic pressure and outcompeting rivals persist (Álvarez-Pérez et al. 2019, Vannette 2020). Since most flowers are short-lived, dispersal critically influences microbial species richness, composition, and function. Potential dispersal vectors - wind, water, plant tissues, and flower-visiting animals - each carry distinct microbial groups (Vannette 2020). However, not all arriving microorganisms successfully establish on floral surfaces. Environmental stressors, including UV radiation, desiccation, patchy nutrient availability, and microbial competition, limit colonization (Herrera et al. 2010). For instance, the bumble bee pathogen *Crithidia bombi* remains infectious on petals and sepals but survives only a few hours under environmental exposure (Figueroa et al. 2019), underscoring how abiotic and biotic factors shape floral microbial communities.

Microorganisms can colonize flowers not only from external sources but also from within the plant itself. Endophytes, microorganisms living within plant tissues without causing visible harm, along with surface pathogens and epiphytic microorganisms, can migrate into floral tissues. For example, fungal hyphae have been observed moving from vegetative tissues to flowers in grasses (Hinton and Bacon 1985). Bacteria can also travel through the plant's vascular system, moving between flowers and other tissues (An et al. 2020, Kim et al. 2019, Vanneste 2000). It is hypothesized that certain species of bacteria that are endemic to the xylem may be able to penetrate nectar through secreted droplets. Nevertheless, this assertion is not yet substantiated by empirical evidence (An et al. 2020, Roy et al. 2017).

Microorganisms also colonize flowers through interactions with honey bees, which act as vectors for microbial transfer (Shi et al. 2025). When honey bees visit flowers to collect nectar and pollen, microbes from the honey bees or hive environments can adhere to floral surfaces and nectar (Hietaranta et al. 2022). Honey bees visitation transfers microbes from the insect to the flower, shaping the floral microbiome (Lignon et al. 2024). The presence of honey bees significantly alters the community composition of both bacteria and fungi on inflorescences, highlighting their role in structuring floral microbial ecosystems (Hietaranta et al. 2022). This dynamic interplay underscores the ecological importance of honey bees in mediating plant-microbe-pollinator interactions (Shi et al. 2025).

Epiphytic microorganisms can reach flowers from leaves, or from a shared external source, via wind, water, rain splash, or insects. Bacterial communities on petals and leaves often overlap, but floral microorganisms tend to be less diverse and unevenly distributed (Junker et al. 2014, Massoni et al. 2020, Wei and Ashman 2018). This supports the idea that microorganisms move from leaves (or a common source) to flowers. While microbial transfer from flowers back to leaves is possible, it is likely rarer due to flowers' shorter lifespans.

Although leaves and flowers often host similar microbial species, it remains unclear whether the strains differ between these tissues. Future studies could use single-cell tracking or comparative genomics to trace microbial origins and adaptations to floral environments (Vannette 2020).

A specialized group of microorganisms thrives in floral environments, often reaching high abundances on flower tissues but rarely on leaves or other plant parts. Though these bacteria and fungi also appear in honey bee crops and stored pollen, they are typically scarce outside of flowers (Brysch-Herzberg 2004, Pozo et al. 2012). Their limited distribution beyond nectar strongly suggests ecological specialization. Additionally, they exhibit traits that may be adaptations to floral life, including rapid growth, efficient nutrient assimilation (Dhami et al. 2016), tolerance to osmotic stress (Herrera et al. 2010), and the ability to metabolize floral-specific compounds (Lachance et al. 2001, Pozo and Jacquemyn 2019). However, further comparative and experimental studies are needed to confirm which traits truly represent floral adaptations (Pozo et al. 2012).

Flowers are not sterile, even at early developmental stages. Microorganisms are nearly always present on some floral tissues, though their abundance and composition vary widely. Detection methods differ in their biases, but studies consistently identify three key patterns in floral microbiome origins (Vannette 2020, Ilyasov et al. 2024).

Firstly, it has been determined that bacteria and fungi manifest during the initial phases of floral development, in some cases even preceding the opening of buds (Shade et al. 2013, von Arx et al. 2019). Newly opened petals and nectar-containing apple blossoms often harbor culturable microorganisms, though detection rates can be low (8–35% at anthesis) (Pusey et al. 2009). Filamentous fungi and bacteria have also been found in grass ovaries (Hinton and Bacon 1985) and herbaceous plant pollen (Hodgson et al. 2014) during early floral development (Vannette 2020).

Second, microbial abundance in flowers often increases over time. Strong evidence comes from studies of nectar and pistil surfaces. For example, in the nocturnal plants *Datura wrightii* and *Agave palmeri,* bacterial and fungal colony-forming units (CFUs) in nectar were low before flowers opened but rose exponentially afterward (von Arx et al. 2019). Similarly, in *Mimulus aurantiacus*, yeast detection increased from 20% in one-day-old flowers to 60–80% in older flowers (Peay et al. 2011). Bacteria also became more frequent and abundant over time in *Epilobium canum* nectar (Morris et al. 2020) and on apple flower stigmas (Pusey et al. 2009). While most studies focus on nectar, data on pollen and petal microbiomes remain scarce. To confirm whether this trend applies broadly, future research should use quantitative methods like qPCR or microscopy (Vannette 2020, Ilyasov et al. 2024).

Third, pollinators shape the nectar microbiome. Although microorganisms can occur in unvisited flowers, insects are key vectors for microbial transmission. As early as 1884, Boutroux observed more fermentative yeasts in honey bee-visited flowers than in untouched ones. Recent studies confirm that ascomycetous yeasts thrive in pollinator-visited nectar but are absent when honey bees and other large pollinators are excluded (Belisle et al. 2012, Herrera et al. 2008, 2010). Some pollinators even disperse specific microorganisms - hummingbirds and large insects boost bacterial abundance in *Mimulus aurantiacus* and *Epilobium canum* (Vannette and Fukami 2017, Morris et al. 2020), while Nitidulid beetles spread large-spored yeasts (Lachance et al. 2001), and ants carry diverse bacteria and yeasts (de Vega and Herrera 2012, Samuni-Blank et al. 2014). Smaller insects, like thrips, may also play an overlooked role, as they harbor bacteria (*Rosenbergiella*, *Pantoea*) commonly found in flowers (von Arx et al. 2019) and pollen (Manirajan et al. 2016).

Insect-associated microorganisms commonly colonize flowers. These bacteria, fungi, and viruses - often isolated from insect digestive tracts (Corby-Harris et al. 2014), mouthparts (Belisle et al. 2012, Pozo et al. 2012), or nest environments (Brysch-Herzberg 2004, McFrederick et al. 2012, 2017) - can be beneficial, neutral, or pathogenic to pollinators and other floral visitors. Although they rarely reach high abundances on flowers (Lachance et al. 2001), they persist long enough on floral surfaces to transfer to new hosts. While pollinators can acquire pathogens from flowers, the role of floral transmission compared to other routes remains unclear (Vannette 2020).

Pollinators shape microbial communities across floral tissues. Though most research focuses on nectar-inhabiting microorganisms, floral visitors also influence microbial composition on petals, stigmas, pollen, and other structures. Bumble bees, for instance, deposit bacteria primarily on petals and stamens (Russell et al. 2019). Bee feces, rich in microorganisms, often contaminate inner and outer corollas, bracts, and nearby leaves, with deposition patterns depending on flower morphology and bee species (Figueroa et al. 2019). Insects also alter the pollen microbiome's species composition (Manirajan et al. 2016, Vannette 2020, Ilyasov et al. 2024).

Tracking microbial life cycles remains challenging. Short rRNA gene regions used in sequencing often fail to distinguish between closely related species or strains, masking ecologically distinct taxa (Dhami et al. 2018). Additionally, detecting environmental microorganisms is complex, making presence/absence determinations difficult. Microbial lineages likely vary from animal- to plant-associated, both ecologically and evolutionarily. For example, *Lactobacillus* (McFrederick et al. 2012), *Acinetobacter*, and *Starmerella* yeasts (Lachance et al. 2001, Rosa et al. 2003) include species specialized for animals or flowers, yet the frequency of host shifts remains poorly understood (Vannette 2020).

Pollinator-mediated shaping of nectar and honey microbial communities

Nectar serves as a unique habitat for microorganisms, fostering interactions between fungi, bacteria, and the nectar's chemical environment. Studies on nectar microbiomes have primarily examined bacteria and fungi, the dominant components of these communities (Vannette 2020, Herrera et al. 2009, Fridman et al. 2012). The composition of these microbial communities varies significantly depending on pollinator type, suggesting that pollinators not only transfer pollen but also disperse microorganisms, shaping distinct nectar microbiomes (Belisle et al. 2012, Morris et al. 2020, Vannette 2020). Interactions between these microorganisms further influence species diversity, highlighting the complexity of these microecosystems (Vannette and Fukami 2017).

Microbial abundance in nectar increases over the flower's lifespan, reaching densities of up to 10^5 yeast cells and 10^7 bacterial cells per microliter (Herrera et al. 2009, Fridman et al. 2012). In temperate regions, nectar microbiomes typically exhibit low diversity, often dominated by a single yeast or bacterial species (Belisle et al. 2012, Pozo et al. 2011). This limited diversity likely results from dispersal constraints, competition, and harsh conditions like high osmotic pressure and low nitrogen availability (Peay et al. 2011, Vannette and Fukami 2014). In contrast, subtropical and tropical regions may support greater microbial diversity due to extended flower availability, higher plant diversity, and shorter flower lifespans that reduce competitive exclusion (Mittelbach et al. 2015, Canto et al. 2017).

Floral nectar hosts specialized microbial communities dominated by osmotolerant and low-nitrogen-adapted species. The most common are ascomycete yeasts, including *Metschnikowia* (e.g., *M. reukaufii* and *M. gruessii*), *Wickerhamiella*, *Starmerella*, and *Kodamaea*, as well as bacteria like *Acinetobacter* and *Rosenbergiella*. These microorganisms thrive under nectar's high osmotic pressure and nutrient scarcity, outcompeting less-adapted species (Lachance et al. 2018, Santos et al. 2018, Vannette 2020, Martin et al. 2022).

Pollinator activity shapes nectar microbial composition: flowers visited by insects favor ascomycetous yeasts, while isolated flowers show higher basidiomycetous yeast abundance (Lachance et al. 2001, Herrera et al. 2008, 2010, Belisle et al. 2012, Hietaranta et al. 2022). Filamentous fungi, by contrast, are rare in nectar (Taniwaki et al. 2015). Although non-specialized microorganisms from soil, water, or plant surfaces occasionally enter nectar, they typically persist at low levels due to poor stress tolerance (de Vega and Herrera 2012, Martin et al. 2022, Ilyasov et al. 2024).

Microorganisms in floral nectar compete for resources, often excluding each other through niche preemption or modification (Fukami 2015). Bees ingest these nectar-dwelling bacteria, which then colonize their gut and influence the microbial diversity of their colonies - sometimes beneficially, sometimes harmfully (Anderson et al. 2013). Many of these microorganisms produce antibiotics, suppressing pathogens in nectar, stored food, or honey bee gut (McCormack et al. 1994, Parret and De Mot, 2002, Pozo et al. 2020). For instance, bacteria like *Lactobacillus kunkeei* - found in nectar and honey - can inhibit honey bee pathogens such as *Paenibacillus larvae* and *Nosema ceranae* (Arredondo et al. 2018, Nowak et al. 2021).

The honey bee gut microbiome also metabolizes nectar and pollen, producing lactic, acetic, and citric acids, which support energy metabolism, and suppress pathogens (Ricigliano and Anderson 2020). When honey bees eat pollen-deficient diets, their microbiome diversity declines, reducing organic acid production and weakening immunity. However, supplementing their diet with lactic acid can restore some resistance to infections (Ricigliano and Anderson 2020).

Species of *Lactobacillus*, the dominant genus in honey bee gut microbiota (Romanovich et al. 2020), drive carbohydrate fermentation, producing lactic acid that energizes honey bees and suppresses pathogenic *Enterobacteriaceae* by acidifying the gut environment (Ricigliano and Anderson 2020). Deficiencies in these organic acids are linked to impaired foraging behavior, including reduced activity and social interactions in hives (Ricigliano and Anderson 2020).

Although floral nectar is often dominated by single microbial species (Peay et al. 2011, Belisle et al. 2012, Vannette and Fukami 2014, Tucker and Fukami 2014), diverse microorganisms frequently co-occur (de Vega et al. 2021). For example, *Metschnikowia* yeasts and *Acinetobacter* bacteria exhibit positive associations, likely due to niche partitioning: *Metschnikowia* ferments glucose, while *Acinetobacter* metabolizes fructose (Álvarez-Pérez and Herrera 2013). Such interactions suggest

microbial communities may have additive or synergistic effects on nectar chemistry and pollinator health. Indeed, yeast-bacteria consortia can enhance nectar aroma, boosting visits by honey bees and hoverflies (Golda et al. 2021), though their impact on bumble bee colony growth remains no greater than that of single species (Pozo et al. 2021).

The honey bee gut microbiome is largely derived from microorganisms in plant nectar, primarily bacteria and yeasts. Studies show that honey's microbial composition varies by geographic location, floral source, and storage conditions (Brudzynski 2021). Common bacterial genera include *Lactobacillus*, *Bifidobacterium*, *Gluconobacter*, and *Asaia*, which can function as beneficial symbionts or potential pathogens depending on their abundance and environmental context (Brudzynski 2021). For instance, *Lactobacillus* and *Bifidobacterium* exhibit probiotic properties and help protect honey bees from pathogens (Vásquez et al. 2012, Ilyasov et al. 2024).

Lactobacillus spp., a well-studied group of lactic acid bacteria in honey, play a key role in fermentation and preservation. They produce antimicrobial compounds such as hydrogen peroxide, bacteriocins, and organic acids like lactic acid. By lowering pH, lactic acid creates an inhospitable environment for pathogens, while hydrogen peroxide broadly inhibits bacterial growth. Certain *Lactobacillus* strains also secrete bacteriocins - peptide-based antimicrobials - such as nisin, which targets pathogens like *Listeria* (Brudzynski 2021).

Bifidobacteria (*Bifidobacterium spp.*), beneficial microorganisms found in honey, exhibit probiotic properties and produce antimicrobial compounds such as acetic acid, lactic acid, and bacteriocins. These substances inhibit pathogens by lowering environmental pH and directly targeting harmful bacteria like *Escherichia coli* and *Salmonella* (Brudzynski 2021).

Acetic acid bacteria (*Gluconobacter* and *Asaia*), also present in honey, contribute to its antimicrobial activity by producing organic acids that suppress pathogen growth. Their antibacterial effects are particularly strong against Gram-negative bacteria (Crotti et al. 2010).

Certain *Bacillus* species in honey generate antimicrobial peptides like bacitracin, which effectively target Gram-positive bacteria, including *Staphylococcus* and *Streptococcus* (Brudzynski 2021).

Yeasts such as *Metschnikowia reukaufii* may not always produce antimicrobials, but some species secrete ethanol and volatile organic compounds (VOCs) that inhibit microbial growth. Additionally, certain yeasts generate acetic and lactic acids, further enhancing honey's protective effects (Good et al. 2014). Although fungi like *Penicillium* are typically linked to honey spoilage, some species produce antibiotics such as penicillin, which combat Gram-positive bacteria (Brudzynski 2021).

Nectar microbiome dynamics and their functional consequences for pollination

Microorganisms in nectar significantly alter its chemical composition, thereby shaping plant-pollinator interactions. Through metabolic activity, they consume sugars - reducing overall sugar concentration - and convert sucrose into glucose and fructose (de Vega et al. 2009, Herrera et al. 2008, Canto et al. 2008, Vannette and Fukami 2017). Additionally, they modify amino acid levels and other nectar components, with effects varying by microbial species (Lenaerts et al. 2017, Rering et al. 2017).

Specialized bacteria such as *Neokomagataea*, *Rosenbergiella nectarea*, and *Acinetobacter spp.* exhibit metabolic effects similar to the yeast *Metschnikowia reukaufii*, reducing sucrose while increasing glucose and fructose (Lenaerts et al. 2017, Rering et al. 2017, Álvarez-Pérez et al. 2021, Kim et al. 2014). *Acinetobacter* species (e.g., *A. nectaris*, *A. boissieri*) preferentially consume fructose and nitrogenous yeast byproducts like ammonia, often co-occurring with yeasts (Álvarez-Pérez and Herrera 2013). While *R. nectarea* and *Acinetobacter* decrease amino acid concentrations, *Neokomagataea* increases them (Martin et al. 2022). These shifts in sugar and amino acid profiles may directly affect pollinator nutrition (Álvarez-Pérez et al. 2021).

It has been demonstrated that generalist bacteria, including *Lactococcus garvieae*, *Apilactobacillus kunkeei* (Zheng et al. 2020), *Erwinia tasmaniensis*, and *Asaia spp.*, also have an influence on the chemistry of nectar (Zheng et al. 2020). *E. tasmaniensis* does not alter sugar or amino acid levels but lowers nectar pH (Martin et al. 2022). In contrast, acetic acid bacteria (*Asaia platycodi*, *A. astilbes*) reduce sucrose and amino acids while increasing monosaccharides and acidity via acetic

acid production (Lenaerts et al. 2017). The lactic acid bacterium *L. garvieae* leaves sugar levels unchanged but raises amino acid concentration and decreases pH through lactic acid secretion (Lenaerts et al. 2017).

Fungi play a key role in shaping nectar chemistry. For example, the black yeast *Aureobasidium pullulans* decreases sucrose concentration and metabolizes fructose, while the yeast *Sporobolomyces roseus* increases amino acid content (Sobhy et al. 2018). Similarly, *Hanseniaspora uvarum* alters the emission of volatile organic compounds (VOCs) (Sobhy et al. 2018). All three of these fungi also lower nectar pH (Sobhy et al. 2018, Ilyasov et al. 2024). Another common nectar yeast, *Metschnikowia*, reduces sucrose and glucose levels while increasing fructose proportions (Herrera et al. 2008, Canto et al. 2015, Pozo et al. 2020). It also produces VOCs (Sobhy et al. 2018) and lowers nectar pH (Vannette et al. 2013, Tucker and Fukami 2014). Additionally, *Metschnikowia reukaufii* depletes amino acids in nectar (Dhami et al. 2016).

It has been demonstrated that specialized nectar microorganisms modify nectar's nutritional properties by consuming sugars, thereby shifting their ratios and acidifying the nectar (Vannette et al. 2013, Tucker and Fukami 2014, Lenaerts et al. 2017, Pozo et al. 2020). They can also metabolize, modify, or synthesize amino acids and secondary metabolites, altering nectar's taste and aroma (Herrera et al. 2013, Schaeffer et al. 2017).

These microorganisms also release VOCs that influence nectar attractiveness. Some bacteria and fungi on flowers produce VOCs that blend with floral scents or break down plant volatiles (Farré-Armengol et al. 2016), ultimately affecting pollinator behavior (Schaeffer et al. 2019). Certain microorganisms - and even plants - produce compounds that repel insects, such as toluenediisocyanate. For instance, *Asaia astilbes* and *Neokomagataea sp.* emit 2,5-dimethylfuran, a potential deterrent (Schaeffer et al. 2019).

Nectar-inhabiting microorganisms, particularly yeasts and bacteria, can alter nectar chemistry and influence pollinator nutrition and foraging behavior. While some microorganisms reduce sugar concentrations, potentially creating competition between microorganisms and pollinators (Herrera et al. 2008), bumble bees often prefer yeast-colonized nectar (Schaeffer and Irwin 2014, Herrera et al. 2013, Yang et al. 2019). This preference may arise from changes in scent and taste, as the yeast *Metschnikowia reukaufii* produces attractive fruity and floral esters (Golonka et al. 2014).

Pollinators rely on visual, olfactory, and gustatory cues to find food, so microbial changes in nectar directly affect their choices. Both bumble bees and honey bees detect and respond to volatile organic compounds (VOCs) emitted by nectar microorganisms. For example, naive bumble bees (*Bombus impatiens* and *Bombus terrestris*) use *M. reukaufii* VOCs to locate nectar (Herrera et al. 2013, Schaeffer et al. 2017) and consume more yeast-infected nectar than sterile nectar (Herrera et al. 2013, Schaeffer et al. 2017). In contrast, their responses to bacteria are often neutral or negative (Good et al. 2014, Junker et al. 2014). Interestingly, while *B. impatiens* is attracted to odors from the bacterium *A. astilbes*, it still consumes more nectar infected with *M. reukaufii* (Schaeffer et al. 2017).

Bees are highly sensitive to changes in sugar composition and concentration. For example, low sugar levels reduce foraging activity in honey bees (Oldroyd et al. 1991, Seeley et al. 2000, Brunner et al. 2014, Stabler et al. 2015, Grund-Mueller et al. 2020). Bumble bees, in particular, prefer sucrose (Mommaerts et al. 2013, Gegear and Thomson, 2004), and immune-challenged workers consume 7.5% more sugars (Dolezal and Toth 2018). Nutrient scarcity can also weaken honey bee immune responses (Brunner et al. 2014, Stabler et al. 2015, Grund-Mueller et al. 2020).

Microorganisms in nectar can change its chemistry, affecting honey bee feeding behavior. Honey bees avoid nectar contaminated with *Asaia astilbes*, *Erwinia tasmaniensis*, and *Lactobacillus kunkeei* but show no aversion to *Metschnikowia reukaufii* (Good et al. 2014). Some bacteria, like *Asaia astilbes*, lower nectar pH while increasing glucose and fructose levels (Good et al. 2014). These chemical changes - rather than the microorganisms themselves - primarily drive honey bee preferences (Brudzynski 2021).

Microorganisms in nectar can increase hydrogen peroxide levels, potentially benefiting honey bee health (reducing pathogenic microorganisms and shaping their normal microbiome) (Vannette et al. 2013, Rothman et al. 2020). Some microorganisms can inhibit pathogens like *Crithidia bombi* in bumble bees (Richardson et al. 2015, Palmer-Young et al. 2019, Folly et al. 2020).

Changes in nectar composition influence adult honey bees, queen egg-laying, and brood health (Steffan et al. 2019). In honey bees and bumble bees, nutrition affects ovary development and egg production (Fine et al. 2018, Lin and Winston 1998). While nectar colonized by *M. reukaufii* does not alter worker bumble bee egg production (Schaeffer et al. 2017), nectar containing bacteria like *P. coronafaciens*, *A. nectaris*, and *R. nectarea* boosts oviposition and brood quantity in bumble bees (Pozo et al. 2021). Overall, microbial effects on bumble bee colony development vary from neutral to positive (Martin et al. 2022).

Specific yeasts and bacteria in nectar can shape bumble bee (*B. terrestris*) development (Pozo et al. 2020, Pozo et al. 2021). Two of five studied yeast species increased worker numbers, likely by enriching nectar micronutrients (Pozo et al. 2020). Each yeast species also produced distinct fructooligosaccharides - compounds with prebiotic properties (Peshev and Van den Ende 2014).

Nectar composition influences the bacterial community structure in honey bee gut. High sugar concentrations and larger nectar volumes increase flower visitation rates (Mallinger and Prasifka 2017). Honey bees fed sucrose-rich nectar develop more diverse midgut communities, whereas those consuming glucose- or fructose-rich nectar show greater hindgut diversity (Wang et al. 2020). Since honey bee gut microbiome affects host health (Hammer et al. 2021, Lee et al. 2015, Zheng et al. 2019, Martin et al. 2022).

Microorganisms also alter the nutritional value of pollen in nectar. For example, *Metschnikowia reukaufii* clusters around pollen grains, consuming their nutrients (Herrera 2017, Pozo and Jacquemyn 2019) and causing pollen rupture (Eisikowitch et al. 1990). Similarly, *Acinetobacter spp.* trigger pollen germination and rupture in *Eschscholzia californica* (Christensen et al. 2021). Some microorganisms degrade pollen quality - the pathogen *Microbotryum violaceum* replaces pollen with spores, disrupting plant reproduction (Alexander and Antonovics 1988). Non-pathogenic fungi and bacteria colonize pollen surfaces, reducing viability, as seen in *Asclepias syriaca* (Eisikowitch et al. 1990), and limiting nutrient availability for pollinators (Roulston and Cane 2000). However, some honey bee-associated bacteria aid digestion by producing pectinases that break down pollen (Engel et al. 2012, Vuong and McFrederick 2019).

Floral microorganisms also modify nectar, pollen, and floral traits that attract pollinators. Pathogens like *Microbotryum violaceum* lower nectar volume and sugar content (Biere and Honders 2006), while *Fusarium verticillioides* extends flowering in *Moussonia deppeana*, prolonging nectar availability (Lara and Ornelas, 2003).

Microorganisms can significantly alter nectar chemistry and properties, as demonstrated in laboratory studies (Vannette et al. 2013, Lenaerts et al. 2017, Vannette and Fukami, 2018). For example, flowers inoculated with *Neokomagataea anthophila* and *Gluconobacter sp.* produced less nectar, likely due to accelerated senescence (Vannette and Fukami, 2018). However, differences between laboratory and field results suggest that host plants may regulate microbial effects on nectar chemistry (Vannette et al. 2013).

Yeast and bacteria form complex microbial communities in nectar through aggregation, biofilm formation, nutrient competition, antagonism, signaling, and horizontal gene transfer (Álvarez-Pérez et al. 2013, Tucker and Fukami 2014, Álvarez-Pérez et al. 2019). Their co-occurrence is often negatively correlated, pointing to competitive interactions like antibiosis (Álvarez-Pérez et al. 2019). While these mechanisms explain some microbial patterns, they do not fully account for global variations in community composition (de Vega et al. 2021, Ilyasov et al. 2024).

Nectar chemistry - including sugars, amino acids, proteins, and alkaloids - plays a significant role in shaping these communities (Nicolson and Thornburg 2007). High osmotic pressure restricts colonization to osmotolerant species (Álvarez-Pérez et al. 2012, Lenaerts et al. 2014, Morris et al. 2020), favoring specific yeasts and bacteria (Adler 2000, Adler et al. 2020, Carter and Thornburg 2004). Yet, nectar chemistry alone does not fully explain microbial diversity (de Vega et al. 2021).

The sugar ratio in nectar, influenced by both plants and microorganisms, can affect pollinator attraction (Colda et al. 2021). Nectar composition often aligns with pollination strategies (Baker and Baker 1983, 1990), but even plants visited by the same pollinators show substantial chemical variation (Barnes et al. 1995, Brown et al. 2010). While sugars contribute to microbial differences, they are not the sole factor (Johnson 2000, Nicolson and Thornburg 2007). Plant-derived antimicrobial compounds may be more decisive in structuring these communities

(Buwa and Van Staden 2006, Aremu and Van Staden 2013, Amoo et al. 2014).

Floral volatile organic compounds (VOCs) can influence microbial growth, but their effects vary. For example, (E)-β-caryophyllene in *Arabidopsis thaliana* suppresses the pathogenic bacterium *Pseudomonas syringae* (Huang et al. 2012), while phenylacetonitrile and linalool have species-specific effects on bacteria (Hua et al. 2014, Junker et al. 2014, Burdon et al. 2018).

Plant secondary metabolites, such as flavonoids and alkaloids, may help structure floral microbial communities by deterring pathogens (Adler 2000, Rivest and Forrest 2019). In *Calluna vulgaris* (heather), the nectar compound callunene inhibits the bumble bee parasite *Crithidia bombi* at natural concentrations, suggesting that other metabolites with similar antimicrobial roles may exist (Koch et al. 2019). However, not all secondary metabolites necessarily evolve for microbial defense, as experimental evidence sometimes contradicts this hypothesis (Fridman et al. 2012, Pozo et al. 2012, Vannette and Fukami 2017).

Floral traits like high nectar osmolarity, antimicrobial-like proteins, elevated oxygen levels, and reactive oxygen species further restrict microbial growth (Herrera et al. 2010, Schmitt et al. 2018), making nectar inhospitable to certain pathogens (Carter et al. 1999, Schmitt et al. 2018). Their precise role in shaping microbial communities remains an important area for future research.

Plants employ induced defense mechanisms to regulate floral microbial communities. For example, pepper flowers respond to *Xanthomonas campestris* infection by producing antimicrobial enzymes faster than leaves (O'Garro and Charlemange 1994). Similarly, apple trees rely on pathogen-defense genes to resist *Erwinia amylovora* (Khan et al. 2012). While plants appear to actively manage floral microbiomes, it is unclear whether these responses target only pathogens or more general microbial regulation (Vannette 2020).

Nectar's antimicrobial compounds shape microbial communities by restricting survival and growth. Certain plants produce substances that specifically inhibit bacteria or yeasts (Ncube et al. 2015), though the full extent of these effects requires further study (de Vega et al. 2021).

Geographic factors influence nectar microbial communities, but plant-pollinator interactions have stronger effects. Nectar microbiomes show greater similarity among plants visited by the same pollinator group across different regions than among co-located plants with different pollinators (de Vega et al. 2021). Yeast communities demonstrate phylogenetic patterns tied to geography, reflecting historical dispersal (Lachance et al. 2005, de Vega et al. 2014), while some insect-associated yeasts show restricted distributions matching their host insects' ranges (de Vega et al. 2021).

Conclusion

Plants, pollinators, and microorganisms form a complex web of interactions, with microorganisms playing a crucial role in shaping these relationships in surprising ways. This review focuses on how nectar-feeding yeasts and bacteria influence the chemistry and physical properties of nectar, affecting pollinator behavior and plant reproduction.

Microorganisms can modify nectar's chemical composition and physical characteristics, making it more or less attractive to pollinators. For example, *Metschnikowia* yeast strains produce fruity esters that attract bumble bees, while bacteria such as *Asaia astilbes* sour nectar and repulse honey bees. These microorganisms demonstrate their ability to guide pollinator foraging decisions.

These interactions are bidirectional. Pollinators transfer microorganisms from one flower to another, but the microorganisms that they carry affect their health and nutrition. Bee gut bacteria, such as *Lactobacillus* and *Bifidobacterium*, help with digestion, boost immunity, and combat pathogens, creating a cycle in which pollinators influence the microbial composition of nectar, which then affects their own well-being.

This interaction reveals microorganisms as crucial players in plant-pollinator relationships, amplifying or moderating benefits for both parties. Geography and ecology add complexity to this system. Flowers in tropical regions, which bloom year-round, have richer microbial communities compared to those in temperate regions. The type of pollinator also matters - hummingbird-pollinated flowers carry different microorganisms than those visited by bees, demonstrating how pollinator behaviors shape the microbial profile of nectar.

Microorganisms play an important role in pollination, but they do more than just guide the process. They can alter nectar in ways that can either benefit or harm plants by attracting or draining nutrients,

respectively. Some plants have developed defenses, like callunene in heather nectar, to fight off microorganisms and maintain their success. Pollinators also benefit from warmer flowers, thanks to yeast-produced heat, and protection against bumble bee pathogens, such as *Crithidia bombi.*

Despite these interactions, there are still many gaps in our understanding of how microorganisms, plants, and pollinators interact. Future research should combine tools such as gene sequencing, chemical analysis, and behavioral tests to better understand the roles of microorganisms in various ecosystems. Additionally, we know little about the effects of pesticides and habitat loss on these intricate relationships.

In short, microorganisms play an active role in shaping plant-pollinator interactions by adjusting nectar composition, supporting pollinator health, and helping them adapt to environmental changes. As the decline of pollinators threatens ecosystems and agricultural production, understanding the role of microorganisms could lead to innovative solutions such as bee probiotics and microbiome-enhanced pollination. By recognizing these tiny actors, we can appreciate the complexity of nature's interconnected systems and the need for broader efforts to protect them.

Acknowledgments: We acknowledge for Government basic research program in IDB RAS No. 0088-2024-0009.

Author Contributions: Conceptualization, R.A.I., D.V.B., S.N.K., A.G.M.; writing review and editing, R.A.I., A.Y.I., V.N.S., V.N.D, M.K., S.R., P.H.T., S.N.K., A.G.M., S.S.P. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

Data Availability Statement: All reviewed and analysed papers are available on their websites.

Informed Consent Statement: Not applicable

Institutional Review Board Statement: The Institutional Review Board checked this Review manuscript and concluded that the article contains reliable data, does not contain data prohibited for publication, and can be published in scientific journal.

Ethical Standards Compliance: This article does not contain any studies involving animals as subjects.

Ethical statement: Ethics committee approval is not required.

Funding: This research was funded by Russian Science Foundation (RSF) grant 24-16-00179 (Rustem Ilyasov).

REFERENCES

Adler L. S. Alkaloid Uptake Increases Fitness in a Hemiparasitic Plant via Reduced Herbivory and Increased Pollination. The American naturalist, 2000; 156(1): 92-99. https://doi.org/10.1086/303374

Adler L. S., Irwin R. E., McArt S. H., & Vannette R. L. Floral traits affecting the transmission of beneficial and pathogenic pollinator–associated microbes. Curr Opin Insect Sci, 2020; 44: 1-7. https://doi.org/10.1016/j.cois.2020.08.006

Aizenberg-Gershtein Y., Izhaki I., & Halpern M. Do Honey bees Shape the Bacterial Community Composition in Floral Nectar? Plos One, 2013; 8(7): e67556. https://doi.org/10.1371/journal.pone.0067556

Aleklett K., Hart M., & Shade A. The microbial ecology of flowers: an emerging frontier in phyllosphere research. Botany, 2014; 92(4): 253-266. https://doi.org/10.1139/cjb-2013-0166

Alexander H., & Antonovics J. Disease spread and population dynamics of anther-smut infection of *Silene alba* caused by the fungus *Ustilago violacea*. Journal of Ecology, 1988; 76(1): 91-104. https://doi.org/10.2307/2260456

Álvarez-Pérez S., & Herrera C. M. Composition, richness and non-concurrent fungal communities in floral nectar of Mediterranean plants. FEMS Microbiology Ecology, 2013; 83(3): 685-699. https://doi.org/10.1111/1574-6941.12027

Álvarez-Pérez S., Herrera C. M., & de Vega C. Zooming-in on floral nectar: A first exploration of nectar-associated bacteria in wild plant communities. FEMS Microbiology Ecology, 2012; 80(3): 591-602. https://doi.org/10.1111/j.1574-6941.2012.01329.x

Álvarez-Pérez S., Lievens B., & Fukami T. Yeast-

bacterium interactions: the next frontier in nectar research. Trends in Plant Science, 2019; 24(5): 393-401. https://doi.org/10.1016/j.tplants.2019.01.012

Álvarez-Pérez S., Lievens B., Jacquemyn H., & Herrera C. M. *Acinetobacter nectaris* sp. nov. and *Acinetobacter boissieri* sp. nov., isolated from floral nectar of wild Mediterranean insect-pollinated plants. International Journal of Systematic and Evolutionary Microbiology, 2013; 63(Pt 4): 1532-1539. https://doi.org/10.1099/ijs.0.043489-0

Álvarez-Pérez S., Tsuji K., Donald M., Van Assche A., Vannette R. L., Herrera C. M., Jacquemyn H., Fukami T., & Lievens B. Nitrogen assimilation varies among clades of nectar- and insect-associated Acinetobacters. Microbial Ecology, 2021; 81(4): 990-1003. https://doi.org/10.1007/s00248-020-01671-x

Amoo S. O., Aremu A. O., & Van Staden J. Unraveling the medicinal potential of South African Aloe species. Journal of Ethnopharmacology, 2014; 153(1): 19-41. https://doi.org/10.1016/j.jep.2014.01.036

An S. Q., Potnis N., Dow M., Vorhölter F. J., He Y. Q., Becker A., Teper D., Li Y., Wang N., Bleris L., & Tang J. L. Mechanistic insights into host adaptation, virulence and epidemiology of the phytopathogen Xanthomonas. FEMS Microbiology Reviews, 2020; 44(1): 1-32. https://doi.org/10.1093/femsre/fuz024

Anderson K. E., Sheehan T. H., Mott B. M., Maes P., Snyder L., Schwan M. R., Walton A., Jones B. M., & Corby-Harris V. Microbial Ecology of the Hive and Pollination Landscape: Bacterial Associates from Floral Nectar, the Alimentary Tract and Stored Food of Honey Bees (*Apis mellifera*). Plos One, 2013; 8(12): e83125. https://doi.org/10.1371/journal.pone.0083125

Aremu A. O., & Van Staden J. The genus Tulbaghia (Alliaceae) – A review of its ethnobotany, pharmacology, phytochemistry and conservation needs. Journal of Ethnopharmacology, 2013; 149(2): 387-400. https://doi.org/10.1016/j.jep.2013.06.046

Arredondo D., Castelli L., Porrini M. P., Garrido P. M., Eguaras M. J., Zunino P., & Antúnez K. *Lactobacillus kunkeei* strains decreased the infection by honey bee pathogens Paenibacillus larvae and Nosema ceranae. Benef Microbes, 2018; 9(2): 279-290. https://doi.org/10.3920/bm2017.0075

Baker H. G., & Baker I. Floral nectar constituents in relation to pollinator type. Handbook of Experimental Pollination Biology, 1983: 117-141.

Baker H. G., & Baker I. The predictive value of nectar chemistry to the recognition of pollinator types. Israel Journal of Plant Sciences, 1990; 39(1-2): 157-166. https://doi.org/10.1080/0021213X.1990.10677140

Barnes K., Nicolson S. W., & van Wyk B. E. Nectar sugar composition in Erica. Biochemical Systematics and Ecology, 1995; 23(4): 419-423. https://doi.org/10.1016/0305-1978(95)00024-O

Belisle M., Peay K. G., & Fukami T. Flowers as islands: spatial distribution of nectar-inhabiting microfungi among plants of *Mimulus aurantiacus*, a hummingbird-pollinated shrub. Microbial Ecology, 2012; 63(4): 711-718. https://doi.org/10.1007/s00248-011-9975-8

Biere A., & Honders S. C. Coping with third parties in a nursery pollination mutualism: *Hadena bicruris* avoids oviposition on pathogen-infected, less rewarding Silene latifolia. New Phytologist, 2006; 169(4): 719-727. https://doi.org/10.1111/j.1469-8137.2005.01511.x

Boutroux L. Conservation des ferments alcooliques dans la nature. Annales des Sci Naturelles, Série IV, Botanique, 1884; 17: 144-209.

Brown M., Downs C. T., & Johnson S. D. Concentration-dependent sugar preferences of the malachite sunbird (*Nectarinia famosa*). Ornithology, 2010; 127(1): 151-155. https://doi.org/10.1525/auk.2009.09054

Brudzynski K. Honey as an Ecological Reservoir of Antibacterial Compounds Produced by Antagonistic Microbial Interactions in Plant Nectars, Honey and Honey Bee. Antibiotics (Basel), 2021; 10(5). https://doi.org/10.3390/antibiotics10050551

Brunner F. S., Schmid-Hempel P., & Barribeau S. M. Protein-poor diet reduces host-specific

immune gene expression in *Bombus terrestris*. Proceedings of the Royal Society B: Biological Sciences, 2014; 281(1786): 20140128. https://doi.org/10.1098/rspb.2014.0128

Brysch-Herzberg M. Ecology of yeasts in plant-bumble bee mutualism in Central Europe. FEMS Microbiology Ecology, 2004; 50(2): 87-100. https://doi.org/10.1016/j.femsec.2004.06.003

Burdon R. C. F., Junker R. R., Scofield D. G., & Parachnowitsch A. L. Bacteria colonising Penstemon digitalis show volatile and tissue-specific responses to a natural concentration range of the floral volatile linalool. Chemoecology, 2018; 28(1): 11-19. https://doi.org/10.1007/s00049-018-0252-x

Buwa L. V., & Van Staden J. Antibacterial and antifungal activity of traditional medicinal plants used against venereal diseases in South Africa. Journal of Ethnopharmacology, 2006; 103(1): 139-142. https://doi.org/10.1016/j.jep.2005.09.020

Canto A., & Herrera C. M. Micro-organisms behind the pollination scenes: Microbial imprint on floral nectar sugar variation in a tropical plant community. Annals of Botany, 2012; 110(6): 1173-1183. https://doi.org/10.1093/aob/mcs183

Canto A., Herrera C. M., & Rodriguez R. Nectar-living yeasts of a tropical host plant community: diversity and effects on community-wide floral nectar traits. PeerJ, 2017; 5: e3517. https://doi.org/10.7717/peerj.3517

Canto A., Herrera C. M., García I. M., García M., & Bazaga P. Comparative effects of two species of floricolous Metschnikowia yeasts on nectar. Anales Del Jardín Botánico de Madrid, 2015; 72(1): e019. https://doi.org/10.3989/ajbm.2396

Canto A., Herrera C. M., Medrano M., Pérez R., & García I. M. Pollinator foraging modifies nectar sugar composition in *Helleborus foetidus* (*Ranunculaceae*): An experimental test. American Journal of Botany, 2008; 95(3): 315-320. https://doi.org/10.3732/ajb.95.3.315

Carter C., & Thornburg R. W. Is the nectar redox cycle a floral defense against microbial attack? Trends in Plant Science, 2004; 9(7): 320-324. https://doi.org/10.1016/j.tplants.2004.05.008

Carter C., Graham R. A., & Thornburg R. W. Nectarin I is a novel, soluble germin-like protein expressed in the nectar of Nicotiana sp. Plant Molecular Biology, 1999; 41(2): 207-216. https://doi.org/10.1023/A:1006363508648

Christensen S. M., Munkres I., & Vannette R. L. Nectar bacteria stimulate pollen germination and bursting to enhance microbial fitness. Current Biology, 2021; 31(19): 4373-4380.e4376. https://doi.org/10.1016/j.cub.2021.07.016

Colda A., Bossaert S., Verreth C., Vanhoutte B., Honnay O., Keulemans W., & Lievens B. Inoculation of pear flowers with Metschnikowia reukaufii and Acinetobacter nectaris enhances attraction of honeybees and hoverflies, but does not increase fruit and seed set. Plos One, 2021; 16(4): e0250203. https://doi.org/10.1371/journal.pone.0250203

Corbet S. A., Willmer P. G., Beament J. W. L., Unwin D. M., & Prŷs-Jones O. E. Post-secretory determinants of sugar concentration in nectar. Plant, Cell & Environment, 1979; 2(4): 293-308. https://doi.org/10.1111/j.1365-3040.1979.tb00084.x

Corby-Harris V., Maes P., & Anderson K. E. The bacterial communities associatedforagers. Plos One, 2014; 9(4): e95056. https://doi.org/10.1371/journal.pone.0095056

Crotti E., Rizzi A., Chouaia B., Ricci I., Favia G., Alma A., Sacchi L., Bourtzis K., Mandrioli M., Cherif A., Bandi C., & Daffonchio D. Acetic Acid Bacteria, Newly Emerging Symbionts of Insects. Applied and Environmental Microbiology, 2010; 76(21): 6963-6970. https://doi.org/10.1128/aem.01336-10

Cullen N. P., Fetters A. M., & Ashman T. L. Integrating microbes into pollination. Curr Opin Insect Sci, 2021; 44: 48-54. https://doi.org/10.1016/j.cois.2020.11.002

de Vega C., & Herrera C. M. Relationships among nectar-dwelling yeasts, flowers and ants: patterns and incidence on nectar traits. Oikos, 2012; 121(11): 1878-1888. https://doi.org/10.1111/j.1600-

0706.2012.20295.x

de Vega C., Albaladejo R. G., Guzmán B., Steenhuisen S., Johnson S. D., Herrera C. M., & Lachance M. Flowers as a reservoir of yeast diversity: description of *Wickerhamiella nectarea* f.a. sp. nov., and *Wickerhamiella natalensis* f.a. sp. nov. from South African flowers and pollinators, and transfer of related Candida species to the genus *Wickerhamiella* as new combinations. FEMS Yeast Research, 2017; 17(5). https://doi.org/10.1093/femsyr/fox054

de Vega C., Álvarez-Pérez S., Albaladejo R. G., Steenhuisen S. L., Lachance M. A., Johnson S. D., & Herrera C. M. The role of plant-pollinator interactions in structuring nectar microbial communities. Journal of Ecology, 2021; 109(9): 3379-3395. https://doi.org/10.1111/1365-2745.13726

de Vega C., Álvarez-Pérez S., Albaladejo R. G., Steenhuisen S., Lachance M., Johnson S. D., & Herrera C. M. The role of plant-pollinator interactions in structuring nectar microbial communities. Journal of Ecology, 2021; 109(9): 3379-3395. https://doi.org/10.1111/1365-2745.13726

de Vega C., Guzman B., Steenhuisen S. L., Johnson S. D., Herrera C. M., & Lachance M. A. *Metschnikowia drakensbergensis* sp. nov. and *Metschnikowia caudata* sp. nov., endemic yeasts associated with Protea flowers in South Africa. International Journal of Systematic and Evolutionary Microbiology, 2014; 64(11): 3724-3732. https://doi.org/10.1099/ijs.0.068445-0

de Vega C., Herrera C. M., & Johnson S. D. Yeasts in floral nectar of some South African plants: quantification and associations with pollinator type and sugar concentration. South African Journal of Botany, 2009; 75(4): 798-806. https://doi.org/10.1016/j.sajb.2009.07.016

Dhami M. K., Hartwig T., & Fukami T. Genetic basis of priority effects: insights from nectar yeast. Proceedings of the Royal Society B: Biological Sciences, 2016; 283(1840): 20161455. https://doi.org/10.1098/rspb.2016.1455

Dhami M. K., Hartwig T., Letten A. D., Banf M., & Fukami T. Genomic diversity of a nectar yeast clusters into metabolically, but not geographically, distinct lineages. Molecular Ecology, 2018; 27(8): 2067-2076. https://doi.org/10.1111/mec.14535

Dolezal A. G., & Toth A. L. Feedbacks between nutrition and disease in honey bee health. Curr Opin Insect Sci, 2018; 26: 114-119. https://doi.org/10.1016/j.cois.2018.02.006

Eisikowitch D., Lachance M. A., Kevan P. G., Willis S., & Collins-Thompson D. L. The effect of the natural assemblage of microorganisms and selected strains of the yeast *Metschnikowia reukaufii* in controlling the germination of pollen of the common milkweed Asclepias syriaca. Canadian Journal of Botany, 1990; 68(5): 1163-1165. https://doi.org/10.1139/b90-147

Engel P., Martinson V. G., & Moran N. A. Functional diversity within the simple gut microbiota of the honey bee. Proceedings of the National Academy of Sciences of the United States of America, 2012; 109(27): 11002-11007. https://doi.org/10.1073/pnas.1202970109

Farré-Armengol G., Filella I., Llusia J., & Peñuelas J. Bidirectional Interaction between Phyllospheric Microbiotas and Plant Volatile Emissions. Trends in Plant Science, 2016; 21(10): 854-860. https://doi.org/10.1016/j.tplants.2016.06.005

Figueroa L. L., Blinder M., Grincavitch C., Jelinek A., Mann E. K., Merva L. A., Metz L. E., Zhao A. Y., Irwin R. E., McArt S. H., & Adler L. S. Bee pathogen transmission dynamics: deposition, persistence and acquisition on flowers. Proceedings of the Royal Society B: Biological Sciences, 2019; 286(1903): 20190603. https://doi.org/10.1098/rspb.2019.0603

Fine J. D., Shpigler H. Y., Ray A. M., Beach N. J., Sankey A. L., Cash-Ahmed A., Huang Z. Y., Astrauskaite I., Chao R., Zhao H., & Robinson G. E. Quantifying the effects of pollen nutrition on honey bee queen egg laying with a new laboratory system. Plos One, 2018; 13(9): e0203444. https://doi.org/10.1371/journal.pone.0203444

Folly A. J., Barton-Navarro M., & Brown M. Exposure to nectar-realistic sugar concentrations negatively impacts the ability of the trypanto infect its bumble bee host. Ecological Entomology, 2020; 45(6): 1495-1498.

https://doi.org/10.1111/een.12901

Fridman S., Izhaki I., Gerchman Y., & Halpern M. Bacterial communities in floral nectar. Environmental Microbiology Reports, 2012; 4(1): 97-104. https://doi.org/10.1111/j.1758-2229.2011.00309.x

Fukami T. Historical contingency in community assembly: integrating niches, species pools, and priority effects. Annual Review of Ecology, Evolution, and Systematics, 2015; 46(1): 1-23. https://doi.org/10.1146/annurev-ecolsys-110411-160340

Gegear R. J., & Thomson J. D. Does the flower constancy of bumble bees reflect foraging economics? Ethology, 2004; 110(10): 793-805. https://doi.org/10.1111/j.1439-0310.2004.01010.x

Golonka A. M., Johnson B. O., Freeman J., & Hinson D. W. Impact of nectarivorous yeasts on Silene caroliniana's scent. Eastern Biologist, 2014; 3: 1-26.

Good A. P., Gauthier M. P. L., Vannette R. L., & Fukami T. Honey bees avoid nectar colonized by three bacterial species, but not by a yeast species, isolated from the bee gut. Plos One, 2014; 9(1): e86494. https://doi.org/10.1371/journal.pone.0086494

Grund-Mueller N., Ruedenauer F. A., Spaethe J., & Leonhardt S. D. Adding amino acids to a sucrose diet is not sufficient to support longevity of adult bumble bees. Insects, 2020; 11(4): 247. https://doi.org/10.3390/insects11040247

Hammer T. J., Le E., Martin A. N., & Moran N. A. The gut microbiota of bumble bees. Insectes Sociaux, 2021; 68(4): 287-301. https://doi.org/10.1007/s00040-021-00837-1

Heiduk A., Brake I., Shuttleworth A., & Johnson S. D. 'Bleeding' flowers of Ceropegia gerrardii (Apocynaceae-asclepiadoideae) mimic wounded insects to attract kleptoparasitic fly pollinators. New Phytologist, 2023; 239(4): 1490-1504. https://doi.org/10.1111/nph.18888

Herrera C. M. Scavengers that fit beneath a microscope lens. Ecology, 2017; 98(10): 2725-2726. https://doi.org/10.1002/ecy.1874

Herrera C. M., & Pozo M. I. Nectar yeasts warm the flowers of a winter-blooming plant. Proceedings of the Royal Society B: Biological Sciences, 2010; 277(1689): 1827-1834. https://doi.org/10.1098/rspb.2009.2252

Herrera C. M., Canto A., Pozo M. I., & Bazaga P. Inhospitable sweetness: Nectar filtering of pollinator-borne inocula leads to impoverished, phylogenetically clustered yeast communities. Proceedings of the Royal Society B: Biological Sciences, 2010; 277(1682): 747-754. https://doi.org/10.1098/rspb.2009.1485

Herrera C. M., De Vega C., Canto A., & Pozo M. I. Yeasts in floral nectar: A quantitative survey. Annals of Botany, 2009; 103(9): 1415-1423. https://doi.org/10.1093/aob/mcp026

Herrera C. M., García I. M., & Pérez R. Invisible floral larcenies: microbial communities degrade floral nectar of bumble bee-pollinated plants. Ecology, 2008; 89(9): 2369-2376. https://doi.org/10.1890/08-0241.1

Herrera C. M., Pozo M. I., & Medrano M. Yeasts in nectar of an early-blooming herb: sought by bumble bees, detrimental to plant fecundity. Ecology, 2013; 94(2): 273-279. https://doi.org/10.1890/12-0595.1

Hietaranta E., Juottonen H., & Kytöviita M. M. Honey bees affect floral microbiome composition in a central food source for wild pollinators in boreal ecosystems. Oecologia, 2022; 201(1): 59-72. https://doi.org/10.1007/s00442-022-05285-7

Hinton D., & Bacon C. The distribution and ultrastructure of the endophyte of toxic tall fescue. Canadian Journal of Botany, 1985; 63(1): 36-42. https://doi.org/10.1139/b85-006

Hodgson S., Cates C., Hodgson J., Morley N., Sutton B., & Gange A. Vertical transmission of fungal endophytes is widespread in forbs. Ecology and Evolution, 2014; 4(8): 1199-1208. https://doi.org/10.1002/ece3.953

Hua S. S. T., Beck J. J., Sarreal S. B. L., & Gee W. The major volatile compound 2-phenylethanol from the biocontrol yeast, *Pichia anomala*, inhibits growth and expression of aflatoxin biosynthetic genes of Aspergillus flavus. Mycotoxin Res, 2014; 30(2): 71-78. https://doi.org/10.1007/s12550-014-0189-z

Huang M., Sanchez-Moreiras A. M., Abel C., Sohrabi R., Lee S., Gershenzon J., & Tholl D. The major volatile organic compound emitted from *Arabidopsis thaliana* flowers, the sesquiterpene (e)-β-caryophyllene, is a defense against a bacterial pathogen. New Phytologist, 2012; 193(4): 997-1008. https://doi.org/10.1111/j.1469-8137.2011.04001.x

Ilyasov R., Boguslavsky D., Ilyasova A., Sattarov V., & Danilenko V. A multifaceted bioactivity of honey: Interactions between bees, plants and microorganisms. Uludağ Arıcılık Dergisi, 2024; 24(2): 356-385. https://doi.org/10.31467/uluaricilik.1511847

Jacquemyn H., Lenaerts M., Brys R., Willems K., Honnay O., & Lievens B. Among-population variation in microbial community structure in the floral nectar of the bee-pollinated forest herb *Pulmonaria officinalis* L. Plos One, 2013; 8(3): e56917. https://doi.org/10.1371/journal.pone.0056917

Jersáková J., Johnson S. D., & Kindlmann P. Mechanisms and evolution of deceptive pollination in orchids. Biological Reviews of the Cambridge Philosophical Society, 2006; 81(2): 219-235. https://doi.org/10.1017/S1464793105006986

Johnson S. D. Batesian mimicry in the non-rewarding orchid *Disa pulchra*, and its consequences for pollinator behavior. Biological Journal of the Linnean Society, 2000; 71(1): 119-132. https://doi.org/10.1111/j.1095-8312.2000.tb01246.x

Junker R. R., Romeike T., Keller A., & Langen D. Density-dependent negative responses by bumble bees to bacteria isolated from flowers. Apidologie, 2014; 45(4): 467-477. https://doi.org/10.1007/s13592-013-0262-1

Keller A., McFrederick Q. S., Dharampal P., Steffan S., Danforth B. N., & Leonhardt S. D. (More than) Hitchhikers through the network: The shared microbiome of bees and flowers. Curr Opin Insect Sci, 2021; 44: 8-15. https://doi.org/10.1016/j.cois.2020.09.007

Khan A., Zhao Y., & Korban S. Molecular Mechanisms of Pathogenesis and Resistance to the Bacterial Pathogen *Erwinia amylovora*, Causal Agent of Fire Blight Disease in Rosaceae. Plant Molecular Biology Reporter, 2012; 30(2): 1-14. https://doi.org/10.1007/s11105-011-0334-1.

Kim D. R., Cho G., Jeon C. W., Weller D. M., Thomashow L. S., Paulitz T. C., & Kwak Y. S. A mutualistic interaction between Streptomyces bacteria, strawberry plants and pollinating bees. Nat Commun, 2019; 10(1): 4802. https://doi.org/10.1038/s41467-019-12785-3.

Kim P. S., Shin N. R., Kim J. Y., Yun J. H., Hyun D. W., & Bae J. W. Acinetobacter apis sp. nov., isolated from the intestinal tract of a honey bee, *Apis mellifera*. Journal of Microbiology, 2014; 52(8): 639-645. https://doi.org/10.1007/s12275-014-4078-0.

Klaps J. Analysis of yeast diversity in floral microhabitats from Metrosideros polymorpha Gaud. KU Leuven. Leuven, 2019, Pages. Retrieved from URL| Available rom Database Provider Name of Database database.| (Document Number)

Klaps J., Lievens B., & Álvarez-Pérez S. Towards a better understanding of the role of nectar-inhabiting yeasts in plant-animal interactions. Fungal Biology and Biotechnology, 2020; 7: 1. https://doi.org/10.1186/s40694-019-0091-8

Koch H., Woodward J., Langat M. K., Brown M. J. F., & Stevenson P. C. Flagellum Removal by a Nectar Metabolite Inhibits Infectivity of a Bumble bee Parasite. Current Biology, 2019; 29(20): 3494-3500.e3495. https://doi.org/10.1016/j.cub.2019.08.037.

Lachance M. A., Ewing C. P., Bowles J. M., & Starmer W. T. *Metschnikowia hamakuensis* sp. nov., *Metschnikowia kamakouana* sp. nov. and *Metschnikowia mauinuiana* sp. nov., three endemic yeasts from Hawaiian nitidulid beetles. International Journal of Systematic and Evolutionary Microbiology, 2005; 55(3): 1369-1377. https://doi.org/10.1099/ijs.0.63615-0.

Lachance M. A., Vale H. M. M., Sperandio E. M., Carvalho A. O. S., Santos A. R. O., Grondin C., Jacques N., Casaregola S., & Rosa C. A. *Wickerhamiella dianesei* f.a., sp. nov. and *Wickerhamiella kurtzmanii* f.a., sp. nov., two yeast species isolated from plants and

insects. International Journal of Systematic and Evolutionary Microbiology, 2018; 68(10): 3351-3355. https://doi.org/10.1099/ijsem.0.003000.

Lachance M., Starmer W., Rosa C., Bowles J., Barker J., & Janzen D. Biogeography of the yeasts of ephemeral flowers and their insects. FEMS Yeast Research, 2001; 1(1): 1-8. https://doi.org/10.1016/s1567-1356(00)00003-9.

Lara C., & Ornelas J. F. Hummingbirds as vectors of fungal spores in *Moussonia deppeana* (Gesneriaceae): Taking advantage of a mutualism? American Journal of Botany, 2003; 90(2): 260-267. https://doi.org/10.3732/ajb.90.2.262

Lee F. J., Rusch D. B., Stewart F. J., Mattila H. R., & Newton I. L. G. Saccharide breakdown and fermentation by the honey bee gut microbiome. Environmental Microbiology, 2014; 17(3): 796-815. https://doi.org/10.1111/1462-2920.12526

Lenaerts M., Álvarez-Pérez S., De Vega C., Van Assche A., Johnson S. D., Willems K. A., Herrera C. M., Jacquemyn H., & Lievens B. *Rosenbergiella australoborealis* sp. nov., *Rosenbergiella collisarenosi* sp. nov. and *Rosenbergiella epipactidis* sp. nov., three novel bacterial species isolated from floral nectar. Systematic and Applied Microbiology, 2014; 37(6): 402-411. https://doi.org/10.1016/j.syapm.2014.03.002

Lenaerts M., Goelen T., Paulussen C., Herrera-Malaver B., Steensels J., Van Den Ende W., Verstrepen K. J., Wäckers F., Jacquemyn H., Lievens B., & Manson J. Nectar bacteria affect life history of a generalist aphid parasitoid by altering nectar chemistry. Functional Ecology, 2017; 31(11): 2061-2069. https://doi.org/10.1111/1365-2435.12933

Lignon V. A., Mas F., Jones E. E., Kaiser C., & Dhami M. K. The floral interface: a playground for interactions between insect pollinators, microbes, and plants. New Zealand Journal of Zoology, 2024; 52(2): 218-237. https://doi.org/10.1080/03014223.2024.2353285

Lin H., & Winston M. L. The role of nutrition and temperature in the ovarian development of the worker honey bee (*Apis mellifera*). The Canadian Entomologist, 1998; 130(6): 883-891. https://doi.org/10.4039/Ent130883-6

Mallinger R. E., & Prasifka J. R. Bee visitation rates to cultivated sunflowers increase with the amount and accessibility of nectar sugars. Journal of Applied Entomology, 2017; 141(7): 561-573. https://doi.org/10.1111/jen.12375

Manirajan B. A., Ratering S., Rusch V., Schwiertz A., Geissler-Plaum R., Cardinale M., & Schnell S. Bacterial microbiota associated with flower pollen is influenced by pollination type, and shows a high degree of diversity and species-specificity. Environmental Microbiology, 2016; 18(12): 5161-5174. https://doi.org/10.1111/1462-2920.13524

Martin V. N., Schaeffer R. N., & Fukami T. Potential effects of nectar microbes on pollinator health. Philosophical Transactions of the Royal Society B: Biological Sciences, 2022; 377(1853): 20210155. https://doi.org/10.1098/rstb.2021.0155

Massoni J., Bortfeld-Miller M., Jardillier L., Salazar G., Sunagawa S., & Vorholt J. A. Consistent host and organ occupancy of phyllosphere bacteria in a community of wild herbaceous plant species. ISME J, 2019; 14(1): 245-258. https://doi.org/10.1038/s41396-019-0531-8

McCormack P. J., Wildman H. G., & Jeffries P. Production of antibacterial compounds by phylloplane-inhabiting yeasts and yeast-like fungi. Applied and Environmental Microbiology, 1994; 60(3): 927-931. https://doi.org/10.1128/aem.60.3.927-931.1994

McFrederick Q. S., Thomas J. M., Neff J. L., Vuong H. Q., Russell K. A., Hale A. R., & Mueller U. G. Flowers and Wild Megachilid Bees Share Microbes. Microbial Ecology, 2016; 73(1): 188-200. https://doi.org/10.1007/s00248-016-0838-1

McFrederick Q. S., Wcislo W. T., Taylor D. R., Ishak H. D., Dowd S. E., & Mueller U. G. Environment or kin: whence do bees obtain acidophilic bacteria? Molecular Ecology, 2012; 21(7): 1754-1768. https://doi.org/10.1111/j.1365-294x.2012.05496.x

McFrederick Q. S., Wcislo W. T., Taylor D. R., Ishak

H. D., Dowd S. E., & Mueller U. G. Environment or kin: whence do bees obtain acidophilic bacteria? Molecular Ecology, 2012; 21(7): 1754-1768. https://doi.org/10.1111/j.1365-294x.2012.05496.x

Miller R., Owens S. J., & Rørslett B. Plants and colour: flowers and pollination. Optics & Laser Technology, 2011; 43(2): 282-294. https://doi.org/10.1016/j.optlastec.2008.12.018

Mittelbach M., Yurkov A. M., Nocentini D., Nepi M., Weigend M., & Begerow D. Nectar sugars and bird visitation define a floral niche for basidiomycetous yeast on the Canary Islands. BMC Ecology, 2015; 15(1): 2-2. https://doi.org/10.1186/s12898-015-0036-x

Mommaerts V., Wäckers F., & Smagghe G. Assessment of gustatory responses to different sugars in harnessed and free-moving bumble bee workers (Bombus terrestris). Chemical Senses, 2013; 38(5): 399-407. https://doi.org/10.1093/chemse/bjt014

Morris M. M., Frixione N. J., Burkert A. C., Dinsdale E. A., & Vannette R. L. Microbial abundance, composition, and function in nectar are shaped by flower visitor identity. FEMS Microbiology Ecology, 2020; 96(3). https://doi.org/10.1093/femsec/fiaa003

Ncube B., Nair J. J., Rárová L., Strnad M., Finnie J. F., & Van Staden J. Seasonal pharmacological properties and alkaloid content in *Cyrtanthus contractus* N.E. Br. South African Journal of Botany, 2015; 97: 69-76. https://doi.org/10.1016/j.sajb.2014.12.005

Nicolson S. W., & Thornburg R. W. Nectar chemistry. In Ed.^Eds. Springer Netherlands, Dordrecht, 2007, p. 215-264. https://doi.org/10.1007/978-1-4020-5937-7_5

Nowak A., Szczuka D., Górczyńska A., Motyl I., & Kręgiel D. Characterization of *Apis mellifera* Gastrointestinal Microbiota and Lactic Acid Bacteria for Honeybee Protection-A Review. Cells, 2021; 10(3): 701-701. https://doi.org/10.3390/cells10030701

O'Garro L., & Charlemange E. Comparison of bacterial growth and activity of glucanase and chitinase in pepper leaf and flower tissue infected with *Xanthomonas campestris* pv. vesicatoria. Physiological and Molecular Plant Pathology, 1994; 45(3): 181-188. https://doi.org/10.1016/S0885-5765(05)80075-X

Oldroyd B. P., Rinderer T. E., & Buco S. M. Intracolonial variance in honey bee foraging behaviour: the effects of sucrose concentration. Journal of Apicultural Research, 1991; 30(3-4): 137-145. https://doi.org/10.1080/00218839.1991.11101248

Palmer-Young E. C., Raffel T. R., & McFrederick Q. S. pH-mediated inhibition of a bumble bee parasite by an intestinal symbiont. Parasitology, 2019; 146(3): 380-388. https://doi.org/10.1017/S0031182018001555

Parret A., & De Mot R. Bacteria killing their own kind: novel bacteriocins of *Pseudomonas* and other γ-*proteobacteria*. Trends in Microbiology, 2002; 10(3): 107-112. https://doi.org/10.1016/S0966-842X(02)02307-7

Peakall R., Ebert D., Poldy J., Barrow R. A., Francke W., Bower C. C., & Schiestl F. P. Pollinator specificity, floral odour chemistry and the phylogeny of Australian sexually deceptive Chiloglottis orchids: implications for pollinator-driven speciation. New Phytologist, 2010; 188(2): 437-450. https://doi.org/10.1111/j.1469-8137.2010.03308.x

Peay K. G., Belisle M., & Fukami T. Phylogenetic relatedness predicts priority effects in nectar yeast communities. Proceedings of the Royal Society B: Biological Sciences, 2011; 279(1729): 749-758. https://doi.org/10.1098/rspb.2011.1230

Peshev D., & Van den Ende W. Fructans: prebiotics and immunomodulators. Journal of Functional Foods, 2014; 8: 348-357. https://doi.org/10.1016/j.jff.2014.04.005

Pozo M. I., & Jacquemyn H. Addition of pollen increases growth of nectar-living yeasts. FEMS Microbiology Letters, 2019; 366(15): fnz191. https://doi.org/10.1093/femsle/fnz191

Pozo M. I., Herrera C. M., & Bazaga P. Species richness of yeast communities in floral nectar of southern Spanish plants. Microbial Ecology, 2011; 61(1): 82-91.

https://doi.org/10.1007/s00248-010-9682-x

Pozo M. I., Lachance M. A., & Herrera C. M. Nectar yeasts of two southern Spanish plants: the roles of immigration and physiological traits in community assembly. FEMS Microbiology Ecology, 2012; 80: 281-293. https://doi.org/10.1111/j.1574-6941.2011.01286.x

Pozo M. I., Mariën T., Van Kemenade G., Wäckers F., & Jacquemyn H. Effects of pollen and nectar inoculation by yeasts, bacteria or both on bumble bee colony development. Oecologia, 2021; 195: 689-703. https://doi.org/10.1007/s00442-021-04872-4

Pozo M. I., Van Kemenade G., Van Oystaeyen A., Aledón-Catalá T., Benavente A., Van Den Ende W., Wäckers F., & Jacquemyn H. The impact of yeast presence in nectar on bumble bee behavior and fitness. Ecological Monographs, 2019; 90(1): 1-10. https://doi.org/10.1002/ecm.1393

Pusey P. L., Stockwell V. O., & Mazzola M. Epiphytic bacteria and yeasts on apple blossoms and their potential as antagonists of *Erwinia amylovora*. Phytopathology, 2009; 99: 571–581. https://doi.org/10.1094/PHYTO-99-5-0571

Pyke G. H. Floral Nectar: Pollinator Attraction or Manipulation? Trends in ecology & evolution, 2016; 31: 339-341. https://doi.org/10.1016/j.tree.2016.02.013

Ramanovich N., Savelieva T., Biruk A., & Shukshyna M. Investigation of the Possibility of Using Bee Microbiotes and Bee Products for Isolation of Lactic Acids and Bifidobacteria. Topical issues of processing of meat and milk raw materials, 2021; 1(15) (15): 55-64. https://doi.org/10.47612/2220-8755-2020-15-55-64

Rands S. A., & Whitney H. M. Floral temperature and optimal foraging: is heat a feasible floral reward for pollinators? Plos One, 2008; 3: e2007. https://doi.org/10.1371/journal.pone.0002007

Rering C. C., Beck J. J., Hall G. W., McCartney M. M., & Vannette R. L. Nectar-inhabiting microorganisms influence nectar volatile composition and attractiveness to a generalist pollinator. New Phytologist, 2017; 220: 750-759. https://doi.org/10.1111/nph.14809

Richardson L. L., Adler L. S., Leonard A. S., Andicoechea J., Regan K. H., Anthony W. E., Manson J. S., & Irwin R. E. Secondary metabolites in floral nectar reduce parasite infections in bumble bees. Proceedings of the Royal Society B: Biological Sciences, 2015; 282: 20142471. https://doi.org/10.1098/rspb.2014.2471

Ricigliano V., & Anderson K. Probing the Honey Bee Diet-Microbiota-Host Axis Using Pollen Restriction and Organic Acid Feeding. Insects, 2020; 11(5): 1-10. https://doi.org/10.3390/insects11050291

Rivest S., & Forrest J. R. K. Defence compounds in pollen: Why do they occur and how do they affect the ecology and evolution of bees? New Phytologist, 2019; 225: 1053-1064. https://doi.org/10.1111/nph.16230

Rosa C. A., Lachance M. A., Silva J. O., Teixeira A. C., Marini M. M., Antonini Y., & Martins R. P. Yeast communities associated with stingless bees. FEMS Yeast Research, 2003; 4: 271-275. https://doi.org/10.1016/s1567-1356(03)00173-9

Rothman J. A., Russell K. A., Leger L., McFrederick Q. S., & Graystock P. The direct and indirect effects of environmental toxicants on the health of bumble bees and their microbiomes. Proceedings of the Royal Society B: Biological Sciences, 2020; 287: 20200980. https://doi.org/10.1098/rspb.2020.0980

Roulston T. A. H., & Cane J. H. Pollen nutritional content and digestibility for animals. Plant Systematics and Evolution, 2000; 222: 187-209. https://doi.org/10.1007/978-3-7091-6306-1_10

Roy R., Schmitt A. J., Thomas J. B., & Carter C. J. Nectar biology: from molecules to ecosystems. Plant Science, 2017; 262: 148-164. https://doi.org/10.1016/j.plantsci.2017.04.012

Russell A. L., Rebolleda-Gómez M., Shaible T. M., & Ashman T. Movers and shakers: Bumble bee foraging behavior shapes the dispersal of microbes among and within flowers. Ecosphere, 2019; 10: e02714. https://doi.org/10.1002/ecs2.2714

Samuni-Blank M., Izhaki I., Laviad S., Bar-Massada A., Gerchman Y., & Halpern M. The Role of Abiotic Environmental Conditions and Herbivory in Shaping Bacterial Community Composition in Floral Nectar. Plos One, 2014; 9(6): e99107. https://doi.org/10.1371/journal.pone.0099107

Sandhu D. K., & Waraich M. K. Yeasts Associated with Pollinating Bees and Flower Nectar. Microbial Ecology, 1985; 11(1): 51–58. https://doi.org/10.1007/BF02015108

Santorelli L. A., Wilkinson T., Abdulmalik R., Rai Y., Creevey C. J., Huws S., & Gutierrez-Merino J. Beehives possess their own distinct microbiomes. Environmental Microbiome, 2023; 18(1): 1. https://doi.org/10.1186/s40793-023-00460-6

Santos A. R. O., Leon M. P., Barros K. O., Freitas L. F. D., Hughes A. F. S., Morais P. B., Lachance M. A., & Rosa C. A. *Starmerella camargoi* f.a., sp. nov., *Starmerella ilheusensis* f.a., sp. nov., *Starmerella litoralis* f.a., sp. nov*., Starmerella opuntiae* f.a., sp. nov., *Starmerella roubikii* f.a., sp. nov. and *Starmerella vitae* f.a., sp. nov., isolated from flowers and bees, and transfer of related Candida species to the genus *Starmerella* as new combinations. International Journal of Systematic and Evolutionary Microbiology, 2018; 68: 1333-1343. https://doi.org/10.1099/ijsem.0.002675

Schaeffer R. N., & Irwin R. E. Yeasts in nectar enhance male fitness in a montane perennial herb. Ecology, 2014; 95: 1792-1798. https://doi.org/10.1890/13-1740.1

Schaeffer R. N., Mei Y. Z., Andicoechea J., Manson J. S., Irwin R. E., & Kudo G. Consequences of a nectar yeast for pollinator preference and performance. Functional Ecology, 2016; 31: 613-621. https://doi.org/10.1111/1365-2435.12762

Schaeffer R. N., Rering C. C., Maalouf I., Beck J. J., & Vannette R. L. Microbial metabolites elicit distinct olfactory and gustatory preferences in bumble bees. Biology Letters, 2019; 15: 20190132. https://doi.org/10.1098/rsbl.2019.0132

Schaeffer R. N., Rering C. C., Maalouf I., Beck J. J., & Vannette R. L. Microbial metabolites elicit distinct olfactory and gustatory preferences in bumble bees. Biology Letters, 2019; 15: 20190132. https://doi.org/10.1098/rsbl.2019.0132

Schmitt A. J., Sathoff A. E., Holl C., Bauer B., Samac D. A., & Carter C. J. The major nectar protein of *Brassica rapa* is a non-specific lipid transfer protein, BrLTP2.1, with strong antifungal activity. Journal of Experimental Botany, 2018; 69: 5587-5597. https://doi.org/10.1093/jxb/ery319

Seeley T. D., Mikheyev A. S., & Pagano G. J. Dancing bees tune both duration and rate of waggle-run production in relation to nectar-source profitability. Journal of comparative physiology. A, Sensory, neural, and behavioral physiology, 2000; 186: 813-819. https://doi.org/10.1007/s003590000134

Seymour R. S., & Schultze-Motel P. Physiological temperature regulation by flowers of the sacred lotus. Philos Trans R Soc Lon B, 1998; 353: 935-943. https://doi.org/10.1098/rstb.1998.0258

Seymour R. S., White C. R., & Gibernau M. Environmental biology: heat reward for insect pollinators. Nature, 2003; 426: 243-244. https://doi.org/10.1038/426243a

Shade A., McManus P. S., & Handelsman J. Unexpected diversity during community succession in the apple flower microbiome. mBio, 2013; 4: e00602-00612. https://doi.org/10.1128/mBio.00602-12

Sharaby Y., Rodríguez-Martínez S., Lalzar M., Halpern M., & Izhaki I. Geographic partitioning or environmental selection: What governs the global distribution of bacterial communities inhabiting floral nectar? The Science of the total environment, 2020; 749: 142305. https://doi.org/10.1016/j.scitotenv.2020.142305

Shi H., Ratering S., Schneider B., & Schnell S. Microbiome of honey bee corbicular pollen: Factors influencing its structure and potential for studying pathogen transmission. The Science of the total environment, 2025; 958: 178107. https://doi.org/10.1016/j.scitotenv.2024.178107

Sobhy I. S., Baets D., Goelen T., Herrera-Malaver B., Bosmans L., Van Den Ende W.,

Verstrepen K. J., Wäckers F., Jacquemyn H., & Lievens B. Sweet Scents: Nectar Specialist Yeasts Enhance Nectar Attraction of a Generalist Aphid Parasitoid Without Affecting Survival. Frontiers in plant science, 2018; 9: 1009. https://doi.org/10.3389/fpls.2018.01009

Sobral M., Veiga T., Domínguez P., Guitián J. A., Guitián P., & Guitián J. M. Selective Pressures Explain Differences in Flower Color among Gentiana lutea Populations. Plos One, 2015; 10(7): e0132522. https://doi.org/10.1371/journal.pone.0132522

Stabler D., Paoli P. P., Nicolson S. W., & Wright G. A. Nutrient balancing of the adult worker bumble bee *(Bombus terrestris*) depends on the dietary source of essential amino acids. Journal of experimental Biology, 2015; 218(5): 793-802. https://doi.org/10.1242/jeb.114249

Steffan S. A., Dharampal P. S., Danforth B. N., Gaines-Day H. R., Takizawa Y., & Chikaraishi Y. Omnivory in Bees: Elevated Trophic Positions among All Major Bee Families. The American naturalist, 2019; 194(3): 414-421. https://doi.org/10.1086/704281

Stökl J., Strutz A., Dafni A., Svatos A., Doubsky J., Knaden M., Sachse S., Hansson B. S., & Stensmyr M. C. A Deceptive Pollination System Targeting Drosophilids through Olfactory Mimicry of Yeast. Current Biology, 2010; 20(20): 1846-1852. https://doi.org/10.1016/j.cub.2010.09.033

Taniwaki M. H., Pitt J. I., Iamanaka B. T., Massi F. P., Fungaro M. H. P., & Frisvad J. C. Penicillium excelsum sp. nov from the Brazil Nut Tree Ecosystem in the Amazon Basin. Plos One, 2015; 10(12): e0143189. https://doi.org/10.1371/journal.pone.0143189

Teixido A. L., Barrio M., & Valladares F. Size Matters: Understanding the Conflict Faced by Large Flowers in Mediterranean Environments. Botanical Review, 2016; 82: 204-228. https://doi.org/10.1007/s12229-016-9168-

Tucker C. M., & Fukami T. Environmental variability counteracts priority effects to facilitate species coexistence: evidence from nectar microbes. Proceedings of the Royal Society B: Biological Sciences, 2014; 281(1778): 20132637. https://doi.org/10.1098/rspb.2013.2637

Ushio M., Yamasaki E., Takasu H., Nagano A. J., Fujinaga S., Honjo M. N., Ikemoto M., Sakai S., & Kudoh H. Microbial communities on flower surfaces act as signatures of pollinator visitation. Scientific Reports, 2015; 5(1): 8695. https://doi.org/10.1038/srep08695

Vanneste J. L. Fire Blight: The Disease and Its Causative Agent, *Erwinia amylovora*. CABI Publ, New York, 2000, p. https://doi.org/10.1079/9780851992945.0000

Vannette R. L. The floral microbiome: plant, pollinator, and microbial perspectives. Annual Review of Ecology, Evolution, and Systematics, 2020; 51(1): 363-386. https://doi.org/10.1146/annurev-ecolsys-011720-013401

Vannette R. L., & Fukami T. Contrasting effects of yeasts and bacteria on floral nectar traits. Annals of Botany, 2018; 121(7): 1343-1349. https://doi.org/10.1093/aob/mcy032

Vannette R. L., & Fukami T. Dispersal enhances beta diversity in nectar microbes. Ecology Letters, 2017; 20(7): 901-910. https://doi.org/10.1111/ele.12787

Vannette R. L., & Fukami T. Historical contingency in species interactions: towards niche-based predictions. Ecology Letters, 2014; 17(1): 115-124. https://doi.org/10.1111/ele.12204

Vannette R. L., Gauthier M.-P. L., & Fukami T. Nectar bacteria, but not yeast, weaken a plant-pollinator mutualism. Proceedings of the Royal Society B: Biological Sciences, 2013; 280(1752): 20122601. https://doi.org/10.1098/rspb.2012.2601

Vásquez A., Forsgren E., Fries I., Paxton R. J., Flaberg E., Szekely L., & Olofsson T. C. Symbionts as major modulators of insect health: lactic acid bacteria and honeybees. Plos One, 2012; 7(7): e33188. https://doi.org/10.1371/annotation/3ac2b867-c013-4504-9e06-bebf3fa039d1

Von Arx M., Moore A., Davidowitz G., & Arnold A. E. Diversity and distribution of microbial communities in floral nectar of two night-blooming plants of the Sonoran Desert. Plos One, 2019; 14(12): e0225309. https://doi.org/10.1371/journal.pone.0225309

Vuong H. Q., & McFrederick Q. S. Comparative

genomics of wild bee and flower isolated *Lactobacillus reveals* potential adaptation to the bee host. Genome Biology and Evolution, 2019; 11(8): 2151-2161. https://doi.org/10.1093/gbe/evz136

Wang H., Liu C., Liu Z., Wang Y., Ma L., & Xu B. The different dietary sugars modulate the composition of the gut microbiota in honeybee during overwintering. BMC Microbiology, 2020; 20(1): 61. https://doi.org/10.1186/s12866-020-01726-6

Wei N., & Ashman T. L. The effects of host species and sexual dimorphism differ among root, leaf and flower microbiomes of wild strawberries in situ. Scientific Reports, 2018; 8(1): 5195. https://doi.org/10.1038/s41598-018-23518-9

Wiens F., Zitzmann A., Lachance M. A., Yegles M., Pragst F., Wurst F. M., Von Holst D., Guan S. L., & Spanagel R. Chronic intake of fermented floral nectar by wild treeshrews. Proceedings of the National Academy of Sciences, 2008; 105(30): 10426-10431. https://doi.org/10.1073/pnas.0801628105

Willmer P. The Effects of Insect Visitors on Nectar Constituents in Temperate Plants. Oecologia, 1980; 47(2): 270-277. https://doi.org/10.1007/BF00346832

Yan J., Wang G., Sui Y., Wang M., & Zhang L. Pollinator responses to floral colour change, nectar and scent promote reproductive fitness in *Quisqualis indica* (Combretaceae). Scientific Reports, 2016; 6(1): 24408. https://doi.org/10.1038/srep24408

Yang M., Deng G. C., Gong Y. B., & Huang S. Q. Nectar yeasts enhance the interaction between Clematis akebioides and its bumble bee pollinator. Plant Biology, 2019; 21(4): 732-737. https://doi.org/10.1111/plb.12957

Zariman A., Omar N., & Nurul Huda A. Plant Attractants and Rewards for Pollinators: Their Significance to Successful Crop Pollination. International Journal of Life Sciences and Biotechnology, 2022; 5(2): 270-293. https://doi.org/10.38001/ijlsb.1069254

Zarraonaindia I., Owens S. M., Weisenhorn P., West K., Hampton-Marcell J., Lax S., Bokulich N. A., Mills D. A., Martin G., Taghavi S., Van Der Lelie D., & Gilbert J. A. The Soil Microbiome Influences Grapevine-Associated Microbiota. mBio, 2015; 6(2). https://doi.org/10.1128/mbio.02527-14

Zheng H., Perreau J., Powell J. E., Han B., Zhang Z., Kwong W. K., Tringe S. G., & Moran N. A. Division of labor in honey bee gut microbiota for plant polysaccharide digestion. Proceedings of the National Academy of Sciences, 2019; 116(51): 25909-25916. https://doi.org/10.1073/pnas.1916224116

Zheng J., Wittouck S., Salvetti E., Franz C. M. a. P., Harris H. M. B., Mattarelli P., O'Toole P. W., Pot B., Vandamme P., Walter J., Watanabe K., Wuyts S., Felis G. E., Gänzle M. G., & Lebeer S. A taxonomic note on the genus *Lactobacillus*: Description of 23 novel genera, emended description of the genus *Lactobacillus* Beijerinck 1901, and union of Lactobacillaceae and Leuconostocaceae. International Journal of Systematic and Evolutionary Microbiology, 2020; 70(4): 2782-2858. https://doi.org/10.1099/ijsem.0.004107