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ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

ANTIMICROBIAL ACTIVITY OF EGYPTIAN SIDR HONEY AND ITS SYNERGISTIC ACTION WITH ANTIMICROBIAL AGENTS

Mısır Sidr Balının Antimikrobiyal Aktivitesi ve Antimikrobiyal Ajanlarla Sinerjik Etkisi

Nageh, S.M. OMRAN[1], Mostafa M. M. HASSAN[2*], Mohamed F. ABDEL-RAHMAN[2], Abd El-Aleem S.S. DESOKY[1], Sayed M. HAMOUDA[3]

[1]Plant Protection Department, Faculty of Agriculture, Sohag University, EGYPT, E-posta: nagehomran@yahoo.com. ORCID No: 0009-0006- 8285- 2558, E-posta: abdelalem2011@gmail.com, ORCID No: 0009- 0003- 1738- 5189.

[*2]Bee Research Department, Plant Protection Research Institute, Agriculture Research Center, Assiut, EGYPT, Corresponding author: E-mail: m65866236@gmail.com, ORCID No: 0000-0002-1592-0948, E-posta: m_fathalla70@yahoo.com, ORCID No: 0000-0002-0721-9756.

[3]Assiut Regional Lab., Animal Health Research Institute, Agriculture Center, Assiut, EGYPT, E-posta: smhamuda60@gmail.com. ORCID No: 0000-0002-3382-5637.

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ABSTRACT

Determine the *in vitro* antibacterial potential activity sidr honey produced in upper Egypt against five references bacterial strains (Gram positive and Gram negative strains) and its synergistic effect with some antimicrobial agents. Material & Methods: fifteen Sidr honey samples were collected from three Governorates in Upper Egypt. Honey samples were diluted and tested against *Staphylococcus aureus, Streptococcus agalactiae, Escherichia coli, Klebsiella pneumoniae and Bacillus cereus* by agar dilution method. Post determination of the minimum inhibitory concentration (MIC) values, six honey samples were examined for their synergistic action with the ineffective antimicrobial agents. Results: *In vitro* antimicrobial sensitivity test, all bacterial strains showed multidrug resistance action against the 13 tested antimicrobial agents with high multiple antibiotic resistance (MAR) index, it was ≥ 0.38. All Sidr bee honey samples showing antibacterial activity against the five tested references bacterial strains. All Sidr bee honey samples, showed better synergistic effect with all antimicrobial agents against.

Key words: Egyptian Sidr honey, Antimicrobial impact, Minimum inhibitory concentration, Synergistic effect

ÖZ

Bu çalışmanın amacı, Yukarı Mısır'da üretilen Sidr balının beş referans bakteri suşuna (Gram pozitif ve Gram negatif suşlar) karşı in vitro antibakteriyel potansiyel aktivitesini ve bazı antimikrobiyal ajanlarla sinerjistik etkisini belirlemektir. Yukarı Mısır'daki üç valilikten on beş Sidr balı örneği toplanmıştır. Bal örnekleri seyreltilmiş ve agar seyreltme yöntemiyle Staphylococcus aureus, Streptococcus agalactiae, Escherichia coli, Klebsiella pneumoniae ve Bacillus cereus'a karşı test edilmiştir. Minimum inhibitör konsantrasyon (MİK) değerlerinin belirlenmesinden sonra, altı bal örneği etkisiz antimikrobiyal ajanlarla sinerjik etkileri açısından incelenmiştir. In vitro antimikrobiyal duyarlılık testinde, tüm bakteri suşları test edilen 13 antimikrobiyal ajana karşı yüksek çoklu antibiyotik direnci (MAR) indeksi ile çoklu ilaç direnci etkisi göstermiştir, bu değer ≥ 0.38'dir. Tüm Sidr arı balı örnekleri, test edilen beş referans bakteri suşuna karşı antibakteriyel aktivite göstermiştir. Tüm Sidr arı balı örnekleri, tüm antimikrobiyal ajanlara karşı daha iyi sinerjik etki göstermiştir.

Anahtar kelimeler: Mısır Sidr balı, Antimikrobiyal etki, Minimum inhibitör konsantrasyon, Sinerjistik etki

GENİŞLETILMİŞ ÖZET

Giriş: Artık dünyanın bulaşıcı hastalıklardan kurtulma umudu daha etkili ve güvenli antimikrobiyal ajanlara ulaşmaktır. Düşük bakım maliyeti ve yerel olarak bulunabilirliği bala alternatif bir antimikrobiyal tedavi olarak kullanım için değerli avantajlar sağlamaktadır. Arı balının antimikrobiyal aktivitesi fiziksel ve kimyasal özelliklerine, toplam fenol içeriğine, coğrafi bölgeye ve bitki kaynaklarına bağlıdır. Bu süre zarfında, Yukarı Mısır'da üretilen sidr balı (UESH) ticari olarak üretilmeye başlanmıştır; konu, çok ilaca dirençli (MDR) farklı bakteri suşlarına karşı antimikrobiyal aktivitesinin belirlenmesini gerektirmektedir. Tatlı bir materyal olan UESH, antimikrobiyal etkiye ve antimikrobiyal ajanlarla sinerjik etkiye sahiptir, bu nedenle bakteriyel enfeksiyon vakalarının tedavisinde tamamlayıcı ve alternatif ilaç olarak kullanılabilir.

Amaç: Bu çalışmanın amacı, Yukarı Mısır Sidr arı balının beş referans (3 Gram +ve ve 2 Gram -ve) bakteri suşuna karşı in vitro antibakteriyel yeteneğini ve bazı antimikrobiyal ajanlarla sinerjik etkisini belirlemektir.

Gereç ve Yöntem: Yukarı Mısır'daki üç farklı valilikte (Sohag, Qena ve Luxor) bulunan çeşitli arılıklardan on beş Sidr balı örneği toplanmıştır. Bal örnekleri agar difüzyon yöntemi kullanılarak 5 referans bakteri suşuna (çoklu ilaca dirençli bakteriler olarak bilinen Staphylococcus aureus AUMC B - 261, Streptococcus agalactiae AUMC B - 253, Escherichia coli AUMC B - 243 ve Klebsiella pneumoniae AUMC B - 257; ve gıda toksisitesine neden olan Bacillus cereus AUMC B - 100) karşı test edilmiştir. MİK değerlerinin belirlenmesinden sonra, altı bal örneği etkisiz antimikrobiyal ajanlarla sinerjik etkileri açısından incelenmiştir.

Bulgular: In vitro antimikrobiyal duyarlılık testinde tüm referans bakteri suşları, test edilen 13 antimikrobiyal ajana karşı çoklu antibiyotik direnci (MAR) indeksi ≥ 0.38 olarak yüksek çoklu ilaç direnci (MDR) göstermiştir. Tüm Sidr bal örneklerine karşı test edildiklerinde, genel ortalama %15,16 ± 3,77 olmak üzere farklı MİK değerleri (%11,6 - 18,8) ile büyüme inhibisyonu elde etmişlerdir. Sohag'dan toplanan Sidr balı örnekleri, in vitro testlerde %13,6 MIC değeri gösterdiğinden en iyi antimikrobiyal etkiyi ortaya koymuş, bunu sırasıyla %14,36 ve 17,96 ile Qena ve Luxor örnekleri izlemiştir. Tüm Sidr arı balı örnekleri, tüm antimikrobiyal ajanlarla en iyi sinerjik etkiyi göstermiştir.

Sonuç: Çalışma, tüm Yukarı Mısır Sidr arı ballarının %11,6 ila %18,8 (v/v) arasında değişen in vitro en iyi minimum inhibitör konsantrasyon değerlerine sahip olduğu sonucuna varmıştır. Çalışılan tüm Sidr balı örneklerinin antimikrobiyal etkisi, çoklu ilaca dirençli Gram pozitif veya Gram negatif bakterilere karşı bile umut verici minimum inhibitör konsantrasyon değerlerine sahipti ve Sohag vilayetinden toplanan Sidr balı örnekleri en iyi antimikrobiyal etki göstermiştir. Ayrıca, Sidr balı örnekleri dirençli bakterilere karşı etkili olmayan antibiyotik ajanlarla sinerjik aktivite göstererek onları duyarlı hale getirmiştir. Elde edilen sonuçlar, Yukarı Mısır Sidr arı balının tüm api-terapötik kullanımlar için kullanılabileceğini göstermiştir.

Tüm UESH örnekleri, test edilen tüm Gram pozitif veya negatif bakteriler üzerinde büyümeyi engelleyici etkiye sahiptir ve en çok test edilen antimikrobiyal ajanlarla sinerjik etki göstermiştir. Yerel olarak temin edilebilen UESH balının antibakteriyel aktivitesi, çoklu ilaca dirençli ve gıda kaynaklı bakterilere karşı terapötik ajan olarak kullanılabilir.

INTRODUCTION

Honey antimicrobial activity is proved in ancient medicine where the primary characteristic of "Active Honey" is the presence and concentration of antibacterial compounds (Brudzynsk 2021). The difference in antibacterial activity of bee honey against different pathogens attributes to seasonality of tested honey types**,** difference in floral origin, physical and chemical properties, total phenol contents, geographical area as well as plant resources (Elbanna et al. 2014, Almasaudi 2021).

Determination of in vitro honey antimicrobial activity is widely documented (Hegazi 2011, Abdul-Hafeez et al. 2021) but with different potencies owing to the action of honey macro-components (sucrose, glucose) and the bioactive micro-components flavonoids (Go´rniak et al. 2019), also, polyphenols that seems to contribute to the most antibacterial activity (Stagos et al. 2018), in addition to definsin (Bucekova et al. 2017) and hydrogen peroxidase and catalase enzymes (Scripcă et al. 2019), these components are acting chemically resulting in production of new created bioactive micro-components (Almasaudi 2021, Hamouda et al. 2019).

The high osmatic pressure and high acidity are the main two factors of antibacterial activity of undiluted honey while the activity of diluted honey is peroxide - dependent. (Zainol et al. 2013). This activity is highly complex and still remains not fully recognized which is determined by agar dilution or serial diffusion methods (Szweda 2017).

The application of antibiotics with honey yielded potential synergistic effects against microbes multidrug resistance bacterial strains even biofilm producers (Liu et al. 2018, Abdul-Hafeez et al. 2021, Almasaudi 2021). The low maintenance cost, local production and low price give honey good advantage to use as an alternative antimicrobial treatment (Mandal and Mandal 2012).

Sidr and Talah honey samples contain great potential as an antimicrobial against and Gram-negative bacteria (Gram + ve) and dermatophytes regardless of the origin of the sample. Where found that Gram-negative more resistant to the tested bacteria than Gram (Owayss et al. 2019).

In vitro there was synergistic affect between honey and antibiotics against methicillin resistance *Staphylococcus aureus* (MRSA) and clinical isolates of *Staphylococcus. aureus*. In addition, the honey and antibiotics combination stopped the appearance of antibiotics resistant *S. aureus*. The susceptibility of isolated bacterial strains revealed the synergistic effect of added honey to the antibiotic discs tested. Also, when honey was added with the antibiotic discs, there was a highly synergistic increase in the mean areas of inhibition (Abdul-Hafeez et al. 2021).

So, the present work aimed to study *in vitro* antibacterial activity sidr honey produced in upper Egypt (UESH) from different Governorates against five bacterial references strains (three Gram positive and two Gram negative strains) and their synergistic action with antimicrobial agents**.**

MATERIAL AND METHODS

Honey Samples:

The present study was carried out through flow season of Sidr honey, during the year 2021, collected in three Governorates in Upper Egypt (Sohag, Qena & Luxor). The antimicrobial activity of selected monofloral 15 Sidr honey samples was tested against 5 reference bacterial strains.

The quantitative and qualitative evaluation of pollen grains was carried out under microscopy slide. The results of the study showed that Egyptian Sidr honey is considered a mono - floral honey with a high content of Sidr honey pollen grains with an average more than 45000 sidr pollen grains per 10 gm of honey (Louveaux et al.1978).

Bacterial Strains:

Five reference bacterial strains known to be pathogenic to human were chosen to evaluate the antimicrobial activity of monofloral 15 Upper Egyptian Sidr bee honey samples. The selected tested strains included pathogenic Gram-positive and Gram-negative bacteria- which were provided kindly from Assiut University Mycological Center (AUMC), Assiut, Egypt-(*Bacillus cereus* AUMC B-100*, Staphylococcus aureus* B- 261, *Streptococcus agalactiae* AUMC B- 253, *Escherichia coli* AUMC B-243 and *Klebsiella pneumoniae* AUMC B- 257 and *Bacillus cereus* AUMC B - 100; that causing food toxicity. The bacterial study carried in Assiut Regional Lab., Animal Health Researcher Institute; Agriculture Researcher Center, Egypt. All tested bacterial were used as freshly prepared to be adjusted to a 0.5 McFarland opacity standard in each bacterial inoculation all over the study to obtain approximately $5x10^5$ CFU/ml (Quinn et al. 2004).

Antimicrobial Sensitivity Testing: The antibiotic susceptibility of the examined isolated bacteria was evaluated to calculate multi antibiotic resistance (MAR) index of examined strains. Overnight broth cultured was compared with 0.5 McFarland standards, using the Kirby-Bauer disc spread sensitivity method as described in the National Laboratory Standards Committee guidelines (2000). The above mentioned five reference bacterial strains (*Staphylococcus aureus* B-261, *Streptococcus agalactiae* AUMC B-253, *Escherichia coli* AUMC B-243, *Klebsiella pneumoniae* AUMC B-257 and *Bacillus cereus* AUMC B-100) were tested against 13 antimicrobial agents [Cloxacillin (CX) 1µg, Erythromycin (E) 15µg, Norfloxacin (Nor) 5µg; Cefotaxime (CTX) 30µg, Levofloxacin (Lev) 5µg, Tobramycin (TOB) 10µg; Vancomycin (VA) 30µg, Tetracycline (TE) 30µg, Gentamicin; (CN) 10µg, Ofloxacin (Ofx) 5µg; Amoxycillin (AML) 10µg; Amoxycillin/ Clavulanic acid (AMC) 30µg; and Trimethoprim - Sulfamethoxazole (SXT) (25µg-1.25/23.75µg); Oxoid -England] For Cloxacillin inhibition zones diameter around the disc were measured after 24 and 48 h using the following

breakpoints: susceptible ≥ 13mm and resistance ≤11mm (Quinn et al. 2004). Multiple antibiotic resistance (MAR) index of each tested bacterial strain was calculated; to determine the MAR index that was defined as a/b, where (a) represents the number of antibiotics that isolated strain was resistant and (b) represents the number of all tested antibiotics (Subramani and Vignesh 2012).

Honey Minimum Inhibitory Concentration (MIC) Determination: It was *performed according to* Cooper *et al*. 2002 method by an agar dilution method.

Synergism between Sidr honeys and ineffective antimicrobial agents:

Synergy assay was based on described procedures by Stepanović et al. (2003), where the five strains, six Sidr honey samples and inactive antibacterial agents was evaluated. As follows, by determination of the MIC of honey, different dilutions of samples are less than 1% minimum inhibitory concentration of honey (sub-inhibitory concentration) prepared (allowing bacteria to grow).

RESULTS

In vitro the present antimicrobial susceptibility testing showed that all tested strains were highly sensitive to Levofloxacin, Norfloxacin, Gentamycin, Tetracycline and Ofloxacin (100%). While the tested five reference bacterial strains were resisted Tobramycin and Vancomycin with percentage 60% followed by 40% towered Erythromycin and Trimethoprim/ Sulphamethoxazole. But the highest percentage of resistance was to Amoxycillin, Cloxacillin, Amoxycillin/ Clavulanic acid and Cefotaxime (100%); Table (1) and Plate (1) Multiple Antimicrobial resistance index (MAR) among the tested five reference bacterial strains; *Escherichia coli, Staphylococcus aureus* and *Streptococcus agalactiae* showed the highest value as 0.54, while *Bacillus cereus* and *Klebsiella pneumoniae* showed lowest value 0.38; for each. Table (1) and Plate (1) the all-tested strains had very high MAR index value since MAR index value just ≥ 0.2 was considered high.

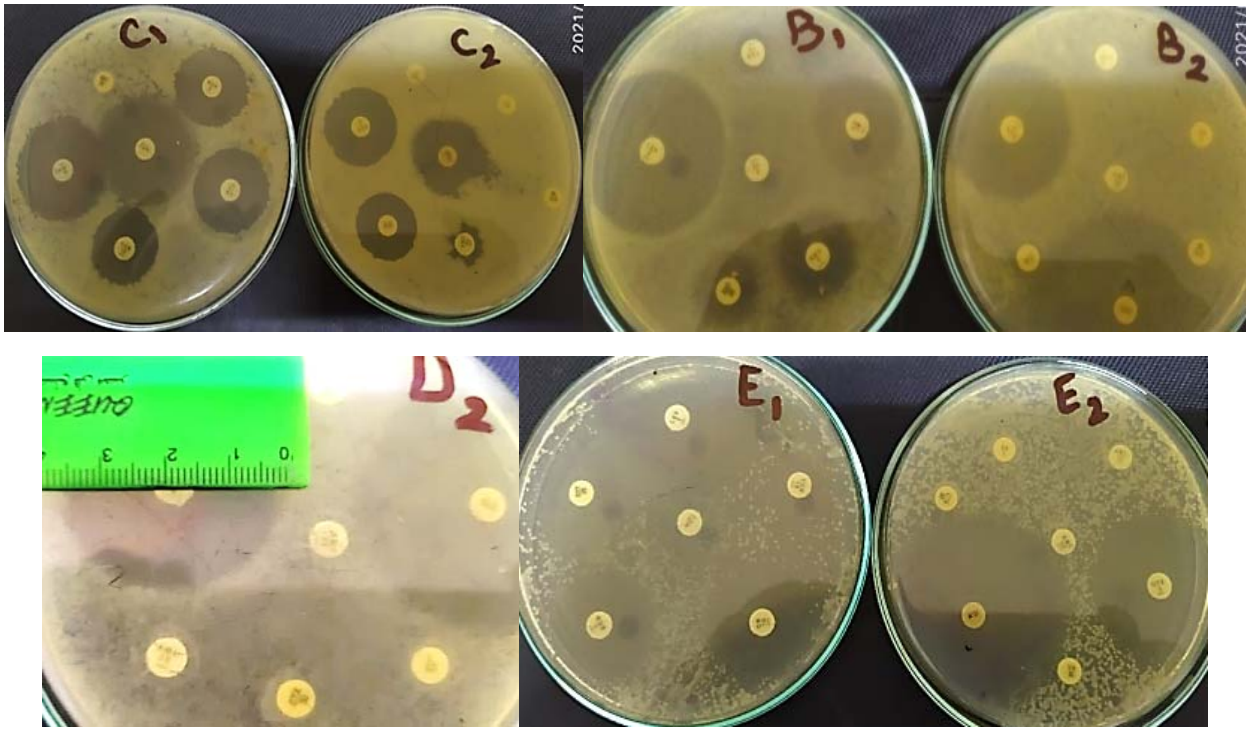

Plate 1: *In Vitro antimicrobial susceptibility testing of Escherichia coli (B), Klebsiella pneumoniae (C), Staphylococcus aureus (D) and Streptococcus agalactiae (E) against 13 antimicrobial agents showing multi-drug resistance.*

In the present study, the minimum inhibitory concentration (MIC) of Sidr bee honey samples collected from Upper Egypt and the antibacterial activity was evaluated against five reference bacterial strains, showing varying antibacterial activity against the various tested bacterial strains. Minimum inhibitory concentration of Egyptian Sidr bee honey samples, from Upper Egypt, against *Bacillus cereus, Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus and Streptococcus agalactia*e showing varying antibacterial activity were 14.2±2.36, 15.0±1.6, 15.4±2.56, 15.4±2.18 and 15.8±2.27 %, respectively. The highest MIC value (15.8±2.27 %) was found against *Streptococcus agalactia*e. On the contrary, the lowest MIC value (14.2±2.36 %) was showed against *Bacillus cereus*, with overall mean 15.16±2.19%, (Table 2 & Diagram 1, 2 & Fig. 1–4).

Table 1: In vitro antimicrobial susceptibility test of the tested bacterial strains and multiple antibiotics resistanc Index (MAR).

Bacterial strains	AMC (30 mcg)	CTX (30 mcg)	CX (1 mcg)	AML (10 mcg	OFX (5 mcg)	VA (30 mcg)	TE (30 mcg)	TOB (30 mcg)	CN (10 mcg)	NOR (5 Mcg)	SXT (1.25 /23.75 mcg)	LEV (5 mcg)	E (15 mcg)	MAR (multiple antibiotics Resistance Index)
	The diameter of inhibition zone in mm													
Bacillus cereus	14	Zero	Zero	11	25	20	25	19	24	21	Zero	28	30	SXT, AML CX, CTX, AMC(0.38)
	R	R	R	R	S	S	S	S	S	S	R	S	S	
Escherichia coli	Zero	Zero	Zero	Zero	30	8	>30	11	25	21	10	40	25	SXT, TOP, VA, AML, CX, CTX, AMC, (0.54)
	R	R	R	R	S	R	S	R	S	S	R	S	S	
Klebsiella pneumoniae	10	Zero	Zero	Zero	25	20	24	20	24	25	Zero	30	25	SXT, AML, CX, CTX, AMC (0.38)
	R	R	R	R	S	S	S	S	S	S	R	S	S	
Staphylococcus aureus	Zero	Zero	Zero	Zero	35	Zero	>30	12	25	25	22	40	Zero	E, TOP, VA, AML, CX, CTX, AMC, (0.54)
	R	R	R	R	S	R	S	R	S	S	S	S	R	
Streptococcus agalactiae	Zero	Zero	Zero	Zero	>33	Zero	>40	12	23	23	22	>30	Zero	E, TOP, VA, AML, CX, CTX, AMC, (0.54)
	R	R	R	R	S	R	S	R	S	S	S	S	R	

AMC= Amoxycillin/Clavulanic acid, CTX= Cefotaxime, CX= Cloxacillin, AML= Amoxycillin, OFX= Ofloxacin, VA=Vancomycin, TE= Tetracycline, E= Erythromycin, LEV= Levofloxacin, NOR= Norfloxacin, CN=Gentamicin, TOB=Tobramycin, SXT=Trimethoprim/Sulfamethoxa

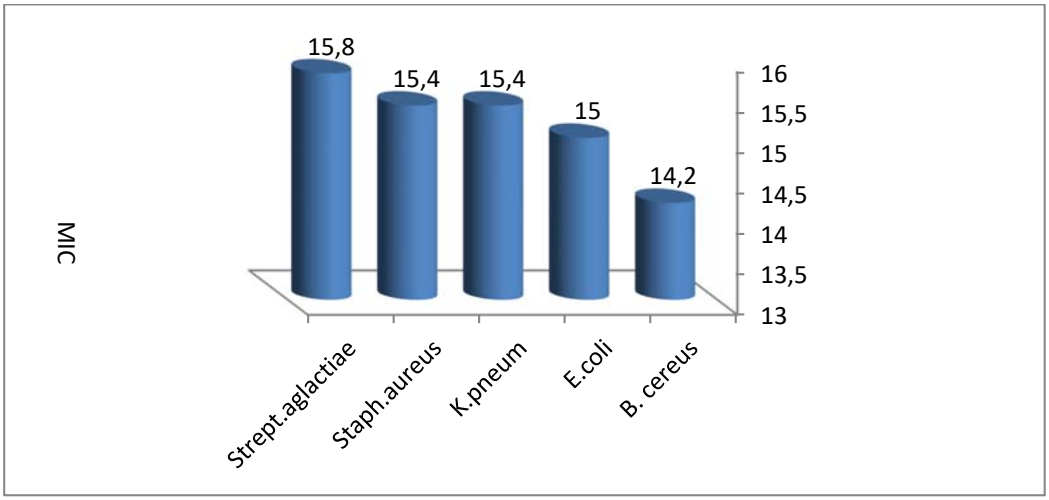

Diagram (1): Percentage of the minimum inhibitory concentration (MIC) of Upper Egyptian Sidr bee honey samples against the tested bacterial strains.

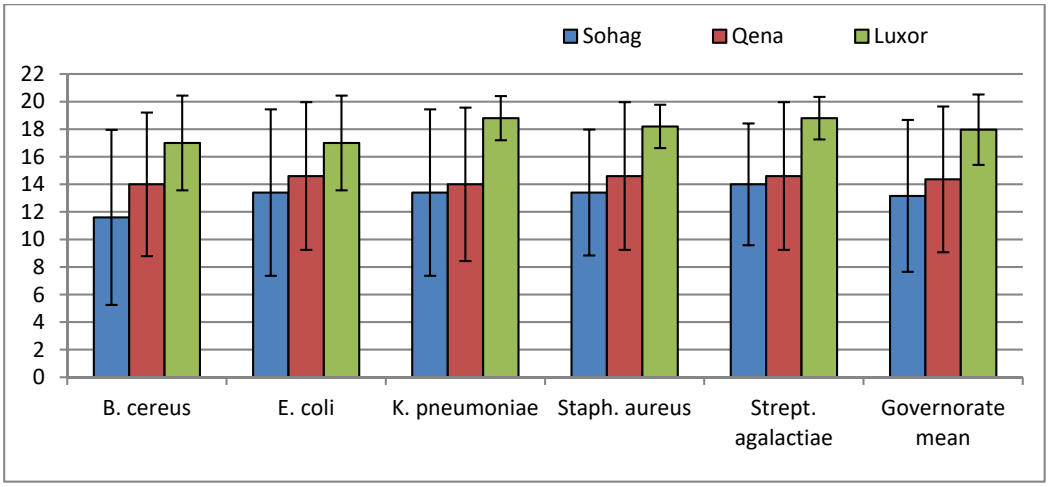

Diagram (2): Percentage of the minimum inhibitory concentration (MIC %) of Sidr honey samples from different locations of Upper Egypt against the tested bacterial strains.

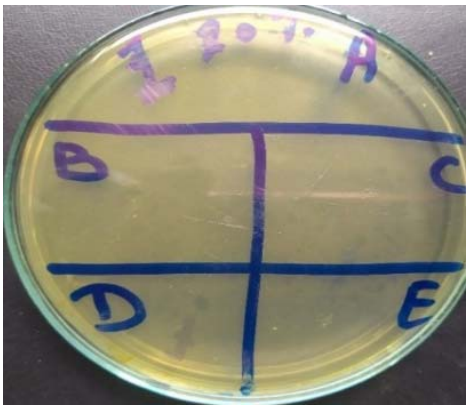

Figure 1: In Vitro honey (20%) showing no growth against all five reference strains

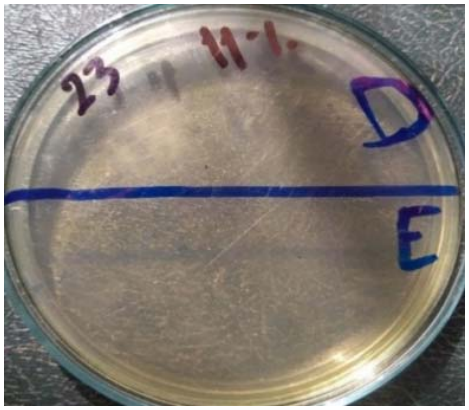

Figure 2: In Vitro honey (11%) showing no growth against *Staph. aureus (D) and Strept. agalactiae (E)*.

Figure 3: In Vitro honey at (10%) all reference strains showing growth

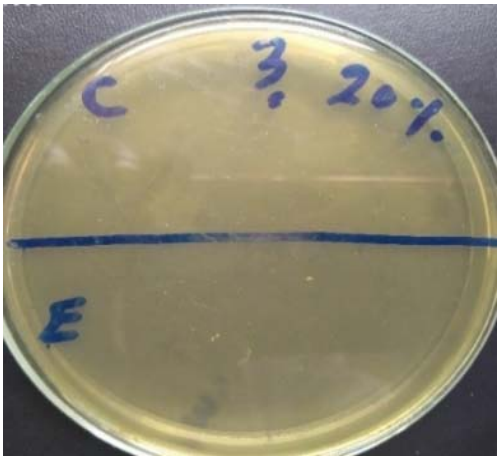

Figure 4: In Vitro honey (20%) showing no growth all five against K. pneumonia (C), and Strept. agalactiae (E)

Table 2. Percentage of the minimum inhibitory concentration (MIC %) of Sidr honey samples from different locations of Upper Egypt against the tested bacterial strains.

Location	MIC percentage v/v against different bacterial strains					
	Bacillus cereus (B.100)	*Escherichia coli (B243)*	*Klebsiella pneumonia (B257)*	*Staphylococcus aureus (B251)*	*Streptococcus (B253)*	Mean of location
Sohag	11.6±0.24	13.4±0.42	13.4±0.23	13.4±0.19	14.0±0.26	13.6±0.225 C
Qena	14.0±0.33	14.6±0.12	14.0±0.17	14.6±0.39	14.6±0.17	14.36±0.375 B
Luxor	17.0±0.44	17.0±0.17	18.8±0.09	18.2±0.33	18.8±0.12	17.96±0.872 A
Mean of bacterial strain	14.2±2.36 *D*	15.0±1.6 *C*	15.4±2.56 *B*	15.4±2.18 *B*	15.8±2.27 *A*	15.16±2.19
Range	11.6-17.0	13.4-17.0	13.0-18.8	13.4-18.2	14.0-18.8	-

In this study, the high antibacterial activity of honey samples against all test strains support the assessment of synergistic action of honey and antibiotics. Honey interacts synergistically with antibiotics at sub-lethal concentrations (sub-MIC), so the present work tested this activity for six brands of Sidr honey. with the ineffective antimicrobial agents.

All Sidr honey brands, in the present study, showed best synergistic action with different antimicrobial agents against *Staphylococcus aureus* (B 261), *Klebsiella pneumoniae* (B 257), *Bacillus cereus* (B100) and *Streptococcus agalactia*e (B 253), Table (3) Fig. (5-7).

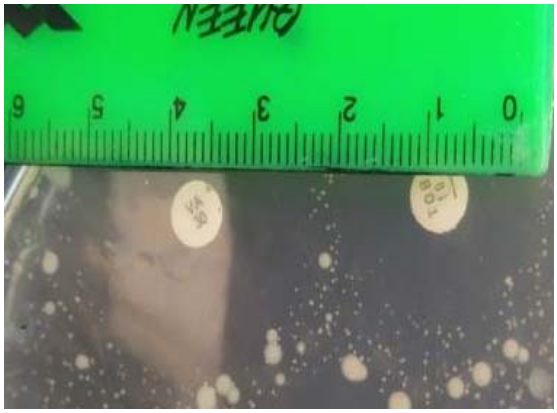

Figure 5: Synergistic action of Sidr honey TOB and VA against E. coli.

Figure 6: Synergistic action of Sidr honey with AMC against K. pneumonia.

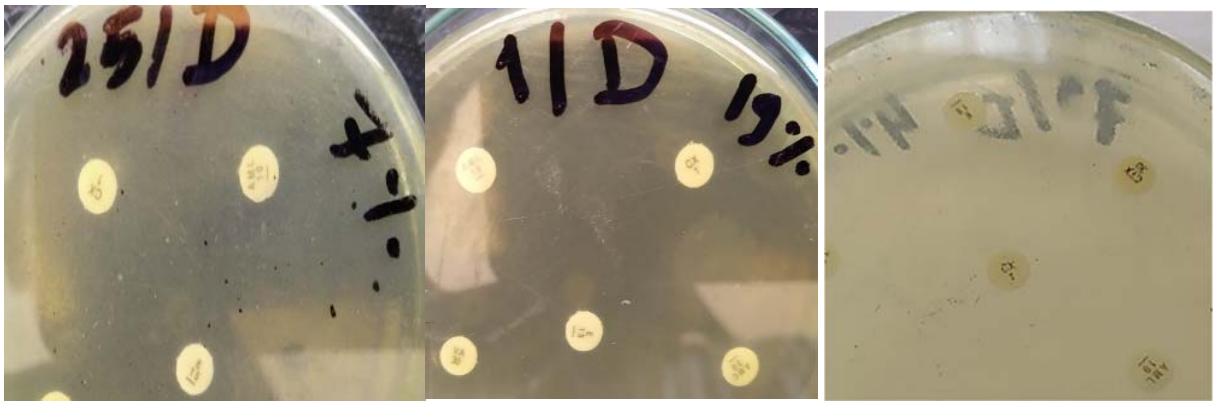

Figure 7: Different types of Sidr honeys (A, B and C) restored methicillin resistant S. aureus (MRSA) sensitive to Cloxacillin (MSSA).

Table 3: Synergistic action of Sidr bee honey and the ineffective antimicrobial agents against both Staph. aureus (B 261) and Strept. agalactiae (B 253)

Antimicrobial agents	Breakpoint ≥ mm	zone of inhibition of antimicrobial disc only	zone of inhibition of tested Sidr honey with antimicrobial disc in mm					
			I	II	III	IV	V	VI
Amoxycillin	29 mm	Zero mm	33 mm	35 mm	33 mm	33 mm	33 mm	35 mm
Amoxycillin / Clavulanic	20 mm	Zero mm	25 mm	22 mm	24 mm	22 mm	23 mm	22 mm
Vancomycin	12 mm	Zero mm	19 mm	26 mm	20 mm	19 mm	24 mm	25 mm
Cloxacillin	13 mm	Zero mm	16 mm	15 mm	18 mm	17mm	15 mm	18 mm
Tobramycin	15 mm	12 mm	23 mm	20 mm	18 mm	21 mm	19 mm	22 mm
Erythromycin	23 mm	Zero mm	26 mm	28 mm	30 mm	28 mm	27 mm	29 mm
Cefotaxime	23 mm	zero mm	25 mm	27 mm	29 mm	25 mm	29 mm	30 mm

DISCUSSION

In the present study, the tested bacterial strains showed strong multidrug resistance action (Table 1 & Plate 1) against different antimicrobial groups; β-lactam (amoxycillin, cloxacillin, amoxycillin/ clavulanic acid) or against the third-generation cephalosporin (cefotaxime) showed resistance 100% resulting that the tested *Staphylococcus aureus* B – 261 was methicillin resistance *Staphylococcus aureus* (MRSA). But against quinolone compound (tobramycin) and glycopeptide (vancomycin) showed resistance 60%, while against macrolide (erythromycin) and sulfonamides (Trimethoprim - Sulfamethoxazole) was only 40% resistance.

But all tested strains were highly sensitive to Levofloxacin, Norfloxacin, Gentamycin, Tetracycline and Ofloxacin (100%), (Table 1). These obtained resistance results were with high value of multiple antibiotic resistance (MAR) index as it was ≥ 0.38 [0.38- 0.54] since MAR index ≥ 0.2 is considered high (Subramani and Vignesh 2012). These results go in parallel with that obtained (Abdul-Hafeez et al. 2021, Sayed et al. 2011).

But fortunately, there is no bacterial resistance against honey as that antibiotics (Blair et al. 2009; Cooper et al. 2010). In the present study, the minimum inhibitory concentration (MIC) of Sidr honey samples collected from Upper Egypt showing varying antibacterial activity against the various tested bacterial strains.

All tested Upper Egyptian Sidr honey (UESH) samples, in present work, have different potencies of antibacterial effects against all five reference bacterial strains with a percentage of minimum inhibitory concentration. (MIC%) values were 13.6 - 17.96% (v/v), with the mean percentage minimum inhibitory concentration (MIC%) values as 15.16% (v/v), (Table 2 & Diagram 1); Generally, the percentage of the minimum inhibitory concentration of Upper Egyptian sider honey samples against *Bacillus cereus* AUMC B- 100*, Escherichia coli* AUMC B- 243 and *Klebsiella pneumoniae* AUMC B-257*Staphylococcus aureus* B- 261, *and Streptococcus agalactiae* AUMC B- 253, were 14.2±5.97, 15±5.18, 15.4±5.32, 15.4±4.56 and 15.8±4.56%, respectively. The highest MIC% value (15.8±4.56%) was found against *Streptococcus agalactiae*. While the lowest MIC value (14.2±5.97%) was showed against *Bacillus cereus* (Table 2, Fig. 1 - 4 & Diagram 1). In the present work, Sider honey showed broad-spectrum antimicrobial activity against Gram+ve and Gram-ve bacteria, which is agreement With previous findings where different types of honey from diverse botanical origins were reported with widespread activity against Gram+ve and Gram-ve bacteria (Irish et al. 2011, Hegazi & Abd Allah 2012, Elbanna et al. 2014, Almasaudi et al. 2017). honey samples collected from Sohag were having goodly antibacterial efficacy against the tested bacterial strains revealed MIC% was 13.6 % followed by samples of Qena and Luxor as 14.36 & 17.96 %, respectively (Table 2 & Diagram 2).

The antibacterial potency differences among honey samples could be attributed to the natural variations the different geographical locations and floral sources of nectar (Alzahrani et al. 2012, Da Silvaet al. 2016). The difference in antimicrobial potency between different types of honey can be more than 100-fold, depending on its geographical, seasonal and botanical source (Molan and Cooper 2000). Bacterial strains showed differential sensitivity as Gram-positive bacteria were more sensitive than Gram-negative bacteria (Owayss et al, 2019). Gram-positive and Gram-negative bacteria were inhibited by 5-10 and 10 to 20 % (w/v), respectively (Eman and Mohammed 2011).

These results are According to other authors, Sidr honey has been used as an antimicrobial agent. The average MIC values of Sidr honey was 15%, 20%, 10% & 20% (v/v) (Alqurashi et al. 2013, Almasaudi et al. 2017, Mohammed & Jayashankar 2020), respectively. The present obtained MIC values of Upper Egyptian Sidr bee honey were much less than MIC values of either Egyptian Sidr Honey recorded formerly as 20% (Hamouda et al. 2019) or the fully studied Saudi Sidr Honey as 20% (Hegazi 2011, Almasaudi et al. 2017, Hegazi et al. 2017).

Sidr honey possessed MIC value against *Streptococcus pyogenes* 20% (w/v), as Sidr honey is effective at inducing lysis of bacterial cell and identifies targets genes, at the genetic level (Mohammad et al. 2021). While the another study showed high MIC that at a concentration 50% (w/v), where *Staphylococcus aureus* ATCC 29213 was susceptibility to two out of the three Sidr honey types tested, while *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27453 were completely unaffected by the three Sidr honeys tested (Dash et al. 2016). Also, Sidr honey sample showing MIC on *Staphylococcus heamolyticus* more

than 25% v/v (Mohammed et al. 2016). Nigerian honey at concentration 40% v/v gave better antimicrobial activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus, Klebsiella pneumoniae and Escherichia coli* (Braide et al. 2012).

Recent study of Saudi Sidr Honey showed higher MIC value as 33% w/v. Saudi Talh and Sidr honey The water-diluted honeys caused an increase in antimicrobial activity compared to the undiluted natural honey. (Owayss et al. 2019). Consequently**,** MIC of the Upper Egyptian Sidr bee honey revealed the best *in vitro* antimicrobial action of all available studied Sidr honey.

The potential therapeutic agent of honey is increasingly due to its antibacterial activity as the most researched biological property of honey through the influence of its phytochemical contents that vary geographically or seasonally. Sider honey has several antibacterial components and property is likely to explain why, unlike antibiotics, it does not induce resistance in bacteria. Commercial development of new classes of antibiotics has been diminished and few pharmaceutical companies still active in this area (European Center for Disease Prevention and Control 2009).

Honey interacts synergistically with antibiotics at sub-lethal (sub-MIC) concentrations (Masoud et al. 2015, Brown et al. 2020), so the present work tested this activity of six Upper Egyptian Sidr bee honey samples with the eight ineffective antimicrobial agents. All tested Upper Egyptian Sidr bee honey samples showed synergistic effect with different antimicrobial agents against the five tested bacterial strains and these resistant bacteria become sensitive (Table 3 & Fig. 5 & 6); and the best synergistic action was against *Staphylococcus aureus* (B 261) and *Streptococcus agalactiae* (B 253). Meanwhile, all tested Sidr honey samples restored *Staphylococcus aureus* sensitive to cloxacillin, Table (3) and Fig. (7).

It was documented that manuka (Jenkins and Cooper 2012, Müller et al. 2013) or Egyptian fennel honey (Abdul-Hafeez et al. 2021) restored methicillin resistant *Staphylococcus aureus* (MRSA) to methicillin sensitive *Staphylococcus aureus* (MSSA) since honey interacts synergistically with antibiotics at sub-lethal (sub-MIC) indicated by significant decline in minimum inhibitory concentration or sub-lethal concentration (Enany et al. 2018). This action is obtained by the down regulation of the honey on methicillin resistant *Staphylococcus aureus* (MRSA) accessory gene regulator genes (*agr B, agr C, agr D*) responsible for virulence (Jenkins and Cooper 2012) and *cid B* gene responsible for bacterial cell division and reduced expression of *mecR1* (responsible for methicillin resistance) (Jenkins et al. 2014) and decreased transcription of the MRSA-specific penicillin binding protein (PBP2A) that has markedly reduced affinity to β-lactams compared to endogenous *Staphylococcus aureus* PBP enzymes (Liu et al. 2015). But, in the present study, *Escherichia coli* (B 243) and *Klebsiella pneumoniae* (B257) still resistant the trimethoprim – sulfamethoxazole and sub-MIC of one Upper Egyptian Sidr bee honey (UESH) sample and *Bacillus cereus* (B100) still resistant to two samples It was evident that both the antimicrobial strength and fairness brands of honey had variable synergistic activity against *Escherichia coli, Klebsiella pneumonia* and *Streptococcus agalactiae* (Abdul-Hafeez et al. 2021). These findings provide a strong basis for the use of Upper Egyptian Sidr honey in treatment of bacterial infections. In addition of mixing of honey and antibiotics have synergistic activity against biofilms producing bacteria. The micro-organisms may not develop resistance against honey in the same way as they develop for other commonly used antimicrobial agents. All these features may make the honey a promising alternative to the commonly used antibiotics.

Conclusion:

The study concluded that all Upper Egyptian Sidr bee honey have the best minimum inhibitory concentration values *in vitro* ranged from 11.6 to 18.8% (v/v). Antimicrobial action of all available studied Sidr honey samples even against multi- drug resistant Gram- positive or Gram- negative bacteria was with promising minimum inhibitory concentration values, where Sidr honey samples collected from Sohag governorate showed the best antimicrobial action. Moreover, Sidr honey samples achieved synergistic activity with the non-effective antibiotic agents against resistant bacteria restoring them sensitive. The obtained results recommended that Upper Egyptian Sidr bee honey can be used for all api-therapeutic usage.

Authors' contributions: The authors contributed equally in the study. They designed, performed, analyzed the data, wrote and revised the manuscript.

Conflict of interest: Authors declare that they have no any conflict of interests to be reported.

Data availability: All data and materials used and/or analyzed during the current study are available in this manuscript.

Ethical issue: Not applicable because this study on honey and not animals or humans

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REFERENCES

Abdul-Hafeez, MM., Hamouda, SM and Abd El Rahman, MF. Synergistic antimicrobial effect of Egyptian honey with antibiotics and its capability to restore MRSA sensitive to oxacillin. International Journal of Science and Research. 2021; 10 (3):1389-1395. DOI: 10.21275/SR21313001535.

Almasaudi, BS, Alaa, AM, Al-Nahari, El, Sayed M. Antimicrobial effect of different types of honey on *Staphylococcus aureus*. Saudi Journal of Biological Sciences. 2017; 24:1255–1261.

Almasaudi, BS. The antibacterial activities of honey. Saudi Journal of Biological Sciences. 2021;28: 2188–2196. doi: 10.1016/j.sjbs.2020.10.017.

Alqurashi, AM, Masoud, EA, Alamin, MA. Antibacterial activity of Saudi honey against Gram negative bacteria. J. of Microbiology and Antimicrobials. 2013; 5(1):1- 5. DOI: 10.5897/JMA2012.0235.

Alzahrani, HA, Alsabehi R, Boukraâ L, Abdellah F, Bellik Y. Antibacterial and antioxidant potency of floral honeys from different botanical and geographical origins. Molecules. 2012; 17: 10540-10549. doi: 10.3390/molecules170910540.

Blair, S, Cokcetin, N, Harry E, Carter, D. The unusual antibacterial activity of medical-grade Leptospermum honey: antibacterial spectrum, resistance and transcriptome analysis. European journal of clinical microbiology & infectious diseases. 2009; 28: 1199–1208. doi: 10.1007/s10096-009-0763.

Braide, W, Oranusi, S, Akaluka, C. Antibacterial efficacy of crude and diluted honey on four wound isolates. Global Advanced Research J. of Microbiology. 2012; 1(1): 1–4.

Brown, HL, Georgie, M, Matthew, D. Antibacterial and antivirulence activity of Manuka Honey against genetically diverse *Staphylococcus pseudintermedius* strains. Applied and Environmental Microbiology. 2020; 86 (20): e01768-20.

Brudzynsk, K. Honey as an Ecological Reservoir of Antibacterial Compounds Produced by Antagonistic Microbial Interactions in Plant Nectars, Honey and Honey Bee. Antibiotics. 2021; 10: 551. doi: 10.3390/antibiotics10050551.

Bucekova, M, Martin S, Ivana V. Bee-derived antibacterial peptide, efensing-1, promotes wound reepithelialisation *in vitro* and *in vivo*. Scientific Reports. 2017; 7: 7340. doi:10.1038/s41598-017-07494-0.

Cooper, R, Jenkins, L, Henriques, AFM, Duggan, R, Burton, N. Absence of bacterial resistance to medical-grade manuka honey. European j. of clinical microbiology & infectious diseases. 2010; 1–5. doi: 10.1007/s10096-010-0992.

Cooper, RA, Molan, PC, Harding, KG. The sensitivity to honey of Gram-positive cocci of clinical significance isolated from wounds. J. Appl. Microbiol. 2002; 93(5): 857-863. doi:org/10.1046/i.1365-2672.2002.01761.x

Da Silva, PM, Gauche, C, Gonzaga, LV, Costa, ACO, Fett R. “Honey: Chemical Composition, Stability and Authenticity” in Food Chemistry. 2016; 196: 309-323. doi: 10.1016/j.foodchem.2015.09.051.

Dash, N, Debadatta, P, Mansour, Al-Zarouni. Antimicrobial Effect of Honey from the Arabian Gulf Region against Bacterial Isolates from Pus and Wound swabs. Advances in Microbiology. 2016; 6: 745-752. DOI: 10.4236/aim.2016.610073

Elbanna, K, Attalla, K, Elbadry, M, Abdeltawab, A, Gamal-Eldin, H, Ramadan, MF. Impact of floral sources and processing on the antimicrobial activities of different unifloral honeys. Asian Pacific Journal of Tropical Disease. 2014; 4(3): 194–200. doi: 10.1016/S2222-1808(14)60504-1.

Eman, MH, Mohammed, MS. Shaoka and Sidr honeys surpass in their antibacterial activity local and imported honeys available in Saudi markets against pathogenic and food spoilage bacteria. Australian Journal of Basic and Applied Sciences. 2011; 5(4): 187-191.

Enany, M E, Algammal, AM, Shagar, GI. Molecular typing and evaluation of Sidr honey inhibitory effect on virulence genes of MRSA strains isolated from catfish in Egypt. Pak. J. Pharm. Sci. 2018; 31, (5):1865-1870.

European Center for Disease Prevention and Control (ECDCE). The bacterial challenge: time to react. Stockholm: European Center for Disease Prevention and Control. 2009.

Go´rniak, I, Rafał, B, Jarosław, K. Comprehensive review of antimicrobial activities of plant flavonoids. Phytochem Rev. 2019; 18:241–272. doi10.1007/s11101-018-9591.

Hamouda, SM, Heba, MMK, Mahmoud, A, Magda, MA, Haroun, AY. Apitherapy of Septic Metacarpal and Metatarsal Wounds (An Experimental Study on Donkeys). Clinical Medicine Research. 2019; 8(4): 77-84. doi:10.11648/j.cmr.20190804.12.

Hegazi, AG, Abd Allah, FM. Antimicrobial activity of different Saudi Arabia honeys. Global Veterinaria. 2012; 9(1): 53–59.

Hegazi, AG, Al Guthami, FM, Al Gethami, AFM. Potential antibacterial activity of some Saudi Arabia honey. Veterinary World. 2017; 10(2): 233-237. DOI:10.14202/vetworld.2017.233-237.

Hegazi, AG. Antimicrobial activity of different Egyptian honeys as comparison of Saudi Arabia honey. Res J Microbiol. 2011; 6:488–495. doi: 10.17311/jm.2011.488.495.

Irish, J, Blair, S, Carter, DA. The antibacterial activity of honey derived from Australian flora. PLoS ONE. 2011; 6(3): e18229. https ://doi. org/10.1371/journ al.pone.0018229.

Jaktaji, PR, Ghalamfarsa, F. Antibacterial activity of honeys and potential synergism of honeys with antibiotics and alkaloid extract of *Sophora alopecuroides* plant against antibiotic-resistant *Escherichia coli* mutant. Iran J Basic Med Sci. 2021; 24(5):623-628. doi: 10.22038/ijbms.2021.54224.12179.

Jenkins, R, Burton, N, Cooper, R. Proteomic and genomic analysis of methicillin resistant *Staphylococcus aureus* (MRSA) exposed to manuka honey in vitro demonstrated down-regulation of virulence markers. J. Antimicrob. Chemother.2014; 9(3):603-615. doi: 0.1093/jac/dkt430.

Jenkins, RE, Cooper, R. Synergy between oxacillin and manuka honey sensitizes methicillin-resistant *Staphylococcus aureus* to oxacillin. J. Antimicrob. Chemother. 2012; 67:1405–1407. DOI:10.1093/jac/dks071.

Liu, M, Lu, J, Müller, P. Antibiotic specific differences in the response of *Staphylococcus aureus* to treatment with antimicrobials combined with manuka honey. Frontiers in microbiology. 2015; 5 (Article 779):1-9. doi:10. 3389 / fmicb. 00779.

Liu, MY, Nural C, Jing L. Rifampicin - Manuka honey combinations are superior to other antibiotic-Manuka honey combinations in eradicating *Staphylococcus aureus* Biofilms. FrontiesMicrobiol.2018; 8(11):1-12.doi.org/10.3389/fmicb.2017.02653.

Louveaux J, Maurizio A. and Vorwohl, G. Methods of melissopalynology. Bee World. 1978; 59:139-157.

Mandal, MD, Mandal, S. Honey: its medicinal property and antibacterial activity. Asian Pacific Journal of Tropical Biomedicine. 2012; 1(2): 154-160.

Masoud, EA, Alqurashi, AM, Alamin, AA. Synergistic effects of honeys and commonly used antibiotics on Gram Positive Bacteria. Egypt. Acad. J. Biolog. Sci. 2015; 7(1): 101–109. DOI:10.21608/eajbsg.2015.16491.

Mohammad, A, Al-Kafaween, MHA, Hamid ANA. Potential antibacterial activity of Yemeni Sidr honey against *Pseudomonas aeruginosa* and *Streptococcus pyogenes*. Anti-Infective Agents in Medicinal Chemistry. 2021;19(4):1-15.DOI:10.2174/2211352519666210319100204.

Mohammed, AAA, Sailh, MK, Ali AF, Nusaybah, KHS. *In Vitro* antibacterial activity of some natural and trade iraqi honey against MRSA *Staphylococcus Heamolyticus* isolated from some burned patients in Misan City. American

Journal of Microbiological Research. 2016; 4(5): 159-163. DOI:10.12691/AJMR-4-5-6.

Mohammed, AS, Jayashankar, M. Evaluation of antibacterial activity of some Indian and Yemeni honey against few bacterial isolates from human patients. Egypt. J. Microbiol. 2020; 55: 21-28. doi: 10.21608/EJM.2020.1135.

Molan, PC, Cooper, RA. Honey and sugar as a dressing for wounds and ulcers. Trop. Doct. 2000; 30:249-250. doi: 10.1177/004947550003000101.

Müller, P, Alber, DG, Turnbull, L. Synergism between Medihoney and Rifampicin against Methicillin-Resistant *Staphylococcus aureus* (MRSA). PLOS ONE. 2013; 8(2): doi: 10.1371/journal.pone.0057679.

National Committee for Laboratory Standards (NCCLS) 2000. Performance Standards for Antimicrobial Disk Susceptibility Test. Approved Standard M2 – A7, M100 – S10. PA.

Owayss AA, Khaled E, Javaid I. In vitro antimicrobial activities of Saudi honeys originating from Ziziphus spina-christi L. and Acacia gerrardii Benth. Trees. Food Sci. Nutr. 2019; 8:390–401. doi: org/10.1002/fsn3.1320.

Quinn PJ, Carter ME, Markey B, Carter GR. Bacteriology: Clinical veterinary microbiology. 2004; (6thEd.) Mosby Edinburgh London New York Oxford Philadelphia St Louis Sydney Toronto.

Sayed, MS, Maryam FM, El Berbawy SM. Bacteriological studies on sub-clinical mastitis in cows and buffaloes with trails for its treatment. Assiut Veterinary Medical Journal. 2011; 57, (129):185-200. doi:10.21608/AVMJ.2011.174542.

Scripcă, LA, Liliana N, Sonia A. Comparison of physicochemical, microbiological properties and bioactive compounds content of grassland honey and other floral origin honeys. Molecules. 2019; 24:1-17, 2932. doi:10.3390/molecules24162932.

Stagos, D, Soulitsiotis, N, Tsadila, C. Antibacterial and antioxidant activity of different types of honey derived from Mount Olympus in Greece. International Journal of Molecular Medicine. 2018; 42(2):726–734. doi:10.3892/ijmm.2018.3656.

Stepanović S, Antić N, Dakić I. *In vitro* antimicrobial activity of propolis and synergism between propolis and antimicrobial drugs. Microbiol. Res. 2003; 158(4):353-357. doi:10.1078/0944-5013-00215.

Subramani, S, Vignesh, S. MAR index study and MDR character analysis of a few golden Staph isolates. Asian Journal of Pharmacy and Life Science. 2012; 2(2).

Szweda, P. Antimicrobial Activity of Honey. Honey Analysis. 2017; pp. 215-232. doi:10.1155/2019/2464507.

Zainol, MI, Kamaruddin, MY, Mohd, YMY. Antibacterial activity of selected Malaysian honey. BMC Complementary and Alternative Medicine. 2013; 13:129.

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ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

A COMPARATIVE STUDY ON THE QUALITY OF HONEY BEE (*Apis mellifera*) QUEENS DEVELOPED FROM LARVAE AFTER THE COLLECTION OF ROYAL JELLY

Bal Arılarında (*Apis mellifera*) Arı Sütü Toplama Sonrası Larvalardan Geliştirilen Ana Arıların Kalitesi Üzerine Karşılaştırmalı bir Çalışma

Hossam F. ABOU-SHAARA

Department of Plant Protection, Faculty of Agriculture, Damanhour University, Damanhour, 22516, EGYPT, ORCID No: 0000-0001-7208-6526, E-posta: hossam.farag@agr.dmu.edu.eg

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ABSTRACT

Rearing bee queens is almost done utilizing grafting young larvae while the effects of grafting using old larvae after the collection of royal jelly on the quality of queens are not known. In fact, the production of royal jelly depends on grafting, then discarding the larvae to collect the royal jelly. This study aimed to investigate this point by grafting old larvae after removing them from their original cells without food. Larvae at age about 2 days were grafted into plastic queen cell cups (selection and grafting method or S&G method) leaving royal jelly behind and then resultant queens were compared with naturally reared ones (or NQ). The study showed the absence of significant variations between the queens reared from the two methods in characteristics of queens and cells. Meanwhile, no significant differences were found in regard to the performance of colonies. The colonies with queens from S&G method had slightly higher performance than those with NQ. The study concluded that grafting using old larvae without their original food does not impair the quality of queens. During the production of royal jelly, larvae may be grafted into new cells to continue their normal development instead of discarding them.

Key Words: *Apis mellifera*, morphology, performance, rearing, cells

ÖZ

Ana arıların yetiştirilmesi genç larvaların aşılanmasıyla neredeyse tamamlanırken, arı sütünün toplanmasından sonra eski larvaların aşılanmasının kraliçe arıların kalitesine etkisi bilinmemektedir. Aslında, arı sütü üretimi aşılamaya ve ardından arı sütünü toplamak için larvaları atmaya bağlıdır. Bu çalışma, eski larvaları yemeksiz olarak orijinal hücrelerinden çıkardıktan sonra aşılayarak bu noktayı araştırmayı amaçlamıştır. Yaklaşık 2 günlük olan larvalar, geride arı sütü bırakarak plastik kraliçe hücre kaplarına (seçme ve aşılama yöntemi veya S&G yöntemi) aşılandı ve ardından ortaya çıkan kraliçeler, doğal olarak yetiştirilenlerle (veya NQ) karşılaştırıldı. Çalışma, iki yöntemden yetiştirilen ana arılar arasında ana arı ve hücre özelliklerinde önemli farklılıkların olmadığını gösterdi. Bu arada, kolonilerin performansı açısından önemli bir fark bulunamadı. S&G yönteminden kraliçeleri olan koloniler, NQ'ya sahip olanlardan biraz daha yüksek performans gösterdi. Çalışma, orijinal besinleri olmadan eski larvaları kullanarak aşılamanın kraliçelerin kalitesini bozmadığı sonucuna varmıştır. Arı sütünün üretimi sırasında larvalar, onları atmak yerine normal gelişimlerini sürdürmek için yeni hücrelere aşılanabilir.

Anahtar Kelimeler: *Apis mellifera*, morfoloji, performans, yetiştirme, hücreler

GENİŞLETİLMİŞ ÖZET

Çalışmanın amacı: Ana arı yetiştiriciliği genç larvaların aşılanmasıyla neredeyse tamamlanırken, arı sütü toplandıktan sonra eski larvaların aşılanmasının kraliçe arıların kalitesine etkisi bilinmemektedir. Aslında, arı sütü üretimi aşılamaya ve ardından arı sütünü toplamak için larvaları atmaya bağlıdır. Bu çalışma, eski larvaları yemeksiz olarak orijinal hücrelerinden çıkardıktan sonra aşılayarak bu noktayı araştırmayı amaçlamıştır.
Gereçler ve yöntemler: yaklaşık 2 günlük larvalar, geride arı sütü bırakarak plastik kraliçe hücre kaplarına aşılandı (seçme ve aşılama yöntemi veya S&G yöntemi) ve ardından ortaya çıkan kraliçeler, doğal olarak yetiştirilenlerle (veya NQ) karşılaştırıldı. Bu çalışmada kullanılan koloniler, ana arı içermeyen besin peteklerinin yanında yumurta içeren kuluçka peteklerine sahipti. Kraliçe hücrelerinin özellikleri, uzunluk, taban genişliği ve uç genişliği dahil olmak üzere ölçüldü. Ortaya çıkan ana arıların taze ağırlığı, göğüs genişliği, ön kanat uzunluğu ve ön kanat genişliği dahil olmak üzere ana arı özellikleri incelenmiştir. Ayrıca, arılarla kaplı peteklerin sayısı sayılmış ve kapalı kuluçka, depolanmış bal ve depolanmış arı ekmeği alanları ölçülmüştür.
Bulgular: Çalışma, iki yöntemle yetiştirilen ana arılar arasında ana arı ve hücre özelliklerinde önemli farklılıkların olmadığını gösterdi. S&G ve NQ arasındaki fark sırasıyla vücut ağırlığı, ön kanat uzunluğu, ön kanat genişliği ve göğüs genişliği için sadece 1,2 mg, 0,01 mm, 0,02 mm ve 0,1 mm ve hücre tabanı, hücre için 0,56, 0,38 ve 0,04 mm idi. sırasıyla uzunluk ve uç genişliği. Bu arada, kolonilerin performansı açısından iki yöntem arasında anlamlı bir fark bulunamadı. S&G yönteminden ana arılı koloniler, petek sayısı, kapalı kuluçka alanı, depolanan bal alanı ve depolanan arı ekmeği alanı için sırasıyla 0,4 petek, 69,67, 45,17 ve 246,45 cm2 ile NQ'lu kolonilerden biraz daha yüksek ortalamalara sahipti. S&G yönteminden kraliçeleri olan koloniler, NQ'ya sahip olanlardan biraz daha yüksek performans gösterdi. Bu, S&G'den elde edilen kraliçe arıların kalitesinin doğal olarak yetiştirilenlere benzer olduğunu gösterdi.
Sonuç: Çalışma, orijinal besinleri olmadan eski larvaları kullanarak aşılamanın, ortaya çıkan bal arısı kraliçelerinin kalitesini bozmadığı sonucuna varmıştır. Ayrıca, bu yöntemle yetiştirilen ana arılarla yönetilen kolonilerin performansı etkilenmez. Arı sütünün üretimi sırasında larvalar, ekonomik faydaları en üst düzeye çıkarmak için onları atmak yerine normal gelişimlerini sürdürmek için yeni hücrelere aşılanabilir.

INTRODUCTION

Honey bee colonies headed with good young queens are expected to yield better productivity than those headed with older ones (Akyol et al. 2008, Hatjina et al. 2014, Junus 2019). There are various methods that can be employed by beekeepers to produce queens such as grafting (Zawislak and Burns 2012, Büchler et al. 2013, Given 2021). Also, grafting is widely used during the production of royal jelly (Zheng et al. 2011, Al-Kahtani and Taha 2020, Gemeda et al. 2020) and larvae are mostly discarded after the collection of royal jelly from cells. It is not known if these larvae can be used to obtain good queens instead of discarding them. Looking at the literature, comparing queen rearing methods was the main focus of some previous studies (Cengiz et al. 2009, Kumar 2018, Dhaliwal et al. 2019), while other studies focused on age of grafted larvae (Mahbobi et al. 2012, Okuyan and Akyol 2018), queen cell size and numbers (Al-Fattah et al. 2011, Wu et al. 2018, Adgaba et al. 2019), rearing months and seasons (Koç and Karacaoğlu 2011, Kamel et al. 2013, Önk et al. 2016), and grafting methods (Gene et al. 2005, Rafique et al. 2019). There are no studies however on grafting old larvae without their royal jelly.

In fact, queen rearing is regulated by bee workers (Hatch et al. 1999, Tarpy et al. 2004). In queenless colonies, orphan workers build many emergency queen cells (Abou-Shaara et al. 2021). However, the queen cells may be built over old larvae (Fell and Morse 1984, Tofilski and Czekonska 2004), especially bee workers select larvae for emergency queen rearing based on their nutritional status (Sagili et al. 2018). In the present study, to be able to compare naturally reared larvae and those removed from their cells, the age of larvae over which queen cells were built was controlled. Also, grafting was used to move larvae from their queen cells into new plastic cell cups. Then, the quality of the emerged queens was assessed in comparison with the naturally reared ones.

Parameters commonly considered to compare queens resulting from various methods include cell characteristics, body weight, and morphological characteristics of queens (Al-Ghzawi and Zaitoun 2008, Cengiz et al. 2009, Al-Fattah et al. 2011, Mahbobi et al. 2012, Kamel et al. 2013, Önk et al. 2016, Okuyan and Akyol 2018, Mattiello et al. 2022), and performance of colonies including brood rearing and colony productivity (Gençer et al. 2000, Mahbobi

et al. 2014, Kumar 2018). Such parameters were included in the comparison between queens naturally reared in their cells and those grafted away from their original cells. This study presents also some insights into the development of honey bee queens and provides a method to utilize queen larvae after the collection of royal jelly instead of discarding the larvae.

MATERIALS AND METHODS

Grafting using old larvae

In this study, queenless colonies (Carniolan hybrid bees) were allowed to rear their own queens from combs containing eggs followed by grafting. Firstly, the queens were removed from the colonies. Secondly, queen cells containing larvae at age of about two days were selected. Thirdly, these larvae were moved from their natural cells into plastic queen cell cups. Then, grafted queens were left inside the colonies until the sealing of cells, and then the cells were placed in an incubator (34±1°C, and 80% RH) until hatching. This method was applied in particular to be able to compare queens reared under the same conditions and meanwhile to mimic the process of royal jelly production by removing the larvae without their food. To simplify the study, this method of removing larvae from their cells into new cups was named selection and grafting method (S&G).

Experimental setup.

Queens reared from S&G method were compared with naturally reared queens (NQ) in the colonies. To do this, Carniolan hybrid colonies with the same strength (each with 10 combs covered with bees: 6 combs each with brood and 4 combs each with stored pollen/honey) from an apiary at Damanhour city were used in the study during March – July. There are good sources for nectar/pollen during this period at the study area. The study started with eight colonies but finally, five colonies were considered in the study. Combs with capped brood and old larvae were replaced by new empty combs to ensure the availability of eggs before splitting the colonies. Then, each colony was divided into two small colonies (each with 5 bee combs: 3 brood combs containing mainly eggs and 2 food combs covered completely with bees) placed in 10-frame Langstroth beehives without queens. All colonies were supplied temporarily with 2 combs of capped brood to increase their strength. The first 5 small colonies were allowed to rear queens naturally (NQ) while their sister 5 small colonies were used to obtain queens using S&G method. Each colony was able to rear >13 queen cells, and 10 of them were used in measuring the following parameters.

Cell characteristics

A digital caliper was used to measure the length, base width, and tip width of queen cells for 10 cells from each colony (a total of 50 cells per group).

Queen characteristics

The fresh weight of the emerged queens was determined using an electronic balance. Also, body characteristics related to body size were measured including thorax width, forewing length, and forewing width. The thorax width was measured using a digital caliper while forewing length and width were measured according to Ruttner et al. (1978) using Scan Photo Technique (El-Aw et al., 2012) after scanning the wings at 1200 dpi using a scanner (Canon LiDE 110, k10352, Vietnam). The measurements were taken for 10 queens per colony with a total of 50 queens per group.

Colony performance

The number of combs covered with bees was counted. While a frame divided into grids of cm^2 (Jeffree 1958) was used to measure areas of sealed brood, stored honey, and stored bee bread. These areas were measured after two months from the start of egg laying by the new queens in the 10 colonies (5 per each group) as an indicator for the performance of queens reared by the two methods.

Statistical analysis

The measured parameters for the two groups were compared using t-test. The variations were considered significant when $P \leq 0.05$. The analysis was done using SPSS v. 16 (SPSS for Windows 2007, Chicago, USA).

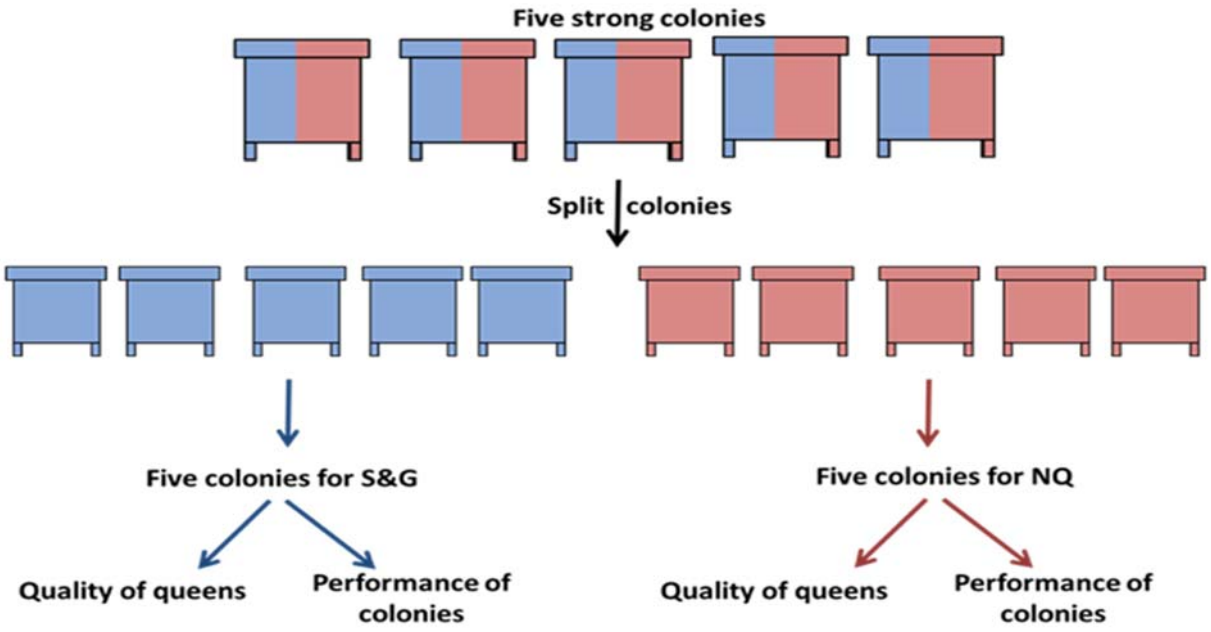

Fig.1: The experimental setup of the study to compare between naturally reared queens (NQ) and queens reared by selection and grafting method (S&G).

RESULTS

Cell characteristics

No significant differences (P>0.05) were found between the two groups in cell length and tip width while base width differed significantly (P<0.05) between them as shown in Table 1. The measured characteristics of cells were slightly higher in S&G method than NQ, except base width. The difference between the two groups was 0.56, 0.38, and 0.04 mm for base width, cell length, and tip width, respectively.

Table 1: Cell characteristics (Mean±SE) of natural queens (NQ) and queens from selection and grafting (S&G) method.

Characteristics	Mean±SE (mm)		t-test
	NQ	S&G	
Base width	11.56±0.16	11.00±0.00	(t=3.35, P=0.001)
Length	17.56±0.25	17.94±0.21	(t=1.1, P=0.25)
Tip width	6.42±0.11	6.46±0.12	(t=0.22, P=0.81)

Queen characteristics.

No significant differences (P>0.05) were found between the two groups in all characteristics (Table 2). Naturally reared queens had slightly higher values in the measured characteristics than S&G queens. The difference between queen characteristics of the two groups was 1.2 mg, 0.01 mm, 0.02 mm, and 0.1 mm for body weight, forewing length, forewing width, and thorax width, respectively.

Table 2: Queen characteristics (Mean±SE) of natural queens (NQ) and queens from selection and grafting (S&G) method.

	Mean±SE		
Characteristics	**NQ**	**S&G**	**t-test**
Weight (mg)	198.34±1.35	197.14±1.27	(t=0.64, P=0.51)
Forewing length (mm)	9.11±0.007	9.10±0.008	(t=1.42, P=0.15)
Forewing width (mm)	3.01±0.010	2.99±0.011	(t=0.99, P=0.32)
Thorax width (mm)	4.82±0.05	4.72±0.06	(t=1.18, P=0.23)

Colony performance.

No significant differences (P>0.05) were found between them in measured parameters (Tables 3). The colonies headed with NQ or queens from S&G method showed approximately the same performance level. The number of combs covered with bees, after two months, ranged from 3 to 5 combs for the both groups. The colonies with queens from S&G method had slightly higher means than colonies with NQ by 0.4 comb, 69.67, 45.17, and 246.45 cm^2 for the number of combs covered with bees, sealed brood area, stored honey area, and stored bee bread area, respectively.

Table 3: Parameters (Mean±SE) of colonies headed with natural queens (NQ) and queens reared from selection and grafting (S&G) method.

	Mean±SE		
Parameters	**NQ**	**S&G**	**t-test**
Number of combs	3.80±0.37	4.20±0.49	(t=0.64, P=0.53)
Sealed brood area (cm^2)	1406.45±119.22	1476.12±225.24	(t=0.27, P=0.79)
Stored honey area (cm^2)	709.67±145.34	754.84±338.87	(t=0.12, P=0.91)
Stored bee bread area (cm^2)	529.03±120.95	775.48±177.92	(t=1.14, P=0.28)

DISCUSSION

The best quality of queens was found when queens were reared from 1 to 2 days old larvae (Gençer et al. 2000, Mahbobi et al. 2012, Okuyan and Akyol 2018, Dhaliwal et al. 2019). In the present study, workers in queenless colonies accepted the grafted old larvae when moved into new plastic queen cell cups. Accordingly, Staron et al. (2019) recorded low death rates when grafting old larvae, suggesting the good survival of old larvae after grafting. The two methods used in this study had approximately similar cell characteristics without significant differences in cell length and tip width. This indicates the lack of any negative impacts of the S&G method on the ability of bees to construct normal cells on older grafted larvae. The significant differences between the two methods in cell base can be explained by using plastic cups with fixed width in S&G method than the naturally built queen cells.

The measured queen characteristics proved that queens from S&G method were not different from those reared naturally. This supports the idea that grafting old queen larvae into new cells did not affect the subsequent development of queens. Based on bee subspecies, the queen weight of 190-200 mg can be considered as moderate queens (Kahya et al. 2008) or heavy queens (Al-Fattah et al. 2011, Dhaliwal et al. 2019); therefore, queens developed from the two rearing methods can be considered at least as moderate queens. This indicates the good quality of queens; especially queen weight is a good indicator for colony productivity (De Souza et al. 2013) and queen quality (Wilkinson and Brown 2002, Kahya et al. 2008, Hatjina et al. 2014), and large queens have large spermatheca and can store more sperms (Collins and Pettis 2013). In line with the obtained results, the weight of queens from grafting method (189.80 mg) was higher than naturally reared queens in queenless colonies (Kumar 2018). The measurements of forewing length and width recorded in this study for queens from the two groups were similar to those recorded by Kamel et al. (2013) for bee queens in Egypt reared during different months.

Despite its importance, queen weight has no role in the acceptance of queens by bee workers as well as the beginning of oviposition (Medina and Goncalves 2001). The commencement of egg laying after mating is impacted by many factors (Woyke and Jasinski 1990, Schlüns et al. 2005), and can range from a few days to one month (Moritz and Kuhnert 1984, Cobey 2007). In the present study, all queens developed from the two groups were observed to lay eggs within the first two weeks after emergence and had similar performance. The insignificant variations between performance parameters reflected that queens from the two methods were able to naturally mate in a similar way. It is known that poor queen mating can impair its egg laying ability, and subsequently colony development (Abou-Shaara et al. 2021). But in this study colonies were developed over two months in a similar way.

The obtained results are somewhat supported by a previous study, wherein the queens from the grafting method were better than naturally reared queens in the studied parameters including areas of brood, pollen, and honey (Kumar, 2018). The higher brood area in S&G group can be explained by the higher area of stored food than NQ group. Accordingly, a relationship was found between stored pollen area and brood rearing activity (Abou-Shaara et al. 2013). Gençer et al. (2000) recorded significant differences in brood rearing activity and the number of combs covered with bees in colonies headed with heavy and light queens. On the contrary, all queens from the two groups in this study were approximately with the same weight, and thus had no variations in their performances.

Conclusion

This study tested the effects of grafting old larvae without their food (S&G method) on the quality of queens in comparison with naturally reared ones. To mimic the situation during royal jelly production as larvae are discarded after royal jelly collection, but here the larvae were grafted again into new plastic cups. This method yields queens with similar quality to those reared naturally inside hives. The results proved that the transportation of old larvae into new cells (i.e. plastic queen cell cups) did not affect negatively on the quality of queens and colony performance. The comparison between naturally reared queens and queens reared using S&G showed the absence of high variations in queen cell characteristics and queen morphology. Also, the performance of colonies headed with queens from the two methods showed insignificant variations. On the beekeeping scale, royal jelly producers may plan to utilize the larvae in queen rearing instead of discarding them but accelerate the process of royal jelly collection to be done with younger larvae (< 3 days). This study also shows that the interruption in the feeding of larvae may not pose serious effects on their development and quality. More studies using different honey bee subspecies are advised.

Author contribution: The author designed, performed, analyzed the data, wrote and revised the manuscript.

Conflict of Interest: No conflict of interests to be reported.

Ethical issue: Not applicable because this study on honey bees and not animals or humans.

Source of Finance: Not applicable.

REFERENCES

Abou-Shaara, HF., Adgaba, N., Al-Ghamdi, AA. 2021. Current knowledge about behaviors of honey bee queens with highlighting of the importance future studies. Journal of Basic and Applied Zoology 82, 1-7.

Abou-Shaara, HF., Al-Ghamdi, AA., Mohamed, AA. 2013. Honey bee colonies performance enhance by newly modified beehives. Journal of Apicultural Science 57, 45-57.

Adgaba, N., Al-Ghamdi, A., Tadesse, Y., Alsarhan, R., Single, A., Mohammed, SE., Khan, KA. 2019. The responses of *Apis mellifera jemenitica* to different artificial queen rearing techniques. Saudi Journal of Biological Sciences 26, 1649-1654.

Akyol, E., Yeninar, H., Korkmaz, A., Çakmak, I. 2008. An observation study on the effects of queen age on some characteristics of honey bee colonies. Italian Journal of Animal Science. 7, 19-25.

Al-Fattah, MAA., Mazeed, AM., Al-Hady, NA. 2011. Quality and quantity of honeybee queens as affected by the number and distribution of queen cells within queen rearing colonies. Journal of Apicultural Science 55, 31-41.

Al-Ghzawi, AAM, Zaitoun S. 2008. Origin and rearing season of honeybee queens affect

some of their physiological and reproductive characteristics. Entomological Research 38, 139-148.

Al-Kahtani SN., Taha EKA. 2020. Post grafting time significantly influences royal jelly yield and content of macro and trace elements. PloS one 15(9), e0238751.

Büchler, R., Andonov, S., Bienefeld, K., Costa, C., Hatjina, F., Kezic, N., Kryger, P., Spivak, M., Uzunov, A., Wilde, J. 2013. Standard methods for rearing and selection of *Apis mellifera* queens. Journal of Apicultural Research 52(1), 1-30.

Cengiz, M., Emsen, B., Dodologlu, A. 2009. Some characteristics of queen bees (*Apis mellifera* L.) rearing in queenright and queenless colonies. Journal of Animal Veterinary advances 8, 1083-1085.

Cobey, SW. 2007. Comparison studies of instrumentally inseminated and naturally mated honey bee queens and factors affecting their performance. Apidologie 38, 390-410.

Collins, AM., Pettis, JS. 2013. Correlation of queen size and spermathecal contents and effects of miticide exposure during development. Apidologie 44, 351-356.

De Souza, DA., Bezzera-Laure, MAF., Francoy, TM., Gonçalves, LS. 2013. Experimental evaluation of the reproductive quality of Africanized queen bees (*Apis mellifera*) on the basis of body weight at emergence. Genetics and Molecular Research 12, 5382-5391.

Dhaliwal, NK., Singh, J., Chhuneja, PK. 2019. Effect of rearing method, age of brood and queenliness of cell-builder colony on weight of *Apis mellifera* Linnaeus queen bees. Journal of Entomology and Zoology Studies 7, 1260-1262.

El-Aw, MAM., Draz, KAA., Eid, KS., Abou-Shaara, HF. 2012. Measuring the morphological characters of honey bee (*Apis mellifera* L.) using a simple semi-automatic technique. Journal of American Science 8, 558-564.

Fell RD., Morse, RA. 1984. Emergency queen cell production in the honey bee colony. Insectes Sociaux 31, 221-237.

Gemeda, M., Legesse, G., Damto, T., Kebaba, D. 2020. Harvesting Royal Jelly Using Splitting and Grafting Queen Rearing Methods in Ethiopia. Bee World 97(4), 114-116.

Gençer, HV., Shah, SQ., Firatli, Ç. 2000. Effects of supplemental feeding of queen rearing colonies and larval age on the acceptance of grafted larvae and queen traits. Pakistan Journal of Biological Sciences 3, 1319-1322.

Gene, F., Emsen, B., Dodologlu, A. 2005. Effects of rearing period and grafting method on the queen bee rearing. Journal of Applied Animal Research 27, 45-48.

Given, K. 2021. Queen rearing and bee breeding. In Honey Bee Medicine for the Veterinary Practitioner, chapter 29, 363-366.

Hatch, S., Tarpy, DR., Fletcher, DJC. 1999. Worker regulation of emergency queen rearing in honey bee colonies and the resultant variation in queen quality. Insectes Sociaux 46, 372-377.

Hatjina, F., Bieńkowska, M., Charistos, L., Chlebo, R., Costa, C., Dražić, M. M., Filipi, J., Gregorc, A., Ivanova, F. N., Kezić, N., Kopernicky, J., Kryger, P.,Lodesani, M., Lokar, V., Mladenovic, M., Panasiuk, P., Petrov, P. P., Rašic, S., Skerl, M. I. S., Vejsnæs, F., Wilde, J. 2014. A review of methods used in some European countries for assessing the quality of honey bee queens through their physical characters and the performance of their colonies. Journal of Apicultural Research 53, 337-363.

Jeffree, EP. 1958. A shaped wire grid for estimating quantities of brood and pollen in combs. Bee World 58, 105-110.

Junus, M. 2019. The influence of queen bee age, the number of brood combs, and the use of a queen excluder on comb brood size in Apis mellifera bees during the blossom season. Bulgarian Journal of Agricultural Science 25, 1271–1276.

Kahya, Y., Gençer, H.V., Woyke, J. 2008. Weight at emergence of honey bee (Apis mellifera caucasica) queens and its effect on live weights at the pre and post mating periods. Journal of Apicultural Research 47, 118-125.

Kamel, SM., Osman, MAM., Mahmoud, MF., Mohamed, KM., Allah, SA. 2013. Morphometric study of newly emerged unmated queens of honey bee *Apis mellifera* L. in Ismailia Governorate, Egypt. Arthropds 2, 80-88.

Koç, AU., Karacaoğlu, M. 2011. Effects of queen rearing period on reproductive features of Italian (*Apis mellifera ligustica*), Caucasian (*Apis mellifera caucasica*), and Aegean ecotype of Anatolian honey bee (*Apis mellifera anatoliaca*) queens. Turkish Journal of Veterinary and Animal 35, 271-276.

Kumar, N. 2018. Evaluation of larval grafted queen and natural reared queen of Italian honey bees (*Apis mellifera* L.). Journal of Pharmacognosy and Phytochemistry 1, 3181-3183.

Mahbobi, A., Farshineh-Adl, M., Woyke, J., Abbasi, S. 2012. Effects of the age of grafted larvae and the effects of supplemental feeding on some morphological characteristics of Iranian queen honey bees (*Apis mellifera meda* Skorikov, 1929). Apiculture Science 56, 93-98.

Mahbobi, A., Woyke, J., Abbasi, S., Farshineh-Adl, M., Malakzadegan, A. 2014. The effects of age of grafted larvae and of supplemental feeding on performance of Iranian honey bee colonies (*Apis mellifera meda*). Journal of Apicultural Science 58, 113.

Mattiello, S., Rizzi, R., Cattaneo, M., Martino, PA., Mortarino, M. 2022. Effect of queen cell size on morphometric characteristics of queen honey bees (*Apis mellifera ligustica*). Italian Journal of Animal Science 21, 532-538.

Medina, LM., Goncalves, LS. 2001. Effect of weight at emergence of Africanized (*Apis mellifera* L.) virgin queens on their acceptance and beginning of oviposition. American Bee Journal 141, 213-215.

Moritz, RFA., Kuhnert, M. 1984. Seasonal effects of artificial insemination of honey bee queens (*Apis mellifera* L.). Apidologie 15, 223-231.

Okuyan, S., Akyol, E. 2018. The Effects of age and number of grafted larvae on some physical characteristics of queen bees and acceptance rate of queen bee cell. Turkish Journal of Food and Agriculture Sciences 6, 1556-1561.

Önk, K., Cengiz, MM., Yazici, K., Kirmizibayrak, T. 2016. Effects of rearing periods on some reproductive characteristics of Caucasian (*Apis mellifera caucasica*) Queen Bees. Atatürk Üniversitesi Veteriner Bilimleri Dergisi 11(3), 259-266.

Rafique, MK., Mahmood, R., Qadir, ZA., Farid Asifshaheen, IB. 2019. Effects of rearing interlude and grafting technique on honeybee Apis mellifera L. queen under field conditions. Pakistan Journal of Zoology 51, 2369-2372.

Ruttner, F., Tassencourt, L., Louveaux, J. 1978. Biometrical-statistical analysis of the geographic variability of Apis mellifera LI Material and methods. Apidologie 9, 363-381.

Sagili, RR., Metz, BN., Lucas, HM., Chakrabarti, P., Breece, CR. 2018. Honey bees consider larval nutritional status rather than genetic relatedness when selecting larvae for emergency queen rearing. Scientific Reports 8, 7679.

Schlüns, H., Moritz, RFA., Neumann, P., Kryger, P., Koeniger, G. 2005. Multiple nuptial flights, sperm transfer and the evolution of extreme polyandry in honeybee queens. Animal Behaviour 70, 125-131.

Staron, M., Sabo, R., Staroňová, D., Sabová, L., Abou-Shaara, HF. 2019. The age of honey bee larvae at grafting can affect survival during larval tests. Environmental and Experimental Biology 17: 1–4.

Tarpy, DR., Gilley, DC., Seeley, TD. 2004. Levels of selection in a social insect: a review of conflict and cooperation during honey bee (*Apis mellifera*) queen replacement. Behavioral Ecology and Sociobiology 55, 513-523.

Tofilski, A., Czekonska, K. 2004. Emergency queen rearing in honeybee colonies with brood of known age. Apidologie 35: 275-282.

Wilkinson, D., and Brown, MA. 2002. Rearing queen honey bees in a queenright colony. American Bee Journal 142, 270-274.

Woyke, J., Jasinski, Z. 1990. Effect of the number of attendant worker bees on the initiation of egg laying by instrumentally inseminated

queens kept in small nuclei. Journal of Apicultural Research 29, 101-106.

Wu, X., Zhou, L., Zou, C., Zeng, Z. 2018. Effects of queen cell size and caging days of mother queen on rearing young honey bee queens *Apis mellifera* L. Journal of Apicultural Science 62, 215-222.

Zawislak, J., Burns, D. 2012. Raising quality queen bees (MP518). University of Arkansas Division of Agriculture. Little Rock, AR. Retrieved from: http://www.uaex.edu/publications/pdf/mp518.pdf.

Zheng, HQ., Hu, FL., Dietemann, V. 2011. Changes in composition of royal jelly harvested at different times: consequences for quality standards. Apidologie 42(1), 39-47.

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ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

INVESTIGATION OF HONEY TYPES (CHASTE BERRY, CHESTNUT, LAVENDER, JERUSALEM THORN, ACACIA AND SUNFLOWER) FOR SPECIFIC MACRO AND MICRO ELEMENTS WITH HEAVY METAL POLLUTION

Bal Çeşitlerinin (Hayıt, Kestane, Lavanta, Karaçalı, Akasya ve Çiçek) Belirli Mikro ve Makro Elementler ile Ağır Metal Kirliliği Bakımından İncelenmesi

Metin GULDAS

Department of Nutrition and Dietetics, Faculty of Health Sciences, Bursa Uludağ University, Gorukle Campus, 16059 Nilufer, Bursa, TÜRKİYE, ORCID No: 0000-0002-5187-9380, E-posta: mguldas@uludag.edu.tr

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ABSTRACT

In this research, heavy metal contents (Al, As, Pb and Cd) of 6 honey samples obtained from Marmara and Aegean regions of Turkiye (chaste berry, chestnut, jerusalem torn and sunflower kind of honeys) and 4 honey samples obtained from Bulgaria (lavender, acacia and sunflower kind of honeys) with micro and macro element contents including Ba, Cr, Co, Ni, Fe, Cu, Zn, Mn, Mg, P, B, Na, K, Sr, S and Ca were analyzed by ICP-OES (Inductively Coupled Optical Emission Spectrometer). It was found that the heavy metal contents (Al, As, Cd and Pb) in the investigated honey samples were below the toxic limit values specified by the World Health Organisation and the Turkish Food Codex. In general, the mineral contents of honey samples vary according to the regions where they were taken. Among the honey samples taken from different regions; the contents of Pb, Al, As, Cr, Cu, Ba, Sr, Zn, B, Ca, K, Na, P and S changed at 1% significance level, while Mn, Ni and Fe contents differ at 5% level of significance. It was determined that as the apiary locations from which honey samples were taken approached the urban areas, the Pb content increased statistically by 1%, while the As and Co content increased at the 5% level of significance.

Keywords: Honey, Heavy Metals, Micro Element, Macro Element, ICP-OES

ÖZ

Araştırmada Türkiye Marmara ve Ege bölgelerinden temin edilen 6 bal örneği (hayıt, kestane, karaçalı ve çiçek balları) ile Bulgaristan'dan temin edilen 4 bal örneği (lavanta, akasya ve çiçek balları) ağır metal içerikleri (Al, As, Pb ve Cd) başta olmak üzere; Ba, Cr, Co, Ni, Fe, Cu, Zn, Mn, Mg, P, B, Na, K, Sr, S ve Ca gibi mikro ve makro element içerikleri ICP-OES (İndüktif Eşleşmiş Optik Emisyon Spektrometresi) ile analiz edilmiştir. İncelenen bal örneklerindeki ağır metal düzeylerinin (Al, As, Cd ve Pb) WHO ve Türk Gıda Kodeksi' nde belirtilen toksik limit değerlerinin altında olduğu tespit edilmiştir. Genel olarak, bal örneklerinin mineral içerikleri alındıkları bölgelere göre değişmektedir. Farklı bölgelerden alınan bal örnekleri arasında Pb, Al, As, Cr, Cu, Ba, Sr, Zn, B, Ca, K, Na, P ve S içerikleri %1 önem düzeyinde; Mn, Ni ve Fe içerikleri ise %5 önem düzeyinde farklılık göstermektedir. İstatistiksel analizler sonucunda; bal örneklerinin alındığı arılıklar yerleşim yerine yaklaştıkça Pb %1, As ve Co içerikleri ise %5 önem düzeyinde artış gösterdiği ortaya çıkmıştır.

Anahtar Kelimeler: Bal, Ağır Metaller, Mikro Element, Makro Element, Mineraller, ICP-OES

GENİŞLETİLMİŞ ÖZET

Amaç: Bal, arı sütü, polen ve propolis gibi arı ürünlerinin sağlıklı yaşamı sürdürme ve hastalıklara karşı vücut bağışıklığını güçlendirme gibi insan sağlığı açısından önem taşıyan pek çok faydası vardır. Bu arı ürünleri içerdiği vitaminler, mineraller, enzimler, fenolik bileşenler ve diğer biyoaktif maddelerden dolayı antibakteriyel, antimikrobiyal, antiviral ve antiparaziter gibi birçok sağlık fonksiyonuna sahiptir. Demir, bakır, çinko, kalsiyum, sodyum ve potasyum gibi mineraller balda sıklıkla bulunan ve insan beslenmesi için önem taşımaktadır. Bu faydalı minerallerin yanı sıra bal ve diğer arı ürünleri şehir merkezleri, otoyollar ve endüstriyel kirlilik faktörler nedeniyle insan sağlığına zararlı bazı ağır metaller içerebilmektedir. Çünkü arı kovandaki bal ve diğer ürünlerini 3-4 km çapında bir alandan topladığından, çevresel kirlilik kaynaklarından bazı bulaşanları kovana taşıyabilmektedir. Bu nedenle ballar insan beslenmesine katkı veren besleyici mineraller açısından incelenmesi yanında, gıda güvenliği açısından bölgesel bazda çevre kirliliğinin bir yansıması olan ağır metal içerikleri bakımından da sık sık incelenmelidir. Türkiye ve Bulgaristan arıcılıkta önemli potansiyele sahip iki ülkedir. Her iki ülkenin coğrafi konumu, bitki çeşitliliği, ekolojisi, nektar kaynakları ve koloni varlığı bal üretimi için çok uygundur. Çalışmada, Türkiye'de Ege ve Marmara bölgelerinden 4 farklı bal çeşidine ait 6 ve Bulgaristan'dan temin edilen 3 farklı bal çeşidine ait 4 örnek, 16 farklı mineral (Ba, Cr, Co, Ni, Fe, Cu, Zn, Mn, Mg, P, B, Na, K, Sr, S ve Ca) ve 4 farklı ağır metal (Al, As, Cd ve Pb) incelenmiştir. Bal örneklerinin şehit merkezleri ile otoyolların neden olduğu ağır metal kirlilik düzeylerinden etkilenme durumları ortaya konulmaya çalışılmıştır. Temel analiz yöntemlerinin gelişmesine paralel olarak, son yıllarda besinlerin ağır metal analizlerinde daha güvenilir sonuçların elde edilmesi talep edilmektedir. ICP-OES analiz tekniği, temel araştırmalar için en hassas ve güvenilir bir mineral madde ve ağır metal analizi ölçüm yöntemi olarak kabul edilmektedir. Bu nedenle örneklerin mineral ve ağır metal içeriklerinin incelenmesinde, ICP-OES tekniği kullanılmıştır.

Gereç ve Yöntem: Bu çalışmada Türkiye'nin Marmara Bölgesinde Bursa-Mudanya'nın 4 köyü ile Bursa-Karacabey'in Malkara Köyü kırsal alanından 1 ve Ege Bölgesi'ndeki İzmir Bergama'dan 1 olmak üzere ülke genelinde 6 örnek incelenmiştir. Bulgaristan'ın ise Oblast ili Şumnu, Benkovski, Kayaloba ve Dobromirtsi şehirleri kırsal alanlarından 4 bal örneği temin edilmiş ve incelenmiştir (Tablo 1). Bu bölgelerin bal üretimi için önemli olduğu bilinmektedir. Çalışma boyunca ağır metaller de dahil olmak üzere 20 mineral analiz edilmiştir. Analiz, Bursa Uludağ Üniversitesi Ziraat Fakültesi Toprak Bilimi Bölümü ve Bitki Besleme Araştırma Laboratuvarı'nda ICP-OES cihazı kullanılarak gerçekleştirilmiştir. Bal örnekleri asitte mikrodalga kullanılarak yakılmış ve seyreltme işleminden sonra analiz edilmiştir. Kalibrasyon testleri sonrasında ICP-OES cihazı analize uygun hale getirilmiş ve numuneler standart bir eğri kullanılarak hesaplanmıştır.

Sonuçlar ve Tartışma: Türkiye'den alınan bal örnekleri içerisinde en yüksek Al (4,04±0,20 ppm) ve As (0,39± içerikleri T5 ve T2 (3,60±0,24 ppm) bal örneklerinde ölçülmüştür. Bulgaristan'da ise en yüksek Al içeriğine Kayaloba' dan alınan çiçek balında (B3; 2,06±0,18 ppm), As içeriğine ise B4 örneğinde rastlanılmıştır. Türkiye bal örnekleri içerisinde en yüksek kurşun (Pb) içeriğine İzmir Bergama'dan alınan bal örneğinde (2,79± 0,16 ppm) rastlanırken, Bulgaristan bal örnekleri içinde Pb kalıntı miktarı en yüksek B1 örneğinde (0,84±0,01 ppm) tespit edilmiştir. Kadmiyum içeriği en yüksek Türkiye bal örneği olarak T5 örneği (0,36±0,00 ppm) ve en yüksek Bulgaristan bal örneği olarak B3 (0,13±0,16 ppm) bulunmuştur. Analizler sonucunda tüm bal örneklerinde element içeriklerinin kabul edilebilir düzeyde olduğu, toksik ağır metal (As, Al, Pb ve Cd) içeriklerinin toksik düzeyin altında olduğu belirlenmiştir. Özkul ve diğerleri (2018) de belirlediği gibi, şehir merkezi ve yerleşim alanlarına yaklaşıldıkça bal örneklerindeki kirlenme oranının (Pb ve As içerikleri) arttığı ve bu artışların istatistiksel olarak %1 ve %5 düzeylerinde önem taşıdığı tespit edilmiştir. Araştırma sonunda en yüksek kurşun içeriğine sahip olan hayıt balının bile günlük tüketilebilecek maksimum bal miktarı ile orantılandığında FDA'nın güvenli limit değeri altında kaldığı belirlenmiştir.

Diğer yandan balların besin değeri ve mineral zenginliği bakımından değerlendirildiğinde (Tablo 2); tüm bal örneklerinin kemik sağlığı açısından önemli bir mineral olan Ca içeriklerinin yüksek olduğu ve 67,44±2,21 ile 198,70±1,44 ppm arsında değiştiği bulunmuştur. Balların kansızlık açısından önemli bir element olan Fe içeriği bakımından da zengin bir kaynak olduğu ve bu element içeriğinin ballarda (B1) 29,13±0,36 ppm'e varan düzeylere kadar çıkabildiği belirlenmiştir. Yine bir koenzim faktörü olarak vücutta görev yapan Zn; Türkiye ballarında (T5)

2,94±0,02, Bulgaristan ballarında (B3) ise 3,79±0,01 ppm düzeylerinde ölçülmüş olup balın zengin bir mineral madde kaynağı olarak beslenmede rol oynadığı ortaya konulmuştur.

INTRODUCTION

In recent years, the impact of rapid industrialization on nature in terms of environmental pollution increased significantly in our country parallel to the World. Industrial plants in business enterprises such as paint, automotive and plastic cannot completely prevent toxic and harmful chemicals from production, although waste treatment units are mandatory, toxic substances continue to spread to the environment through water and air (Tchounwou et al. 2012). Bees are accepted as indicator creatures due to their interaction with nature in maintaining the balance in nature. They are living organisms that are indispensable in ensuring the sustainability of plant production by pollination (Sıralı and Cinbirtoğlu 2018). Environmental problems such as heavy metal pollution in nature pose a risk not only to human health and nutrition but also to bee life and health, elevating the risk of pollution of the products such as honey, pollen and royal jelly produced by bees.

Beekeeping is a socio-economic activity that uses plant resources, bees, technical knowledge, and labor to produce various bee products. (Burucu ve Gülse Bal 2018). The orientation towards providing the nutrients and energy elements needed by the body from natural sources with the understanding of healthy life has led to the development of beekeeping activities and to a significant increase in the value of these products. Bee products such as honey, royal jelly, pollen and propolis have great benefits in terms of maintaining a healthy life, preventing certain diseases and strengthening the immune system. These products have antibacterial, antimicrobial, antiviral and antiparasitic health functions due to their antioxidant elements such as vitamins, minerals, enzymes, phenolic components and their bioactive substances. In addition to all these benefits, beekeeping is important in agricultural activities due to the reasons such as earning income in a short time, it can be done with little capital and it does not require a lot of land area (Tepge 2021).

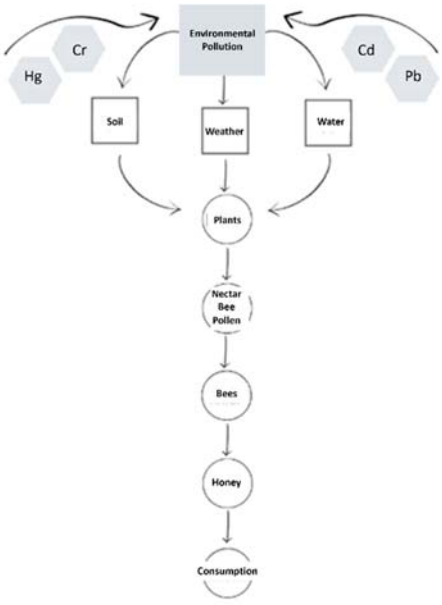
Turkiye has significant potential in beekeeping and is very favorable for honey production due to its geographical location, plant diversity, ecology, nectar reserves and the existence of colonies or hives (Borum 2014). Approximately 5.9% of the honey obtained in the world in 2019 was produced in Turkiye. This brings Turkiye to second place in the world in honey production (Güler 2021). Projection studies show that honey production will increase even more in Turkiye. According to the data of 2021, while around 97 thousand tons of honey was produced in Turkiye (TUIK 2021), honey production is expected to be between 121 thousand and 125 thousand tons in 2023 (Burucu ve Gülse Bal 2017).

Due to the rapid increase in the world population, industrialization and agricultural production activities recently, heavy metal pollution in soil and nature has reached serious levels (Mikhailenko et al. 2020). Soil is an important medium in which plants grow and acts as filtration for pollutants. Since there is an exchange of substances and energy between the three main ecosystems in nature (water, air and soil), the pollutant is able to transport from one ecosystem to another (Figure 1). As a result of the accumulation of heavy metals in the soil, these can be transported to humans through these plants and can play a role as significant risk factors responsible for some chronic diseases (Kara and Kara 2018). The increase in the heavy metal concentration in Turkey's natural resources with each passing day keeps this issue on the agenda and leads to an increase in the number of studies on this subject (Sönmez and Kılıç 2021). In a study conducted in the province of Istanbul, soil samples were examined from 40 different locations and the highest pollution values were determined in the unwashed leaf samples taken from the roadside as 14.90 ± 2.96 µg/g for Pb, 0.65 ± 0.13 µg/g for Cd, 19.94 ± 1.17 µg/g for Cu and 42.53 ± 3.08 µg/g for Zn (Ozturk et al. 2017). It has been determined that there is a linear relationship between heavy metal accumulation, traffic density and proximity to the roadside. In a study carried out in the Altıntaş plain, located in the south of Kütahya, a region where agricultural activities are intense, the heavy metal contents were analyzed by ICP-MS by sampling from 15 points of agricultural lands. (Özkul 2018). As a result of the analysis of soil samples taken from the study area, while the pollution was moderate for Cu, Pb, Sb and Zn heavy metals, Hg, As and Ni were found as the heavy metals that cause the most pollution, respectively. In another study, the heavy metal contents of the soils in the playgrounds in the city center of Kütahya have investigated, and found

significant soil contamination in terms of heavy metal contents of As, Cd, Hg, Ni, Pb, and Zn (Özkul 2019). Among these, it has been observed that the contamination rate of heavy metals, especially As and Cd, has reached serious levels. Pollution caused by heavy metals is easily able to transport between the ecosystems through the food chain. In this respect, heavy metals can be transmitted to humans through many pathways including food production, drinking water, inhalation and skin contact (Figure 1). Pollution sources occurring in the ecosystem causes serious negative effects if it is taken into the metabolism of the living organisms (Vareda et al. 2016). Heavy metals mixed with the soil can be transported to the human body either directly through plants or indirectly through animal meats and products that consume plants (Kara and Kara 2018).

Figure 1: Pollution caused by heavy metals.

Şekil 1: Ağır metallerin sebep olduğu kirlilik.



In Turkiye, which has a wide and diverse flora potential and geography where flowering continues at all times of the year; beekeeping is an agricultural business area that can be done almost anywhere from sea level to high plateaus (Tepge 2021). The high quality of honey in our country is based on the abundance of nectar-producing plant varieties in different climatic conditions and different periods. For these reasons, an important part of the honey produced from these regions is a mixture, that is, multi-floral. In addition, herbal kinds of honey specific to a local plant flora, which are unique to certain regions in Turkiye, can also be produced. **Chasteberry honey**, one of these kind of honey, is one of the only plant kind of honey that is produced intensively in the Aegean and Marmara Regions and consumed in common. Chasteberry (Vitex agnus-castus) is an herb, also known as priest pepper or five-finger plant. The chasteberry plant is a good source of nectar for honey bees and has an estrogenic effect and hormone-balancing characteristics (Uçak Koç et al. 2017).

Jerusalem thorn honey is produced mostly in Marmara and Thrace regions in Turkiye (Malkoç et al. 2019). *Paliurus spina-christi*, known as Jerusalem thorn, is a shrub plant belonging to the *Miller Rhamnaceae* family. The flowers of the Jerusalem thorn plant turn yellow in May and July depending on the weather conditions; medium sweet and slightly bitter honeys that can crystallize very quickly are obtained. Jerusalem thorn herb has traditionally been used for the treatment of diuretic, antirheumatic, hypocholesterolemic, and chronic obstructive pulmonary disease (Şen 2018, Zor et al. 2017).

Lavender honey is among the most admired high-quality honeys for its pleasant aroma and taste. Lavender is a popular aromatic Mediterranean plant belonging to the *Lamiaceae* family (Castro-Vázquez et al. 2014).

Chestnut honey is honey obtained from the extracts collected by the bees from the flowers of the chestnut trees in a certain period. Since chestnut trees (*Castanea sativa*) begin to bloom in June, this honey made by bees that work intensively during this period is produced less frequently in our country, so its economic value is higher than the many other honey varieties (Alkan 2020).

Acacia honey (*Robinia pseudocacia*), with its light yellow appearance, delicate scent and floral aroma, is one of the most popular honey varieties available in the European market (Oroian et al. 2015, Schievano et al. 2019). In order for a honey sample to be classified as real acacia honey, it must contain at least 45% granular acacia pollen as specified in the regulation on honey quality (Soares et al. 2017).

Sunflower honey (*Helianthus annuus*) is one of the most produced monofloral (single flower) honey varieties in Turkiye and it is the most exported honey in the country after pine honey. It is generally used by beekeepers as winter food or bee feed. Sunflower

blooms in July and is harvested in August. Sunflower honey is honey having golden yellow in color, has a unique taste, and crystallizes very quickly. The quick crystallization of this honey is due to its high glucose and pollen content. It looks like a yellow candle when it crystallizes (Sen 2019).

Chasteberry, chestnut and Jerusalem thorn are plants preferred by bees, which naturally reproduce in forest areas far from city centers for honey production. Lavender, on the other hand, is an aromatic plant that is extensively planted as an important raw material for the perfume and cosmetics industry, generally in the fields relatively close to cities and industrial pollution sources compared to other forest plants examined in the research. These plants, which exist both in forest areas and in areas close to cities, are among the plants used extensively by honey bees (*Apis mellifica*) to create unmatured liquid honey. Residue levels in the honey produced by bees collecting liquid honey extracts and pollen from these plants may vary depending on the distance from the environmental pollution sources of the plants where the bee collects the nectar and the area where the beehive is located.

Honey, which is loved and consumed by all age groups, is a functional food that is easy to digest and has a curative or protective effect against some diseases. Honey contains elements such as potassium, sodium, phosphorus, magnesium, sulfur, manganese, chlorine and iron, which are important for human health and nutrition. According to the Turkish Food Codex Honey Communiqué, honey; is defined as a natural bee product created by the collection of plant nectars, the secretions of living parts of plants, or the secretions of plant-sucking insects living on the living parts of plants by honey bees (TGK 2020). For this reason, honey is one of the natural food products whose source is nature and therefore can be affected by environmental pollution sources such as heavy metal pollution in nature.

In the composition of honey; there are main nutritional components such as sugars (31% glucose, 38% fructose), vitamins (ascorbic acid, etc.), moisture (10-20%), elements (potassium, sodium, calcium, magnesium, phosphorus and organic acids (gluconic acid, acetic acid, etc.). The amounts of major and trace elements in honey vary according to the element contents of the sources (soil and vegetation) from which the bee collects the nectar. The elements are generally transported to the honey through the nectars and nectars collected by the bees from the plants through the roots of the flowering plants (Lanjwani and Channa 2019). Apart from environmental pollution, the amount of heavy metals and minerals varies according to the geographical and flora characteristics of honey. Heavy metals are elements with a density higher than 5 g/cm3 in terms of physical properties in three or higher periods in the periodic table. More than 60 metals are in this group, including lead, cadmium, chromium, iron, cobalt, copper, nickel, mercury and zinc. The most common heavy metals determined in polluted soils are Cr, Pb, Cu, Cd, Zn, Hg and As, respectively (Khalid et al. 2017).

In many studies on the element contents in honey, macro elements such as Na, K, Cl, Ca, P, S and Mg, and micro elements such as Al, Mn, Pb, Cd, Cu, Tl, Co, Zn, Rb, Ni, Ba, Bi, Pt, Be, V, Pd, Te, Fe, Mo, Hf, Sb, Sn, U, La, Sm, I, Tb, Th, Dy, Sd, Nd, Pr, Lu, Gd, Yb, Er, Ho, Cr, B, As, Br, Ce, Cd, Se, Sr and Hg have been investigated in detail (Lanjwani and Channa 2019). However, since environmental pollution is a rapidly increasing problem both in our country parallel to the World, it is necessary to frequently examine and evaluate foods such as honey, which is a product obtained from nature, in terms of pesticide and heavy metals which have serious toxic effect on human health.

Bees take heavy metals from plants and water sources on the soil surface while collecting nectar. These heavy metals are transmitted to plants from the soil, pesticides, chemicals and industrial wastes in the region. Although the typical food collection area of the honey bee varies according to the distance of the food and water sources around the hive; most of the time it is collected within a radius of 600-800 of an area. However, they can often fly distances of 2 km to gather food and can even follow a flight route of up to 5 km. It is stated that in cases of food and water deprivation, bees can fly up to a maximum distance of 13 km in order to find food sources and under experimental conditions (Pahl et al. 2011). Metals can contaminate honey by a mechanism defined as migration, not only from the environment and nature but also from the metal surfaces, metal-based containers and cauldrons and equipment used in the production steps (Özcan and AL Juhaimi 2012). Environmental pollution, on the other hand, is a global problem of today, and its effects are increasing day by day and reaching a dangerous dimension. Among the main factors

polluting nature, heavy metals, various pesticides, organic compounds, radioactive substances and hydrocarbon combustion products at first (Doelsch et al. 2006). Due to food safety concerns, studies on the determination of heavy metal content in honey have increased in recent years. It is stated that if these toxic substances are taken by the organism above a certain level, they can harm the metabolism and the body (Bengü and Kutlu 2020).

There is no separate classification regarding the heavy metal limit values in honey. There are regulations published by the World Health Organization (WHO 2007), European Union (EC 2006), The Food and Drug Administration (FDA 2021) regarding heavy metal residue limits in foods. In Turkiye, heavy metal limit values are considered as toxic residue values in foods like in other countries, and there is no separate legal classification for heavy metal contents only in honey (Resmi Gazete 2011).

In this study, 16 different micro and macro elements and 4 heavy metals were tried to be measured in the honey samples (chasteberry, chestnut, jerusalemthorn, and sunflower honeys) obtained from two regions of Turkiye and the honeys (lavender, acacia, and sunflower honeys) from four settlements of Bulgaria. With statistical methods, it has been tried to reveal the level of being affected by environmental pollution according to the distance of the honey to the pollution sources that cause environmental pollution such as city centers and highways.

MATERIALS AND METHODS

Collection of Samples

In the study, 10 honey samples produced in Turkiye and Bulgaria were used. For this purpose, 6 honey samples belonging to the Marmara and Aegean regions of Turkiye and 4 honey samples belonging to 4 settlements of Bulgaria were examined in terms of heavy metals (4) and micro and macro elements (16) (Table 1 and Figure 2). Honey samples without honeycombs (500 g) were brought to the laboratory in clean glass jars, labeled and stored at 4-8 °C until analysis.

Figure 2. Honey sampling locations on the map

Şekil 2. Bal örneklerinin alındıkları bölgeler

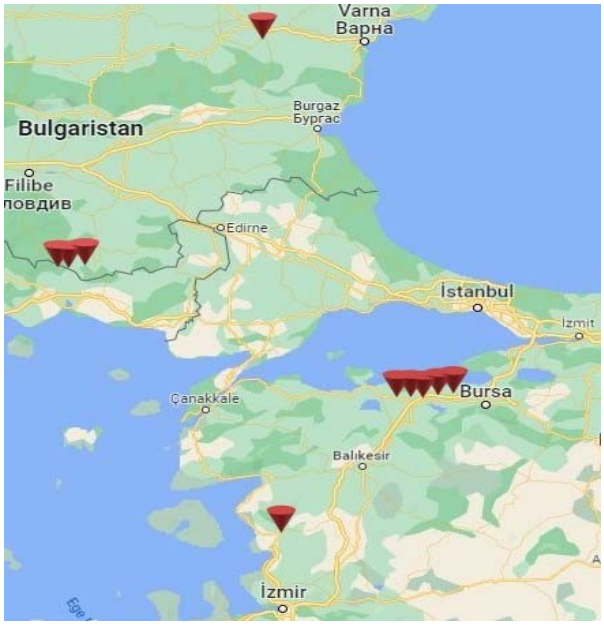


Table 1. Geographical coordinates and regions for honey sampling

Tablo 1. Bal örneklerinin alındıkları bölgeler ve coğrafi koordinatları

Sample Code	Honey Variety	Geographical Position	Coordinates
T1	Chasteberry	Turkiye/Izmir/Bergama	39.29774,27.28536
T2	Chestnut	Turkiye/Bursa/Malkara/Karacabey	40.31248,28.34772
T3	Jerusalemthorn	Turkiye/Bursa/Mudanya/Sogutpinar	40.46111,28.62627
T4	Jerusalemthorn	Turkiye/Bursa/ Mudanya/Mesudiye	40.57991,28.58261
T5	Jerusalemthorn	Turkiye/Bursa/ Mudanya/Camlık	40.45518,28.56538
T6	Sunflower	Turkiye/Bursa/Mudanya/Evciler	40.55469,28.49159
B1	Lavander	Bulgaria/Sumnu	43.45616,26.90330
B2	Acacia	Bulgaria/Benkovski	41.60622,25.22514
B3	Sunflower	Bulgaria/Kayaloba	41.52410,25.17348
B4	Sunflower	Bulgaria/Dobromirtsi	41.46227,25.15832

Sample Preparation and ICP-OES Method

Approximately 1 g of honey samples were weighed and burned with 4 ml of 37% HCl (Honeywell, Germany) and 3 ml of 65% HNO_3 (Merck, Darmstadt, Germany) using a Multiwave 5000 (Anton Paar, Austria) microwave device. The samples were completed with ultrapure water to a final volume of 50 ml. The samples were burned using HNO_3 and H_2O_2 in a Berghof MWS-2 model microwave oven. In the extracts obtained, 4 heavy metal types (Al, As, Pb and Cd) and micro and macro elements (Ba, Cr, Co, Ni, Fe, Cu, Zn, Mn, Mg, P, B, Na, K, Sr, S and Ca) were determined with Perkin Elmer OPTIMA 2100DV model ICP OES apparatus. Measurement conditions applied in the device are given in Table 2.

Table 2. The Measurement Conditions Of ICP-OES

Tablo 2. ICP OES ölçüm koşulları

Reding Time	5 (s)
Rf Power	1.20 (kW)
Stabilization Time	15 (s)

Chemical Analysis

During heavy metal and other element analyses, appropriate calibration standards were used at periodic intervals to maintain measurement accuracy between samples and to minimize productivity loss. For this purpose, the accuracy of the ICP-OES data was checked using blank reagents and calibration standards prepared with standard solutions (Agilent Technologies Co.). Calibrations were generally applied in the range of 0-0.1 ppm for micro elements and 5-80 ppm for macro elements.

Statistical Analysis

Whether the heavy metal contents of honey samples showed normal distribution was analyzed by Kolmogorov-Smirnov test. As a result of the analysis; It was determined that Pb, Al, Cr, Cu, Ba, Zn, B, Ca, Fe, K, Na and S elements showed normal distribution, while As, Cd, Co, Mn, Ni, Sr, Mg and P elements did not show normal distribution. Whether there is a difference between the mean element levels of honeys was analyzed by t-test.

At the same time, whether there is a relationship between heavy metal levels of honey and their distance from settlements and highways was examined with the help of Pearson correlation tests for elements with normal distribution and Spearman correlation tests for elements that do not show normal distribution.

RESULTS

The contents of the heavy metals with micro and macro elements determined in the honey samples are given in Table 3.

Toxic Heavy Metal Contents (Pb, Cd, Al and As) of the Honey Samples

Balların Toksik Ağır Metal (Pb, Cd, Al ve As) İçerikleri

Toxic heavy metal (Pb, Cd, Al and As) contents of the honeys are given in Table 3.

Lead (Pb) is an element with atomic number 82 in the periodic table and has very important in terms of its toxicity and food safety (Bengü and Kutlu, 2020). In the analyzes made, the highest lead content was found in chasteberry honey (T1), which is one of the test samples, with a level of 2.79 ppm. The lead content in other honey samples varies between 0.03-1.70 ppm (Table 3). The sources of lead pollution in nature are usually lead-containing industrial wastes and gasoline-powered car emissions. The maximum amount of lead that can be taken from food is stated by the US Food and Drug Administration (FDA) as 12.5 micrograms per day (FDA 2022). Considering the average daily honey consumption in Turkiye, this limit corresponds to 4.1 ppm lead content that can be taken with honey. When evaluated in terms of honey samples examined in our research; even the chasteberry honey, which has the highest lead content, has a content far below the lead level that can be safely consumed daily.

Cadmium (Cd) is a soft metal known for its bluish color and is a metal that can slowly oxidize due to moisture in the air. The cadmium mineral with atomic number 48 is less than 0.01% in the earth's crust (Bengü and Kutlu 2020). Long-term intake of cadmium causes accumulation in the human organism, especially in the liver and kidney. When cadmium accumulates in the body and its level in the renal cortex reaches 0.2-0.3 mg/g, it causes damage to the tubules. The tolerable weekly dose for an adult (70 kg) is considered to be 0.49 mg of cadmium (Wang et al. 2012). The amount of cadmium in all honey varieties examined in the study varied between 0.01-0.36 ppm (mg/kg), and no significant Cd presence that could cause toxicity in the human organism was found.

Alüminyum (Al) is the third most common element in the world (Rafati Rahimzadeh et al. 2022) and 50-150 mg of aluminum is found in the human body. It is stated that the level of aluminum is higher in aging organisms. The daily intake of aluminum to the body is at the level of 2-10 mg, and the dose that can be absorbed by the gastrointestinal tract is very small, that is, in trace amounts (Wang et al. 2012). It is stated that as a result of environmental pollution, aluminum can pose a great threat to humans, animals and plants. Aluminum, which accumulates in an overdose in the body, can cause damage by increasing oxidative stress in the brain, liver and kidney. This damage can occur in the form of disruption of the mechanism of action of some enzymes, protein synthesis, utilization of nucleic acids and dysfunctioning of cell membrane permeability in the body. It is stated that they can affect triglyceride levels in plasma and fat metabolism in the body (Bengü and Kutlu 2020, Rafati Rahimzadeh et al. 2022). Al was found at the highest level of 4.04 ppm in jerusalemthorn honey (T5) and 3.60 ppm in chestnut honey (T2) in the honey samples examined, and it ranged between 0.06-1.83 ppm in other honey samples.

Table 3. The Contents of Heavy Metals, Micro and Macro Elements in the Honey Samples (ppm)

Çizelge 3. Bal Örneklerindeki Ağır Metal, Mikro ve Makro Element İçerikleri (ppm)

SAMPLES[1]	MINERALS																		
	Al	As	Pb	Cd	Co	Cr	Cu	Mn	Ni	Ba	Sr	Zn	B	Ca	Fe	K	Mg	Na	P	S
T1	LOD[2]	LOD	2,79 ±0,16	0,02 ±0,03	LOD	0,10 ±0,00	0,20 ±0,00	0,27 ±0,03	0,07 ±0,00	LOD	0,12 ±0,01	0,44 ±0,06	6,69 ±2,36	81,87 ±4,13	0,40± 0,05	386,04 ±2,20	15,84 ±0,53	77,27 ±3,24	236,11 ±19,33	21,46 ±0,92
T2	3,60 ±0,24	0,08 ±0,00	1,70 ±0,5	0,01 ±0,00	LOD	0,12 ±0,01	0,59 ±0,03	2,22 ±0,10	0,11 ±0,01	0,18 ±0,04	0,21 ±0,02	1,17 ±0,08	5,16 ±1,04	131,27±3,30	8,42± 0,22	2697,75±0,02	41,00 ±0,75	93,23 ±1,74	294,96 ±9,26	56,86 ±0,30
T3	0,18 ±0,02	0,06 ±0,00	1,67 ±0,13	0,02 ±0,00	LOD	0,09 ±0,00	0,32 ±0,03	0,44 ±0,01	0,15 ±0,01	0,05 ±0,01	0,15 ±0,01	0,76 ±0,11	3,38 ±0,11	67,44 ±2,21	0,70± 0,19	2610,03±82,97	26,40 ±0,23	76,15 ±2,62	221,88 ±27,00	43,76 ±2,19
T4	1,62 ±0,08	0,10 ± 0,08	0,61 ± 0,04	0,03 ± 0,01	0,02 ± 0,02	0,04 ± 0,01	0,35 ± 0,02	0,64 ± 0,00	0,9 ± 0,01	0,18 ± 0,02	0,22 ± 0,00	1,35 ± 0,01	3,26 ±0,55	105,25 ± 0,31	1,43± 0,31	1102,89 ± 5,79	26,93 ± 0,1	54,24 ± 0,29	205,77 ±6,88	32,66 ±1,19
T5	4,04 ±0,20	0,39 ± 0,07	0,23 ± 0,12	0,36 ± 0,01	0,02 ± 0,00	0,04 ± 0,01	0,86 ± 0,16	0,50 ± 0,00	0,28 ± 0,05	0,24 ± 0,01	0,63 ± 0,01	2,94 ± 0,02	3,29 ±0,25	192,08 ± 0,57	1,18± 0,16	1196,09 ± 4,24	68 ±0,18	111,46 ± 0,32	2,30±0,14	27,95 ±1,43
T6	1,29 ± 0,07	0,11 ± 0,01	0,44 ± 0,13	0,07 ± 0,00	0,06 ± 0,00	0,04 ± 0,00	0,29 ± 0,01	0,38 ± 0,00	0,14 ± 0,02	0,165 ± 0,02	0,12 ± 0,00	1,30 ± 0,02	4,42 ±0,19	76,6± 0,37	1,51± 0,39	203,33 ± 1,08	24,89 ±2,37	57,19 ±3,61	205,49 ±3,06	41,86 ±1,50
B1	0,06 ±0,02	0,08 ±0,01	0,84 ±0,01	0,02 ±0,00	LOD	0,10 ±0,00	0,22 ±0,01	0,48 ±0,02	0,04 ±0,00	0,01 ±0,01	0,15 ±0,03	1,69 ±0,16	8,01 ±0,99	132,86±3,54	29,13 ±0,36	966,10 ±14,87	22,33 ±0,48	63,30 ±2,60	205,93 ±10,87	23,90 ±1,02
B2	1,83 ± 0,09	0,12 ± 0,02	0,41 ± 0,14	0,02 ± 0,00	0,02 ± 0,00	0,04 ± 0,01	0,46 ± 0,05	0,36 ± 0,07	0,15 ± 0,03	0,18 ± 0,00	0,18 ± 0,00	2,52 ± 0,01	5,61 ±0,95	131,54 ± 0,19	10,76 ±0,64	589,04 ± 1,20	27,07 ± 0,07	45,56 ± 0,07	148,82 ±28,49	26,04 ±2,54
B3	2,06 ± 0,18	0,14 ± 0,02	0,45 ± 0,09	0,13 ± 0,16	0,37 ± 0,00	0,11 ± 0,02	0,75 ± 0,01	0,32 ± 0,01	0,12 ± 0,04	0,35 ± 0,02	0,18 ± 0,00	3,80 ± 0,01	4,98 ±0,87	198,7 ± 1,43	13,46 ±0,92	594,7 ± 2,24	42,11 ± 0,32	64,46 ± 0,28	210,07 ±7,21	21,75 ±1,53
B4	0,81 ± 0,08	0,15 ± 0,03	0,03 ±0,09	0,05 ± 0,00	0,031 ± 0,01	0,10 ± 0,01	0,17 ± 0,04	0,13 ± 0,00	0,13 ± 0,00	0,17 ± 0,01	0,21 ± 0,00	1,39 ± 0,01	5,81 ±0,86	70,2 ± 0,57	11,02 ±0,33	299,25 ± 1,51	21,68 ± 0,11	41,48 ± 0,15	243,58 ±33,29	33,78 ±2,11

[1] $T_{1,2,3,4,5,6}$ or $B_{1,2,3,4}$: The samples from Turkiye as the "T" and the samples from Bulgaria as the "B" were coded
[2] LOD: Under limit of detection

Arsenic (As) is one of the important risk factors for public health due to its high toxicity level. It can be transmitted to individuals as a result of the direct consumption of food and water contaminated with arsenic or within the scope of the situations covered by the definition of occupational disease in the workplace (Gupta et al. 2017). Arsenic causes neurodegenerative diseases in the body, especially on the nervous system (Mitra et al. 2022). The World Health Organization (WHO) has determined the level of 10 µg/kg as the safe limit for arsenic (Singh et al. 2017). In the study, arsenic was not detected in chasteberry honey (T1), but it was found to be between 0.06-0.39 ppm and at trace level in other honeys. According to these results, it was observed that the honey analyzed in the study was within the safe limits determined by the World Health Organization (WHO) in terms of arsenic content, which has a serious toxic effect on humans.

Contents of Macro and Micro Element (Na, K, Ca, Fe, Cu, Zn, Mn, B, Mg, P, Cr, S, Ba, Ni, Co and Sr) in the Honeys

The macro and micro element contents of honeys (Na, K, Ca, Fe, Cu, Zn, Mn, B, Mg, P, Cr, S, Ba, Ni, Co and Sr) are given in Table 3.

Sodium (Na) was found to be between 41.48-111.46 ppm in the honeys examined and the highest was found in jerusalemthorn honey (T5). When the amount of honey consumed daily and the amount of Na that can be taken into the body are compared, it does not pose any risk. Sodium is a component in the body that regulates the osmotic pressure of the extracellular fluid. It also activates some enzymes such as amylase. The absorption of sodium begins 3 to 6 minutes after ingestion, is quite rapid and is completed within 3 hours. The average daily sodium intake for the body with food is 2.5 g for women and 3.3 g for men. The average requirement for adults is between 1.3–1.6 g/day, which is equivalent to 3.3–4.0 g/day of table salt (NaCl) intake. Too little or too much Na intake into the body can cause serious disorders such as hypertension (Wang et al. 2012).

Potassium (K) is an alkaline and soft metal, compared to the other elements examined, it is an element found in the highest amount in honey. The potassium levels in the honey samples varies between 203.33-2697.75 ppm. Potassium is the basic cationic element in the cell fluid and has an important function in maintaining the acid-base balance. Potassium is an important component of the glycogenesis energy cycle and the regulation of intracellular osmotic pressure in the cell (Bengü and Kutlu 2020). It is an important nutritional element in terms of cell membrane permeability and cofactor functions for some enzymes. The daily amount of potassium taken into the body by diet is 2-5.9 g. The minimum daily requirement is estimated to be around 780-800 mg (Wang et al. 2012). Therefore, when honey samples are considered in terms of nutrition, they can be recommended as one of the important sources in providing the potassium that the body needs for individuals other than diabetics.

Calcium (Ca) is a metallic element of the alkaline earth group. As a result of elemental analysis, when all honey samples are taken into account in the research, it has been determined that it varies between 67.44-198.70 ppm. Calcium is another essential element for healthy nutrition, which plays a role in the regulation of the nervous system and the realization of muscle functions, and is also found as a building block in teeth and bones. In addition to the conversion mechanism of prothrombin to thrombin in blood coagulation; it is a vital element for muscle contraction, nerve conduction and membrane permeability (Bengü and Kutlu 2020).

Iron (Fe) is the most abundant element in the earth's crust, and its total amount in the human body is 4-5 g. Most of the iron in the body is found in the structure of hemoglobin in the blood and myoglobin in the muscle tissue. Iron, which is also found in the structure of enzymes such as hydroxylase, peroxidase, and catalase, is an essential element in terms of maintaining the normal function of the body. For this reason, it is one of the important blood markers sought in determining whether the body maintains a healthy life. Although the iron requirement of the individual varies according to age and gender, it varies between 1.5-2.2 mg/day (Wang et al. 2012). In the study, the highest iron contents were determined as lavender honey (B1) 29.13 ppm and then sunflower honey (B3) 13.46 ppm. It is thought that, among the honeys examined in the research, especially lavender and jerusalem thorn honeys can be used as an important food in the diets of individuals suffering from iron anemia and without a history of diabetes.

Copper (Cu) is an important element that plays a role in iron absorption (Chandra 1990) and its daily requirement is 1-1.5 mg (Wang et al. 2012). The copper content of the honey samples examined in the research ranged between 0.17-0.86 ppm. Although the Cu content of honey is not very high, it

is stated that when taken together with other nutrients in the diet, honey may be one of the food sources that can play a role in meeting the daily Cu requirement.

Zinc (Zn) is an element found in the structure of plant and animal foods and in all living cells. There is a total of 2-4 g of zinc in an adult human body, and the daily requirement of the body is 5-10 mg. This amount can be easily met daily with the foods included in the diet in individuals with a normal diet. Zinc is a cofactor component that plays a key role in maintaining the function of many enzymes such as malate dehydrogenase, alcohol dehydrogenase, lactate dehydrogenase, glutamate dehydrogenase and carboxypeptidase (Wang et al. 2012). Zinc (Zn) was found between 0.44-3.80 ppm in research honeys and is one of the important natural food sources in providing the body's daily zinc requirement in the daily diet.

Manganese (Mn) was found to be the highest in chestnut honey (T2) at 2.22 ppm among the honey types used in the study. It was determined between 0.13-0.64 ppm in the other three honey types (chasteberry, jerusalemthorn and lavender). Manganese is one of the important enzyme cofactors like zinc; it is an essential element for the functioning of decarboxylase, hydrolase and transferase enzymes (Bengü and Kutlu 2020). The amount of manganese in the body is about 10-40 mg in total. The daily manganese requirement of the body is a very small amount, such as 2-5 mg. This requirement can be easily met up to 48 mg per day, depending on the manganese content of the foods consumed in the daily diet. It is stated that even if manganese is taken in excess of the body's requirement, it does not have a toxic effect (Wang et al. 2012).

Boron (B) is an element found between 3.26-8.01 ppm in honey varieties evaluated in the research. Turkiye is a country known for its rich boron reserves (Elevli and Laratte 2022). As can be seen from the boron content of local country honeys such as chasteberry, chestnut and jerusalem thorn honey, which are especially studied, this rich element source is; it is estimated that it is carried to the hive by bees through plants grown in the soil and running water sources. It is stated that the daily boron requirement for the body is 1-2 mg. In addition, it is thought that boron is an element that interacts with calcium, magnesium and vitamin D and has an effect on bone formation (Wang et al. 2012).

Magnesium (Mg), considering all the examined honey varieties, is an element that is detected between 15.84 and 41.00 ppm and can be taken into the body as 300-500 mg in daily nutrition. Magnesium is one of the important enzyme activators like zinc and manganese. It has a function in the stabilization of cell and plasma membranes and nucleic acids. The lack of magnesium in the body can pave the way for important problems in metabolism (Wang et al. 2012).

Phosphorus (P) is an element with a daily requirement of about 0.8–1.2 g. It was found that the phosphorus content of the honey varieties used in the research varied between 205.93 and 294.96 ppm, and chestnut honey had the highest phosphorus content. Phosphorus is present in the structure of bones and teeth, DNA and RNA in the body. It is effective in the bipolarity of membrane lipids and lipoproteins in the bloodstream. Phosphorus also; it is an element involved in many metabolic processes such as energy production (ATP) and conversion in the cell, buffering of blood, regulation of gene transcription, enzyme activation (cofactor), renal system excretion function, immune system activity and signal transmission (Calvo and Lamberg-Allardt 2015).

Chromium (Cr) was found to vary between 0.09-0.12 ppm in honey samples. Chromium, as an enzyme activator (phosphoglucomutase enzyme), is an element that has an effect on increasing the activity of insulin and regulating blood glucose levels. In case of chromium deficiency in the body, glucose tolerance decreases and therefore the risk of cardiovascular disease increases. It is stated that feeding with 25 ppm chromate in experimental mice does not cause any toxic effect (Wang et al. 2012). Considering the maximum amount of honey that can be consumed daily, it is seen that the chromium content in research honeys has a content well below the level that can be toxic.

Sulfur (S) is an element in the macro elements group found in foods. Sulfur is found in the composition of many metabolites that are important for the maintenance of cell structure and the maintenance of biological activities (Bohrer and Takahashi 2016). After calcium and phosphorus, it is the third mineral substance in the body, constituting approximately 0.3% of the human body (Hewlings and Douglas, 2019). It was determined that the sulfur content of the research honeys varied between

21.46-56-86 ppm and was found mostly in chestnut honey.

Barium (Ba) is an element found in the skeletal system (0.5-10 µg/g), teeth (0.1-3 µg/g), heart, lung, kidney and liver in the human body. As a result of excessive Ba intake into the body, especially; health problems such as heart and/or kidney failure, pulmonary edema, respiratory paralysis and bleeding in the stomach and intestinal tract occur. As an orally adult reference dose for barium, 0.2 mg/kg/day has been determined by the U.S. Environmental Protection Agency (Kravchenko et al. 2014). In the study, although barium was not detected in chasteberry honey; the Ba level in chestnut, jerusalemthorn and lavender honeys is between 0.01-0.18 ppm.

In the study, **nickel (Ni)** was found to be 0.07, 0.11, 0.15 and 0.04 ppm at trace levels in chasteberry, chestnut, jerusalem thorn and lavender honeys, respectively. Nickel is an element that is a cofactor for many enzymes and plays a role in increasing insulin activity. Nickel requirement is estimated to be 35-500 µg per day; The amount of Ni that can be taken with the daily diet is at the level of 150-700 µg (Wang et al. 2012).

Strontium (Sr) is a trace element whose function in the human body is not fully understood. In some animal experiments, strontium is thought to be effective in physiological processes such as muscle contraction, blood clotting, and secretion of certain hormones. It is stated that the daily adult dose is about 4 mg (Kołodziejska et al. 2021). In our study, 0.12-0.21 ppm of strontium was found in the honey samples examined (chasteberry, chestnut, jerusalemthorn and lavender), and these levels are far below the daily intake dose.

DISCUSSION

In our study, when the daily honey consumption of people is evaluated in grams of portion, it is seen that the possible heavy metal doses that can be taken into the body with these portions are far below the toxic values. While there are studies on honey that overlap with the results of this research, there are also research results that do not overlap and indicate that heavy metal pollution increases as you get closer to settlements. For example, in 11 different honey samples taken from Bingöl and its surroundings, 18 mineral substances were analyzed (Bengü and Kutlu, 2020) and honey samples were found to be reliable in terms of heavy metal contents, in parallel with this study. But there are also research findings to the contrary. For example, Demirezen et al. (2005) determined that the heavy metal contents of honey samples taken from places close to the residential area were generally higher.

Although the honey used in our study was generally supplied from the Marmara and Aegean regions, where industrialization is intense, the amount of Al contained in some honey samples was even lower than the amounts contained in the honey obtained from Bingöl, which is a city far from industrial and production facilities that may cause environmental pollution. For example, Cd, Zn and Ni concentrations in honey samples taken from Kayseri Erciyes Mountain and its surroundings were determined to be in the range of 0.11-0.18 ppm, 2.2-11 ppm and 0.2-0.8 ppm, respectively (Demirezen and Aksoy, 2005). According to the analysis results, the contents of Cd (0.01-0.02 ppm), Zn (0.44-1.69 ppm) and Ni (0.04-0.15 ppm) in the present study were lower than the honey samples investigated in Kayseri. It is estimated that the difference in the honeys of these two regions, which are not close to the industrial zones, may be due to the metal content and elemental composition in the soil and other natural resources, rather than the pollution caused by the industrial facilities and city centers in nature.

When honey samples were compared in terms of heavy metals and elements examined, the statistical significance level was found 1% between honey samples and their Pb, Al, As, Cr, Cu, Ba, Sr, Zn, B, Ca, K, Na, P and S contents. A significant difference of 5% was found in terms of Mn, Ni and Fe contents. No statistical difference was found between Cd and Co levels.

Based on the coordinates of the apiaries where honey samples were collected, the distance to the highway and the nearest settlement was determined in kilometers. Considering the relationship between the distances of the apiaries from the highway or settlement centers with the contents of elements detected in honey; it has been determined that there is an inversely proportional relationship between the amounts of Pb, As and Co and the distance to the settlements. In other words, Pb, As and Co contents increase as the apiaries from which honey samples are taken get closer to the settlement. The said correlation value is significant at the level of 1% for Pb and 5% for As and Co.

There are many studies on heavy metal and trace element contents in honey produced in Turkiye. However, environmental pollution changes depending on many factors such as geography, climate and pollution sources, it is important for data security to repeat the effects of environmental pollution on people and food as often and in as many different and many examples as possible. Heavy metals, which are an indicator of environmental pollution, seriously pollute the soil, water and air. The increase in the heavy metal level in the soil also increases the heavy metal accumulation in the plants. The bees, which obtain most of their food sources from plants, and their water needs from water sources in nature, carry nectars such as sap, nectar and pollen they collect from plants and heavy metals to the hive. Therefore, heavy metal and element contents in the composition of bee products such as honey, pollen, royal jelly and propolis may vary depending on the heavy metal and element content in environmental sources such as soil, water and air.

Preserving the naturalness of honey produced and consumed in different regions of our country is very important for our health. Honey, which is an important food for human health in nutrition, can be exposed to many sources of contamination during the processes after it is produced.

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REFERENCES

Alkan PE. Pollen Analysis of Chestnut Honey in Some Provinces of the Black Sea Region, Turkiye. Mellifera 2020;20(2):18-31.

Bengü AŞ, Kutlu MA. Bingöl'den Temin Edilen Ballarda Icp-Ms ile Bazı Temel ve Toksik Elementlerin Analizi. U. Arı D.-U. Bee J 2020;20(1):1-12, doi:10.31467/uluaricilik.648631

Bohrer AS, Takahashi H. Compartmentalization and Regulation of Sulfate Assimilation Pathways in Plants. Int Rev Cell Mol Biol 2016;326:1-31, doi:10.1016/bs.ircmb.2016.03.001

Borum E. Arılarin Yavru Çürüklüğü İnfeksiyonlarinda Doğru Teşhis, Mücadele Ve Korunma Yöntemleri. U. Arı D.-U Bee J 2014;14(1):44-55, doi:10.31467/uluaricilik.376732

Burucu V, Gülse Bal HS. Türkiye'de Arıcılığın Mevcut Durumu ve Bal Üretim Öngörüsü. T. Eko Arş D.- J. Agri Eco Res 2017;3(1): 28-37, http://dergipark.gov.tr/tead/issue/29947/322443 adresinden erişildi (Date of access:18.10.2022).

Burucu V, Gülse Bal HS. Arıcılık İşletmelerinin Pazarlama Olanakları: Kastamonu İli Azdavay İlçesi Örneği. T. Eko Arş D.- J. Agri Eco Res 2018;4(1): 23-35.

Calvo MS, Lamberg-Allardt CJ. Phosphorus. Adv Nutr 2015;6(6):860-862, doi:10.3945/an.115.008516

Castro-Vázquez L, Leon-Ruiz V, Alañon ME, Pérez-Coello MS, González-Porto AV. Floral origin markers for authenticating Lavandin honey (Lavandula angustifolia x latifolia). Discrimination from Lavender honey (Lavandula latifolia). Food Control. 2014;37:362-370, doi:10.1016/j.foodcont.2013.09.003

Chandra RK. Micronutrients and Immune Functions. Ann Ny Acad Sci 1990;587(1):9-16, doi:10.1111/j.1749-6632.1990.tb00128.x

Demirezen D, Aksoy A. Determination of Heavy Metals in Bee Honey Using By Inductively Coupled Plasma Optical Emission Spectrometry (Icp-Oes). Gazi U J Sci 2005;18(4):569-575.

Doelsch E, Saint Macary H, Van de Kerchove V. Sources of very high heavy metal content in soils of volcanic island (La Réunion). J Geochem Explor 2006;88(1-3):194-197, doi:10.1016/j.gexplo.2005.08.037

Elevli B, Laratte B. Estimation of the Turkish Boron

Exportation to Europe. Mining 2022;2(2):155-169.

EC (European Comission) 2006. European Union Regulation EC1881/2006 Maximum levels for certain contaminants in foodstuffs. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:02006R1881-20150731 (Date of access:31.10.2022)

FDA (US Food and Drug Administration) 2021. https://www.fda.gov/food/metals-and-your-food/lead-food-foodwares-and-dietarysupplements#:~:text=The%20FDA's%20current%20IRL%20is,age%20(updated%20in%202022 (Date of access:01.11.2022).

Güler D.Türkiye'de İllere Göre Arıcılık Etkinliğinin Veri Zarflama Analizi ile Belirlenmesi. U. Arı D.-U. Bee J 2021;21(2):146-156, doi:10.31467/uluaricilik.940167

Gupta DK, Tiwari S, Razafindrabe BHN, Chatterjee S. Arsenic Contamination from Historical Aspects to the Present. Arsenic contamination from historical aspects to the present. In Arsenic Contamination in the Environment. Springer, Cham 2017;1-12., doi:10.1007/978-3-319-54356-7_1

Hewlings S, Douglas K. Sulfur and Human Health. Ec Nutr, 2019;785-791. https://www.researchgate.net/publication/335653705_Sulfur_and_Human_Health adresinden erişildi (Date of access: 18.10.2022).

Kara EE, Kara E. Toprakta Ağır Metal Kirliliğinin İnsan Sağlığına Etkileri ve Çözüm Önerileri. Türk Bil Der Der-Turk J Sci Rev 2018;11(1):56-62.

Khalid S, Shahid M, Niazi NK, Murtaza B, Bibi I, Dumat C. A comparison of technologies for remediation of heavy metal contaminated soils. J Geochem Explor 2017;182:247-268, doi:10.1016/j.gexplo.2016.11.021

Kołodziejska B, Stępień N, Kolmas J. The Influence of Strontium on Bone Tissue Metabolism and Its Application in Osteoporosis Treatment. Int J Mol Sci 2021;22(12):6564, doi:10.3390/ijms22126564

Kravchenko J, Darrah TH, Miller RK, Kim Lyerly H, Vengosh A, Kravchenko J, Vd. A review of the health impacts of barium from natural and anthropogenic exposure. Environ Geo Health 2014;36:797-814, doi:10.1007/s10653-014-9622-7

Lanjwani MF, Channa FA. Minerals content in different types of local and branded honey in Sindh, Pakistan. Heliyon 2019;5(7):e02042, doi:10.1016/j.heliyon.2019.e02042

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

Mikhailenko AV, Ruban DA, Ermolaev VA, Van Loon T. Cadmium pollution in the tourism environment: A literature review. Geosciences 2020;10(6):242, doi:10.3390/geosciences10060242

Mitra S, Chakraborty AJ, Tareq AM, Emran T, Bin Nainu F, Khusro A, Vd. Impact of heavy metals on the environment and human health: Novel therapeutic insights to counter the toxicity. J King Saud U- Sci 2022;34(3):101865, doi:10.1016/j.jksus.2022.101865

Özcan MM, AL Juhaimi FY. Determination of heavy metals in bee honey with connected and not connected metal wires using inductively coupled plasma atomic emission spectrometry (ICP–AES). Environm Moni Asses 2012;184(4):2373-2375, doi:10.1007/s10661-011-2123-6

Özkul C. Kütahya Şehir Merkezinde Yer Alan Çocuk Parklarındaki Toprakların Ağır Metal Kirliliğinin Belirlenmesi. Afyon Kocatepe U Jour Sci Eng 2019;19(1):226-240, doi:10.35414/akufemubid.408653

Ozkul C, Acar RU, Köprübaşı N Er AE, Kızılkaya H İ, Metin M, Şenel MN. Altintaş (KütahyaTürkiye) Ovasi Tarim Topraklarinda Ağir MKirlîliğin Araştirilmasi, Öncel Çalişma. Uyg Yerbil Der 2018;17(1):13-26, doi:10.30706/uybd.426408

Oroian M, Amariei S, Rosu A, Gutt G. Classification of unifloral honeys using multivariate analysis, J Essent Oil Res 2015;27(6):533-544, doi.org/10.1080/10412905.2015.1073183

Ozturk A, Yarci C, Ozyigit II. Assessment of heavy metal pollution in Istanbul using plant (Celtis australis L.) and soil assays. Biotechnol Bio Equ 2017;31(5):948-954, doi:10.1080/13102818.2017.1353922

Pahl M, Zhu H, Tautz J, Zhang S. Large Scale Homing in Honeybees. PLoS ONE 2011;6(5):19669, doi:10.1371/journal.pone.0019669

Pires J, Estevinho ML, Feás X, Cantalapiedra J, Iglesias A. Pollen spectrum and physico-chemical attributes of heather (Erica sp.) honeys of north Portugal. J Sci Food Agr 2009;89(11):1862-1870,

doi.org/10.1002/jsfa.3663

Rafati Rahimzadeh M, Rafati Rahimzadeh M, Kazemi S, Jafarian Amiri R, Pirzadeh M, Akbar Moghadamnia A. Aluminum Poisoning with Emphasis on Its Mechanism and Treatment of Intoxication. Emerg Med Inter 2022, doi:10.1155/2022/1480553

Resmi Gazete 2011. Türk Gıda Kodeksi Bulaşanlar Yönetmeliği (29 Aralık 2011). https://www.resmigazete.gov.tr/eskiler/2011/12/20111229M3-8.htm (Date of access: 01.11.2022)

Schievano E, Stocchero M, Zuccato V, Conti I, Piana L. NMR assessment of European acacia honey origin and composition of EU–blend based on geographical floral markers, Food Chem 2019;288:96-101, doi.org/10.1016/j.foodchem.2019.02.062

Sıralı R, Cinbirtoğlu Ş. Bal arilarinin tozlaşmadaki ve bi̇tki̇sel üreti̇mdeki̇ önemi̇. Arı Arş (August) 2018;28-33.

Singh SP, Kaur S, Singh D. Food toxicology-past, present, and the future (the Indian perspective). Food Safety in the 21st Century: Public Health Perspective 2017;1:91-110, doi:10.1016/B978-0-12-801773-9.00008-X

Soares S, Amaral JS, Oliveira MBP, Mafra IA. comprehensive review on the main honey authentication issues: Production and origin, Comp Rev Food Sci Food Safety 2017;16(5):1072–1100, doi.org/10.1111/1541-4337.12278

Sönmez O, Kılıç FN. Toprakta Ağır Metal Kirliliği ve Giderim Yöntemleri. Turk J Agri Eng Res 2021;2(2):493-507, doi:10.46592/turkager.2021.v02i02.020

Şen A. Antioxidant and anti-inflammatory activity of fruit, leaf and branch extracts of Paliurus spina-christi P. Mill. Marmara Pharma J 2018;22(2):328-333, doi:10.12991/mpj.2018.71

Şen, K. Trakya yöresi ayçiçeği balı, meşe balı ve karaçalı balı'nın çeşitli kalite özellikleri üzerine bir araştırma (Master's thesis, Namık Kemal Üniversitesi) 2019.

Tchounwou PB, Yedjou CG, Patlolla AK, Sutton DJ, Tchounwou PB, Yedjou CG, Vd. Heavy Metal Toxicity and the Environment. Exper Supp 2012;101, doi:10.1007/978-3-7643-8340-4_6

Tepge TE, PGE. Ürün Raporu: Arıcılık 2021. https://arastirma.tarimorman.gov.tr/tepge/Belgeler/PDF Ürün Raporları/2021 Ürün Raporları/Arıcılık Ürün Raporu 2021-320 TEPGE.pdf adresinden erişildi (Date of access:18.10.22).

TGK, Turkish Food Codex. Honey Communiqué, Number: 2020/7. https://www.tarimorman.gov.tr/HAYGEM/KAGEM/Link/8/Bal-Tebligi (Date of access: 19.10.2022)

TUIK, Hayvansal Üretim İstatistikleri, (Aralık) 2021. https://data.tuik.gov.tr/Bulten/Index?p=Hayvansal-%C3%9Cretim-%C4%B0statistikleri-Aral%C4%B1k-2021-45593&dil=1#:~:text=Bir%20%C3%B6nceki%20y%C4%B1la%20g%C3%B6re%20yapa%C4%9F%C4%B1,96%20bin%20344%20ton%20oldu.

Uçak Koç A, Karacaoğlu M, Doğan M. Hayıt (Vitex agnus-castus), Çam ve Karışım Çiçek Balının Bazı Kalite Kriterleri Açısından Karşılaştırılması. Adnan Menderes U Ziraat Fak D 2017;14(1):17-21, doi:10.25308/aduziraat.294889

Vareda JP, Valente AJM, Durães L. Heavy metals in Iberian soils: Removal by current adsorbents/amendments and prospective for aerogels. Adv Coll Interface Sci 2016;237:28-42, doi:10.1016/j.cis.2016.08.009

Wang D, Lin H, Kan J, Liu L, Zeng X, Shen S. Food Chemistry. Nova Science Publishers, Inc., Hauppauge, NewYork, USA, 2012, p.1-370, doi:10.1201/b18894-16.

WHO (World Health Organization) 2007. Exposure of children to chemical hazards in food. Fact Sheet No.4.4. chrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/https://www.euro.who.int/__data/assets/pdf_file/0003/97446/4.4.pdf (Date of access: 01.11.2022)

Zor M, Aydin S, Güner ND, Başaran N, Başaran AA. Antigenotoxic properties of Paliurus spina-christi Mill fruits and their active compounds. Bmc Complem Altern M 2017;17(1):229, doi:10.1186/s12906-017-1732-1.

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ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

INVESTIGATION OF CHEMICAL CONTENT AND ANTIMICROBIAL ACTIVITIES OF DIFFERENT PLANT SOURCES OF ANATOLIAN PROPOLIS SAMPLES

Farklı Bitki Kaynaklı Anadolu Propolis Örneklerinin Kimyasal İçeriği ve Antimikrobial Aktivitelerinin Araştırılması

Emine SÖNMEZ

Düzce University, Beekeeping Research Development and Aplication Centre, 81620 Düzce, TÜRKİYE, E-posta: eminesonmez@duzce.edu.tr, ORCID No: 0000-0003-4418-5599

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ABSTRACT

The ethnopharmacological approach combined with chemical and biological methods can be a useful model in the field of pharmacology. One of these approaches, apitherapy, is the use of bee and hive products for therapeutic purposes. Propolis is among the best known of these bee products. The chemical composition of propolis varies according to the local or endemic flora, bee species, geographical origin and season. This study is to determine the antimicrobial activity differences between chestnut and polyfloral origin propolis against various pathogenic bacterial species. First of all, the Liquid Chromatography-Mass Spectrometry (LC-MS/MS) method was used for the determination of bioactive components known to be responsible for antimicrobial activity. Folin-Ciocalteu method and colorimetric aluminum chloride assay were used to determine the total phenolic (TP) and flavonoid (TF) amounts. 19 different pathogenic microorganisms were selected to test the antimicrobial activity levels of propolis samples with agar well diffusion and minimum inhibitory concentration (MIC) methods. TP and TF values of chestnut propolis (71.06 mg GAE/mL-11.75 mg QE/mL) were significantly higher than polyfloral sample (36.84 mg GAE/mL-7.04 mg QE/mL). Chrysin, a flavone derivative, was the most abundant compound in both samples. The MIC values of chestnut propolis ranged from 19.5 to 2500 µg/mL, while the MIC value of polyfloral origin propolis was between 39.06 and 5000 µg/mL. The most susceptible strain was *Mycobacterium smegmatis* for both samples with different concentration. Notably, it was observed that the botanical origins affect the chemical composition of propolis, and this situation can also be effect antibacterial and antifungal activity in respective propolis because of the different amount and diversity of bioactive compounds. Consequently, chestnut propolis is a promising candidate for drug discovery that can be used to treat some infectious diseases, including diseases related with resistant bacteria.

Keywords: Chestnut propolis, total phenolic, flavonoid, phenolic composition, antimicrobial and antifungal activity

ÖZ

Kimyasal ve biyolojik yöntemlerin entegre çalışılması ile oluşturulan etnofarmakolojik yaklaşım, farmakoloji alanında faydalı bir model olabilir. Bu yaklaşımlardan biri olan apiterapi, arı ve kovan ürünlerinin tedavi amaçlı kullanılmasıdır. Bu arıcılık ürünleri içinde propolis, en iyi bilinenler arasındadır. Propolisin kimyasal bileşiminin yerel veya endemik floraya, arı ırkına, coğrafi kökene ve mevsime göre değiştiği bilinmektedir. Bu bilgiler doğrultusunda çalışma, kestane ve polifloral orijinli propolis örneklerinin farklı patojenik mikroorganizma suşlarına karşı antimikrobiyal aktivite

farklılıklarını belirlemek amacıyla yapılmıştır. Antimikrobiyal aktiviteden sorumlu olduğu bilinen biyoaktif bileşenlerin tayini için öncelikle Sıvı Kromatografi-Kütle Spektrometresi (LC-MS/MS) yöntemi kullanıldı. Toplam fenolik (TP) ve flavonoid (TF) miktarlarını belirlemek için Folin-Ciocalteau yöntemi ve kolorimetrik alüminyum klorür testleri kullanıldı. Propolis örneklerinin antimikrobiyal aktivite düzeyleri seçilen 19 farklı patojenik mikroorganizmaya karşı agar kuyu difüzyonu ve minimum inhibitör konsantrasyon (MIC) yöntemleri ile belirlendi. Kestane propolisinin TP ve TF değerleri (71.06 mg GAE/mL-11.75 mg QE/mL), polifloral örnekle (36.84 mg GAE/mL-7.04 mg QE/mL) kıyaslandığında anlamlı olarak yüksek bulunmuştur. Bir flavon türevi olan Chrysin, her iki örnekte de en yüksek oranda bulunan bileşik olarak tespit edildi. Kestane propolisinin MİK değerleri 19,5 ile 2500 µg/mL arasında değişirken, polifloral orijinli propolisin MİK değeri 39,06 ile 5000 µg/mL arasında belirlendi. Her iki örneğe karşı farklı konsantrasyonlarda en duyarlı suş *Mycobacterium smegmatis*'di. Bu çalışma ile botanik orijinlerin propolisin kimyasal bileşimini etkilediği ve bu durumun biyoaktif bileşiklerin farklı miktar ve çeşitliliğinden dolayı ilgili propoliste antibakteriyel ve antifungal aktiviteyi de etkileyebileceği doğrulandı. Sonuç olarak, kestane propolisi, dirençli bakteriler de dahil olmak üzere bazı bulaşıcı hastalıkları tedavi etmek amacıyla kullanılabilecek ilaç geliştirme çalışmaları için umut vaad edici bir aday olarak kullanılabileceği önerilmektedir.

Anahtar Kelimeler: Kestane propolisi, toplam fenolik madde, flavonoid, fenolik kompozisyon, antimikrobiyal ve antifungal aktivite

GENİŞLETİLMİŞ ÖZET

Amaç: Bu çalışmanın amacı farklı orjinli propolis örneklerinin biyoaktif çeşitliliğini ve miktarını belirleyerek, seçilen farklı patojen mikroorganizmalara karşı antimikrobiyal aktivite düzeylerini karşılaştırmaktır.

Giriş: Dünya genelinde artan antibiyotik direnci sebebiyle insanlar sentetik ürünler yerine doğal ürünlere yönelmektedir. Doğalürünler, tarih boyunca geleneksel tıpta kullanılmış ve potansiyel bir yeni ilaç kaynağı olmuştur. Propolis, eski Mısırlılar ve Yunanlılar zamanından beri bilinen ve bazı hastalıkların tedavisinde kullanılanantimikrobiyal ajan örneğidir. Propolisin antimikrobiyal aktivitesi, farklı araştırmacılar tarafından kapsamlı bir şekilde incelenmiş; Gram pozitif veya Gram negatif bakterilerin yanı sıra mayalar ve küfler gibi çok çeşitli mikroorganizmaların büyümesini inhibe veya kontrol edebildiği bildirilmiştir. Propolis, polifenol (flavonoidler, fenolik asitler ve esterler), fenolik aldehitler ve ketonlar gibi 300'den fazla farklı bileşenden oluşur. Polifenoller ve terpenoidler de en aktif grup olarak kabul edilir. Bu biyoaktif bileşiklerin sayısı ve konsantrasyonu bal arısının yaşadığı coğrafyaya, mevsime, arı ırkına ve kovanının belirli bitki kaynaklarına yakınlığına bağlı olarak değişkenlik gösterir.

Gereç ve Yöntem: Bu çalışmada etkinliği araştırılan propolis örnekleri Düzce Üniversitesi Arıcılık Uygulama ve Araştırma Merkezi'nden (DAGEM) temin edildi. Örnekler Haziran ve Temmuz aylarında propolis tuzakları kullanılarak kovanlardan toplandı. Labaratuvara getirilen ham propolis örnekleri (Kestane ve polifloral orjinli) öğütüldükten sonra etanolik ekstraksiyon metoduna tabi tutuldu. Kullanıma hazır hale gelen örneklerin toplam fenolik (TP) miktarları Folin-Ciocalteau yöntemi ile toplam flavonoid (TF) miktarları ise kolorimetrik alüminyum klorür testi ile tespit edildi. Propolis örneklerinin biyoaktif bileşenlerinin tespiti için Sıvı Kromatografi-Kütle Spektrometresi (LC-MS/MS) yöntemi kullanıldı. Seçilen 19 farklı patojene karşı örneklerin antimikrobiyal aktivite düzeylerini belirlemek için ilk basamakta agar kuyucuk, ardından minimal inhibisyon konsantrasyonu (MİK) deneyleri yapıldı.

Bulgular: Araştırmalar sonucunda kestane propolisinin polifloral örneğe göre daha yüksek oranda antimikrobiyal aktivite sergilediği tespit edilmiştir. Her iki örneğe karşı da farklı konsantrasyonlarda en duyarlı suş *Mycobacterium smegmatis* olarak belirlenmiştir. Bu yüksek etkinliğin de içeriğindeki biyoaktif bileşenlerin farklılığından kaynaklandığı düşünülmektedir. Kestane propolisinin toplam fenolik ve flavonoid miktarı polifloral örneğe göre anlamlı düzeyde farklılık göstermiştir. Her iki propolis örneğinde de en yüksek oranda tespit edilen bileşik bir flavon türevi olan Chrysin'dir. Kestane propolisinde hesperidin ve protokatekuik asit saptanmazken, polifloral orijinli propoliste bu bileşenler tespit edilmiştir. (±)-Kateşin, siringik asit, (-)-epikateşin ve rutin polifloral kökenli propolis bileşenlerinde tespit edilemezken, bu biyoaktif maddelerin konsantrasyonları kestane

propolisinde kayda değer düzeyde tespit edilmiştir. Sadece bir flavonoid türevi olan daidzein her iki numunede de bulunamamıştır.

Sonuç: Bu çalışmanın sonuçları propolis içeriğinin orjinlendiği bitki kaynaklarına göre değiştiği bilgisini doğrulamaktadır. Kestane propolisinin seçilen patojenlere karşı çok düşük dozlarda etkili olması, bulaşıcı hastalıkların önlenmesinde ve tedavisinde kullanım potansiyeline sahip olduğunu gösteren önemli bir sonuç olarak değerlendirilmektedir.

INTRODUCTION

Propolis, which has great potential as a medicine and has many biological properties, is more effective than medicinal plant extracts, because its composition is extraordinarily variable. The bioactive components of the propolis samples may vary according to the different geographic origin, race, climate, flora and bud exudates (Bankova et al. 2000, Kartal et al. 2003). Propolis consists mainly of polyphenols (phenolic aldehydes, phenolic acids and their esters, flavonoid aglycones, alcohols and ketones), but it also contains terpenoids, amino acids, steroids and inorganic substances (Moreno et al. 2000). It is known that bees collect secretion from buds of poplar (*Populus* spp.), alder (*Alnus* spp.) in Poland and Central Europe (Przybyłek and Karpiński 2019). In other European countries such as Albania, Bulgaria, Hungary, different types of poplar are known as sources of propolis (Zabaiou et al. 2017). In some regions of Turkiye, chestnut (*Castanea sativa*) trees are common and honey bees often use these trees to produce propolis (Kekecoglu et al. 2021). Many previous studies have shown that different types of propolis exhibits great potential as an antioxidant, antimicrobial and antiviral agent because of the its rich content (Fatima et al. 2014, Al-Juhaimi et al. 2022, Kekecoglu et al. 2021, Yıldız 2020, Uçar 2021). It is thought that the main source of antimicrobial activity originates from pinocembrin, galangin and caffeic acid phenethyl esters, and this effect is caused by the inhibition of bacterial RNA-polymerase by phenolic compounds (Takaisi-Kikuni and Schilcher 1994). In this process, where the incidence of antimicrobial resistance is constantly increasing, the demand for natural products is increasing rapidly. Propolis is effective on many microorganisms such as viruses, fungi, including resistant bacteria (Bankova et al. 1996, Koru et al. 2007). For example, Veiga et al. (2017) showed that poplar propolis had antimicrobial activity against both Gram-positive and Gram-negative bacteria, including Methicillin-resistant *Staphylococcus aureus* (MRSA). In addition, it is known that ethanolic extracts of propolis have antifungal effect against different strain of yeast (Bankova et al. 2014). In addition to the therapeutic properties of propolis, it has reported that it has no side effects in animals or humans as a result of toxicity tests (Demir et al. 2016).

All countries have honeybee races of local ecotypes that adapt to its own ecological conditions. Although there are different bee races in our country, ecotypes of these races have spread in different areas (Ruttner, 2013, Kekecoglu, 2018). Honey bees have some characteristics that are different from each other in every race. Accordingly, propolis collection behavior also varies according to different honey bee races and ecotypes (Eroglu et al. 2021). *Apis mellifera anatoliaca*, which is found in Yıgılca district of Düzce province, is a special ecotype belonging to this region.

The aim of this study is to investigate and compare the bioactive components and antimicrobial activities against pathogenic microorganisms including resistant bacteria of propolis samples obtained from different botanical origins. Secondly, to test whether the Yığılca ecotype, a special bee subspecies, affects this biological activity.

MATERIAL AND METHODS

Sample collection, Extraction and Preparation

Propolis samples were collected from Duzce University Beekeeping Research and Development Center (DAGEM) located in the north-east area of Duzce. Propolis samples were collected with propolis traps placed in hives in June and July. The samples were kept in a dry place and stored at 4℃ until its complete process. For extraction the samples were disintegrated with a grinder and 30 g of the propolis mixed in 90 mL of 96% ethanol and shaken at 30 ℃ for two weeks. Then, centrifuged at 26,000× g for 30 min and the supernatant was filtered twice with Whatman No. 4. The remaining ethanol was allowed to evaporate to obtain a completely dry sample from this final solution. The sample was kept at 4 ℃ in the dark until use.

Liquid Chromatography-Mass Spectrometry (LC-MS/MS)

LC-MS/MS method was used in the content analysis of the samples, as it is a reliable and successful technique for the characterization of active compounds in biological products such as propolis. For component analysis of the samples Thermo-Scientific LC coupled with a TSQ Quantum Access Max triple-stage quadruple-mass spectrometer (San Jose, CA, USA) was used. LC separations were performed in a C18 analytical column (15 cm x 3 mm x 5 µm; Torrance, California, USA). The run time was 5.5 minutes, the temperature of the column was 40°C, and the injection volume was 10µL. The mass-spectrometer was working with an electrospray ion source (ESI) in negative mode under the selected ion monitoring (SRM) condition (Nichitoi et al. 2020).

Determination of Total Phenolic (TP) and Flavonoid (TF) Content

The total phenolic content of both propolis samples was determined using the Folin–Ciocalteu colorimetric method mentioned in Singleton and Rossi (1965) with minor modifications. First, 20 µL of propolis extract was mixed with 680 µL of distilled water. 400 µL of 0.2 N Folin-Ciocalteu was added to this mixture and vortexed, this mixture was incubated for 2 minutes. After incubation, 400 µL of Na_2CO_3 (10%) was added, the mixture was shaken at regular intervals and incubated for 2 hours at room temperature. The absorbance of the mixture was measured at 760 nm and the total amount of phenolic substance was calculated as mg gallic acid equivalent per gram sample.

The total flavonoid amount of propolis samples was determined by making minor changes in the aluminum chloride colorimetric method described by Fukumoto and Mazza (2000). Quercetin was used as a standard to generate the calibration curve. The results were expressed as mg of quercetin equivalents (QE) per g pollen sample.

Test Microorganisms

For determination of the antimicrobial activityof propolis samples, seven Gram-negative, nine Gram positive and three yeast-like fungi were used. Gram-negative bacteria consisted of *Aeromonas sobria* ATCC 43979, *Aeromonas hydrophila* ATCC 7966, *Klebsiella pneumoniae* subsp. *pneumoniae* ATCC 18883, *Escherichia coli* ATCC 25922, *Vibrio* sp. Clinic strain, *Yersinia pseudotuberculosis* ATCC 911, *Pseudomonas aeruginosa* ATCC 27853, while Gram positive bacteria consisted of *Bacillus* sp. Clinic strain, *Bacillus subtilis* ATCC 6633, *Bacillus cereus* 702 Roma, *Staphylococcus aureus* MRSA Clinic strain, *Staphylococcus aureus* ATCC 25923, *Streptococcus pyogenes* Clinic strain, *Listeria monocytogenes* ATCC 11994, *Mycobacterium smegmatis* ATCC 607 and *Enterococcus faecalis* ATCC 29212. Yeast-like fungi group contained *Candida tropicalis* ATCC 13803, *Candida albicans* ATCC 60193, *Saccharomyces cerevisiae* RSKK 251

Culture media and preparation of inoculum

All bacteria were transferred from stock cultures to Tryptic Soy Agar (TSA) (Merck), Blood base agar (for *S. pyogenes*) and Brain Heart Infusion (BHI) Agar (for *M. smegmatis*) and incubated overnight at 37 °C. Potato Dextrose Agar (PDA) (Merck) was preferred for the growth of yeast-like fungi. Single colonies from plates were transferred into tubes containing 2 ml of Mueller Hinton Broth (MHB), except *M. smegmatis*. Yeast-like fungi were inoculated in tubes which include 2 ml Malt extract broth. All tubes were incubated at 37 ℃ and 120 rpm for 1-3 hours. The turbidity of the suspensions were adjusted spectrophotometrically to the McFarland 0.5 turbidity standart (1.5 x 10^8 colony forming unit per ml (cfu/ml) for bacteria, 6 x 10^8cfu/ml for yeast fungi).

Test for antimicrobial activity

Agar well diffusion method

Test plates were prepared with suitable medium and wells of 6 mm in diameter were punched in the agar plates by using sterile glass tube. Overnight cultures (100 µL) spread on the petri surface with a sterile swap. 50 µL of propolis extracts were transferred to each well. Negative control was %96 ethanol and standard controls were Ampicillin (10 µg) for bacteria, streptomycin (10 µg) for *M. smegmatis* and fluconazole (5 µg) for the yeasts. Propolis extracts were tested at 4 different concentrations (1/2, 1/4, 1/8, 1/16) in the agar well method. Zones of inhibition formed by the extracts were determined using caliper after incubation and those that formed larger than 6 mm were used in the MIC experiment (Kuppulakshmi et al., 2008).

Evaluation of Minimum Inhibitory Concentrations (MIC)

For determination of MIC values, inoculum suspensions were prepared from 24 h overnight cultures. 100 µL of propolis extracts were diluted

with the liquid medium to reach a final bacterial and yeast-like fungi count in ELISA plates (96-Well ELISA Microplates) by microdilution technique. The final concentration of propolis samples ranged from 5000 to 39 µg/mL. The MIC values were determined as the lowest concentration of propolis extracts that inhibit microbial population growth.

Statistical Analysis

The analyses results of bioactive compounds in propolis samples were expressed in mean + standard deviation by using Microsoft Office Excel 2019 (Microsoft Corporation, Redmond, WA, USA). Significant differences between means were determined by T-test (SPSS version 25 for Windows 11; post hoc-one way ANOVA).

RESULTS

The bioactive components of ethanol extracts of propolis samples used in the study are given in Table 1. LC-MS/MS analysis showed that different amounts of bioactive components were detected in both propolis samples. Chrysin which is the flavone derivative, was the most abundant of all these components. While hesperidin and protocatechuic acid were not detectable in chestnut propolis, they were present in polyfloral origin propolis. (±)-Catechin, syringic acid, (-)-epicatechin and rutin were absent in polyfloral origin propolis components. Only daidzein, which is a flavonoid derivative, was not found in both samples.

Table 1. Analysis of phenolic composition in propolis samples (µg/ml)

Tablo 1. Propolis örneklerinin fenolik kompozisyonu (µg/ml)

Compounds	**Chestnut Propolis (µg/ml) (MEAN±SD)**	**Polyfloral Propolis (µg/ml) (MEAN±SD)**
Gallic acid	0,422±0,002	*nd
Protocatheuic acid	nd	1,46±0,04
Benzoic acid	95,7±0,011	2,31±0,005
(±)-Catechin	31,33±0,06	nd
Caffeic acid phenethyl ester	725,3±0,02	94,82±0,007
Syringic acid	18,12±0,03	nd
(−)-Epicatechin	1,77±0,024	nd
p- Coumaric acid	375,73±0,03	65,85±0,01
Ferulic acid	633,26±,07	69,17±10,3
Rutin	4734,5±0,47	nd
Myricetin	2596,37±0,025	2,07±0,056
Resveratrol	737,27±0,025	176,28±0,017
Daidzein	nd	nd
Luteolin	90,57±0,03	14,65±0,004
trans-Cinnamic acid	219,43±0,06	103,63±0,003
Hesperidin	nd	12,87±0,004
Chrysin	7214,42±0,07	2301,65±0,005
Pinocembrin	2272,72±0,04	910,4±0,006
CAPE	3593,27±0,06	1209,99±0,008

*nd: not detected

The TPC of the samples, measured by the Folin–Ciocalteu method, the TFC of measured by the aluminum chloride colorimetric method. After the necessary dilution of the ethanolic propolis extracts, the total amount of phenolic and flavonoid substances were determined according to the gallic acid and quercetin standard respevtively. When the data is evaluated the TPC value of chestnut propolis was nearly two times polyfloral origin propolis sample (Table 2). The obtained TPC value was 71,06±1,4 mgGAE/mL for chestnut propolis, 36,84±1,4 mgGAE/mL for polyfloral origin sample. The total flavonoid amounts of the samples were different from each other. The amount of TFC of chestnut propolis was higher than the polyfloral origin propolis sample. (t-test should be added to explain differences between two samples)

Table 2. Total phenolic and flavonoid content of propolis extracts

Tablo 2. Propolis ekstraktlarının toplam fenolik ve flavonoid madde içeriği

	Total Phenolic (mg GAE/mL)	Total flavonoids (mgQE/mL)
Chestnut propolis	71,06±1,40	11,75±0,15
Polyfloral propolis	36,84±1,40	7,04±0,30

Propolis samples obtained from two different sources were effective against all selected test microorganisms. Agar well diffusion and MIC values of propolis samples are summarized in Table 5. As a result of one-way variance analysis (ANOVA), it was seen that there was statistical differences in terms of inhibition zones ($F_{chestnut\ propolis}$=4,300, p<0,05; $F_{polyfloral\ propolis}$=7,420, p<0,05). As a result of the multiple comparison analysis, it was seen that there were significant differences in the effectiveness of chestnut propolis between Gr (-) and Gr (+) bacteria according to the results of the agar well method (x= 4,50; p<,030). Similarly, it was observed that there were significant differences in the effect of the polyfloral propolis sample against Gram (+) and Gram (-) bacteria (x= 4,010; p<,017) (Table 3). According the agar well diffusion method with four different concentration (1/2, 1/4, 1/8, 1/16) among the samples obtained by ethanolic extraction, we obtained the highest antimicrobial activity from chestnut propolis. The microorganism in which both propolis samples were most effective was *M. smegmatis* and their effect zones sizes were differed. The highest susceptible zone was obtained from *M. smegmatis* with the value of 26 and 22 mm for chestnut and polyfloral origin popolis respectively. Gram positive bacteria were more sensitive than Gram-negative one for both propolis samples.

Table 3. Statistical analysis of chestnut and polyfloral samples' inhibition zone between microorganism groups

Tablo 3. Kestane ve polifloral propolis örneklerine ait inhibisyon zonlarının mikroorganizma grupları arasındaki istatistiksel analiz sonuçları

Inhibition Zone	Factors	s	Mean Differences	F	P
Chestnut Propolis	Gr (-)/Gr (+)	1,470	4,500	4,300	,030*
	Gr (+)/Yeast fungi	1,570	2,220		,376
	Gr (-)/Yeast fungi	1,480	2,280		,511
Polyfloral propolis	Gr (-)/Gr (+)	1,270	4,010	7,420	,017*
	Gr (+)/Yeast fungi	1,680	1,330		,306
	Gr (-)/Yeast fungi	1,740	2,670		,715

* Statistical significance was defined as *P*<0.05

Table 4. Statistical analysis of chestnut and polyfloral samples' MIC value between microorganism groups

Tablo 4. Kestane ve polifloral propolis örneklerinin MIC değerlerinin mikroorganizma grupları arasındaki istatistiksel analiz sonuçları

MIC (µg/ml)	Factors	s	Mean Differences	F	P
Chestnut Propolis	Gr (-)/Gr (+)	300,500	1347,660	10,450	,001*
	Gr (+)/Yeast fungi	397,300	253,910		,801
	Gr (-)/Yeast fungi	411,500	1093,740		,047*
Polyfloral propolis	Gr (-)/Gr (+)	610,410	2460, 930	8,680	,003*
	Gr (+)/Yeast fungi	807,500	273,430		,939
	Gr (-)/Yeast fungi	835,840	2187,500		,041*

* Statistical significance was defined as *P*<0.05

Table 5. Agar well diffusion and MIC values of the Propolis extracts against the tested microorganisms

Tablo 5. Propolis ekstraktlarının test edilen mikroorganizmalara karşı agar kuyucuk difüzyonu ve MİK değerleri.

	Microorganisms	Chestnut propolis		Polyfloral propolis		Antibiotics*	
		Inhibition zone (mm)	MIC (µg/ml)	Inhibition zone (mm)	MIC (µg/ml)	Inhibition Zone (mm)	MIC (µg/ml)
Gr (-)	*Escherichia coli*	10	2500	8	5000	10	10
	Klebsiella pneumoniae subsp. *pneumoniae*	10	2500	9	5000	10	32
	Yersinia pseudotuberculosis	12	1250	10	2500	10	32
	Vibrio sp.	14	312.5	13	625	NT	NT
	Aeromonas hydrophila	10	2500	8	5000	NT	NT
	Aeromonas sobria	12	1250	10	2500	NT	NT
	Pseudomonas aeruginosa	14	625	12	1250	18	>128
Gr (+)	*Enterococcus faecalis*	14	312.5	12	1250	10	2
	Listeria monosytogenes	16	156.25	14	312,5	NT	NT
	Streptococcus pyogenes	15	156.25	12	1250	NT	NT
	Staphylococcus aureus	18	39.06	14	312.5	35	2
	S. aureus MRSA+	15	156.25	13	625	NT	NT
	Bacillus subtilis	15	156.25	13	625	NT	NT
	Bacillus sp.	14	312.5	14	312.5	NT	NT
	Bacillus cereus	13	625	12	1250	NT	NT
	Mycobacterium smegmatis	26	19.5	22	39.06	35	<1
Yeast fungi	*Candida albicans*	13	625	12	1250	25	<8
	Candida tropicalis	13	625	12	1250	25	<8
	Saccharomyces cerevisiae	16	156.25	14	312.5	25	<8

*The test control antibiotics used: Ampicillin for Gram (-) and Gram (+) bacteria (10 µg/ml), Streptomycin for ARB+ bacteria (10 µg/ml), and Fluconazole for the yeast fungi (5 µg/ml). (-): No activity, NT, Not tested.

*Kullanılan kontrol antibiyotikleri: Gram (-) ve Gram (+) bakteriler için ampisilin (10 µg/ml), ARB+ bakterileri için Streptomisin (10 µg/ml) ve maya mantarları için Flukonazol (5 µg/ml). (-): Etkinlik yok, NT, Test edilmedi.

According to the MIC results obtained from the propolis samples, significant differences were obtained among the microorganisms ($F_{chestnut\ propolis}$=10,450, p<0,05; $F_{polyfloral\ propolis}$=8,680, p<0,05). When the MIC results of chestnut propolis were evaluated, significant differences were observed between the activity values between Gram (+) and Gram (-) bacteria (x = 1347,660; p<,001). Similarly, significant differences were observed between the efficacy values of the MIC results of polyfloral propolis (x = 2460,930; p<,003). The differences between other groups (Gram (+) /Yeast fungi, Gram (-) /Yeast fungi) are summarized in table 4. The efficacy dose of chestnut propolis was between 19,5 and 2500 µg/mL while the polyfloral origin propolis sample was between 39,06 and 5000 µg/mL. Chestnut propolis showed remarkable bactericidal effect against *M. smegmatis* with the dose of 19,5 µg/mL. The most resistant strains were *E. coli*, K. *pneumoniae* subsp. *pneumoniae* and *A. hydrophila* with inhibition dose of 5000 µg/mL. Two propolis samples exhibited moderate antifungal activity against selected yeast like fungi. Most resistant yeast were *C. albicans* and *C. tropicalis* with the dose of 2500 for polyfloral origin propolis and 1250 for chestnut propolis, most sensitive was *S. cerevisiae* (Table 5).

DISCUSSION

It is known that the chemical content of propolis depends on the origin of the plant, geographical location and the harvest season (Al-Ani et al. 2018). In this study, content differences due to the plant origin of propolis samples were observed. While some bioactive components were found in chestnut propolis, some of them were not detected in the polyfloral origin propolis sample. In addition, the amounts of the analyzed components were different from each other. Previous studies have shown that European, African and Asian propolis mostly contains phenolics and flavonoids such as pinocembrin, *p*-coumaric acid, cinnamic acid, chrysin, naringenin, galangin, quercetin, apigenin, pinobanksin, kaempferol, caffeine (Huang et al. 2014; De Groot et al. 2013). Among these components polyphenols and terpenoids are the most active group (Pimenta et al. 2015). The flavonoid group consists of chrysin, pinostrobin, galangin, pinocembrin, quercetin, apigenin, kaempferol and other components (Przybyłek, and Karpiński 2019). Our chestnut and polyfloral origin propolis samples contained the highest rate of chrysin, which is a flavonoid derivative. Another critical group constituting the content of propolis is aromatic acids, among which cinnamic, ferulic, caffeic, *p*-coumaric and benzoic acids are the most common (Kędzia and Hołderna-Kędzia 2017; Bankova 2000). Almost all of these aromatic esters were detected at high rates in chestnut propolis.

The present study aimed to investigate the antimicrobial properties of chestnut and polyfloral origin propolis samples. The influence of ethanol extraction in different concentrations on the growth of bacteria and fungi was determined. Incubation of propolis samples with higher concentrations resulted in higher inhibition of growth zones. Some researchers reported that propolis samples were only effective against Gram-positive bacteria and fungi, while others reported that the activity was not high against Gram-negative bacteria (Nieva et al.1999; Kujumgiev et al. 1999; Sforcin et al. 2000) . In this study, it was confirmed that Gram-positive bacteria were sensitive to low concentrations for both samples and that Gram-negative bacteria growth was inhibited to a lesser extent than Gram-positive bacteria. Chestnut propolis was the most effective against test microorganisms, followed by polyfloral origin sample. In previous studies, the best anti-staphylococcal effect levels of propolis ethanolic extract were reported for extracts derived from Turkey (8 µg/mL), Oman (42 µg/mL) and Ireland (80 µg/mL) (Uzel et al. 2005; Popova et al. 2013; AL-Ani et al. 2018). The antimicrobial activity of chestnut propolis against this bacterial species that causes pneumonia, osteomyelitis, septic arthritis, bacteremia, endocarditis and various skin infections is quite low as compared to previous studies (39.06 µg/mL). It is known that the presence of phenolic compounds in the chemical structure of chrysin is responsible for the antibacterial effects of propolis, as well as other flavonoids (Warfvinge et al.1985; Sforcin and Bankova, 2011; Sharifi et al. 2020). The slightlyhigh detection of chrysin in our chestnut propolis sample may be explained bylow MIC concentration against *S. aureus*.

We obtained strong antimicrobial activity from both propolis samples against *M. smegmatis* which is a saprophytic acid-resistant bacterium that also causes skin diseases. It has been reported in previous studies that pinocembrin and its 3-OH analog galangin, flavonoids such as quercetin, myricetin and rutin are the components responsible for the most potent microbicidal compounds via

increasing bacterial membrane permeability (Vică et al. 2022; Das et al. 2015; Stepanovic et al. 2003; Kosalec et al. 2003). Otherbioactive compounds which are identified and studied inpropolis are caffeic acid phenethyl ester (CAPE), which exhibit good antimicrobial properties by inhibiting bacterial RNA polymerase (Šuran et al. 2021; Speciale et al. 2006). Considering that the most abundant bioactive components as a result of LC-MS/MS analyzes of the chestnut propolis was chrysin, rutin, CAPE, myricetin and pinocembrin. It is possible to obtain MIC values at such low concentrations against *M. smegmatis.* Because one of these components, rutin, cannot be detected in the polyfloral origin propolis sample, while myricetin is present in trace amounts. These results demonstrated that chestnut propolis is a promising candidate for using as an antimicrobial product.

Ristivojević et al. (2016) tested the efficacy of 53 propolis samples on *L. monocytogenes* and reported the lowest efficacy dose as 100 µg/mL and the highest as 10.600 m/mL. The MIC values of chestnut and polyfloral origin propolis, whose antimicrobial activity levels were investigated in this study, against this bacterium causing meningitis, septicemia and monocytosis were 156.25 µg/mL and 312.5 µg/mL respectively. The fact that the propolis samples have such a low MIC values can be explained by the synergistic effect of the phenolic compounds with high level in the samples or special bee subspecies of Yığılca ecotype that collect propolis. Al-Ani et al. (2018) investigated the effect of different propolis samples against *S. pyogenes*, which causes dermal diseases such as impetigo and necrotizing fasciitis and they obtained different MIC values ranging from 80 to 600 µg/mL. The effect concentration of chestnut propolis against this bacterium is still quite low (156.25), which is below the average dose compared to previous studies.

Previous studies reported that different propolis samples have significant antifungal activity against a wide range of pathogen like Candida species which were isolated from patients and show antibiotic resistance (De Castro 2001; Cornara et al. 2017; Vica et al. 2021; Lan et al. 2016). AL-Ani et al. (2018) evaluated the effect of propolis samples from Germany, Ireland and Czech against different Candida species and reported the effective values against *C. albicans* as 5000, 600 and 1200 µg/mL, respectively. MIC values of the same samples against *C. tropicalis* were reported as 5000, 200 and 600 µg /mL, respectively. Chestnut propolis, which antifungal effect was tested in this study, showed a very low activity value on the same yeast-like fungus, and MIC values were determined as 625 µg /mL against both Candida species. It has been previously reported that the amount of CAPE in propolis significantly affects the antifungal activity (Cornara et al. 2017). Considering the CAPE amount of chestnut propolis, it is not surprising that such a low MIC value was obtained.

Conclusion

The antimicrobial activities of two different floral origin propolis from Anatolia against various pathogenic bacterial strains were determined by a MIC method. It was confirmed that chestnut propolis sample has higherphenolic and flavonoid contents and also it was found to be more effective against both Gram positive and Gram-negative bacteria. In the study, it was determined that the most abundant bioactive component in chestnut propolis samples, wwere chrysin followed by rutin, CAPE, myricetin and pinocembrin. The results suggest that the high content of bioactive components inhibit the growth and proliferation of bacteria by acting alone or synergistically. It was concluded that MIC values were obtained at lower concentrations from chestnut propolis than other sample according to this reason. Among the Gram-positive strains, *M. smegmatis* was the most susceptible strain for chestnut propolis, while the most resistant strains were *E. coli*, *Klebsiella pneumoniae* subsp. *pneumoniae* and *A. hydrophila*. The knowledge gained through this study may be a comparative analysis of the content to attribute the antimicrobial activity of propolis to specific chemical compounds and to confirm that these components are related to the floral origin.

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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, 2018; 5(1):2. doi.org/10.3390/medicines5010002

Al-Juhaimi FY, Özcan MM, Mohamed Ahmed IA, Alsawmahia ON, Özcan MM, Ghafoor K, Babiker EE. Bioactive compounds, antioxidant activity, fatty acid composition, and antimicrobial activity of propolis from different locations in Turkey. Journal of Apic. Res., 2022;61(2):246-254. doi.org/10.1080/00218839.2021.1898785

Bankova V, Marcucci MC, Simonova S, Nikolova N, Kujumgiev A. Antibacterial diterpenic acids from Brazilian propolis. Z Naturforsch. 1996;51:277–80. doi.org/10.1515/znc-1996-5-602

Bankova VS, de Castro SL, Marcucci MC. Propolis: recent advances in chemistry and plant origin. Apidologie, 2000;31(1):3–15. doi: 10.1051/apido:2000102

Bankova V, Popova M, Trusheva B. Propolis volatile compounds: chemical diversity and biological activity: a review. Chemistry Central Journal, 2014;8(1):1-8. doi.org/10.1186/1752-153X-8-28

Cornara L, Biagi M, Xiao J, Burlando B. Therapeutic properties of bioactive compounds from different honeybee products. Frontiers in pharmacology, 2017;412. doi.org/10.3389/fphar.2017.00412

Das A, Datta S, Mukherjee S, Bose S, Ghosh S, Dhar P. Evaluation of antioxidative, antibacterial and probiotic growth stimulatory activities of Sesamum indicum honey containing phenolic compounds and lignans. LWT-Food Science and Technology, 2015;61(1):244-250. doi.org/10.1016/j.lwt.2014.11.044

De Castro SL. Propolis: Biological and pharmacological activities. Therapeutic uses of this bee-product. Annual Review of Biomedical Sciences, 2001;3:49-83. doi.org/10.5016/1806-8774.2001v3p49

De Groot, AC. Propolis: a review of properties, applications, chemical composition, contact allergy, and other adverse effects. Dermatitis, 2013;24(6):263-282. doi.org/10.1097/DER.0000000000000011

Demir S, Aliyazicioglu Y, Turan I, Misir S, Mentese A, Yaman SO, Deger O. Antiproliferative and proapoptotic activity of Turkish propolis on human lung cancer cell line. Nutrition and cancer, 2016;68(1):165-172. doi.org/10.1080/01635581.2016.1115096

Eroğlu N, Kambur M, Kekeçoğlu M. The Investigation Propolis Foraging Preference of Different Honey Bee. Yüzüncü Yıl Üniversitesi Tarım Bilimleri Dergisi, 2021;31(1):133-141. doi.org/10.29133/yyutbd.785911

Fatima J, Baserisalehi M, Nima B. Antimicrobial activity and chemical screening of propolis extracts. American Journal of Life Sciences, 2014;2(2):72–75. doi:10.11648/j.ajls.20140202.16

Fukumoto LR, Mazza G. Assessing antioxidant and prooxidant activities of phenolic compounds. Journal of Agricultural and Food Chemistry, 2000;48(8):3597–3604. doi.org/10.1021/jf000220w

Huang S, Zhang CP, Wang K, Li GQ, Hu FL. Recent advances in the chemical composition of propolis. Molecules, 2014;19(12):19610–19632. doi.org/10.3390/molecules191219610

Kędzia B, Hołderna-Kędzia E. Pinocembrin–flavonoid component of domestic propolis with delaying effect of the development of Alzheimer's disease. Postępy Fitoterapii, 2017;3:223-228. Doi: 10.25121/PF.2017.18.3.223

Kartal M, Yıldız S, Kaya S, Kurucu S, Topçu G. Antimicrobial activity of propolis samples from two different regions of Anatolia. Journal of ethnopharmacology, 2003;86(1):69-73. doi.org/10.1016/S0378-8741(03)00042-4

Kekecoglu M, Sonmez E, Acar MK, Karaoglu SA. Pollen Analysis, Chemical Composition and Antibacterial Activity of Anatolian Chestnut Propolis Collected From Yıgılca Region. Biology Bulletin, 2021;48(6):721-728. doi.org/10.1134/S106235902106011X

Kekecoglu M. Morphometric divergence of anatolian honey bees through loss of original traits: A dangerous outcome of Turkish apiculture, Sociobiology. 2018;65(2):232-243. doi.org/10.13102/sociobiology.v65i2.1895

Koru O, Toksoy F, Acikel CH, Tunca YM, Baysallar M, Guclu AU, ... & Salih B. In vitro antimicrobial activity of propolis samples from different geographical origins against certain oral pathogens. Anaerobe, 2007;13(3-4):140-145. doi.org/10.1016/j.anaerobe.2007.02.001

Kosalec I, Bakmaz M, Pepeljnjak STJEPAN. Analysis of propolis from continental and Adriatic region of Croatia. Acta Pharmaceutıca-Zagreb, 2003;53(4):275-286.

Kujumgiev A, Tsvetkova I, Serkedjieva Yu, Bankova VS, Christov R, Popov S. Antibacterial, antifungal and antiviral activity of propolis of different geographic origin. Journal of ethnopharmacology, 1999;64(3):235-240. doi.org/10.1016/S0378-8741(98)00131-7

Lan X, Wang W, Li Q, Wang J. The Natural Flavonoid Pinocembrin: Molecular Targets and Potential Therapeutic Applications. Molecular neurobiology, 2016;53(3):1794-1801. doı 10.1007/s12035-015-9125-2

Moreno MIN, Isla MI, Sampietro AR, Vattuone MA. Comparison of the free radical-scavenging activity of propolis from several regions of Argentina. Journal of Ethnopharmacology, 2000;7(1):109–114. doi.org/10.1016/S0378-8741(99)00189-0

Nieva MMI, Isla MI, Cudmani NG, Vattuone MA, Sampietro AR. Screening of antibacterial activity of Amaicha del Valle (Tucuman, Argentina) propolis. Journal of ethnopharmacology, 1999;68(1-3):97-102. doi.org/10.1016/S0378-8741(99)00051-3

Pimenta HC, Violante IMP, Musıs CRD, Borges AH, Aranha AMF. In vitro effectiveness of Brazilian brown propolis against *Enterococcus faecalis*. Brazilian oral research, 2015;29:1-6. doi.org/10.1590/1807-3107BOR-2015.vol29.0058

Popova M, Dimitrova R, Al-Lawati HT, Tsvetkova I, Najdenski H, Bankova V. Omani propolis: chemical profiling, antibacterial activity and new propolis plant sources. Chemistry Central Journal, 2013;7(1):1-8. doi:10.1186/1752-153X-7-158

Przybyłek I, Karpiński TM. Antibacterial properties of propolis. Molecules. 2019;24(11):2047. doi.org/10.3390/molecules24112047

Ristivojević P, Dimkić I, Trifković J, Berić T, Vovk I, Milojković-Opsenica D, Stanković S. Antimicrobial activity of Serbian propolis evaluated by means of MIC, HPTLC, bioautography and chemometrics. PloS one, 2016;11(6):e0157097. doi.org/10.1371/journal.pone.0157097

Ruttner F. Biogeography and taxonomy of honeybees. Springer Science & Business Media, 2013.

Sforcin JM, Fernandes Jr A, Lopes CAM, Bankova V, Funari SRC. Seasonal effect on Brazilian propolis antibacterial activity. Journal of ethnopharmacology, 2000;73(1-2):243-249. doi.org/10.1016/S0378-8741(00)00320-2

Sforcin JM, Bankova V. Propolis: is there a potential for the development of new drugs?. Journal of ethnopharmacology, 2011;133(2):253-260. doi.org/10.1016/j.jep.2010.10.032

Sharifi S, Fathi N, Memar MY, Hosseiniyan Khatibi SM, Khalilov R, Negahdari R, ... & Maleki Dizaj S. Anti-microbial activity of curcumin nanoformulations: New trends and future perspectives. Phytotherapy Research, 2020;34(8):1926-1946. doi.org/10.1002/ptr.6658

Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic ccid reagents. American Journal of Enology and Viticulture, 1965;16(3):144–158. doi: 10.5344/ajev.1965.16.3.144

Speciale A, Costanzo R, Puglisi S, Musumeci R, Catania MR, Caccamo F, et al. Antibacterial activity of propolis and its active principles alone and in combination with macrolides, beta-lactams and fluoroquinolones against microorganisms responsible for respiratory infections. Journal of chemotherapy, 2006;18(2):164-171. doi.org/10.1179/joc.2006.18.2.164

Stepanovic S, Antic N, Dakic I, Svabic-Vlahovic M. In vitro antimicrobial activity of propolis and

synergism between propolis and antimicrobial drugs. Microbiological Research, 2003;158(4): 353-357. doi.org/10.1078/0944-5013-00215

Šuran J, Cepanec I, Mašek T, Radić B, Radić S, Tlak Gajger I, Vlainić J. Propolis extract and its bioactive compounds—From traditional to modern extraction technologies. Molecules, 2021;26(10):2930. doi.org/10.3390/molecules26102930

Takaisi-Kikuni NB, Schilcher H. Electron microscopic and microcalorimetric investigations of the possible mechanism of the antibacterial action of a defined propolis provenance. Planta medica, 1994;60(03):222-227. doi: 10.1055/s-2006-959463

Uzel A, Sorkun K, Önçag Ö, Çogulu D, Gençay Ö, Salih B. Chemical compositions and antimicrobial activities of four different Anatolian propolis samples. Microbiological Research. 2005;160(2): 189–195. doi.org/10.1016/j.micres.2005.01.002.

Veiga RS, De Mendonça S, Mendes PB, Paulino N, Mimica MJ, Lagareiro Netto AA, Lira IS, López BGC, Negrão V, Marcucci M.C. Artepillin C and phenolic compounds responsible for antimicrobial and antioxidant activity of green propolis and Baccharis dracunculifolia DC. Journal of Applied Microbiology, 2017;122(4):911-920. doi.org/10.1111/jam.13400

Vica ML, Glevitzky M, Tit DM, Behl T, Heghedus-Mîndru RC, Zaha DC, Ursu F, Popa M, Glevitzky I, Bungau S. The antimicrobial activity of honey and propolis extracts from the central region of Romania. Food Bioscience, 2021;41:101014. doi.org/10.1016/j.fbio.2021.101014

Vică ML, Glevitzky M, Heghedűş-Mîndru RC, Glevitzky I, Matei HV, Balici S, ... Teodoru CA. Potential Effects of Romanian Propolis Extracts against Pathogen Strains. International Journal of Environmental Research and Public Health, 2022;19(5):2640. doi.org/10.3390/ijerph19052640

Warfvinge J, Dahlen G, Bergenholtz G. Dental pulp response to bacterial cell wall material. Journal of Dental Research, 1985;64(8):1046-1050. doi.org/10.1177/00220345850640080401

Zabaiou N, Fouache A, Trousson A, Baron S, Zellagui A, Lahouel M, Lobaccaro JA. Biological properties of propolis extracts: Something new from an ancient product. Chemistry and physics of lipids, 2017;207:214-222. doi.org/10.1016/j.chemphyslip.2017.04.005

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ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

DISCOVERING THE CHEMICAL FACTORS BEHIND REGIONAL ROYAL JELLY DIFFERENCES VIA MACHINE LEARNING

Makine Öğrenimi Yoluyla Bölgesel Arı Sütü Farklarının Arkasındaki Kimyasal Faktörleri Keşfetmek

Aslı ÖZKÖK[1*], Merve KESKİN[2], Aslı Elif TANUĞUR SAMANCI[3], Elif YORULMAZ ÖNDER[3], Gökhan SİLAHTAROĞLU[4]

[*1]Bee and Bee Products Application and Research Center (HARUM), Hacettepe University, Ankara, TÜRKİYE, Yazışma Yazarı / Corresponding author: E-posta: aozkok@hacettepe.edu.tr, ORCID No: 0000-0002-7336-2892.

[2]Vocational School of Health Services, Bilecik Şeyh Edebali University, Bilecik, TÜRKİYE, E-posta: merveozdemirkeskin@gmail.com, ORCID No: 0000-0001-9365-334X.

[3]SBS Scientific Bio Solutions Co Bee&You Propolis, İstanbul, TÜRKİYE, E-posta: asli@sbs-turkey.com, ORCID No: 0000-0003-1639-6495, E-posta: elif.onder@sbs-turkey.com, ORCID No: 0000-0003-1990-0693

[4]Department of Management Information Systems, School of Business, Istanbul Medipol University, İstanbul, TÜRKİYE, E-posta: gsilahtaroglu@medipol.edu.tr, ORCID No: 0000-0001-8863-8348

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ÖZ

Bu çalışmanın amacı, makine öğrenmesi yoluyla arı sütünün bölgesini belirlemek için ayırt edici kimyasal faktörleri keşfetmektir. Çalışmada, Türkiye'nin 13 farklı bölgesinden 84 numune kullanılmış ve nem, pH, asitlik ve 10-hidroksi-2-dekanoik asit (10-HDA) kimyasal parametreleri incelenmiştir. 13 yerden toplanan arı sütleri arasında dört kimyasal değer açısından farklılık olup olmadığı ANOVA testi ile incelenmiştir. İstatistiksel testlere ek olarak, arı sütlerini birbirinden neyin ayırdığını keşfetmek için bir makine öğrenimi modeli kullanılmıştır. Arı sütü, kimyasal analiz sonuçlarının tanımlayıcı istatistikleri sırasıyla, nem %63,05±2,99, pH 3,67±0,08, asitlik 45,32±3,55 ve 10-HDA 2,40±0,24 olarak bulunmuştur. Şaşırtıcı bir şekilde, makine öğrenimi modeli, 10-HDA'nın arı sütünün bölgesini belirlemek için en belirgin parametre olabileceğini öne sürmektedir. Bu bilgi, arı sütünün doğruluğunun tespitini daha kolay öğrenmemize yardımcı olacaktır.

Anahtar Kelimeler: Arı sütü, bal arısı, makine öğrenimi, 10-HDA

ABSTRACT

This study aims to discover the characteristic chemical factors for determining the region of royal jelly using machine learning. 84 samples from 13 different regions of Turkey were used for the study, and the chemical parameters of moisture, pH, acidity, and 10-hydroxy-2-decanoic acid (10-HDA) were investigated. ANOVA test was conducted to determine whether there are differences between royal jelly from 13 locations concerning the four chemical values. In addition to the statistical tests, a machine learning model was used to find out what makes royal jelly different from each other. The descriptive statistics of the chemical analysis results of royal jelly showed the following values: moisture 63.05%±2.99, pH 3.67±0.08, acidity 45.32±3.55, and 10-HDA 2.40±0.24. Surprisingly, the machine learning model suggests that 10-HDA may be the most prominent parameter for determining the region of royal jelly. This information will help us identify royal jelly's authenticity more easily.

Keywords: Royal jelly, honeybee, machine learning, 10-HDA

GENİŞLETİLMİŞ ÖZET

Amaç: Bu çalışmanın amacı, arı sütünün bölgesel farklılıklarının ardındaki kimyasal faktörleri makine öğrenimi kullanarak keşfetmektir. Bu amaçla Türkiye'nin farklı lokasyonlarından toplanan arı sütü ürünlerinin kimyasal analiz sonuçları karşılaştırılmıştır.

Gereç ve yöntem: 2020 yılında Türkiye'nin 13 farklı ilinden toplam 84 adet arı sütü örneği toplanmıştır. Toplanan örnekler analize kadar -18°C'de saklanmıştır. Daha sonra kimyasal analizler yapılmıştır. Bu çalışmada kullanılan veriler arı sütünün kimyasal analiz sonuçlarından oluşmaktadır. Şekil 1'deki haritada gösterilen 13 şehir ismi hedef değişken olarak yapay zeka makine öğrenmesi algoritmalarına verilmiştir. Kullanılan kimyasal değerler nem, pH, asitlik ve 10-hidroksi-2-dekanoik asittir (10-HDA). Her spesifikasyon, karşılık gelen analizle etiketlenmiştir. Diğer bir deyişle, makine analizi biliyordu ve öğrenme sırasında dikkate alıyordu. Arı sütleri arasında kimyasal analiz sonuçları açısından anlamlı istatistiksel fark olup olmadığını belirlemek için ANOVA testi yapılmıştır. Nem, 10-HDA (%), pH ve asitlik açısından bölgeler arasında anlamlı bir fark olup olmadığını görmek için tek taraflı ANOVA testi yapılmıştır. Daha sonra, 13 farklı lokasyondan toplanan arı sütlerinin özelliklerinin ardındaki gizli kuralları çıkarmak için karar ağaçlarına sahip makine öğrenme algoritması kullanılmıştır. SMOTE, makine öğrenimi çalışmalarında yaygın olarak kullanılan sentetik bir veri geliştirme tekniğidir (Silahtaroğlu 2009). Veri seti %30 / %70 oranında bölünmüştür. Bu, verilerin %70'inin makineyi eğitmek için ve %30'unun sonuçları test etmek ve değerlendirmek için kullanıldığı anlamına gelmektedir. Doğruluk, kesinlik, özgüllük, Cronbach' Kappa ve F-değeri, makinenin arı sütünün yerini tahmin etmek için ne kadar iyi eğitildiğini ölçmek için kullanılmıştır.

Bulgular ve tartışma: Arı sütü, kimyasal analiz sonuçlarının tanımlayıcı istatistikleri Tablo 1'de gösterilmiştir. Tablo 1'e göre, aşağıdaki ortalama kimyasal analiz değerleri elde edilmiştir: Nem %63,05±2,99, pH 3,67±0,08, asitlik 45,32±3,55 ve 10-HDA 2,40 ±0,24. Arı sütünün bileşiminin mevsime, ekolojik koşullara ve toplandığı bölgenin özelliklerine bağlı olduğu bildirilmiştir (Zheng ve Hu 2010). Fransız arı sütü üzerinde yapılan araştırmaya göre nem içeriği %60-70, 10-HDA değeri ise %1,4 ile %3,7 arasında değişmektedir (Wytrychowski vd. 2013). Başka bir çalışmada Anadolu arı sütünün 10-HDA değerlerinin %1,0 ile %3,9 arasında, nem içeriğinin ise %62,6 ile %73 arasında değiştiği saptanmıştır (Kolaylı vd. 2015). Ayrıca arı sütünün uluslararası standardı olan ISO/DIS 12824 (2016) uyarınca arı sütünün 10-HDA değeri minimum %1,4, nem içeriği minimum %62 ile maksimum %68,5 arasında ve asitlik 30 ila 53 m.q g/kg arasında değişmektedir. Keskin vd. (2020), arı sütü örneklerinin 10-HDA içeriğinin %2,1 ile %2,6 arasında, nem içeriğinin ise %62,6 ile %66,5 arasında değiştiğini tespit etmiştir. Tüm bu çalışmaların sonuçları ile bizim çalışmamız benzerdir (Tablo 1).

Tablo 2'de görülebileceği gibi, Levene test istatistikleri, dört parametrenin tümü için eşit varyansların varsayılabileceğini göstermektedir. Öte yandan, Tablo 3'teki test istatistikleri, 10-HDA hariç, H1 için sıfır hipotezinin reddedildiğini, yani pH, asitlik ve nem açısından araçlar arasındaki tüm farkların istatistiksel olarak anlamlı olduğunu göstermektedir. Bununla birlikte, bir p-değeri = 0,296 ile sıfır hipotezi reddedilemez, dolayısıyla 10-HDA'ya göre popülasyon araçlarının hepsinin aynı olduğu sonucuna varırız. Şekil 2, oluşturulan karar ağacını göstermektedir. Makine tarafından oluşturulan karar ağacının kökü 10-HDA'dır (%). Değerinin 2,59'dan büyük olup olmamasına göre sola veya sağa yaylanır. Bu, arı sütünün yerini tahmin etmede en önemli faktörün 10-HDA (%) olduğu anlamına gelir. Bu parametrenin eşik değeri de 2,59'dur (Şekil 2). Ağaç, arı sütünün Balıkesir'de mi yoksa Konya'da mı hasat edildiğini tek başına 10-HDA (%) değerleri ile tahmin etmenin mümkün olduğunu göstermektedir. %2,59'a bağlı olarak 10-HDA, arı sütünün hasat edildiği yeri tahmin etmede en önemli faktördür. Ağaç değerlendirme istatistikleri Tablo 4'te gösterilmektedir. İstatistiklerin minimum %75 doğrulukla tatmin edici olduğu görülmektedir. Cohen'in kappa'sı da 0,50'den büyüktür, yani 0,73'tür.

Sonuç: Klasik tek yönlü analiz ANOVA, %10-HDA açısından siteler arasında ortalama bir fark olmadığını öne sürse de, makine öğrenimi karar ağacı, %10-HDA'nın siteler arasında ayrım yapması için eşik olarak 2,59 verir. Bu durum arı sütünün Balıkesir veya Konya'da %10-HDA değeri kullanılarak üretilip hasat edildiğini gösterebilir. Bu, arı sütünün orijinalliğini daha kolay tespit etmemize yardımcı olacaktır. Burada %10-HDA değeri arasındaki ilişkiyi ortaya koyabilmek için daha fazla veri içeren ileri çalışmalara ihtiyaç vardır. Çok fazla veriye sahip bir yapay zeka modeli, bu konuda daha

fazla bilgi sağlayabilir ve gelecekte yapay zeka aracılığıyla arı ürünlerinin doğruluğunu belirlemek için kullanılabilir.

INTRODUCTION

People's interest in natural products is increasing rapidly worldwide, increasing the popularity of bee products. Bee products are rare natural products with high biological activities and therapeutic properties. Royal jelly is also one of the important bee products and is a homogeneous, white or yellow colored, sharp smelling, sour taste, water-soluble substance secreted from the hypopharyngeal and mandibular glands of worker bees (Chinou 2014, Krell 1996, Melliou and Schmidt 1997, Tamura et al. 2009, Wytrychowski et al. 2013).

It was determined that the queen bee was constantly fed with royal jelly by Huber, and for this reason, it was named "royal jelly", which means queen meal in English for the first time in Switzerland in 1793 (Crane 1997). While worker bees are fed royal jelly in the first three days of their larval stages, the only food of the queen bee is royal jelly. As a result of feeding honey bee larvae with royal jelly, a productive and long-lived adult queen develops morphological and functional characteristics that other adult individuals in the hive do not have (Krell 1996, Melliou and Chinou 2014, Schmidt 1997, Wytrychowski et al. 2013). Throughout history, human beings have always been affected by this special effect of royal jelly on bees, and for this reason, royal jelly has been used as a food supplement and therapeutic for centuries.

Fresh royal jelly contains water (60-70%), proteins (9-18%), carbohydrates (7-18%), fatty acids and lipids (3-8%), minerals (about 1.5%), and small amounts of polyphenols and vitamins (Melliou and Chinou 2014). On the other hand, lyophilized royal jelly contains <5% water, 27-41% protein, 22-31% carbohydrates, and 15-30% fat (Melliou and Chinou 2014). The composition of royal jelly is similar when compared with different bee colonies and bee breeds (Krell 1996).

The biological activities of royal jelly vary depending on the number of trace elements in its content. Anti-aging (Salazar-Olivo and Paz-Gonzalez 2005), antioxidant (Liu et al. 2008), antibacterial (Fujiwara et al. 1990; Melliou and Chinou 2005), antitumor (Salazar-Olivo and Paz-Gonzalez 2005, Tamura et al. 1987) antihypertensive (Tokunaga et al. 2004), immunomodulatory (Vucevic et al. 2007), anti-inflammatory (Kohno et al. 2004), liver protective (Kanbur et al. 2009), osteoporosis preventative (Hidaka et al. 2006) properties have attracted attention in recent years. In addition, it is known that people who use royal jelly develop a general sense of well-being in a short time and develop higher learning capacity, fatigue resistance, and better memory (Krell, 1996). In other words, royal jelly appears to be a general stimulant that improves the immune system and general body functions (Krell, 1996).

Royal jelly contains short-chain hydroxy fatty acids, which are not found in other foods, and are claimed to have anti-tumor, anti-bacterial and immunoregulatory activity and hormonal activity (Ramadan and Al-Ghamdi 2012, Terada et al. 2011). Royal jelly fatty acids are mostly short-chain fatty acids, such as dicarboxylic acids with 8-10 carbons, which are not found in most plants and animals (Ramadan and Al-Ghamdi 2012). Trans-10-hydroxy-2-decanoic acid (10-HDA), which is considered the major fatty acid of royal jelly, is considered a marker for royal jelly and is found in royal jelly at a rate of 0.5-3.5% of its dry weight (Garcia- Amoedo and Almeida-Muradian 2007, Ramadan and Al-Ghamdi 2012, Kamakura et al. 2001, Kanelis et al. 2015, Liu et al. 2008, Yukunc 2019). However, it is also stated that in a quality royal jelly, the 10-HDA should be at least 1.4 and the moisture content should be between 60% and 70% (Bogdanov and Gallmann 2008).

The term machine learning was coined in 1959 by American Arthur Samuel, a pioneer in computer games and artificial intelligence and an IBM employee, and machine learning or machine learning refers to the design and development processes of algorithms that enable computers to learn based on data types such as sensor data or databases. It is a science that deals with the subject. The focus of machine learning research is to give computers the ability to detect complex patterns and make rational decisions based on data. This shows that machine learning is closely related to fields such as statistics, probability theory, data mining, pattern recognition, artificial intelligence, adaptive control, and theoretical computer science (Alpaydin 2020, Arthur 1959, Kohavi and Provost 1998).

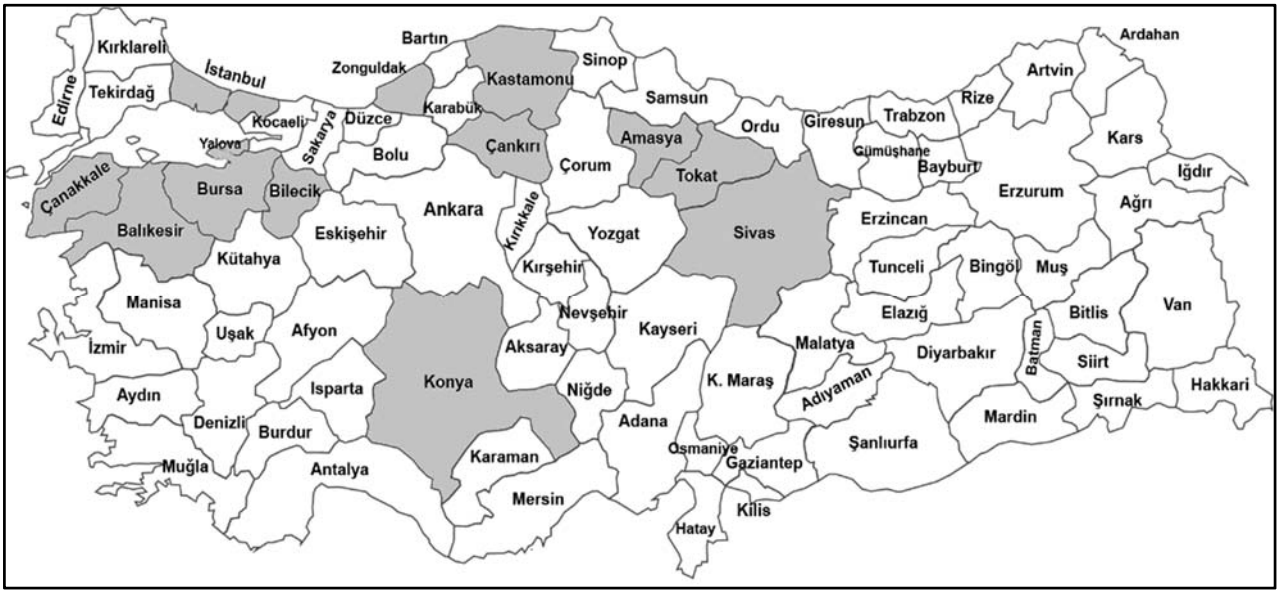
This study aims to discover the chemical factors behind the regional differences of royal jelly through machine learning. To achieve this, we collected,

compared, and evaluated the results of chemical analysis of royal jelly products harvested in various locations in Turkey.

MATERIAL AND METHODS

Collection and storage of royal jelly samples

A total of 84 royal jelly samples were collected from 13 different provinces of Turkey in 2020 (Figure 1). Collected samples were stored at -18°C until analysis. Then, chemical analyses were made.



Şekil 1. Arı sütü toplanan bölgeler

Figure 1. Royal jelly collected regions

Chemical analysis of samples and data collection

The data used in this study consists of the results of the chemical analysis of royal jelly. The 13 city names shown on the map in Figure 1, are given as target variables to artificial intelligence machine learning algorithms. The chemical values used are moisture, pH, acidity, and 10-hydroxy-2-decanoic acid (10-HDA). Each data was marked with the corresponding analysis.

Moisture analysis

For moisture determination, 1 g of each sample was weighed. It was then incubated at 105°C for 3 hours. At the end of the period, the royal jelly samples were cooled in the desiccator until they reached a constant weight. This process was repeated three times for each sample (Horwitz and Latimer 2000).

pH analysis

The pH and acidity of the royal jelly samples were determined according to ISO/DIS 12824 (2016). 1 g of royal jelly sample was weighed and placed in a 100 mL beaker and 75 mL of boiled and cooled distilled water was added. It was titrated with sodium hydroxide standard solution (c = 0.1 mol/L). The endpoint was obtained when the acidometer showed a pH of 8.3.

Acidity analysis

To obtain the sample's acidity, the milliliter amount of sodium hydroxide standard solution consumed in the titration is multiplied by the concentration value (mol/L), divided by the sample's mass, and multiplied by 100. The acidity of royal jelly is calculated with the following equation:

Acidity [(1 mol/l NaOH) mL/100g] = (V x c x 100) / m

V is the volume of 0.1 mol/L NaOH standard solution consumed in the titration in milliliters. c is the concentration of the NaOH standard solution in mol/L; m is the mass of the sample in grams.

10-Hydroxy-2-decanoic acid (10-HDA) analysis

The amount of 10-HDA was determined by ultraviolet absorbing detector high-performance liquid chromatography (HPLC, VWR Hitachi Chromaster HPLC-UV). Measurements were made at 215 nm to obtain the highest absorbance value (Kim and Lee 2010). The mobile phase of the C-18 (150 x 4.6 mm) column was optimized for methanol, water, and phosphoric acid (55:45:2.2) and the flow rate was 1.0 mL min-1 and the injection volume was 3 µL. Liquid methyl 4-hydroxybenzoate (MHB) was used as a standard. Calibration solutions were obtained by diluting 160 µg mL^{-1} of stock 10-HDA solution (0.1, 0.5, 1.0, 5.0, 10, 20, 40, 80, and 160 µg mL-1). The R^2 value of the obtained calibration graph was determined as 0.9998.

Statistical analysis and machine learning

ANOVA test was performed to see if there was a statistically significant difference between royal jellies in terms of chemical analysis results. One-way ANOVA was performed to test whether there was a significant difference between locations in terms of humidity, 10-HDA (%), pH, and acidity.

H0: There is a significant difference between locations in terms of humidity, 10-HDA (%), pH, and acidity averages.

H1: There is no statistically significant difference between the means.

Then the hidden rules behind the properties of the royal jelly harvested from 13 different locations were extracted using the Decision Tree Machine Learning algorithm.

The purpose of the study analysis is to find hidden rules that will allow the machine to predict the location of the royal jelly using the four chemical analysis values. For this purpose, Gini and Gain (entropy-based) decision tree algorithms were used. Decision trees are widely used in machine learning to enable machine learning and predict class value. They are preferred to "deep learning" and "support vector machines" because they create rules that humans can understand. Decision Trees are among the most common and powerful algorithms used especially in classification and regression tasks. They are used to build models that can predict the value of the target variable based on input properties. Decision trees are created by dividing the data using a hierarchical structure according to different feature values and making predictions based on the resulting datasets. Decision Trees are used in two main types: “Classification Trees” and “Regression Trees”. Classification Trees are used for categorical or discrete target variables, while Regression Trees are used for continuous target variables. Both types of trees are created using a similar process. This process is done by separating the data into smaller subsets based on different property values. There are many pollution calculations for decision trees. Gini and entropy are two of them. Their ability to handle missing data and their ability to be used for feature selection is a great advantage (Schug et al. 2005, Shafer et al. 1996, Silahtaroğlu 2013). For this reason, these algorithms were preferred in the study.

Since the number of royal jellies was not evenly distributed across the 13 locations, SMOTE was used to eliminate the class imbalance. SMOTE is a synthetic data augmentation technique that is widely used in Machine Learning studies (Silahtaroğlu 2009). The dataset is partitioned at 30% / 70%. This means that 70% of the data is used to train the machine and 30% is used to test and evaluate the results. Accuracy, Sensitivity, Specificity, Cronbach Kappa, and F-Value were used to measure how well the machine was trained to estimate the location of royal jelly.

RESULTS

Descriptive statistics of the chemical analysis results of royal jelly are given in Table 1. According to Table 1, the mean chemical analysis results were found as follows, respectively: Humidity 63.05% ±2.99, pH 3.67±0.08, acidity 45.32 ±3.55 and 10-HDA 2.40±0.24.

Table 1. Descripitve statistics of the royal jelly samples

Tablo 1. Arı sütü örneklerinin tanımlayıcı istatistikleri

Parameter	Min.	Mean	Max.	Std. Dev.	Skewness	Kurtosis	No. Missing
Nem (%)	53.29	63.05	69.58	2.99	-0.59	1.18	0
pH	3.50	3.67	3.8	0.08	-0.52	-0.05	0
Asitlik (m.q. g/kg)	38.0	45.32	58	3.55	0.76	1.06	0
10-HDA (%)	1.46	2.40	3.06	0.24	-0.49	2.16	0

As can be seen in Table 2, Levene's test statistics show that equal variances can be assumed for all four parameters. On the other hand, the test statistics in Table 3 show that the null hypothesis is rejected on behalf of H1, excluding 10-HDA, that is, any of the differences between the means are statistically significant in terms of pH, acidity, and humidity. However, with a p-value = 0.296, we fail to reject the null hypothesis and conclude that the population means are all equal in terms of 10-HDA.

Table 2. Test of homogeneity of variances

Tablo 2. Varyansların homojenliği testi

		Levene Statistic	df1	df2	Sig.
Moisture	**Based on Mean**	1.244	9	70	.283
	Based on Median	.825	9	70	.595
	Based on the Median and with adjusted df	.825	9	54.611	.596
	Based on trimmed mean	1.191	9	70	.314
pH	**Based on Mean**	1.050	9	70	.410
	Based on Median	.467	9	70	.892
	Based on the Median and with adjusted df	.467	9	57.491	.891
	Based on trimmed mean	.980	9	70	.464
Acidity	**Based on Mean**	.639	9	70	.760
	Based on Median	.604	9	70	.789
	Based on the Median and with adjusted df	.604	9	53.631	.788
	Based on trimmed mean	.626	9	70	.771
10-HDA	**Based on Mean**	1.871	9	70	.071
	Based on Median	1.141	9	70	.346
	Based on the Median and with adjusted df	1.141	9	39.333	.358
	Based on trimmed mean	1.840	9	70	.076

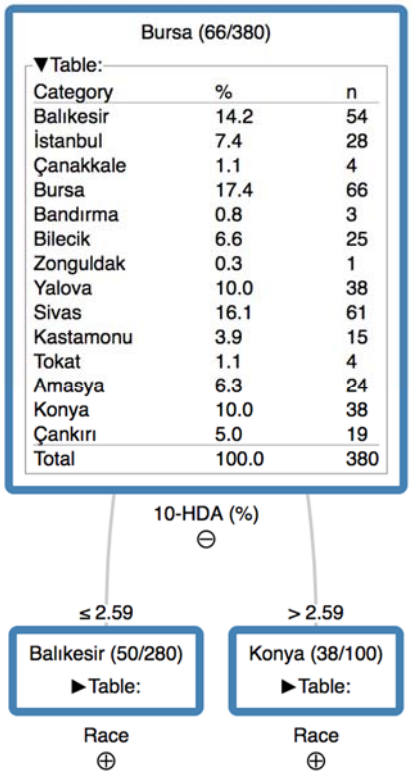
Table 3. ANOVA results
Tablo 3. ANOVA sonuçları

		Sum of Squares	df	Mean Square	F	Sig.
Moisture	**Between Groups**	218.312	13	16.793	2.237	.016
	Within Groups	525.389	70	7.506		
	Total	743.701	83			
pH	**Between Groups**	.179	13	.014	2.550	.006
	Within Groups	.379	70	.005		
	Total	.558	83			
Acidity	**Between Groups**	289.463	13	22.266	2.065	.027
	Within Groups	754.859	70	10.784		
	Total	1044.321	83			
10-HDA	**Between Groups**	.908	13	.070	1.203	.296
	Within Groups	4.065	70	.058		
	Total	4.973	83			

Table 4. Decision tree results
Tablo 4 Karar ağacı sonuçları

Region	True Pos	False Pos	True Neg	False Neg	Recall	Precision	Sensitivity	Specifity	F- Measure
Balıkesir	7.00	0.00	84.00	2.00	0.78	1.00	0.78	1.00	0.88
Tokat	3.00	4.00	81.00	5.00	0.38	0.43	0.38	0.95	0.40
Bursa	6.00	0.00	82.00	5.00	0.55	1.00	0.55	1.00	0.71
Bandırma	1.00	5.00	84.00	3.00	0.25	0.17	0.25	0.94	0.20
Zonguldak	0.00	6.00	83.00	0.00	0.00	0.00	0.00	0.93	NaN
Sivas	6.00	0.00	87.00	0.00	1.00	1.00	1.00	1.00	1.00
Kastamonu	6.00	1.00	86.00	0.00	1.00	0.86	1.00	0.99	0.92
Çanakkale	3.00	4.00	85.00	1.00	0.75	0.43	0.75	0.96	0.55
Konya	7.00	0.00	85.00	1.00	0.88	1.00	0.88	1.00	0.93
Çankırı	5.00	1.00	86.00	1.00	0.83	0.83	0.83	0.99	0.83
Amasya	5.00	2.00	86.00	0.00	1.00	0.71	1.00	0.98	0.83
Bilecik	7.00	0.00	86.00	0.00	1.00	1.00	1.00	1.00	1.00
Yalova	7.00	0.00	85.00	1.00	0.88	1.00	0.88	1.00	0.93
İstanbul	7.00	0.00	86.00	0.00	1.00	1.00	1.00	1.00	1.00

Table 4 and Figure 2 show the machine-generated decision tree results. The root of the machine-generated decision tree is 10-HDA. The tree arcs to the right or left depending on whether the percentage value is greater than 2.59 or not. This means that the most important factor for estimating the location of royal jelly is 10-HDA, and the cut-off point of this parameter is 2.59 (Figure 2). Tree suggests that with 10-HDA values alone it is possible to predict whether royal jelly was harvested in Balikesir or Konya. Based on 2.59%, 10-HDA is the most important factor in estimating the location of royal jelly.



Şekil 2. Karar ağacının kökü

Figure 2. The root of the decision tree

Tree evaluation statistics are shown in Table 5. The statistics appear to be satisfactory with a minimum accuracy of 75%. Cohen's kappa is also greater than 0.50, meaning 0.73.

Table 5. Decision tree evaluation statistics

Tablo 5. Karar ağacı değerlendirme istatistikleri

Precision	Sensivity	Specifity
0.74	0.73	0.98
F-measure	Accuracy	Cohen's kappa
0.78	0.75	0.73

DISCUSSION

It is stated that the composition of royal jelly depends on the season, ecological conditions, and the characteristics of the region where it is collected (Zheng and Hu 2010). In a study conducted with French royal jelly, it was reported that the moisture content varies between 60% and 70%, and the 10-HDA value varies between 1.4% and 3.7% (Wytrychowski et al. 2013). In another study, it was stated that the 10-HDA values of Anatolian royal jelly varied between 1.0% and 3.9%, and the moisture content between 62.6% and 73% (Kolaylı et al. 2015). In addition, according to the international royal jelly standard ISO/DIS 12824 (2016), the 10-HDA value of royal jelly should be between at least 1.4%, its moisture value between at least 62% and a maximum of 68.5%, and its acidity value between 30 and 53 m.q. It is stated that it should be between g / kg. Keskin et al. (2020), it was determined that the 10-HDA content of royal jelly samples varied between 2.1% and 2.6%, and the moisture content varied between 62.6% and 66.5%. The results of all these studies and ours are similar (Table 1).

A study by Yavuz and Gürel (2017) determined that the 10-HDA content of commercial royal jelly ranged between 0.75% and 3.11%, and the moisture content between 63.1% and 73.5%. However, according to the international royal jelly standard (ISO/DIS 12824, 2016), the 10-HDA value of royal

jelly should be at least 1.4%. It has been stated that royal jelly containing 10-HDA below this value is likely to be a product with additives or adulteration (Yavuz and Gürel 2017). In this case, the machine learning method can be a guide in determining the accuracy of royal jelly.

As the success of data mining and machine learning algorithms has increased, these data analysis methods and tools have been used in many fields such as agriculture, beekeeping, and quality and yield estimation in recent years. These studies cover both supervised learning (clustering) and unsupervised learning (classification and prediction) models of machine learning and data mining. In one of the studies conducted in the field of beekeeping in recent years, a face-to-face survey was conducted with 62 beekeepers and the decision tree model, which is one of the data mining methods, was used to determine the factors affecting the cooperative membership of beekeepers. As a result of the statistical analysis, of the cooperative members of the beekeepers; It was concluded that credit utilization status, education level, and beekeeping support have a significant effect on the status (Çukur and Çukur 2022). In another study, the decision tree method was used to monitor honey bee health (Edwards-Murphy et al. 2016). Aksoy et al. (2018) conducted a study on the estimation of honey production in beekeeping enterprises located in eastern Turkey. In this study, they aimed to determine the main factors contributing to honey production and to develop a model that can accurately predict honey production in the region by using different data such as colony number, cultivated land area, and plant species. In the study using Multiple Adaptive Regression Splines (MARS), Classification and Regression Tree (CART), and (Chi-squared Automatic Interaction Detector) CHAID algorithms in honey yield per hive, it was determined that MARS (r=0.920) outperformed other approaches. In a study by Karadas and Kadirhanogullari (2017), data mining and artificial neural network algorithms were used to determine the amount of honey production. In another study, a method based on clustering and classification techniques was developed to develop seasonal honey bee models that can be customized and integrated into the computer system for remote monitoring of beehives. In the model, a clustering technique was applied that monitors the brood temperature, relative humidity, and weight of the beehives. Naive Bayes, k-NN, and Random Forest algorithms were compared to propose a high-accuracy classification model (99.67%) that recommends seasonal honey bee patterns for the remote monitoring system (Rafael Braga et al. 2020).

Beekeepers perform inspection procedures that include identifying potential problems or overall colony health in the colonies. If the inspections are not done properly, it may disrupt the microclimate balance in the hive and the work of the worker bees. Classification algorithms were used in the study, which states that these controls can be made and the health status of honey bee colonies can be predicted with high precision (Rafael Braga et al. 2020, Robles-Guerrero et al. 2017).

Data mining and machine learning algorithms are also used to detect fatal diseases of honey bees. In a study, artificial neural networks and Support Vector Machines (SVM) algorithms were used in the detection of Nosema, which is known as a common fatal disease, and disease detection was successfully performed with artificial neural networks with an accuracy of 96.25% (Dghim et al. 2021).

In our study, it has been shown that the location of the harvest and other ecological conditions can be determined by examining the composition of royal jelly. For this purpose, moisture, pH, acidity, and 10-HDA values, which are among the components of royal jelly, were used. The study has developed and introduced a smart system that predicts the harvested location with high accuracy using the composition values of the collected royal jelly samples with the decision tree method, which is one of the machine learning methods. In the results, it was seen that the 10-HDA value from the royal jelly components is effective in determining the geographical location and the harvested location can be determined with more than 75% accuracy by using the 10-HDA value. Thanks to the developed smart system, the location of the received royal jelly sample could be determined within seconds.

Conclusion

Although classical one-way ANOVA suggests no mean difference between locations in terms of 10%-HDA, the machine learning decision tree gives 2.59 as a cut-off point for 10%-HDA in distinguishing locations. This situation can reveal that royal jelly was produced and harvested in Balıkesir or Konya using a 10%-HDA value. Thus, it will contribute to our learning of the originality of royal jelly more

easily. Here, more studies with more data are needed to reveal the correlation between the 10%-HDA value. This preliminary study, which we have done on the adaptation of bee products to the machine learning path, is the first study conducted in Turkey. An artificial intelligence model with a larger amount of data can provide more information on this issue and can be used in the future to determine the accuracy of artificial intelligence bee products.

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REFERENCES

Aksoy A, Ertürk YE, Erdoğan S, Eyduran E, Tariq MM. Estimation of honey production in beekeeping enterprises from the eastern part of Turkey through some data mining algorithms. Pak. J. Zool. 2018;50(6):2199-2207, http://dx.doi.org/10.17582/journal.pjz/2018.50.6.2199.2207.

Alpaydin E. Introduction to machine learning (Fourth bas.) MIT. Ss.xix, 2020, 1-3, 13-18.

Arthur S. Some studies in machine learning using the game of checkers. IBM J. Res. Devep. 1959;3(3):210-229, doi:10.1147/rd.33.0210.

Bogdanov S, Gallmann P. Authenticity of Honey and Other Bee Products State of the Art, ALP Science Switzerland, 2008, No.520,p.1-12.

Crane E. The Past and Present Importance of Bee Products to Man, A. Mizrahi, Y. Lensky. (eds.), Bee Products. Springer Science & Business Media New York. 1997,p.1-15.

Çukur T, Çukur F. Determining Factors Affecting Cooperative Membership of the Beekeepers Using Decision Tree Algorithms. Tarim Bilim. Derg. 2022;28(1):25-32, https://doi.org/10.15832/ankutbd.739230

Dghim S, Travieso-González CM, Burget R. Analysis of the Nosema cells identification for microscopic images. Sensors, 2021;21(9):3068, https://doi.org/10.3390/s21093068.

Edwards-Murphy F, Magno M, Whelan PM, O'Halloran J, Popovici EM. b+ WSN: Smart beehive with preliminary decision tree analysis for agriculture and honey bee health monitoring. Comput. Electron. Agric. 2016;124:211-219, https://doi.org/10.1016/j.compag.2016.04.008.

Fujiwara S, Imai J, Fujiwara M, Yaeshima T, Kawashima T, Kobayashi K. A potent antibacterial protein in royal jelly. Purification and determination of the primary structure of royalisin. J. Biol. Chem. 1990;265(19):11333-11337.

Garcia-Amoedo LH, Almeida-Muradian LB. Physicochemical composition of pure and adulterated royal jelly. Quím. Nova, 2007;30(2):257-259, https://doi.org/10.1590/S0100-40422007000200002.

Hidaka S, Okamoto Y, Uchiyama S, Nakatsuma A, Hashimoto K, Ohnishi ST, Yamaguchi M. Royal jelly prevents osteoporosis in rats: beneficial effects in ovariectomy model and in bone tissue culture model. Evid. Based Complementary and Altern. Med. 2006;3(3):339-48, doi: 10.1093/ecam/nel019

Horwitz W, Latimer G. Official methods of analysis of AOAC International. Gaithersburg MA, USA: Association of Official Analytical Chemist. 2000.

Kamakura M, Mitani N, Fukuda T, Fukushima M. Antifatigue effect of fresh royal jelly in mice. J. Nutr. Sci. Vitaminol. 2001;47(6):394-401, doi: 10.3177/jnsv.47.394.

Kanbur M, Eraslan G, Beyaz L, Silici S, Liman BC, Altinordulu S, Atasever A. The effects of royal jelly on liver damage induced by paracetamol in mice. Exp. Toxicol. Pathol. 2009;61(2):123-132, doi: 10.1016/j.etp.2008.06.003.

Kanelis D, Tananaki C, Liolios V, Dimou M, Goras G, Rodopoulou MA, Karazafiris E, Thrasyvoulou A. A suggestion for royal jelly

specifications. Arh. Hig. Rada. Toksikol. 2015;66(4):275-284, doi: 10.1515/aiht-2015-66-2651.

Karadas K, Kadirhanogullari IH. Predicting honey production using data mining and artificial neural network algorithms in apiculture. Pak. J. Zool. 2017;49(5):1611-1619, DOI:10.17582/journal.pjz/2017.49.5.1611.1619.

Keskin M, Özkök A, Karahalil F, Kolaylı S. What should be the amount of 10-Hydroxi-2-Decanoic Acid (10-HDA) in royal jelly? Mediterr. Agric. Sci. 2020;33(3):347-350, https://doi.org/10.29136/mediterranean.698926.

Kim J, Lee J. Quantitative analysis of trans-10-hydroxy decanoic acid in royal jelly products purchased in the USA by high-performance liquid chromatography. J. Agric. Food Chem. 2010; 54:77-86.

Kohavi R, Provost F. Glossary of terms. Mach. Learn. 1998;20(2-3):271-274.

Kohno K, Okamoto I, Sano O, Arai N, Iwaki K, Ikeda M, Kurimoto M. Royal jelly inhibits the production of proinflammatory cytokines by activated macrophages. J. Biosci. Biotechnol. Biochem. 2004;68(1):138-145, doi: 10.1271/bbb.68.138.

Kolaylı S, Sahin H, Can Z, Yildiz O, Malkoc M, Asadov A. Member of complementary medicinal food: Anatolian royal jellies, their chemical compositions, and antioxidant properties. Evid. Based Complementary and Altern. Med. 2015;21(4):43-48, doi: 10.1177/2156587215618832.

Krell, R. Value-Added Products from Beekeeping, Fao Agricultural Services Bulletin No. 124, Food and Agriculture Organization of the United Nations Rome. 1996.

Liu JR, Yang YC, Shi L, Peng C. Antioxidant properties of royal jelly associated with larval age and time of harvest. J. Agric. Food Chem. 2008;56(23):11447-11452, doi: 10.1021/jf802494e.

Melliou E, Chinou I. Chemistry and bioactivities of royal jelly. Stud. Nat. Prod. Chem., 2014;43: 261-290, https://doi.org/10.1016/B978-0-444-63430-6.00008-4.

Melliou E, Chinou I. Chemistry and bioactivity of royal jelly from Greece. J. Agric. Food Chem. 2005;53(23):8987-8992, doi: 10.1021/jf051550p.

Rafael Braga A, Gomes DG, Freitas BM, Cazier JA. A cluster-classification method for accurate mining of seasonal honey bee patterns. Ecol. Inform. 2020;59:101107, DOI:10.1016/j.ecoinf.2020.101107

Ramadan MF, Al-Ghamdi A. Bioactive compounds and health-promoting properties of royal jelly: A Review. J. Funct. Foods. 2012;4(1):39-52, https://doi.org/10.1016/j.jff.2011.12.007

Robles-Guerrero A, Saucedo-Anaya T, González-Ramérez E, Galván-Tejada CE. Frequency Analysis of Honey Bee Buzz for Automatic Recognition of Health Status: A Preliminary Study. Res. Comput. Sci. 2017;142:89-98, DOI:10.13053/RCS-412-1-9.

Salazar-Olivo LA, Paz-Gonzalez V.Screening of biological activities present in honeybee (*Apis mellifera*) royal jelly. Toxicol. In Vitro, 2005;19(5):645-651, doi: 10.1016/j.tiv.2005.03.001.

Schmidt JO. Bee Products: Chemical Composition and Application, A. Mizrahi&Y. Lensky. (eds.), Bee Products. Springer Science&Business Media New York; 1997, p.15-25.

Schug J, Schuller WP, Kappen C, Salbaum JM, Bucan M, Stoeckert CJ. Promoter features related to tissue specificity as measured by Shannon entropy. Genome Biol. 2005;6(4):1-24.

Shafer J, Agrawal R, Mehta M. SPRINT: A scalable parallel classifier for data mining. VLDB, 1996;96: 544-555.

Silahtaroğlu G. An attribute-center-based decision tree classification algorithm. World Academy of Science, J. Eng. Technol. 2009;56:302-306, https://doi.org/10.5281/zenodo.1084171

Silahtaroğlu G. Veri madenciliği: Kavram ve algoritmaları. Papatya Bilim, 2013, s.300.

Tamura S, Amano S, Kono T, Kondoh J, Yamaguchi K, Kobayashi S. Molecular characteristics and physiological functions of major royal jelly protein 1 oligomer. *Proteomics,* 2009;9:5534-5543.

Tamura T, Fujii A, Kuboyama N. Antitumor effects of royal jelly (RJ). Nihon Yakurigaku Zasshi, 1987; 89(2):73-80.

Terada Y, Narukawa M, Watanabe T. Specific

hydroxy fatty acids in royal jelly activate TRPA1. J. Agric. Food Chem. 2011;59(6):2627-2635, DOI: 10.1021/jf1041646

Tokunaga KH, Yoshida C, Suzuki KM, Maruyama H, Futamura Y, Araki Y, Mishima S. Antihypertensive effect of peptides from royal jelly in spontaneously hypertensive rats. Biol. Pharm. Bull. 2004;27(2):189-192, DOI: 10.1248/bpb.27.189

Vucevic D, Melliou E, Vasilijic S, Gasic S, Ivanovski P, Chinou I, Colic M. Fatty acids isolated from royal jelly modulate the dendritic cell-mediated immune response in vitro. Int. Immunopharmacol. 2007;7(9):1211-1220, DOI: 10.1016/j.intimp.2007.05.005

Wytrychowski M, Chenavas S, Daniele G, Casabianca H, Batteau M, Guibert S, Brion B. Physicochemical characterization of French royal jelly: Comparison with commercial royal jellies and royal jellies produced through artificial bee-feeding. J. Food Compos. Anal. 2013;29(2):126-133, DOI: 10.1016/j.jfca.2012.12.002

Yavuz İ, Gürel F. Chemical properties of the royal jellies in Turkish markets. Mediterr. Agric. Sci. 2017.30(3): 281-285.

Yukunc GO. Royal jelly: Proteins and peptides. J. Apit. Nat. 2019;2(2):59-70.

Zheng HQ, Hu FL, Dietemann, V. Changes in the composition of royal jelly harvested at different times: Consequences for quality standards. Apidologie, 2010;2(1):39-47.

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ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

COĞRAFİ BİLGİ SİSTEMİ ve AHP ile ARICILIK FAALİYET ALANLARI İÇİN ARAZİ UYGUNLUK DEĞERLENDİRMESİ: BİTLİS/TÜRKİYE ÖRNEĞİ

Land Suitability Assessment for Apiculture (Beekeeping) Activity Areas Using Geographic Information System and AHP: A Case Study Bitlis/Türkiye

Çağrı MERCAN

Mardin Artuklu Üniversitesi, Savur Meslek Yüksekokulu, Harita ve Kadastro Programı, 47860, Mardin, TÜRKİYE, ORCID No: 0000-0003-1694-0024, E-posta: cagrimercan@artuklu.edu.tr

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ÖZ

Arıcılık, biyoçeşitliliğe katkı sunarak kırsal kalkınmaya sağladığı destekten ötürü önemli bir faaliyet türüdür. Arıcılıktan elde edilen verimin arttırılabilmesi ve sürdürülebilirlik için bu faaliyet türünün yapılabileceği uygun yerlerin belirlenmesi gerekmektedir. Yapılan bu çalışmada, Bitlis ilinde Coğrafi Bilgi Sistemi (CBS) ve Çok Kriterli Karar Verme (ÇKKV) yöntemleri kullanılarak arıcılık için bir yer seçimi değerlendirme modeli önerilmektedir. Çalışmanın amacı yerel arıcılık faaliyetleri ile uğraşan kişilerin yanı sıra literatür verilerini de dikkate alan çok kriterli değerlendirmeye dayalı mekânsal bir karar destek sistemi oluşturmaktır. Çalışma ile Bitlis ili için arıcılığın yapılabileceği uygun alanlar belirlenmiştir. Çalışmada 11 kriter (84 alt kriter) seçilmiştir. Arıcılık faaliyetlerini olumsuz etkileyeceği için 5 alt kriter ise sınırlandırıcı olarak değerlendirilmiştir. Çalışmada arazi kullanımı/örtüsü, akarsulara mesafe, ortalama sıcaklık (mayıs-ağustos), NDVI, rüzgâr hızı (mayıs-ağustos), bakı, yükseklik, yağış (mayıs-ağustos), eğim, yola uzaklık ve elektrik hatlarına uzaklık kriterleri kullanılmıştır ve bu kriterlere ait tematik haritalar oluşturulmuştur. Değerlendirme kriterlerinin ağırlıklarının hesaplanmasında AHP yöntemi kullanılmıştır ve CBS ortamında ağırlıklı bindirme yöntemi ile arazi uygunluk haritası elde edilmiştir. Arazi uygunluk haritasında arıcılığın yapılabileceği çok uygun ve uygun alanların sırasıyla 1.620,02 km^2 ve 2.003,81 km^2, yüzey alanlarına sahip olduğu belirlenmiştir. Oluşturulan uygunluk haritasında en uygun yerlerin sırasıyla Mutki, Merkez, Hizan, Tatvan, Ahlat, Güroymak ve Adilcevaz ilçelerinde olduğu belirlenmiştir. Bu çalışma, arıcılık faaliyetleri ile uğraşan insanların haricinde sürdürülebilir tarım ve hayvancılık stratejilerinin oluşturulmasında, karar vericiler için de önemli bir kılavuz olacaktır.

Anahtar Kelimeler: Analitik Hiyerarşi Yöntemi (AHP), Arıcılık, Çok Kriterli Karar Verme, Uygunluk Analizi

ABSTRACT

Apiculture (Beekeeping) is a type of activity that stands out because it supports rural development by contributing to biodiversity. In order to increase the efficiency and sustainability of beekeeping, it is necessary to determine the suitable areas where this type of activity can be carried out. This study proposes a site selection evaluation model for beekeeping using Geographic Information System (GIS), and Multi-Criteria Decision Making (MCDM) methods in Bitlis province. The study aims to create a spatial decision support system based on the multi-criteria evaluation that considers the literature data and experts dealing with local beekeeping activities. The study determined suitable areas where beekeeping can be done in the province of Bitlis. 11 criteria (84 sub-criteria) were determined in the study. Five sub-criteria were considered restricted as they would adversely affect beekeeping

activities. In the study, land use/cover, distance to rivers, average temperature (May to August), NDVI, wind speed (May to August), aspect, elevation, precipitation (May to August), slope, distance to road, and distance to power lines were used. Thematic maps belonging to the criteria were created. AHP was used to calculate the weights of the evaluation criteria, and a land suitability map was obtained using the weighted overlay method in the GIS environment. In the land suitability map, it has been determined that very suitable and suitable areas where beekeeping can be done have surface areas of 1.620,02 km^2 **and** 2.003,81 km^2**, respectively. The suitability map created determined that the most suitable places were in Mutki, Merkez, Hizan, Tatvan, Ahlat, Güroymak and Adilcevaz districts, respectively. This study will be an essential guide for decision-makers in creating sustainable agriculture and livestock strategies, apart from people dealing with beekeeping activities.**

Keywords: Analytical Hierarchy Process (AHP), Beekeeping, Multi-Criteria Decision Making, Suitability Analysis

EXTENDED ABSTRACT

Aim: Apiculture (Beekeeping) is an essential type of activity due to many reasons, such as low cost and labor requirements, easy marketing, and helping pollinate plants. Increasing production efficiency due to this activity is very important for sustainable rural development. For this purpose, it is crucial to determine the suitable beekeeping areas. This study aims to propose a site selection evaluation model for beekeeping using Geographic Information System (GIS), and Multi-Criteria Decision Making (MCDM) methods. The study aims to create a spatial decision support system based on the multi-criteria evaluation that considers the data of the people dealing with local beekeeping activities and the literature. This study it is aimed to provide the most suitable beekeeping places to people who carry out local beekeeping activities to increase their productivity and support rural development. This study will also guide people who plan for the future in agriculture and animal husbandry.

Material and Method: Many criteria affect the selection of places where beekeeping activities can be carried out most appropriately. Evaluating these criteria and their sub-criteria and producing a result is a complicated process. Multi-Criteria Decision Making methods can ease challenges by providing an analytical decision-making solution. Analytical Hierarchy Process (AHP) is a Multi-Criteria Decision Making method widely used in the land suitable analysis. This study was determined by 11 criteria (84 sub-criteria) based on local experts and literature data. Five sub-criteria that may be negative for beekeeping activities were determined and evaluated as limiting criteria (restricted). Land use/cover, distance to rivers, average temperature (May-August), NDVI, wind speed (May-August), aspect, elevation, precipitation (May-August), slope, distance to road, and distance to power lines were used in the study. The weights of the evaluation criteria were made according to the AHP method. Criteria weights were determined according to local experts and literature data. The land suitability map was obtained using the weighted overlay method in the GIS environment.

Result and Discussion: This study was prepared according to beekeeping requirements in Bitlis province. It can be used by being revised for different ecological regions. In order to evaluate the accuracy of the created model, spatial data about the places where beekeeping is carried out are needed. This evaluation could not be made because the data in the appropriate format could not be obtained. Honey production amounts of the districts of Bitlis show that the current potential of the district of Mutki does not fully correspond to the production data. Incentives and studies for developing beekeeping activities in the Mutki district are essential regarding rural development and honey production values. Honey production values of other counties are compatible with their current potential. The compatibility of production values and existing potentials is an essential indicator of the study's accuracy.

Conclusion: This study determined suitable places for beekeeping in Bitlis, where endemic plant species are rich. It shows that suitable and very suitable areas for beekeeping activities are in Mutki, Merkez, Hizan, Tatvan, Ahlat, Güroymak, and Adilcevaz districts, respectively. The suitability map is divided into five suitability classes. Very suitable areas cover 1.620,02 km^2, suitable areas 2.003,81 km^2, suitable medium areas 537.60 km^2, less suitable areas 593.63 km^2, and significantly less suitable areas 468.15 km^2. The places where

beekeeping cannot be done legally or technically and the yield may be low are restricted. These areas are 3.069,39 km^2.

GİRİŞ

Küresel ısınma, artan nüfus ve çevre kirliliği insanların beslenme kaynaklarını tehdit etmektedir (Mercan ve Arpağ 2020). Bu sorun tarımsal alanları tehdit ederek hayvansal üretime de zarar vermektedir (Kutlu vd. 2016). Artan nüfusun beslenme ihtiyacının giderilebilmesi için bitkisel üretimin yanında hayvansal üretimin de verimli bir şekilde arttırılması gerekmektedir. Hayvansal üretim kollarından birisi ise arıcılıktır. Arıcılık faaliyetleri sonucunda bal, arı zehri, propolis, balmumu ve arı sütü gibi katma değeri yüksek ürünler üretilebilmektedir (Estoque ve Murayama, 2010). Çoğunlukla kırsal kesimde yaşamını sürdüren insanlara gelir kazandırması, kolay pazarlanabilmesi, bozulmadan uzun süre saklanabilmesi, bitkilerdeki tozlaşmaya yardımcı olması, az masraf ve işgücü gerektirmesi, kullanım alanlarının fazla olması gibi pek çok faydalı nedenden ötürü arıcılık, yaygınlaştırılması gereken önemli bir hayvansal üretim şeklidir (Estoque ve Murayama 2010, 2011, Sıralı, 2010, Çevrimli ve Sakarya 2019, Fernandez vd. 2016, Sarı vd. 2020b, Elmastaş vd. 2022).

Birleşmiş Milletler Gıda ve Tarım Örgütünün (FAO), 2021 yılı dünya bal üretim verileri incelendiği zaman Türkiye'nin önemli bir bal üreticisi ülke olduğu görülmektedir (FAO 2023). 2021 yılında dünyadaki toplam 2.108.564 ton bal üretiminin %4,57'si (96.344 ton) Türkiye'de gerçekleşmiştir. Türkiye, Çin'den sonra dünya bal üretiminde ikinci sırada yer almaktadır (FAO 2023). Türkiye'nin sahip olduğu ekolojik çeşitlilik, zengin bitki örtüsü ve farklı genetik çeşitliliğe sahip bal arısı popülasyonlarının olması onu arıcılık açısından potansiyel bir ülke yapmaktadır (Sıralı 2010). Bu çalışmanın yapıldığı Bitlis ili de iklimi, topografyası ve endemik bitki örtüsünün zenginliği ile arıcılık açısından potansiyel bir havzadır (Çağlıyan 2015). Buna karşın, Türkiye İstatistik Kurumunun (TÜİK) bal üretim istatistiklerinde bu zenginlik yeterli ölçüde üretim verilerine yansımamaktadır. Bitlis, 2022 yılı için, Türkiye'nin en yüksek bal üretimi yapan 25. şehridir (TÜİK, 2023). TÜİK'in 2022 yılı bal üretim istatistiğine göre Bitlis'in toplam bal üretimi 1.210,35 tondur ve bu değer Türkiye'deki toplam üretimin %1,02'sine tekabül etmektedir (TÜİK 2023). Bitlis gibi Türkiye'deki pek çok şehir, her ne kadar arıcılık için elverişli şartlara sahip olsa da bu potansiyel tam olarak değerlendirilememektedir (Çevrimli ve Sakarya 2019, Çağlıyan 2015).

İllerin doğal kaynaklarının ve ekolojik özelliklerinin rasyonel bir şekilde ortaya konularak arazi uygunluk analizlerinin yapılması bu sorunun çözümüne katkı sunabilir. Arazi uygunluk değerlendirmeleri neticesinde arazinin istenen özellik için en uygun olduğu yerler tespit edilebilmektedir. Böylelikle, üretimdeki verim artırarak kırsal kalkınmaya destek sağlanabilmektedir (Estoque ve Murayama 2011, Sarı vd. 2020b). Arazi uygunluk analizleri birçok değişkenin etkilerini ve ağırlıklarını kullanarak, istenilen amaç için en uygun yerlerin bulunmasını sağlamaktadır (Everest ve Gür 2022). Böylelikle üreticilerin yer seçim tercihlerine alternatifler sunularak potansiyel açıdan verimli arazileri tercih etmeleri sağlanabilir. Bu kapsamda Coğrafi Bilgi Sistemleri (CBS) ile entegre Çok Kriterli Karar Verme (ÇKKV) yöntemleri, uygun yerlerin tespit edilmesi konusunda yaygın bir şekilde kullanılmaktadır (Estoque ve Murayama 2010, 2011, Abou-Shaara 2013, Fernandez vd. 2016, Widiatmaka vd. 2016, Sarı vd. 2020b, Yılmaz vd. 2021, Elmastaş vd. 2022). Bu yöntemler ile araştırmacılar hedefleri doğrultusunda en iyi alternatifi elde edebilirken, karmaşık karar verme sürecini ise basite indirgerler (Jankowski 1995). ÇKKV yöntemlerinden birisi ise Analitik Hiyerarşi Yöntemi (AHP: Analytic Hierarchy Process)'dir.

Arıcılık faaliyetlerinden elde edilen ürünlerin verimi başta ekolojik şartlar olmak üzere pek çok unsurdan etkilenmektedir (Çağlıyan 2015, Kutlu vd. 2016, Sarı vd. 2020b). Arıcılık için uygun alanların belirlenmesinde ekolojik şartları dikkate alarak, ÇKKV yöntemlerini kullanarak uygun yer analizini yapan birçok çalışma bulunmaktadır (Tablo-1). Bu çalışmalarda AHP (Estoque ve Murayama 2010, 2011, Abou-Shaara vd. 2013, Ceylan ve Sarı 2017, Yalçın vd. 2019, Sarı vd. 2020a, 2020b), TOPSIS (Sarı vd. 2020a), VIKOR (Sarı vd. 2020a), ve PROMETHEE (Sarı vd.2020b) yöntemleri kullanılmıştır. Yapılan çalışmalarda kriter olarak, arazi örtüsü, yağış, yükselti, eğim, bakı, su, yol ve yerleşim alanlarına mesafenin yoğun olarak kullanıldığı görülmektedir (Tablo 1).

Tablo 1. Arıcılık için uygun yer analizi yapan araştırmacıların çalışmalarında kullanmış oldukları kriterler

Table 1. The criteria used by the researchers who make suitable site analyses for beekeeping in their studies.

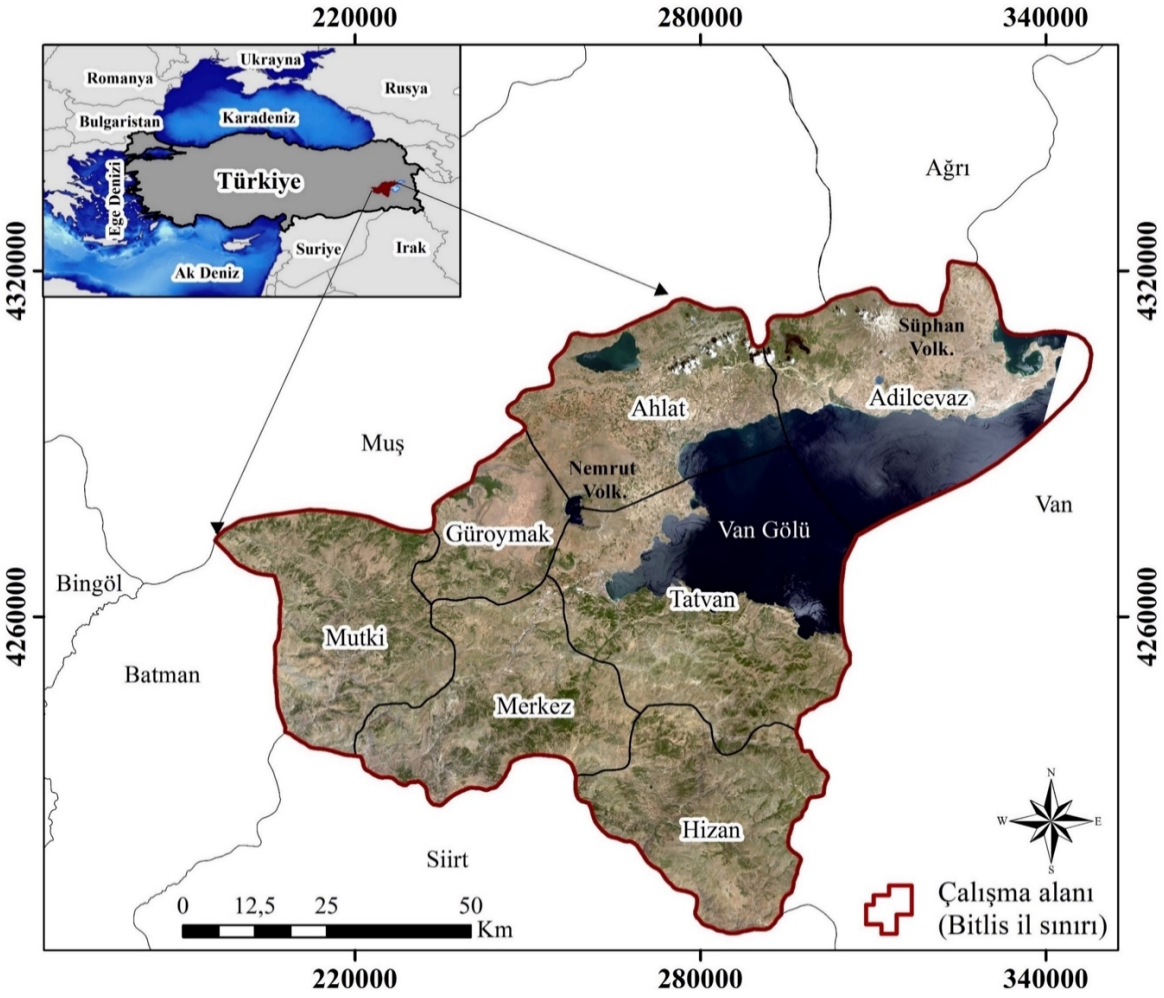
Kriterler / Araştırmacılar	Estoque ve Murayama (2010)	Amiri ve Shariff (2012)	Abou-Shaara vd. (2013)	Abou-Shaara (2013)	Fernandez vd. (2016)	Widiatmaka vd. (2016)	Ceylan ve Sarı (2017)	Zoccali vd. (2017)	Yalçın vd. (2019)	Sarı vd. (2020a)	Sarı vd. (2020b)	Yılmaz vd. (2021)	Elmastaş vd. (2022)
Arazi Örtüsü	+	+	+	+	+	+	+	+	+	+	+	+	+
Yağış		+		+		+	+		+	+	+	+	+
Yükselti	+					+	+	+	+	+	+	+	+
Bakı							+		+	+	+	+	+
Eğim				+			+		+	+	+	+	+
Sıcaklık		+	+	+		+		+					
Yola uzaklık	+	+		+	+	+	+	+	+	+	+	+	+
Su kaynaklarına uzaklık	+	+	+	+	+	+	+	+	+	+	+	+	+
Yerleşim alanlarına uzaklık					+	+	+		+	+	+	+	
Elektromanyetik alanlara uzaklık					+						+		
Bitkilere uzaklık				+									
Pazara uzaklık						+							
Tren ağına uzaklık											+		
Doğal afet		+									+		
Nem			+										
Güneş Radyasyonu					+								

Bu makalenin temel amacı, Coğrafi Bilgi Sistemi (CBS) ve ÇKKV yöntemlerini kullanarak arıcılık için uygun alanların haritalanmasıdır. Önerilen kriterler ve ağırlıkları benzer ekolojik özelliklere sahip bölgeler için de kullanılabilir. Kriterlere ait verilerin açık erişimli kaynaklardan elde edilebilmesi ise farklı bölgeler için benzer çalışmaların yapılabilmesini olanaklı kılmaktadır.

GEREÇ VE YÖNTEM

Çalışma Alanı

Doğu Anadolu bölgesinde yer alan Bitlis ili, Yukarı Murat-Van bölümü ile, Yukarı Fırat bölümleri içerisinde yer almaktadır. Bitlis ilinin doğusunda Van, kuzeyinde Muş ve Ağrı, güneyinde Siirt ve batısında Batman bulunmaktadır (Şekil 1). Bitlis ilinin ilçeleri, Güroymak, Hizan, Merkez, Mutki, Ahlat, Adilcevaz ve Tatvan'dır. Bitlis, çoğunlukla dağlık arazilerden oluşmaktadır. Bu dağlık arazilerin yanında çeşitli plato ve ovalar da şehirde yer almaktadır. Bitlis ilinde Nemrut ve Süphan olmak üzere iki büyük volkanik dağ vardır. Bitlis ili içerisinde Bitlis, Botan ve Garzan Çayı, Karasu, Oranz deresi, Güzeldere gibi birçok akarsu yer alır. İlde Van Gölü, Nazik, Nemrut, Arin ve Aygır Gölü bulunmaktadır. Bitlis ilinde sert karasal iklim şartları görülmektedir (Çağlıyan 2015). Meteoroloji Genel Müdürlüğünün (MGM) istatistiklerine göre, ilin 1991-2020 yılları arasındaki ortalama yıllık sıcaklığı 9 °C ve ortalama yağışı 1.046,6 mm'dir (MGM, 2023). Bitlis ilinde yaşayan insanlar geçimlerini çoğunlukla tarım ve hayvancılık ile sağlamaktadır. Bunlar arasında balıkçılık ve arıcılık önemli gelir kaynaklarındandır. Bitlis ilinde 2021 yılında 2.056,23 ton, 2022 yılında ise 1.210,35 ton bal üretilmiştir (TÜİK 2023). İlde sabit ve gezginci arıcılık faaliyetleri yürütülmektedir. Bunlardan gezginci arıcıların, bal verimleri sabit olanlara göre daha yüksektir (Çağlıyan 2015). Çalışma alanında, Bitlis arı yetiştiricileri birliği ve Hizan bal üreticileri birliği gibi mesleki örgütlenmelerin bulunması arıcılık faaliyetleri açısından önemlidir.



Şekil 1. Çalışma alanının lokasyon haritası (Bitlis il sınırları içerisinde, 2022 yılına ait Landsat uydu görüntüsünün doğal renk kombinasyonu kullanılmıştır).

Figure 1. Location map of the study area (within the provincial borders of Bitlis, the natural colour combination of the Landsat satellite image of 2022 was used).

Çalışmanın Genel Çerçevesi

Yapılan bu çalışmada izlenilen yol Şekil 2'te özetlenmiştir. Öncelikle bal arılarının ekolojik gereksinimleri literatür ve uzman görüşleri dikkate alınarak 11 kriter olarak belirlenmiştir. Uzman görüşü alınan kişiler, bölgede arıcılık faaliyeti yürüten 5 arıcı ve 1 ziraat mühendisinden oluşmaktadır. Çalışmada belirlenen kriterlere ait veriler öncelikle aynı projeksiyon sistemine (Universal Transverse Mercator (UTM), WGS 84, 38. dilim) dönüştürülerek tematik haritaları oluşturulmuştur (Şekil 4). Kullanılan kriterlerin veri kaynaklarına ait bilgiler Tablo 2'de sunulmuştur. Analiz yapılırken kriterlere ait tüm verilerin mekânsal çözünürlüğü 30 metreye yeniden örneklendirilmiştir. Böylelikle analizi yapılan tüm kriterler aynı projeksiyon sistemine ve mekânsal çözünürlüğe sahip olmuştur. CBS ortamında kaydedilen bu veriler, AHP metodolojisi (Saaty 1977; 1980) kullanılarak ikili karşılaştırmaya tabi tutulmuş ve her bir kriterin ağırlığı belirlenmiştir (Şekil 3).

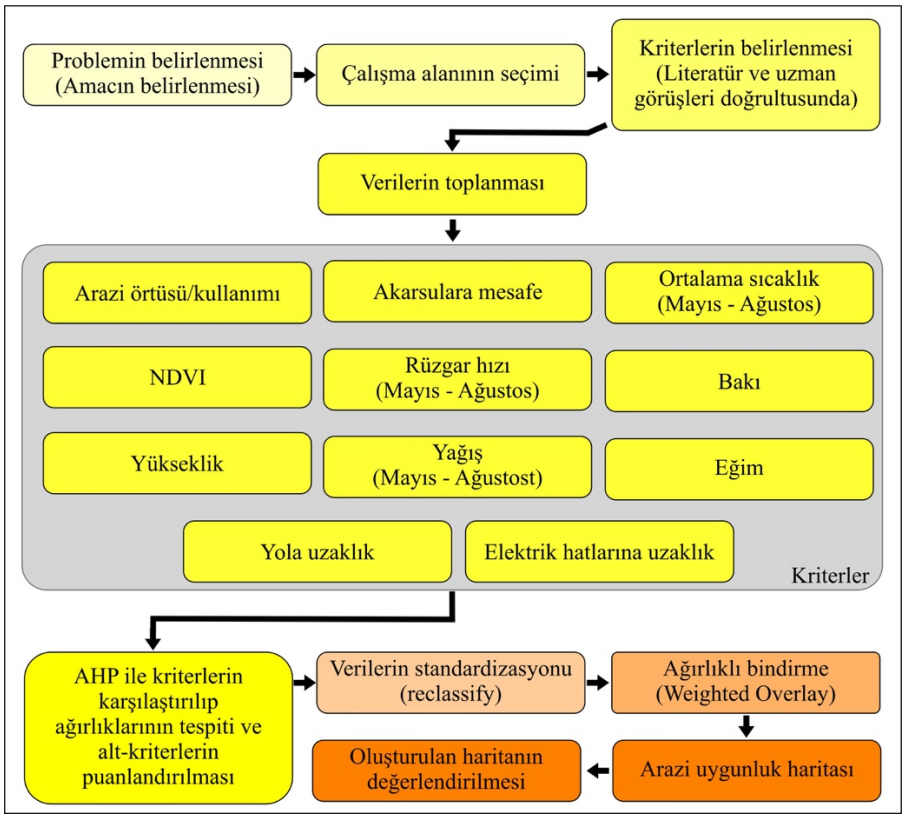
Yapılan bu ikili karşılaştırmalar sonucunda bazı düzeylerde tutarsızlıklar oluşabileceği için matrisin tutarlılığının kontrol edilmesi gerekmektedir. Bundan ötürü Saaty (1980) tarafından önerilen tutarlılık oranı (CR) kullanılır (Saaty 1997, 1980, Malczewski, 1999; Akıncı vd. 2013, Orhan, 2021). Elde edilen değerin 0,1'den küçük olması matrisin tutarlı olduğunu

gösterir. Yapılan bu çalışmadaki tutarlılık oranı (CR) 0,0391 olarak hesaplanmıştır ve bu değer tutarlıdır. Seçilen kriterlerin alt kriterleri ise arıcılık açısından önem derecesine göre 1'den 9'a kadar olan rakamlarla puanlandırılmıştır. Arıcılık için önemli olan alt kriterler yüksek puanlandırılırken önemsiz olanlar ise düşük puanlandırılmıştır. Bazı alt kriterler ise bu aşamada sınırlandırılmıştır. Sınırlandırma yapılan alt kriterler arıcılık faaliyetlerini olumsuz etkilemektedir. Kriterlere ait veriler, belirlenen ağırlıklarına ve puanlarına göre ArcGIS v.10.8 programındaki ağırlıklı bindirme (weigthed overlay) metodolojisi ile birleştirilmiş ve uygunluk haritası oluşturulmuştur (Şekil 5).

Tablo 2. Çalışmada kullanılan veriler ve kaynakları

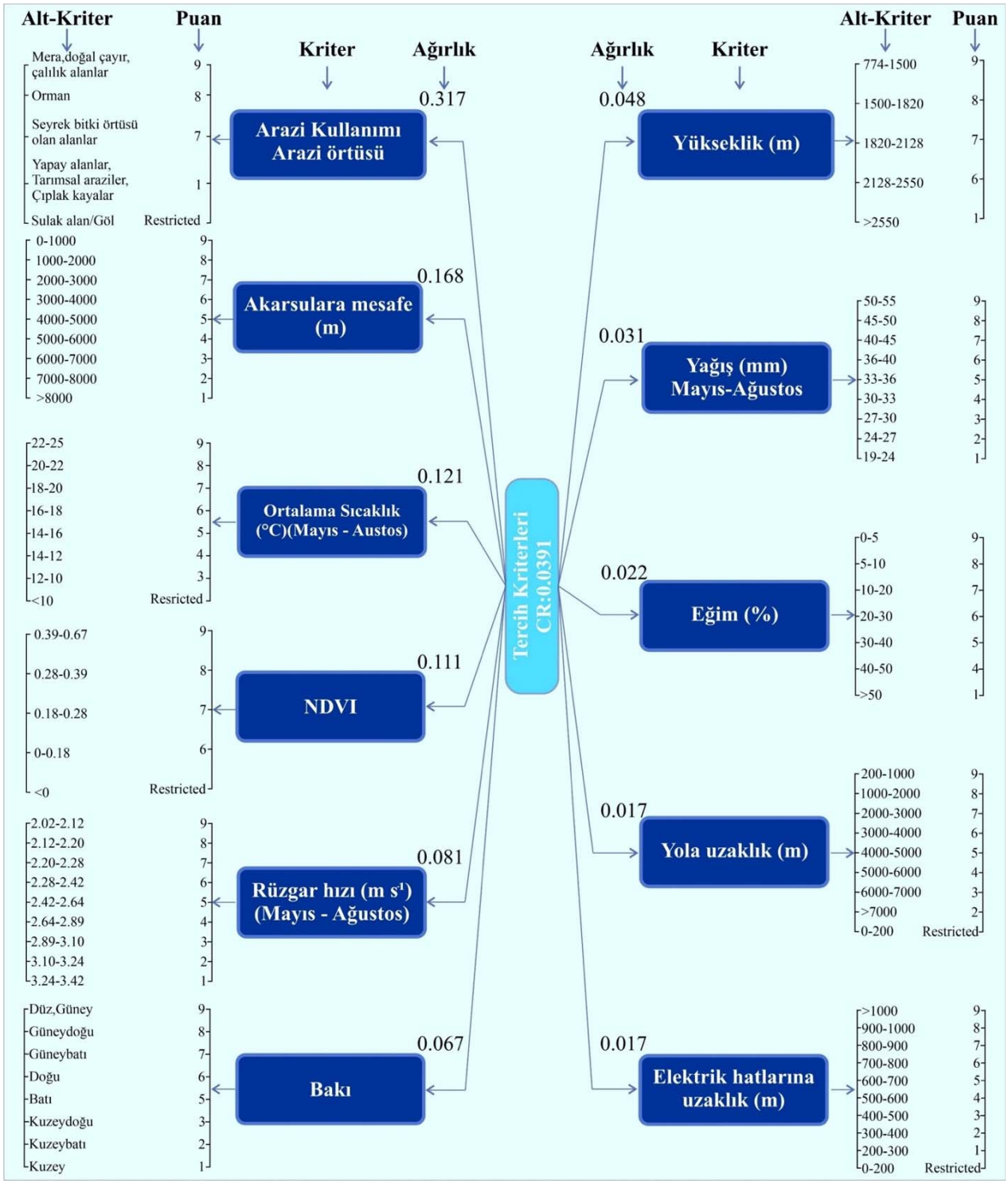
Table 2. Data and data sources used in the study

Kriterler	Veri Türü	Kaynak
Arazi Kullanımı/Örtüsü	Vektör	CORINE-2018 (https://land.copernicus.eu/pan-european/corine-land-cover)
Akarsulara mesafe	Vektör	Copernicus River Data (https://land.copernicus.eu/imagery-in-situ/eu-hydro/eu-hydro-river-network-database?tab=download)
Sıcaklık, Rüzgâr ve Yağış	Raster	WorldClim Data (Fick ve Hijmans 2017) (https://www.worldclim.org/)
NDVI	Raster	USGS Earth Explorer (https://earthexplorer.usgs.gov/)
Yükselti	Raster	ALOS PALSAR (https://search.asf.alaska.edu/#/)
Eğim ve Bakı	Raster	Yükselti verisinden üretilmiştir
Yol ve Elektrik hatlarına uzaklık	Vektör	Open Street Map (https://www.openstreetmap.org/#map=7/39.031/35.252)



Şekil 2. İş akış şeması

Figure 2. Work flow chart



Şekil 3. Arazi uygunluk değerlendirmesi için kullanılan kriterlerinin/alt-kriterlerin ağırlıkları ve puanları

Figure 3. Weights and scores of criteria/sub-criteria used for land suitability assessment

Kriterlerin Seçimi

Arazi Kullanımı/Örtüsü: Arazi örtüsü (AÖ) terimi yerin yüzeyinde (toprak, kaya, bitki örtüsü gibi) bulunan bileşenleri ifade ederken, arazi kullanımı (AK) ise insanların bu örtüyü yönteme ve kullanma şekli olarak ifade edilmektedir (Mercan vd. 2022). Geçmişte bu terimler ayrı ayrı kullanılsa da günümüzde beraber (AKAÖ) kullanılmaktadır (Selçuk vd. 2021). AKAÖ arıcılık faaliyetlerinde verim, kalite ve balın çeşidini etkilediğinden önemli bir kriterdir (Estoque ve Murayama 2010, Elmastaş vd. 2022). Bu kritere ait veri seti 2018 yılına ait CORINE (Coordination of Information on the Environment) verisinden oluşturulmuştur. Arılar faaliyetlerini sürdürebilmek için çiçekli bitkilerden elde edebildikleri nektara ve polene ihtiyaç duyarlar. Bu doğrultuda AKAÖ verilerinde mera, doğal çayırlık ve çalılık bölgeler, orman ve seyrek bitkili alanlar yüksek puanlandırılmıştır. Bölgedeki meralar ile çayırlık ve çalılık alanlar yonca, korunga, geven, kekik ve ballıbaba gibi bitkiler açısından oldukça zengin olduğundan arıların beslenmesi için en uygun alanlardır (Çağlıyan, 2015). Beşeri faaliyetlerin yoğun olduğu yapay alanlar hava kirliliği ve evsel/sanayi atıklarının varlığından ötürü düşük puanlandırılmıştır (Yılmaz vd. 2021). Arazinin çıplak olduğu bölgeler arıcılık için verimsiz olduğundan düşük puanlandırılmıştır. Tarımsal arazilerde bitkilerin yetişebilmesi için bir dizi zirai ilaç kullanılabilmektedir. Kullanılan bu ilaçlar arılar için zararlıdır (Çakmak vd. 2003). Bundan ötürü bu bölgeler düşük puanlandırılmıştır. Göl yüzeyleri üzerinde arıcılık faaliyetinin yapılamasına olanak sağlamadığı için sınırlandırılmıştır.

Akarsuya Uzaklık: Arıcılık faaliyetlerindeki önemli kriterlerden birisi ise arıların su ihtiyacının karşılanabilmesidir (Abou-Shaara vd. 2013, Çağlıyan 2015, Elmastaş vd. 2022). Bu gereksinim bazen yapay sulukların yapılmasıyla karşılanırken, temiz su kaynağı olan akarsulara yakın alanlar seçilerek de bu ihtiyaç giderilebilmektedir. Yapay sulukların yapılması durumunda da sulukların sürekli olarak temiz su ile doldurulabilmesi için bir su kaynağına ihtiyaç vardır. Bundan ötürü akarsuya uzaklık kriteri bu çalışmada kullanılmıştır. Su bal üretimi ve bal arılarının yaşayabilmesi için temel bir ihtiyaçtır (Amiri ve Shariff 2012). Arılar bal üretmek için kovanlarından kilometrelerce uzaklıktaki mesafelere uçabilmektedirler. Ancak gereksinimlerini kovana yakın alanlardan karşılamaları daha verimlidir. Bu nedenle arıcılık yapılan yerin suya yakın olması istenilen bir durumdur (Zoccali vd. 2017, Yılmaz vd. 2021). Bu bağlamda akarsulara yakın olan bölgeler yüksek puanlandırılırken, uzak alanlar düşük puanlandırılmıştır.

Sıcaklık: Arılar bulundukları ortamın sıcaklığına karşı hassas oldukları için, arıcılık için uygun alanlarının belirlenmesinde kullanılması gereken önemli kriterlerden birisi sıcaklıktır (Abou-Shaara vd. 2013, Zoccali vd. 2017). Sıcaklığın 10 °C'nin altına inmesi veya 37 °C'nin üzerine çıkması sonucu arıların faaliyetleri durur (Tunçel 1992). Bundan dolayı sıcaklığın 10 °C'nin altına düştüğü alanlar sınırlandırılmıştır. Maksimum sıcak değerlerinin ise arıcılık faaliyetlerini olumsuz etkileyecek düzeyde olmamasından ötürü herhangi bir sınırlama yapılmamıştır. Bitlis ilinde arıcılığın yoğun olarak yapıldığı aylar mayıs-ağustos dönemleridir (Çağlıyan 2015). Bu aylara ait sıcaklık verileri WorldClim data setinden elde edilmiştir (Fick ve Hijmans 2017). Dört aya ait sıcaklık verilerinin ortalaması alınarak kullanılmıştır. Puanlama yapılırken yüksek sıcaklıklara yüksek puanlar verilmiş, sıcaklık azaldıkça puanlarda azaltılmıştır.

NDVI (Normalized Difference Vegetation Index): NDVI (normalize edilmiş fark bitki indeksi) bir bölgedeki canlı bitki örtüsünün tespitini kolaylaştırmaktadır ve uzaktan algılama çalışmaları sonucunda üretilen görüntüler ile hesaplanabilmektedir (Mercan 2020). NDVI değerleri bölgedeki canlı bitki yoğunluğunu ortaya koysa da bu bitkilerin bal üretimi için uygunluk durumlarını gösterememektedir. Arıcılar, yer seçimi konusunda bitkilerin yoğun olduğu alanları, bitkinin az olduğu veya olmadığı yerlere göre daha çok tercih ettiklerini belirtmektedir. Bitki miktarının artması, nektar ve polen miktarını etkileyebileceği için bu veri seti kullanılmıştır. NDVI değerlerinin hesaplanabilmesi için Amerika Jeoloji Araştırma Kurumu (USGS) tarafından sunulan Landsat uydu görüntüleri kullanılmıştır. Bu verinin hazırlanması için 30/06/2022 tarihine ait, 30m mekânsal çözünürlüklü, bulutluluk oranının %10'un altında olduğu Landsat 9 OLI-2 uydu görüntüleri kullanılmıştır. Bu uydu görüntüsünden hesaplanan NDVI değerleri formül 1'e göre yapılmıştır (Mercan 2020).

$$NDVI = \frac{(\text{Bant } 5) - (\text{Bant}4)}{(\text{Bant } 5) + (\text{Bant } 4)} \qquad (1)$$

Formülde belirtilen Bant 5 yakın kızılötesi bandı, Bant 4 ise kırmızı bandı ifade etmektedir. NDVI değerinin yüksek olması bitki yoğunluğunun arttığını,

düşmesi ise azaldığını göstermektedir. Değerlerin negatif olması ise bitkinin hiç olmaması veya su yüzeyi gibi arıcılık için uygun olmayacak alanları ifade etmektedir. Bundan ötürü NDVI değerlerinin yüksek olduğu yerlere yüksek puanlar verilirken, değerin azaldığı yerlerde puanlar azaltılmıştır. NDVI değerlerin negatif olduğu yerler ise sınırlandırılmıştır.

Rüzgâr: Rüzgâr, arıların kovan dışındaki faaliyetlerini etkileyen önemli unsurlardan biridir (Çağlıyan 2015, Yalçın vd. 2019). Rüzgâra açık alanlarda yapılan arıcılık faaliyetlerinde bal verimi düşmektedir (Tunçel 1992). Bu nedenle arıcılığın yoğun olarak yapıldığı mayıs-ağustos dönemlerine ait rüzgâr verileri, WorldClim data setinden alınmıştır (Fick ve Hijmans 2017). Bu dört aya ait rüzgâr verilerinin ortalaması kullanılmıştır. Arılar için rüzgârın az olduğu yerler daha tercih edilebilir olduğundan, bu alanlara daha yüksek puanlar verilmiştir. Rüzgâr hızının arttığı yerlerde ise puanlar azaltılmıştır.

Yükselti: Topografyanın denizden yükseklik değerleri bitkilerin dağılımını ve sıcaklığı etkilediği için arıların bal verimini de etkileyebilmektedir (Elmastaş vd. 2022). Alaska Satellite Facility (ASF DAAC) tarafından sunulan 12.5 m mekânsal çözünürlüklü ALOS PALSAR veri seti yükselti haritasını oluşturmak için kullanılmıştır. Bölgedeki arıcılar mevsimsel olarak bitkilerin çiçek açma zamanlarına göre farklı yükselti değerlerinde faaliyet gösterebilseler de yükseltinin artması ile birlikte sıcaklık değerleri düşmektedir. Bundan dolayı yükseltinin düşük olduğu alanlar yüksek puanlandırılırken, yükseltinin arttığı yerlerde puanlar azaltılmıştır.

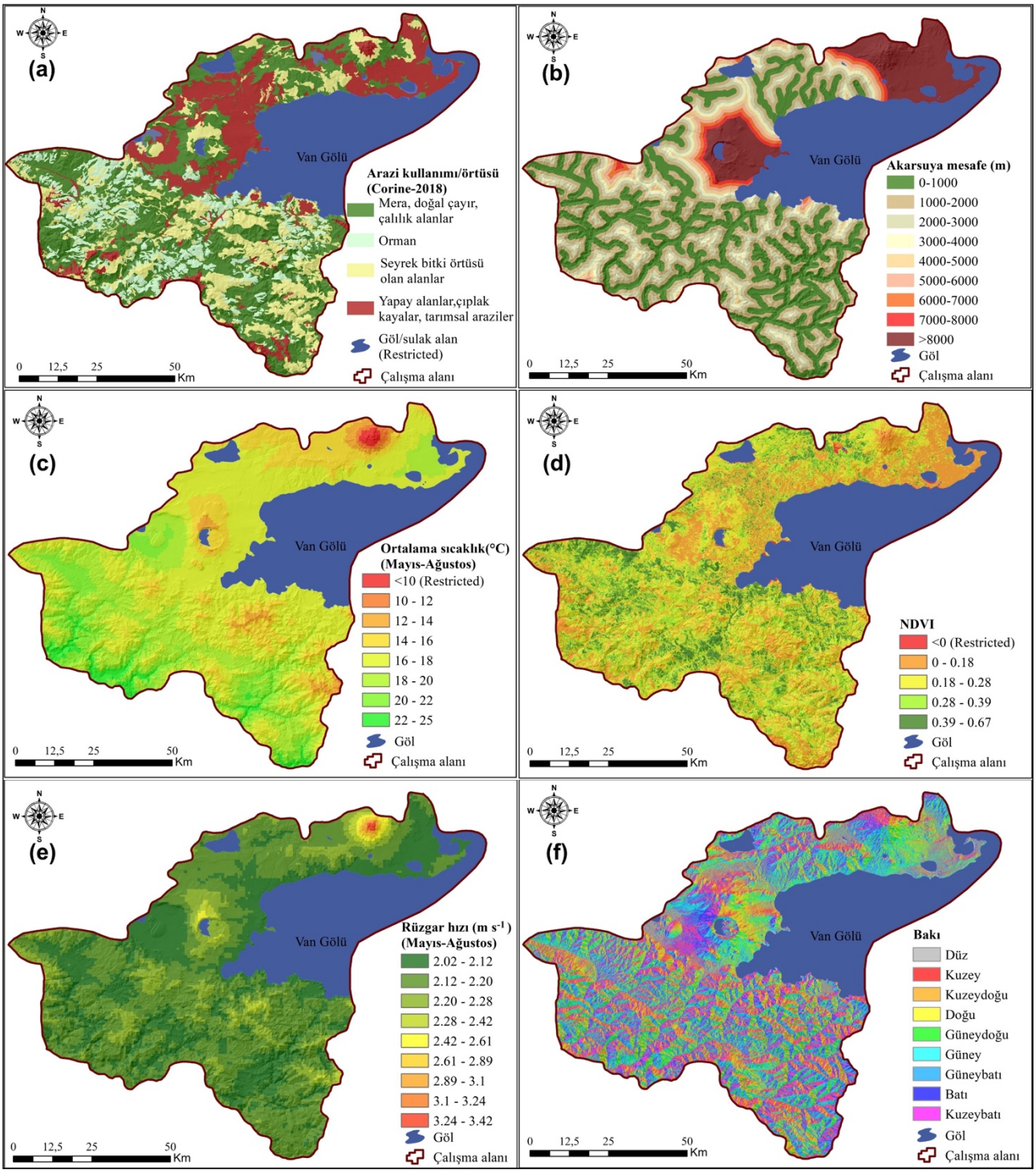
Bakı: Bakı haritası 12.5 m mekânsal çözünürlüklü sayısal yükselti haritasından oluşturulmuştur. Arı kovanlarının konulduğu bakı yönleri bal verimini etkileyebilmektedir (Elmastaş vd. 2022). Güney alanlar arıcılık için daha çok tercih edilebilirken, kuzey alanlar daha az tercih edilmektedir. Kovanların konulduğu yerin doğuya bakması, arıların daha erken saatlerde çalışmaya başlamasını sağlamaktadır ve batı yönüne göre daha uygun olmaktadır (Yalçın vd. 2019). Tüm bu hususlar dikkate alınarak bakı yönlerine, güney bakılarda yüksek, kuzey bakılarda ise düşük puanlar verilmiştir. Doğu yönü ise batıya göre daha yüksek puanlandırılmıştır.

Yağış: Yağış verileri, bal üretiminin yoğun olarak yapıldığı mayıs-ağustos dönemlerine ait verilerden üretilmiştir. Bu veriler WorldClim data setinden alınmıştır (Fick ve Hijmans 2017). Bal üretiminin yoğun olarak gerçekleştirildiği dört aya ait verilerin ortalaması alınarak oluşturulmuştur. Yağış, bitkilerin yoğunluğunu ve çeşitliliğini etkileyen bir faktör olduğu için yağışın fazla olduğu yerler yüksek, az olduğu yerler ise düşük puanlandırılmıştır.

Eğim (%): Eğim değerleri yükselti gibi arıcılık faaliyetlerini etkileyen faktörlerdendir (Abou-Shaara 2013, Yılmaz vd. 2021, Elmastaş vd. 2022). Eğimin düşük olduğu yerler yüksek puanlandırılırken, eğimin arttığı alanlar düşük puanlandırılmıştır.

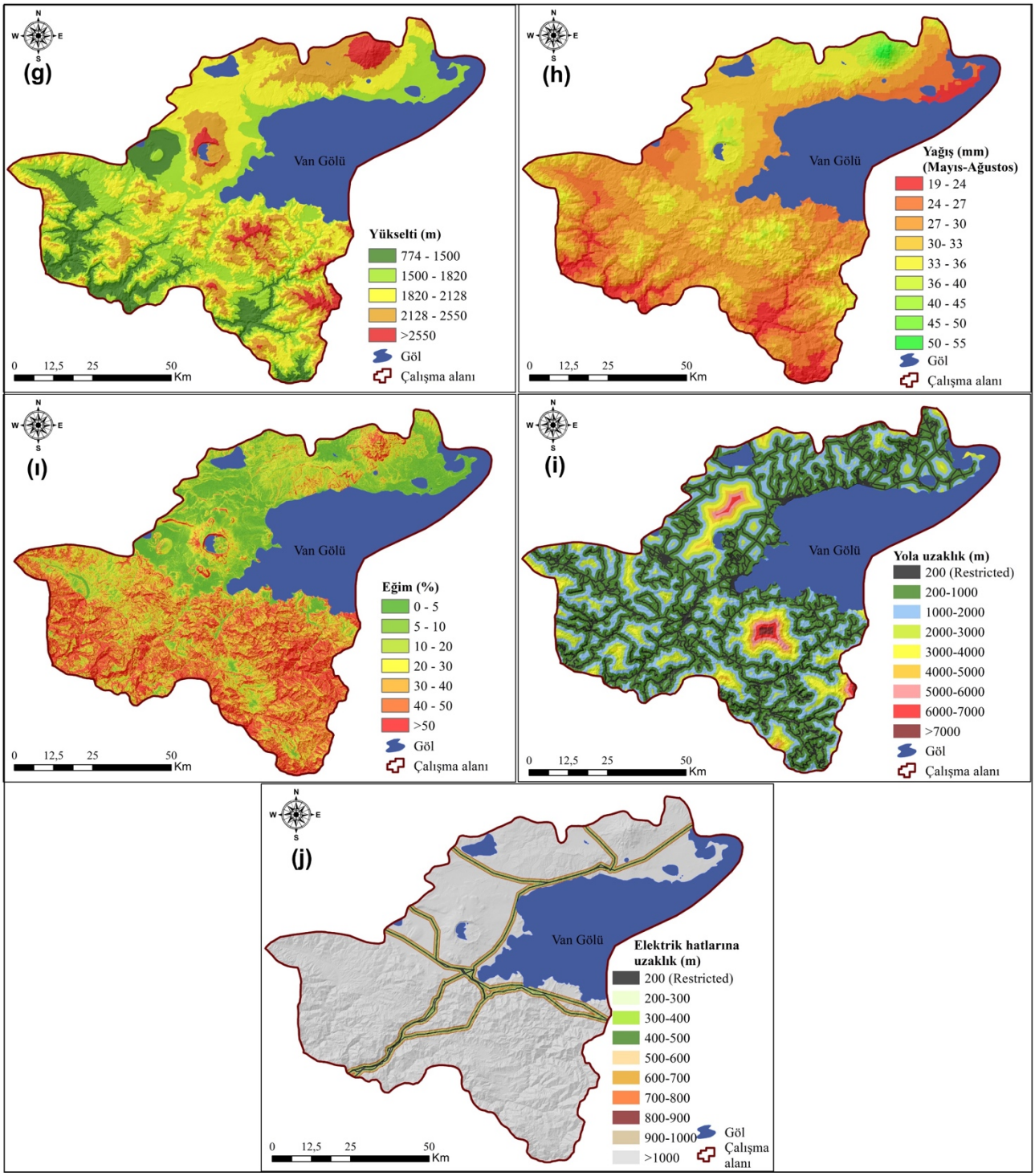
Yola Uzaklık: Yol veri seti Open Street Map'ten vektör formatında elde edilmiştir (https://www.openstreetmap.org/). Gezginci arıcılık ile ilgili yasal mevzuatta trafiğin yoğun olduğu alanlarda, kovanların yoldan 200 m, trafiğin az olduğu yerlerde ise en az 30 m uzakta olması gerektiği belirtilmiştir (Yılmaz vd. 2021). Bu çalışmada da yasal mevzuat gereği yola 200 m'lik tampon alanlar arıcılık için sınırlandırılmıştır. Bu sınırlandırma yapılırken yollardaki trafiğin yoğunluk durumu dikkate alınmamıştır. Arıcılıkla uğraşan kişilerin ulaşım gereksinimlerini sağlamak için yola yakın olmak tercih edilebilir bir durum olarak değerlendirilmiştir (Zoccali vd. 2017, Çevrimli ve Sakarya 2019). Bu amaçla sınırlandırılmış alandan sonra yola yakın kesimler yüksek, uzak kesimler ise düşük puanlandırılmıştır.

Elektrik Hatlarına Uzaklık: Bu veri seti Open Street Map'ten vektör formatında elde edilmiştir (https://www.openstreetmap.org/). Yüksek gerilimli elektrik hatlarının yaydığı elektromanyetik alanlar arıları olumsuz etkileyebilmektedir (Sarı vd. 2020b). Elektrik hatlarına çok yakın bölgelerin sınırlandırılması arıların sağlığı ve bal verimi açısından önemlidir. Bu amaçla elektrik hatlarının her iki yanı boyunca 200 m'lik tampon alanlar arıcılık faaliyetleri açısından sınırlandırılmıştır. Puanlamada elektrik hatlarından uzaklaştıkça puanlar arttırılmıştır.



Şekil 4. Kullanılan kriterlerin tematik haritaları. (a); arazi örtüsü/kullanımı (b); akarsuya mesafe, (c); ortalama sıcaklık (Mayıs-Ağustos) (d); NDVI (e); rüzgâr hızı (f); bakı

Figure 4. Thematic maps of the criteria used. (a); land cover/use (b); distance to stream, (c); average temperature (May-August) (d); NDVI (e); wind speed (f); Aspect



Şekil 4 devamı. (g); yükseklik, (h); yağış (Mayıs-Ağustos), (ı); eğim (i); yola uzaklık, (j); elektrik hatlarına uzaklık haritası

Figure 4 continuation. (g); height, (h); precipitation (May-August), (i); slope (i); distance to road, (j); distance map to power lines

BULGULAR

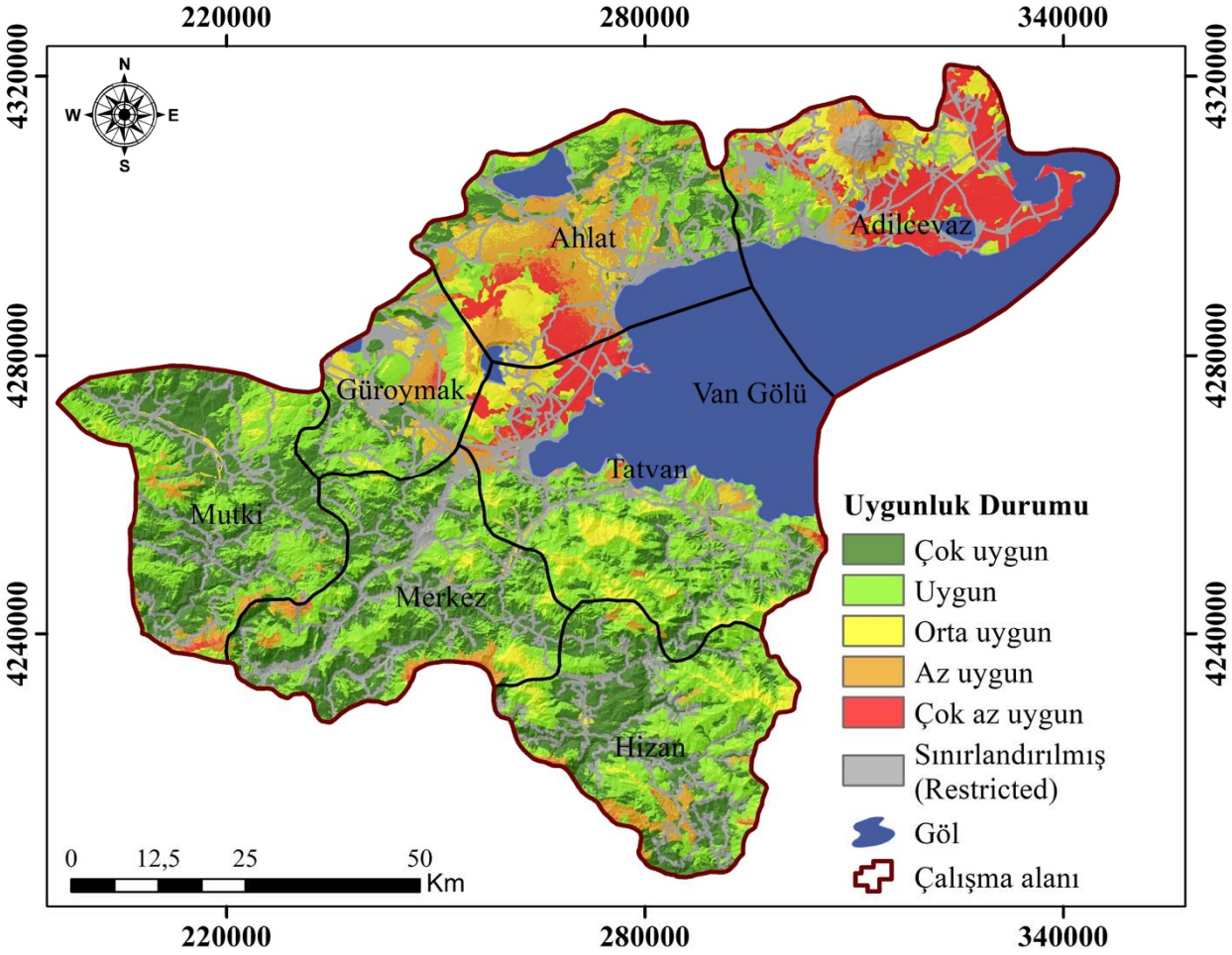
Arıcılık için uygun yerlerin belirlenebilmesi için seçilen kriterlere ait haritalar ve bunların sınıf aralıkları Şekil 4'te sunulmuştur. Arazi kullanımı/örtüsü haritasında, zirai ilaçlamanın olabileceği tarımsal arazilerin ve bitki örtüsü açısından fakir çıplak kaya yüzeylerinin ilin çoğunlukla kuzey kesimlerinde yüzeylendiği görülmektedir. Mera ve çayır alanları birçok yerde bulunurken, ormanlık alanlar daha çok güney bölgelerde yoğunlaşmaktadır (Şekil 4a). Akarsuya mesafe haritasında ilin güney alanlarındaki drenaj ağlarının kuzey bölgelere göre daha yoğun olduğu görülmektedir (Şekil 4b). Ortalama sıcaklık haritasındaki en sıcak bölgeler, ilin güney kesiminde yer alan yükseltinin düşük olduğu kısımlardır. Sıcaklığın en düşük olduğu yer ise Süphan dağının yüksek kesimleridir. Bu bölgelerde yılın büyük bir kısmında buzul örtü bulunabilmektedir ve bu alanlar arıcılık faaliyetleri için sınırlandırılmıştır (Şekil 4c). NDVI haritası bitki örtüsünün daha çok ilin güney bölgelerinde (Mutki, Merkez, Hizan ilçeleri ile Tatvan ve Güroymak'ın güney kesimleri) yoğunlaştığını ve Adilcevaz tarafında ise bitki örtüsünün daha cılız olduğunu göstermektedir (Şekil 4d). Rüzgâr hızı haritasında en yüksek hızın, Süphan dağının zirvesinde olduğu görülmektedir. Rüzgâr hızının yüksek olduğu bu alanlarda aynı zamanda sıcaklık değerleri de düşüktür (Şekil 4e). Yükselti haritasında volkanik dağların olduğu yerlerde yükselti değerlerinin arttığı görülmektedir. Dağların arasında bulunan vadilerde ise yükselti değerlerinin düşük olduğu görülmektedir. Yükseltinin arttığı yerlerde sıcaklık değerleri düşerken, rüzgâr hızı ise artmaktadır (Şekil 4g). Yağış haritasında, yükseltinin arttığı yerlerde yağış değerlerinin de fazlalaştığı görülmektedir. Yağışın en yüksek olduğu bölgeler Süphan ve Nemrut volkanlarının bulunduğu yerlerdir (Şekil 4h). Eğim haritası, ilin güney alanlarının yüksek eğim değerlerine, kuzey kesimlerin ise nispeten daha düşük eğim değerlerine sahip olduğunu göstermektedir. Nemrut kraterinin bulunduğu kısımda ve Süphan dağının yamaçlarında eğimin yine yüksek olduğu görülmektedir. Eğim değerlerinin düşük olduğu kesimler AKAÖ haritasında çoğunlukla tarımsal araziler ve yapay alanlar olarak değerlendirilmiştir (Şekil 4ı).

Şekil 5'de üretilen uygunluk haritasındaki her sınıfın ilçelere göre hesaplanmış alan değerleri Tablo 3'de verilmiştir. Tabloya ayrıca ilçelerin 2021 ve 2022 yılı bal üretim istatistikleri de karşılaştırma yapılabilmesi için eklenmiştir (TÜİK 2023). İlin 2021 ve 2022 yıllarına ait toplam bal üretim değerlerinde ciddi dalgalanmaların olduğu göze çarpmaktadır. Bitlis ilinde arıcılık faaliyetleri için çok uygun ve uygun olarak sınıflandırılan araziler alansal olarak sırasıyla çoktan aza doğru Mutki, Merkez, Hizan, Tatvan, Ahlat, Güroymak ve Adilcevaz ilçeleridir (Tablo 3). Bu ilçelerden Hizan, Merkez ve Mutki ilçelerinin çok büyük bir kısmı arıcılık faaliyetleri için oldukça uygun görünmektedir. Bu ilçeler yapay alanlar, çıplak kayalar, yol ve elektrik hatlarından ötürü sınırlandırılmış bazı alanlar dışında büyük oranda uygun ve çok uygun arazi sınıflarını içermektedir. Tatvan ilçesinin güney kesimleri de (Van Gölünün güney tarafında kalan kısımlar) oldukça uygun araziler bulundurmaktadır.

Tatvan'ın kuzeybatı kesimleri (Van Gölünün batısı ile Nemrut volkanı arasındaki alanlar) ise arıcılık için çoğunlukla uygun olmayan arazileri içermektedir. Bu bölgelerde tarımsal araziler bulunmaktadır ve elektrik ile yol ağları bu kısımlarda yoğunlaşmıştır. Bundan ötürü arıcılık açısından pek uygun görülmemektedir. Güroymak ilçesinin güney kesimleri ile Nemrut volkanının bu ilçe sınırında olan kesimlerinde yer yer uygun araziler bulunmaktadır ancak bunların miktarı fazla değildir. Ahlat ilçesinin özellikle kuzey kesimlerinde uygun ve çok uygun araziler vardır. Nemrut volkanının kuzeyinde yer alan eğimin düşük olduğu ve tarımsal üretimin yapıldığı araziler ise arıcılık açısından pek uygun değildir. Bu durumun nedeni tarımsal arazilerde zirai ilaçların kullanılabilme durumundan dolayıdır. Adilcevaz ilçesi arıcılık açısından en uygun olmayan ilçe olarak görülmektedir. Bu ilçenin yalnızca Ahlat'a sınır olan kesimlerinde uygun olabilecek araziler bulunmaktadır. Adilcevaz ilçesinde akarsu ağının diğer ilçelere göre azlığı, NDVI değerlerinin düşüklüğü ve bu arazilerde mevsimsel olarak tarımsal üretimin yapılıyor olması (zirai ilaçların kullanılma ihtimalinden ötürü) bu kısımları arıcılık açısından uygun kılmamaktadır (Şekil 5).

Tatvan'ın kuzeybatı kesimleri (Van Gölünün batısı ile Nemrut volkanı arasındaki alanlar) ise arıcılık için çoğunlukla uygun olmayan arazileri içermektedir. Bu bölgelerde tarımsal araziler bulunmaktadır ve elektrik ile yol ağları bu kısımlarda yoğunlaşmıştır. Bundan ötürü arıcılık açısından pek uygun görülmemektedir. Güroymak ilçesinin güney kesimleri ile Nemrut volkanının bu ilçe sınırında olan kesimlerinde yer yer uygun araziler bulunmaktadır ancak bunların miktarı fazla değildir. Ahlat ilçesinin özellikle kuzey kesimlerinde uygun ve çok uygun

araziler vardır. Nemrut volkanının kuzeyinde yer alan eğimin düşük olduğu ve tarımsal üretimin yapıldığı araziler ise arıcılık açısından pek uygun değildir. Bu durumun nedeni tarımsal arazilerde zirai ilaçların kullanılabilme durumundan dolayıdır. Adilcevaz ilçesi arıcılık açısından en uygun olmayan ilçe olarak görülmektedir. Bu ilçenin yalnızca Ahlat'a sınır olan kesimlerinde uygun olabilecek araziler bulunmaktadır. Adilcevaz ilçesinde akarsu ağının diğer ilçelere göre azlığı, NDVI değerlerinin düşüklüğü ve bu arazilerde mevsimsel olarak tarımsal üretimin yapılıyor olması (zirai ilaçların kullanılma ihtimalinden ötürü) bu kısımları arıcılık açısından uygun kılmamaktadır (Şekil 5).



Şekil 5. Bitlis ili arıcılık uygunluk haritası

Figure 5. Bitlis province beekeeping suitability map

TARTIŞMA

İlçelerin bal üretim değerleri yüksekten düşüğe doğru Hizan, Merkez, Tatvan, Mutki, Güroymak ve Adilcevaz şeklinde sıralanmaktadır (Tablo 3). Bu ilçelerden Hizan, Merkez ve Tatvan'da görülen yüksek bal üretimi bu bölgelerde görülen uygun ve çok uygun arazilerin miktarları ile oldukça uyumludur. Ahlat ilçesinin kuzey kesimlerindeki uygun arazilerin varlığı bal üretim değerleri ile paraleldir. Güroymak ve Adilcevaz ilçeleri uygun arazi varlığı açısından oldukça fakirdir ve bu durum bal üretim değerlerinde de kendini göstermektedir. Sonuç olarak Mutki dışındaki ilçelerde arıcılık için belirlenen uygun alanların miktarı ile bal üretim değerleri arasında uyum görülmektedir. Yüksek oranda çok uygun ve uygun arazi sınıflarının olduğu

ilçeler, bunların daha az olduğu ilçelere göre bal üretimini daha fazla miktarda yapmaktadır. Bu da yapılan çalışmanın doğruluğu açısından önemli bir göstergedir. Mutki ilçesi çok uygun ve uygun arazi sınıfları bakımından zengin bir yerdir. Bu ilçede görülen düşük bal üretimi ise bu bölgede arıcılığın geliştirilebilmesi için teşviğe ihtiyaç olduğunu göstermektedir. Sağlanacak desteklerle bu ilçedeki arıcılık faaliyetlerinden elde edilen ürün miktarının artması beklenmektedir.

Tablo 3. Bitlis ili bal üretim istatistikleri ve arazi uygunluk sınıflarının ilçelere göre alansal değerleri (TÜİK 2023)

Table 3. Bitlis honey production statistics and areal values of land suitability classes by districts (TUIK 2023)

Uygunluk Sınıfı	Mutki	Merkez	Hizan	Tatvan	Ahlat	Güroymak	Adilcevaz	Toplam
Sınırlandırılmış (km^2)	169,79	219,02	186,93	1.019,11	339,26	138,61	996,67	3.069,39
Çok Az Uygun (km^2)	5,54	3,17	2,37	87,08	89,87	6,65	273,47	468,15
Az Uygun (km^2)	42,30	42,80	55,66	43,67	260,30	71,02	77,88	593,63
Orta Uygun (km^2)	25,55	35,71	60,33	131,76	118,78	52,13	113,34	537,60
Uygun (km^2)	374,51	348,67	377,16	442,26	194,45	162,05	104,71	2.003,81
Çok Uygun (km^2)	451,80	414,78	337,99	161,90	150,24	84,96	18,35	1.620,02
2022 Yılı Bal Üretimi (ton)	48,25	179,56	750,84	110,87	58,43	35,00	27,40	1.210,35
2021 Yılı Bal Üretimi (ton)	46,53	529,00	900,00	442,00	76,00	38,85	23,85	2.056,23

Arıcılık faaliyetlerinde yer seçimini etkileyen pek çok unsur vardır. Bu unsurların tamamının modellenebilmesi zor bir durum olsa da yerel ölçekli bazı temel gereksinimlere göre bu modellerin oluşturulabilmesi mümkündür (Ceylan ve Sarı 2017). Arıcılık için uygun yerlerin belirlenebilmesi amacıyla, Suudi Arabistan (Abou-Shaara vd. 2013), Filipinler (Estonque ve Murayama 2010), İran (Amiri ve Shariff 2012), Mısır (Abou-Shaara 2015) ve Türkiye'de (Ceylan ve Sarı 2017, Sarı vd. 2020a, 2020b, Elmastaş vd. 2022) çeşitli çalışmalar yapılmıştır. Türkiye'de yapılan çalışmalar Konya (Ceylan ve Sarı 2017, Sarı vd. 2020a, 2020b), Adıyaman (Elmastaş vd. 2022), İzmir (Yalçın vd. 2019) ve Artvin (Yılmaz vd. 2021) yörelerinde gerçekleştirilmiştir. Yapılan çalışmalarda kullanılan kriterlerin en önemlisi arazi örtüsü olarak değerlendirilirken diğer kriterler ise bölgelerin kendi gereksinimlerine göre seçilip ağırlıklandırılmıştır. Bu çalışmada seçilen kriterler, yapılan diğer çalışmalardaki kriterler ile benzerlikler göstermektedir (Tablo 1). Sıcaklık, NDVI ve rüzgâr kriterleri ise Türkiye'de yapılan çalışmalardan (Ceylan ve Sarı 2017, Yalçın vd. 2019, Sarı vd. 2020a, 2020b, Yılmaz vd. 2021, Elmastaş vd. 2022) farklı olarak bu çalışmada kullanılmıştır. Üretilen uygunluk haritalarının her biri kendi bölgelerinde arıcılık faaliyetleri için uygun yerleri ortaya koymaktadır ve her biri yapıldığı coğrafyaya özgüdür. Bundan dolayı farklı bölgelerin sonuçlarının kıyaslanması çok uygun değildir (Yılmaz vd. 2021). Yapılan bu çalışmada, Bitlis ili özelinde arıcılık faaliyetleri için çeşitli kriterler değerlendirilerek arazi uygunluk haritası yapılmıştır. Bu haritanın hassasiyeti belirlenen kriterlerin, istenilen amacın gereksinimlerini karşılaması ve doğru bir şekilde ağırlıklandırılıp puanlandırılması ile mümkündür. Kriterlere ait veri kaynaklarının hassasiyeti de uygunluk haritalarının doğruluğunu etkilemektedir. Çalışmada kullanılan kriterler ve bunların ağırlıkları benzer ekolojik özelliklere sahip alanlarda da kullanılabilir niteliktedir. Farklı ekolojik özelliklere sahip yerlerde, belirlenen kriterler ve ağırlıklar bölgelerin gereksinimlerine göre yenilenip değerlendirme yapılabilir.

Yapılan çalışma için gerekli olabilecek bazı kriterler ise verilerin olmayışı veya elde edilememesinden dolayı oluşturulan modele dahil edilememiştir. Bunlardan bazıları biyoçeşitlilik, flora, çiçeklenme dönemlerinin mekânsal ve zamansal değişimidir. Bu verilerin olması, oluşturulan haritayı daha hassas yapacaktır. Bitlis ilinde yapılan gezginci arıcılık faaliyetlerinde, arıcılar çiçeklenme dönemlerine göre kovanlarının yerlerini değiştirmektedir (Çağlıyan 2015). Bölgedeki floranın zamansal ve mekânsal değişimi ile ilgili bir verisinin olması durumunda oluşturulan uygunluk haritası farklı çiçeklenme dönemleri için yapılabilir. Yapılan bu çalışmada elde edilen uygunluk haritasının, mevcut arıcılık yapılan yerler ile uyumunun tespit edilmesi çalışmanın

geliştirilmesi açısından önemlidir. Bunun için mevcut arıcılık yerlerinin mekânsal verilerine ihtiyaç vardır. Bu amaçla mevcut arıcılık yerleri öğrenilmeye çalışılmıştır, ancak köy, mezra isimleri gibi geniş alanları kapsayan kayıtlara ulaşılmıştır. Konumsal dağılımları hakkında doğrulanmış bilgi toplamak mümkün olmadığından bu çalışmanın sonucunda elde edilen arıcılık için uygun alanların mevcut arıcılık sistemiyle karşılaştırması yapılamamıştır.

Sonuç ve Öneriler

1- Yapılan bu çalışmada CBS ve ÇKKV yöntemlerinden AHP metodu kullanılarak ağırlıklı bindirme yöntemi (weighted overlay) ile arıcılık için uygun alanlar haritalandırılmıştır.

2- Oluşturulan uygunluk sınıflarının ilçelere göre alanları hesaplanmış ve bu değerler bal üretim istatistikleri ile kıyaslanmıştır. Bu veriler arasında görülen uyum çalışmanın doğruluğu açısından önemlidir.

3- Başta Mutki ilçesi olmak üzere ilde yapılacak teşvikler ile bal üretim değerlerinin arttırılabileceği düşünülmektedir.

4- Bölgedeki bitkilerin polen ve nektar içeriği bakımından sınıflandırılması, çiçek açma dönemlerinin zamansal ve mekânsal olarak çalışılması farklı çiçek açma dönemleri için uygunluk haritalarının oluşturulabilmesini olanaklı kılacaktır.

5- Tarımsal üretimde zirai ilaçların arılara verdiği zarardan ötürü bu alanlar düşük puanlandırılmıştır. Bu alanlarda kullanılan ilaçların kullanılmaması veya azaltılması durumunda arıcılık için uygun alanların miktarında artış görülebilir. Ayrıca zirai ilaçların kullanım zamanlarının kayıt altına alınarak belirlenmesi durumunda yine bu husus dikkate alınarak dönemsel haritalar oluşturulabilir.

6- Arıcılık yapılan yerlerin mekânsal kayıtlarının oluşturulması yapılan çalışmanın doğrulanması açısından önemlidir. Bu veri ayrıca gezgin arıcılık faaliyeti sürdüren kişilerin belli bölgelerde yoğunlaşmasının önüne geçerek arazinin uygun olduğu, koloni sayısının ise yoğun olmadığı bölgelere arıcıların yönlendirilmesinde kullanılabilinir. Böylelikle arıcılık için uygun yerlerdeki birim alana düşen koloni sayısı kontrol altında tutularak verim kaybı azaltılabilir.

7- Bölgede arıcılığın gelecek yıllarda da sürdürülebilirliği için floranın korunması önemli bir konudur.

Çıkar Çatışması: Yazarın herhangi bir kişisel veya finansal çıkar çatışması bulunamamaktadır.

Etik Belgesi: Bu çalışma için etik belgesi gerekli değildir.

Mali Kaynak: Bu çalışma için sağlanmış mali kaynak bulunmamaktadır.

Veri Sağlama Durumu: Mevcut çalışma sırasında analiz edilen veri kümeleri, makul talep üzerine ilgili yazardan temin edilebilir.

KAYNAKLAR

Abou-Shaara HF. Wintering map for honey bee colonies in El-Behera governorate, Egypt by using Geographical Information System (GIS). Journal of Applied Sciences and Environmental Management. 2013; 17(3): 403-408, http://dx.doi.org/10.4314/jasem.v17i3.9

Abou-Shaara HF, Al-Ghamdi A, Mohamed A. suitability map for keeping honey bees under harsh environmental conditions using geographical information system. Arabia Saudita. *World Appl. Sci. J*, 2013, 22.8: 1099-1105, DOI: 10.5829/idosi.wasj.2013.22.08.7384

Abou-Shaara, HF. Suitability of current and future conditions to apiculture in Egypt using Geographical Information System. Journal of Agricultural Informatics, 2015; 6 (2): 12-22, https://doi.org/10.17700/jai.2015.6.2.189

Akıncı H, Özalp AY, Turgut B. Agricultural land use suitability analysis using GIS and AHP technique. Computers and electronics in agriculture. 2013; 97: 71-82, https://doi.org/10.1016/j.compag.2013.07.006

Amiri F, Shariff ARBM. Application of geographic information systems in land-use suitability evaluation for beekeeping: A case study of Vahregan watershed (Iran). African Journal of Agricultural Research. 2012; 7(1): 89-97, DOI: 10.5897/AJAR10.1037

Ay YE, Yiğit Y. Bal, beslenme ve sağlik. 3rd International Congress on Social Sciences, China to Adriatic, Antalya-Türkiye, Book of Proceedings, 27-30 Ekim 2016.

Ceylan DA, Sarı F. Konya İli İçin Çok Ölçütlü Karar Analizleri ile En Uygun Arıcılık Yerlerinin Belirlenmesi. Uludağ Arıcılık Dergisi. 2017;17(2):59-71, https://doi.org/10.31467/uluaricilik.373637

Çağlıyan A. Bitlis İlinde Arıcılık Faaliyetleri. Coğrafya Dergisi. 2015; 0(30): 1-25.

Çakmak İ, Aydın L, Seven S, Korkut M. Güney Marmara Bölgesi'nde arıcılık anket sonuçları. Uludağ Arıcılık Dergisi. 2003; 3(1): 31-36.

Çevrimli MB, Sakarya E. Arıcılık Ekonomisine Giriş ve Saha Verileri ile Bir Değerlendirme. Veteriner Farmakoloji ve Toksikoloji Derneği Bülteni. 2019;10(1), 40-48.

Elmastaş N, Ölmez İ, Vural E. Suitability Analysis of Apiculture (Beekeeping) Activity Areas with Multi-Criteria Method: A Case Study of Adıyaman. Coğrafya Dergisi. 2022; (44): 19-30, DOI: 10.26650/JGEOG2022-894419

Estoque RC, Murayama Y. Suitability analysis for beekeeping sites in La Union, Philippnes Using GIS and Multi-Crieria Evalution Techniques. Research Journal of Applied Sciences. 2010; 5(3): 242-253.

Estoque RC, Murayama Y. Spatial Analysis and Modeling in Geographical Transformation Process. Ed. Murayama Y, Thapa, RB, "Suitability Analysis for Beekeeping Sites Integrating GIS & MCE Techniques" The GeoJournal Library, 2011, p.215-233, DOI: 10.1007/978-94-007-0671-2_13

Everest T, Gür E. A GIS-based land evaluation model for peach cultivation by using AHP: a case study in NW Turkey. Environmental Monitoring and Assessment. 2022;194(4):1-15. https://doi.org/10.1007/s10661-022-09898-6

FAO (2023). Birleşmiş Milletler Gıda ve Tarım Örgütü, 2021 Yılı Dünya Bal üretim İstatistikleri, https://www.fao.org/faostat/en/#data/QCL (Erişim tarihi: 12/01/2023).

Fernandez P, Roque N, & AnjosO. Spatial multicriteria decision analysis to potential beekeeping assessment. Case study: Montesinho Natural Park (Portugal). 19th AGILE International Conference on Geographic Information Science-Geospatial Data in a Changing World, Helsinki-Finlandiya, Book of Proceedings, 14-17 June 2016.

Fick SE, Hijmans RJ. WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. International journal of climatology. 2017; 37(12): 4302-4315, https://doi.org/10.1002/joc.5086

Jankowski P. Integrating geographical information systems and multiple criteria decision-making methods. International journal of geographical information systems. 1995; 9(3): 251-273, https://doi.org/10.1080/02693799508902036

Kowalski S. Changes of antioxidant activity and formation of 5- hydroxymethylfurfural in honey during thermal and microwave Processing. Food Chemistry. 2013; (141): 1378–1382.

Kutlu MA, Özdemir FA, Kılıç Ö. Hizan İlçesindeki (Bitlis) Arıcılık Faaliyetleri Üzerine Bir Araştırma. Mustafa Kemal Üniversitesi Ziraat Fakültesi Dergisi. 2016; 21(2); 197-206.

Malczewski J. GIS and multicriteria decision analysis, John Wiley & Sons Press, 1999.

Mercan Ç. Yer yüzey sıcaklığının termal uzaktan algılama görüntüleri ile araştırılması: Muş ili örneği. Türkiye Uzaktan Algılama Dergisi. 2020; 2(2), 42-49.

Mercan Ç, Acıbuca V, Ayyıldız AŞ. Mardin İli Tarımında Ekonomik Sürdürülebilirliğe Yönelik Akademik Yaklaşımlar, Editör: Doğan Y, Acıbuca V, "Mardin İli Tarım Arazilerinin 1990-2018 Yılı Arasındaki Mekânsal Değişimi" Mardin Artuklu Üniversitesi Yayınları, Mardin, 2022, p. 73-89.

Mercan Ç, Arpağ S. Coğrafi Bilgi Sistem Analizleri Kullanılarak Toprak ve Arazi Özelliklerinin Değerlendirilmesi: Türkiye, Mardin İli Arazisi. Türkiye Tarımsal Araştırmalar Dergisi. 2020; 7(1), 23-33. https://doi.org/10.19159/tutad.644210

MGM 2023. Meteoroloji Genel Müdürlüğü, Resmi İstatistikler, https://mgm.gov.tr/veridegerlendirme/il-ve-ilceler-istatistik.aspx?k=undefined&m=BITLIS (Erişim tarihi: 10.01.2023).

Orhan O. Land suitability determination for citrus cultivation using a GIS-based multi-criteria analysis in Mersin. Turkey. Computers and Electronics in Agriculture. 2021; 190, 106433, https://doi.org/10.1016/j.compag.2021.106433.

Saaty TL. A scaling method for priorities in hierarchical structures. Journal of mathematical psychology. 1977; 15(3): 234-281, https://doi.org/10.1016/0022-2496(77)90033-5.

Saaty TL. The analytic hierarchy process: Planning, priority setting, resources allocation. McGraw Press, New York, 1980, p. 281.

Saaty RW. The analytic hierarchy process—what it is and how it is used. Mathematical modelling. 1987; 9(3-5): 161-176, https://doi.org/10.1016/0270-0255(87)90473-8.

Sarı F, Ceylan DA, Özcan MM. & Özcan, MM. A comparison of multicriteria decision analysis techniques for determining beekeeping suitability. Apidologie. 2020a; 51: 481-498.

Sarı F, Kandemir İ, Ceylan DA, Gül A. Using AHP and PROMETHEE multi-criteria decision making methods to define suitable apiary locations. Journal of Apicultural Research. 2020b; 59(4): 546-557. DOI: 10.1080/00218839.2020b.1718341.

Selçuk SF, Cebeci MS, Köker B, Yılmaz Z. Konya İli Arazi Kullanım/Örtüsü Değişim Analizi. Türkiye Peyzaj Araştırmaları Dergisi. 2021; 4(2): 100-114.

Sıralı YD. Arıcılığın Türkiye İçin Önemi. Arıcılık Araştırma Dergisi. 2010; 4: 3-4.

TÜİK 2023. Türkiye İstatistik Kurumu, Hayvancılık İstatistikleri, https://biruni.tuik.gov.tr/medas/?kn=101&locale=tr (Erişim tarihi: 11.03.2023).

Tunçel H. Türkiye'de (1966-1986 yılları arasında) arıcılığa genel bir bakış. Türkiye Coğrafyası Uygulama ve Araştırma Merkezi Dergisi. 1992; 1: 97-126.

Widiatmaka., Ambarwulan W, Sjamsudin CE, Syaufina L. Geographic information system and analytical hierarchy process for land use planning of beekeeping in forest margin of Bogor Regency, Indonesia. Jurnal Silvikultur Tropika. 2016; 7(3): S50-S57, https://doi.org/10.29244/j-siltrop.7.3.S50-S57

Yalçın H, Ağaçsapan B, Çabuk A. Coğrafi bilgi sistemleri ile uygun arıcılık yerlerinin belirlenmesi. GSI Journals Serie C: Advancements in Information Sciences and Technologies. 2019; 1(2): 1-15.

Yılmaz E, Sesli FA, & Uzun ÖF. Arıcılık Faaliyetleri İçin Uygun Yerlerin Coğrafi Bilgi Sistemleri ile Belirlenmesi: Şavşat İlçesi Örneği. Black Sea Journal of Engineering and Science. 2021; 4(3): 111-116.

Zoccali P, Malacrinò A, Campolo O, Laudani F. Algeri GM, Giunti G, Palmeri V. A novel GIS-based approach to assess beekeeping suitability of Mediterranean lands. Saudi journal of biological sciences. 2017; 24(5): 1045-1050, https://doi.org/10.1016/j.sjbs.2017.01.062.

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ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

ECONOMIC PERFORMANCE of WOMEN HONEY MARKETERS in ENUGU STATE, NIGERIA

Nijerya Enugu Eyaleti Kadın Bal Piyasacılarının Ekonomik Performansı

Ridwan MUKAILA[1], Abraham FALOLA[2], Sheu-Usman Oladipo AKANBI[3], Festus Eluwande Durojaye AWOYELU[4], Ibrahim Isaac UMARU[5*], Oyeyode Tohib OBALOLA[6], Cosmas Chikwado ONAKU[7]

[1]Department of Agricultural Economics, Faculty of Agriculture, University of Nigeria, Nsukka, NIGERIA, ORCID: 0000-0001-8584-0858, ridwan.mukaila@unn.edu.ng

[2]Department of Agricultural Economics and Farm Management, Faculty of Agriculture, University of Ilorin, Ilorin, NIGERIA, ORCID: 0000-0002--5265-9355, falola.a@unilorin.edu.ng

[3]Department of Agricultural Economics and Farm Management, Faculty of Agriculture, University of Ilorin, Ilorin, NIGERIA, ORCID: 0000-0003-0177-7084, akanbi.so@unilorin.edu.ng

[4]Department of Agricultural Economics, Faculty of Agriculture, University of Nigeria, Nsukka, NIGERIA, ORCID: 0000-0003-1482-0571, festus.awoyelu@unn.edu.ng

[*5]Department of Agricultural Economics, Faculty of Agriculture, University of Nigeria, Nsukka, NIGERIA, ORCID: 0000-0002-4937-0710, Corresponding author's E-mail: ibrahim.umaru@unn.edu.ng

[6]Department of Agricultural Economics, Faculty of Agriculture, Usman Danfodiyo University, Sokoto, NIGERIA, ORCID: 0000-0002-1230-1842, oyeyodeobalola@yahoo.com

[7]Department of Agricultural Economics, Faculty of Agriculture, University of Nigeria, Nsukka, NIGERIA, ORCID: 0000-0002-6901.8220, cosmasonakuc@gmail.com

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ABSTRACT

Honey marketing is an important off-farm economic activity for women's livelihood and sustenance. Despite this, there is scant information in the literature about the economic performance of women honey marketers. Therefore, this study investigates the economic performance of women marketers, its drivers, and its challenges. Data collected from 120 women honey marketers were analyzed using descriptive statistics, gross profit, net profit, benefit-cost ratio, return on capital invested, operating ratio, marketing margin, and multiple regression. We found that honey marketing was a profitable venture, as indicated by the high gross profit (USD 262.08), net income (USD 257.03), marketing margin (56%), benefit-cost ratio (1.72), and return on capital invested (0.72) per 58.14 liters sold. Honey marketing also had a low operating ratio of 0.57. Thus, women honey marketers performed economically well. The significant factors that enhanced the profitability of honey marketing were education, experience in honey marketing, credit, and membership in an association. While age, purchasing costs, and transportation costs reduced honey marketing profitability. Inadequate capital and credit, price fluctuations, a poor road network, high transportation costs, adulteration, and poor marketing information were the major severe constraints faced in honey marketing. These call for the provision of credit, training, and education to honey marketers by the government to enhance the profitability of the enterprise.

Keywords: Economic, Honey marketing, Performance, Profitability, Women marketers

ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

ÖZ

Bal pazarlaması, kadınların geçimi ve geçimi için önemli bir çiftlik dışı ekonomik faaliyettir. Buna rağmen literatürde kadın bal pazarlamacılarının ekonomik performansı hakkında çok az bilgi bulunmaktadır. Bu nedenle, bu çalışma kadın pazarlamacıların ekonomik performansını, itici güçlerini ve zorluklarını araştırıyor. Yüz yirmi kadın bal pazarlamacısından toplanan veriler, tanımlayıcı istatistikler, brüt kâr, net kâr, fayda-maliyet oranı, yatırılan sermaye getirisi, işletme oranı, pazarlama marjı ve çoklu regresyon kullanılarak analiz edildi. Yüksek brüt kar (262,08 ABD Doları), net gelir (257,03 ABD Doları), pazarlama marjı (%56), fayda-maliyet oranı (1,72) ve yatırılan sermaye getirisinin gösterdiği gibi bal pazarlamanın satılan 58,14 litre başına karlı bir girişim olduğunu bulduk (0,72). Bal pazarlaması da 0,57 gibi düşük bir işletme oranına sahipti. Böylece kadın bal pazarlamacıları ekonomik olarak iyi performans gösterdi. Bal pazarlamasının karlılığını artıran önemli faktörler eğitim, bal pazarlama deneyimi, kredi ve dernek üyeliği. Yaş, satın alma maliyetleri ve nakliye maliyetleri bal pazarlama karlılığını azaltmaktadır. Yetersiz sermaye ve kredi, fiyat dalgalanmaları, zayıf bir yol ağı, yüksek nakliye maliyetleri, tağşiş ve yetersiz pazarlama bilgisi, bal pazarlamasında karşılaşılan başlıca ciddi kısıtlamalardı. Bunlar, işletmenin karlılığını artırmak için hükümet tarafından bal pazarlamacılarına kredi, eğitim ve öğretim sağlanmasını gerektirir.

Anahtar kelimeler: Ekonomik, Bal pazarlaması, Performans, Karlılık, Kadın pazarlamacılar

GENİŞLETİLMİŞ ÖZET:

Çalışmanın amaçları: Bal pazarlamasının kadınların geçiminde oynadığı kilit role rağmen, literatürde kadın bal pazarlamacılarının ekonomik performansına ilişkin bilgilerin yetersiz olması nedeniyle, bu çalışma (i) kadın bal pazarlamacılarının ekonomik performansını araştırmış, (ii) kadınlar arasında bal pazarlama karlılığını etkileyen faktörleri incelemiş ve (iii) kadınların bal pazarlamasında karşılaştıkları kısıtlamaları belirlemiştir.

Materyaller ve yöntemler: Çalışma alanı Nijerya'nın Enugu Eyaletidir. Veriler, rastgele seçilen 120 kadın bal pazarlamacısından yapılandırılmış anketlerle toplanmıştır. Veriler tanımlayıcı istatistikler (ortalama, frekans ve yüzde), karlılık analizi (brüt kar, net çiftlik geliri, fayda-maliyet oranı, yatırılan sermaye, işletme oranı ve pazarlama marjı), çoklu regresyon modeli ve Likert ölçekli derecelendirme kullanılarak analiz edilmiştir.

Bulgular: Kadın pazarlamacılar bal satışından ortalama 614,95 ABD doları gelir elde etmiştir. Ortalama 58,14 litre balı 269,69 ABD Doları karşılığında satın almışlardır. Bal pazarlamacılarının toplam değişken maliyeti 352,87 ABD Doları olup, bu tutar 5,05 ABD Doları olan toplam sabit maliyetten daha yüksektir. Ham bal satın alma maliyeti, işçilik maliyeti, nakliye maliyeti ve markalama maliyetleri kadın pazarlamacılar arasında bal pazarlamasının başlıca maliyetleridir. Kadın bal pazarlamacıları, küçük ölçekli faaliyet düzeyleri dikkate alındığında pozitif ve nispeten yüksek brüt kâr (262,08 ABD Doları) ve net kâr (257,03 ABD Doları) elde etmişlerdir. Yüksek bir fayda-maliyet oranına (1,72), yatırılan sermayenin getirisine (0,72) ve pazarlama marjına (%56) sahiptiler. Faaliyet oranları ise 0,57 ile düşüktür. Tüm bu ekonomik performans ölçütleri, bal pazarlamasının kadınlar arasında karlı bir tarımsal işletme olduğunu göstermektedir.

Bal pazarlamasının ekonomik performansını (karlılık) olumlu yönde etkileyen önemli değişkenler eğitim, bal pazarlamasında deneyim, alınan kredi miktarı ve dernek üyeliğidir. Bu durum, bu değişkenlerdeki artışın kadın bal pazarlamacılarının karlılığını artırdığını göstermektedir. Yaş, satın alma maliyeti ve nakliye maliyetleri ise kadın bal pazarlamacılarının ekonomik performansını negatif ve anlamlı olarak etkilemektedir. Bu durum, bu değişkenlerdeki artışın kadın bal pazarlamacılarının karlılığını azalttığını göstermektedir.

Kadın bal pazarlamacılarının bal pazarlama faaliyetlerinde karşılaştıkları kısıtlarla ilgili olarak, yetersiz sermaye ve kredi eksikliği, fiyat dalgalanmaları, balın yüksek maliyeti, zayıf yol altyapısı ve yüksek nakliye maliyetleri sırasıyla kadınların bal pazarlamasında karşılaştıkları birinci, ikinci, üçüncü, dördüncü ve beşinci ciddi kısıtlardır. Bal pazarlamasında karşılaşılan diğer ciddi kısıtlar, önem derecelerine göre, bal pazarlaması için zayıf işletme gelişimi, bazı üreticilerin balda tağşiş yapması, zayıf pazarlama bilgisi, modern bal depolama tesislerinin eksikliği, balın önemi

konusunda tüketici bilincinin zayıf olması ve kalifiye işgücü azlığı ve yüksek işgücü maliyetidir.

Sonuç: Bal pazarlaması, hem kırsal hem de kentli kadınların ekonomik durumlarını ve refahlarını iyileştirmek için kullanılabilecek karlı, ekonomik ve uygulanabilir bir tarım dışı faaliyettir. Bu çalışma, hükümet organlarına, kalkınma ajanslarına ve finans kuruluşlarına, işletmelerinin karlılığını artırmak için kadın pazarlamacılara yardımcı olmaları çağrısında bulunmaktadır. Bu, mali yardım (hibe ve/veya kredi) ve kadın bal pazarlamacılarına pazarlama ve işletme geliştirme konularında eğitim ve öğretim sağlanması şeklinde olabilir.

INTRODUCTION

Honey is a naturally sweet food product, complex in nature, that has extraordinary flavor and aroma, sugars, pollen grains, waxes, pigments, flavonoids, phenols, lipids, vitamins, minerals, enzymes, amino acids, organic acids, and other phytochemicals (Belay et al. 2017, Machado et al. 2018, Mulugeta & Belay 2022). It is made by honeybees from honeydew or nectar and is widely consumed worldwide due to its health benefits (Gebeyehu & Jalata 2023). Globally, honey is known to have several applications and uses in industry, medicine, and nutrition (Gela et al. 2021). It plays a critical role in human health, nutrition, and treatment of diseases (Asari et al. 2022, Cırık & Aksoy 2020; Demirkaya & Sagdicoglu Celep 2022 Ranneh et al. 2021). As a result, it is in high demand locally, is traded globally, and commands a higher premium (García 2018; Gela et al. 2021). Honey also plays an important role in the economic status of both rural and urban dwellers, and it contributes to the nation's economy (Arowolo et al. 2020, Gebeyehu & Jalata 2023; Mulugeta & Belay 2022, Verma et al. 2018). Honey, through production and marketing, is also an important economic activity that can be used to fight against poverty (Shrestha 2017).

In Nigeria, honey marketing is an important off-farm agricultural activity among both rural and urban women, with several benefits and advantages over other agricultural practices. In comparison to other agribusiness enterprises that are highly capital intensive, the honey marketing business, for example, requires little capital as a start-up. Also, it does not require the purchase of land, which makes the women venture into it as most African women have no access to land. It, therefore, serves as a means of livelihood and sustenance for women in Nigeria and other sub-Saharan African nations. Women honey marketers play a significant role in getting honey to consumers through their marketing functions. Marketing functions such as labeling, branding, and packaging show the benefits of honey to consumers, which motivates their decision to purchase (Madas et al. 2020). Honey marketers, therefore, directly and indirectly, contribute to honey producers' well-being and economic status by assisting them to get revenue from their production activities.

Production without an efficient marketing system will lead to the spoilage of goods and economic losses in the agricultural enterprise. Therefore, agricultural marketing is an important agricultural activity as it deals with all activities that happen from the farm gate to the final consumer (Mukaila et al. 2021). Honey farmers' output will remain on the farm without agricultural marketers that distribute the produce to the final consumer who pays for the product. Despite the critical role agricultural marketing plays in honey, most attention has been on the production side.

There exists a large volume of literature on honey production, productivity, economic value, and profitability (Adedeji & Omoba 2016, Ajao & Oladimeji 2015, Akinade 2019, Akinmulewo et al. 2017, Bhatta et al. 2020, Chiemela et al. 2022, DeGrandi-Hoffman et al. 2019, Elzaki & Tian 2020, Masuku 2013, Ogunola et al. 2019, Otim et al. 2019, Onyekuru et al. 2010, Shrestha 2017, Stojanov et al. 2021, Vaziritabar & Esmaeilzade 2016, Verma et al. 2018, Vrabcová & Hájek 2020). These studies have shown that honey production is profitable. Meanwhile, there exist limited studies on the profitability of honey marketing (Arowolo et al. 2020, Mshelia et al. 2013, Yeserah et al. 2019). Arowolo et al. (2020) and Yeserah et al. (2019) assessed honey marketing, structure, and conduct. The analysis of Mshelia et al. (2013) was based on only gross margin, which was not enough to show the profitability or economic performance of honey marketing. These previous studies did not extensively investigate the economic performance of honey marketing and were not focused on women marketers, creating a gap in the honey marketing literature that the current study intends to fill. This study, therefore, contributed to the literature by (i) investigating the economic performance of women honey marketers, (ii) determining the factors influencing honey marketing profitability among

women, and (iii) identifying the constraints faced by women in honey marketing.

This study provides detailed empirical literature on women's honey marketing performance for future studies to build on. As a result of revealing the determinants of women marketers' economic performance and the barriers they faced in efficient marketing, it would serve as a policy framework for interventions. Thus, women marketers would benefit from the outcome of this work, which would consequently increase women's participation in honey marketing, which is needed in light of the current global health challenges.

MATERIALS AND METHODS

Study area

Enugu State was the study area. Enugu state is located in southeastern Nigeria on the coordinates 6.5364° N, 7.4356° E. Women entrepreneurs in the state are engaged in on-farm and off-farm agricultural activities such as honey marketing and processing. They also engaged in other entrepreneurship activities like trading, and artisanship.

Sampling procedure

Three local government areas (LGAs)—Nsukka, Uzouwani, and Enugu South—were purposefully selected from Enugu State, Nigeria, for the study due to the high concentration of honey marketers. From the three selected LGAs, four communities were randomly selected. Finally, ten honey marketers were randomly selected from each community. This resulted in a total of 120 honey marketers.

According to Bannor et al. (2022), a sample size of $n \geq 50+8p$ is sufficient for regression analysis (p is the number of independent variables). For this study, $p = 8$. Following this formula, the minimum sample size (n) for this study is 114. This suggests that the sample size of 120 is adequate for the regression analysis.

Data collection

The primary data were elicited through the use of a structured questionnaire. The data collected include the socio-economic characteristics of women honey marketers, the cost and returns associated with honey marketing, and the constraints faced in honey marketing.

Data analysis

Descriptive statistics: Descriptive statistics such as mean, frequency, and percentage were used to describe the socio-economic characteristics of women honey marketers.

Gross profit: Gross profit is the difference between the total revenue accrued from the marketing enterprise and the total variable cost (Falola et al. 2022a). It is stated as follows:

$$Gross\ profit = Total\ revenue - Total\ variable\ cost$$

Total revenue is generated by multiplying the unit price by the quantity sold by honey marketers. That is P (price) x Q (quantity).

Net farm income: Net farm income was further estimated to show the net profit of honey marketing because gross profit did not include total fixed costs in its estimation (Falola et al. 2022a). The fixed cost was estimated using a straight-line method to depreciate the fixed items used in honey marketing. The NFI is expressed as:

$$Net\ farm\ income = Gross\ profit - Total\ fixed\ cost \quad \text{or}$$

$$Net\ farm\ income = Total\ revenue - Total\ cost$$

Benefit-cost ratio: The benefit-cost ratio is estimated by dividing total revenue by total cost (Falola et al. 2022a; Mukaila et al. 2022). It measures the viability and strength of the honey marketing enterprise and its benefits in monetary terms. It is expressed as:

$$Benefit\ cost\ ratio = \frac{Total\ revenue}{Total\ cost}$$

Return on capital invested: This estimates the amount received by women honey marketers per currency invested and measures the efficiency of the enterprise. It is estimated as "net farm income" divided by the "total cost" (Falola et al. 2022a; Mukaila et al. 2022). It is expressed as:

$$Return\ on\ capital\ invested = \frac{Net\ farm\ income}{Total\ cost}$$

Operating ratio: This is estimated to measure the proportion of honey marketing revenue used as a variable cost (operational cost). A low operating ratio indicates that the marketing enterprise is profitable, and vice versa (Mukaila et al. 2022). Thus, the lower the operating ratio, the higher the profitability of honey marketing among women. It is expressed as:

$$Operating\ ratio = \frac{Total\ variable\ cost}{Total\ revenue}$$

Marketing margin: Marketing margin analysis was used to determine the marketing margins of honey marketing. It depicts the marketers' share of the consumer price (Mukaila et al. 2021; Obetta et al. 2020a). Following Mukaila et al. (2021) and Obetta et al. (2020a), it is determined by finding the difference between the consumer price and the price paid by honey marketers to the producers, dividing it by the consumer price, and taking the result as a percentage. It is expressed as:

$$Marketing\ margin = \frac{Selling\ price - Purchasing\ price}{Selling\ price} X \frac{100}{1}$$

Multiple regressions: The multiple regression model was used to investigate the factors responsible for honey marketing profitability (economic performance). This was used because the dependent variable net income is a continuous variable and other similar studies have used multiple regression in their analysis (Arowolo et al. 2020, Mshelia et al. 2013). Net farm income was used as a proxy for profitability because economic performance depends on the net return from the business enterprise. The multiple regression model is specified as:

$$Y = \beta_0 + \beta_1 A + \beta_2 ED + \beta_3 HS + \beta_4 EX + \beta_5 C + \beta_6 AM + \beta_7 PP + \beta_8 TC + e$$

Where Y = net income from honey marketing (amount in USD); A = age of honey marketers (years); ED = education level (years spent in school); HS = Household size (number of persons in the household); EX = experience in honey marketing (years); C = credit (the amount borrowed in USD); AM = association membership (yes = 1, no = 0); PP = purchasing price; TC = transportation cost; β_0 = constant term; $\beta_{1\text{-}8}$ = coefficients of the regression model; and e = error term.

Likert rating scale: A four points Likert rating scale by Likert (1932) was used to identify the severe

constraints in honey marketing among women. The severity scales used in this study are extremely serious (four points), very serious (three), moderately serious (two), and not at all serious (one). The mean score of the four points scale was calculated to be 2.5 [(4+3+2+1)/4]. This was used to decide if a problem is severe or not. Any Likert score equal to or greater than 2.5 was considered a severe problem, while those less than 2.5 were considered not to be severe constraints faced by women in honey marketing.

RESULTS

Socio-economic characteristics of honey marketers

Table 1 presents the socioeconomic characteristics of women honey marketers. The majority (97.5%) of honey marketers were under 51 years old. They had an average age of 40.3 years, which is an indication that they were still within their economically active age where they can market honey efficiently. Most of the honey marketers had formal education, as only 20% of them had no formal education. This could influence their marketing and profitability positively because education paves the way for marketing information and economic sustainability. The majority of the marketers were married; 15.83% were single; and 5.83% were divorced. Fifty-two percent of the honey marketers had between five and eight household members, while 48% had between one and four household members. They had an average household size of about five people, which could serve as cheap family labor.

Regarding their major occupation, honey marketing was the major occupation of most of the respondents, which is an indication that this study targeted the right population. Only a few had crop farming and trading as their major occupation. The larger proportion had less than or equal to five years of honey marketing experience, followed by six to ten years, between eleven and fifteen years, and above fifteen years. They had an average of 8.75 years of honey marketing experience; thus, they are experience honey marketers. Membership in the association was extremely low among honey marketers, with only 22% belonging to the cooperative association. In the same vein, access to credit was also very low among this group of marketers, as only 26% could access credit. This could affect their level of operation by limiting their activities to a small scale. The honey marketers had an average monthly income of USD 183.63, which is higher than the Nigerian minimum wage. This is an indication that honey marketing serves as a means of income generation for women.

The economic performance of women honey marketers

Table 2 shows the profitability of honey marketing among women, which was used as a proxy for the economic performance of women in the honey marketing enterprise. The average quantity purchased by honey marketers in a month was 58.14 liters at a purchasing price of USD 4.64 per liter. Thus, the average cost of honey purchased was USD 269.69. At an average selling price of USD 10.58, the women marketers received an average revenue of USD 614.95 for the 58.12 liters of honey sold. The total variable cost incurred in honey marketing was USD 352.87, and the total fixed cost incurred was USD 5.05. This gave a total cost of USD 357.92.

The cost of purchasing honey from the honey producers or farmers constituted the highest share of the total cost (Figure 1). The cost of labor which accounted for 15.21% of the total cost had the second largest share of the total cost of honey marketing. The cost of transporting honey from the apiary to the market accounted for 5.6% of the total cost, and branding costs accounted for 1.87% of the total cost. Cost of rent accounted for 0.98%, packaging costs accounted for 0.56%, and marketing taxes or levies accounted for 0.32% of the total cost incurred in honey marketing. In addition, the cost of a bucket, a jerry can, and the sieve used in storing and processing (filtration) honey accounted for 0.32%, 0.03%, and 0.03%, respectively.

Table 1: Socio-economic characteristics of women honey marketers

Variable	Category	Frequency	Percentage
Age (years) Mean = 40.3	≤ 30	26	21.67
	31 – 40	48	40
	41 – 50	43	36
	51 – 60	3	2.5
Educational level	No education	24	20
	Primary	29	24.17
	Secondary	63	52.5
	Tertiary	4	3.33
Marital status	Married	94	78.33
	Single	19	15.83
	Divorced	7	5.83
Household size Mean = 4.59	1 – 4	58	48.33
	5 – 8	62	51.67
Major occupation	Honey marketing	108	90
	Crop farming	5	4.17
	Trading	7	5.83
Honey marketing experience Mean = 8.75	< 5	49	40.83
	6-10	36	30
	11-15	23	19.17
	> 15	12	10
Membership in a cooperative association	Yes	27	22.5
	No	93	77.5
Access to credit	Yes	31	25.83
	No	89	74.17
Monthly income (USD) Mean = USD 183.63	≤ 100	31	25.83
	101 – 200	51	42.50
	201 – 300	32	26.67
	≥ 301	6	5

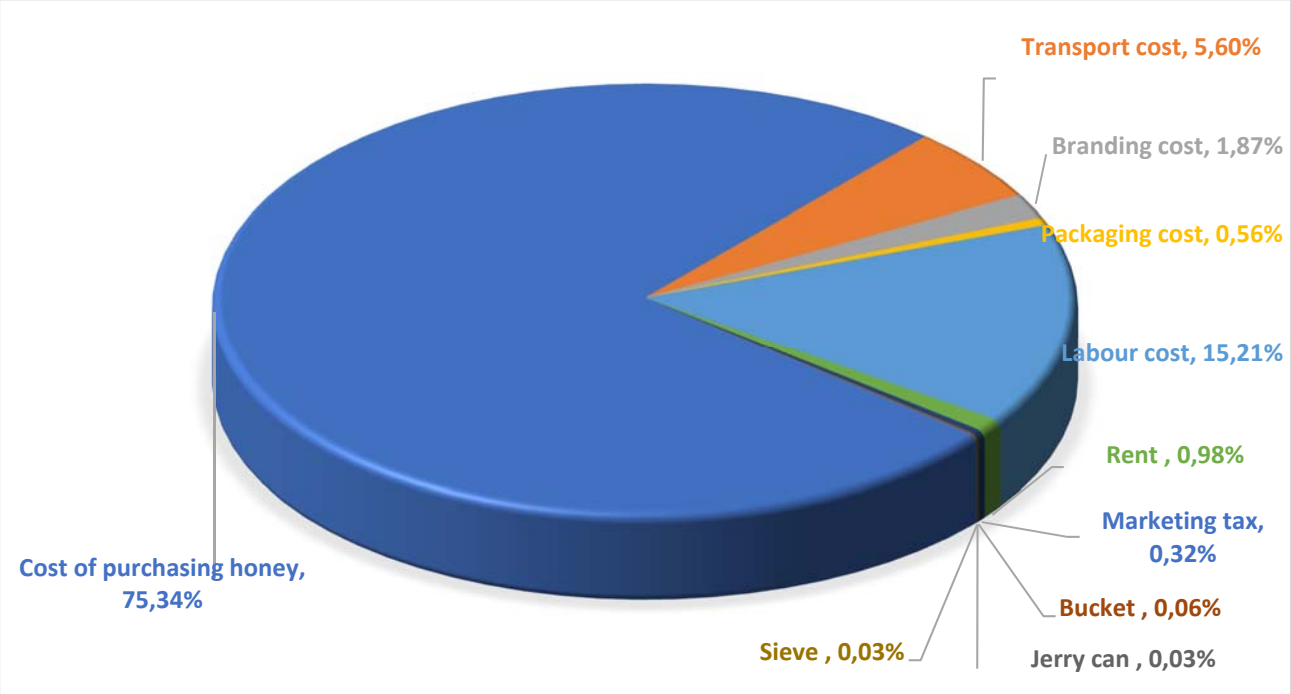

Figure 1: The percentage share of each cost item in the total cost of honey marketing

Table 2 further shows that the women honey marketers made a gross profit of USD 262.08 and a net profit of USD 257.03, respectively. This shows that honey marketing among women was a profitable venture. The women honey marketers had a benefit-cost ratio of 1.72, a return on capital invested of 0.72, an operating ratio of 0.57 and a marketing margin of 56.14%.

Factors Influencing Honey Marketing Profitability

Table 3 shows the results of a multiple regression analysis that was used to investigate the factors influencing honey marketing profitability. The significant variables were age, education, experience in honey marketing, amount of credit borrowed, association membership, purchasing cost, and transportation cost. At the 5% level of significance, the age coefficient had a negative impact on the profitability of honey marketing. The coefficient of education positively influenced the profitability of honey marketing at a 5% level of significance. The coefficient of experience in honey marketing positively influenced the profitability of honey marketing at a 5% level of significance.

Furthermore, the coefficient of credit positively influenced the profitability of honey marketing at a 1% level of significance. The coefficient of association membership positively influenced the profitability of honey marketing at a 10% level of significance. The coefficient of purchasing cost negatively influenced the profitability of honey marketing at the 1% level of significance. At the 5% level of significance, the transportation cost coefficient had a negative impact on the profitability of honey marketing.

Table 2: The cost and returns of honey marketing

Items	Value (USD)
Quantity purchased (Liters)	
Purchasing price	4.64
Selling price	10.58
Revenue	614.95
Variable costs	
Cost of purchasing honey	269.69
Transport cost	20.05
Branding cost	6.69
Packaging cost	2.01
Labor cost	54.44
Total variable cost	352.87
Fixed cost	
Rent	3.50
Marketing tax/levies	1.15
Bucket	0.20
Jerry can	0.09
Sieve	0.11
Total fixed cost	5.05
Total cost	357.92
Gross profit	262.08
Net profit	257.03
Benefit-cost ratio	
Net return on capital invested	
Operating ratio	
Marketing margin (%)	

Table 3: Factors influencing honey marketing profitability

	Coefficient	Standard Error	T	P>t
Age	-5526.808**	2675.709	-2.07	0.045
Education	8436.018**	3415.392	2.47	0.017
Household size	-9470.667	8974.058	-1.06	0.297
Experience in honey marketing	12925.32**	6334.747	2.04	0.048
Credit	.0204718***	.0048976	4.18	0.000
Association membership	40307.85*	22307.61	1.81	0.078
Purchasing price	-6.162657***	1.527352	-4.03	0.000
Transportation cost	-2.531561**	.9517147	-2.66	0.012
Constant	726267.3	171295.2	4.24	0.000
F	5.47			
Prob > F	0.0000			
R-squared	0.4893			
Adj R-squared	0.3992			

*** means significant at 1% level, ** means significant at 5%

Constraints faced in honey marketing

Table 4 presents the constraints faced by women honey marketers in their efficient honey marketing. The first ranked severe constraints in efficient honey marketing among women was inadequate capital and a lack of credit. This was followed by price fluctuation, the high cost of honey, poor road infrastructure and high transportation costs, poor enterprise development for honey marketing, adulteration of honey by some producers, and poor marketing information. Other severe constraints faced in honey marketing, according to their severity, were the lack of modern honey storage equipment, the high cost of labor or lack of skilled labor, and poor awareness of the importance of honey to consumers, which inhibits consumers' willingness to pay more for honey.

Table 4: Constraints in honey marketing

	Mean	Std. Dev	Rank
Inadequate capital and lack of credit	3.94*	0.238	1st
Price fluctuation	3.65*	0.522	2nd
High cost of honey	3.63*	0.528	3rd
Poor road network and high cost of transportation	3.39*	0.777	4th
Poor enterprise development towards honey marketing	3.37*	0.720	5th
Adulteration	3.35*	0.868	6th
Poor marketing information	3.29*	0.576	7th
Lack of modern honey storage equipment	3.12*	0.683	8th
High cost of labor/Lack of labor	2.88*	0.765	9th
Poor awareness of the importance of honey to consumers	2.82*	0.785	10th

* Means severe

DISCUSSION

Regarding the economic performance of women honey marketers, the women marketers received an average revenue of USD 614.95 from the sales of honey in a month. They purchased an average quantity of 58.14 liters for USD 269.69. The honey marketers incurred a total variable cost (USD 352.87) higher than the total fixed cost (USD 5.05). Therefore, it can be inferred from this finding that the variable cost accounted for the highest proportion (98.59%) of the total cost. This supports Mshelia et al. (2013), who found that variable costs accounted for the highest share of total costs in honey marketing. The cost of purchasing honey from the honey producers or farmers constituted the highest share of the total cost incurred in honey marketing. This is in line with Mshelia et al. (2013) that the cost of purchasing honey had the highest share of the total cost in the honey marketing enterprise. This was followed by the cost of labor, the cost of transporting honey from the apiary to the market, and branding costs. Mshelia et al. (2013) discovered that labor and transportation costs accounted for the second and third largest shares of total costs in the honey marketing enterprise, respectively. Other costs incurred in honey marketing among the women in descending order of their contribution were the cost of rent, packaging costs, marketing taxes or levies, the cost of a bucket, the cost of a jerry can, and the cost of a sieve used in storing and processing (filtration) honey. Thus, the cost of purchasing raw honey, the labor cost, and the cost of transportation were the major costs of honey marketing among women marketers. This indicates that any intervention to reduce these costs will go a long way to enhance the economic performance of women honey marketers.

After subtracting the total variable cost and fixed cost from the total revenue, the women honey marketers made a positive and relatively high gross profit (USD 262.08) and net profit (USD 257.03), respectively based on their small-scale level of operation. This shows that honey marketing among women was a profitable enterprise. This is in line with Arowolo et

al. (2020) and Mshelia et al. (2013), who opined that marketing honey was a profitable venture. The benefit-cost ratio (1.72), which was greater than 1, further ascertains the profitability of honey marketing among women. The return on capital invested of 0.72 implies that for every USD 1 invested in honey marketing, USD 0.72 was realized as profit among the women marketers. This high return on investment further shows that honey marketing was a profitable, viable, and economical venture. The operating ratio of 0.57 implies that 57% of the total revenue from honey marketing was used for operational costs, which is relatively low. This further shows that honey marketing was a profitable off-farm agricultural enterprise among women. The marketing margin of 56.14% implies that the honey marketers had a 56% share of the consumer price. This shows that the honey marketers had the larger share of the consumer price, which also indicates that the marketing enterprise gives a higher return.

The significant variables that positively influenced honey marketing economic performance (profitability) were education, experience in honey marketing, amount of credit borrowed, and association membership. While age, purchasing cost, and transportation costs negatively and significantly influence women honey marketers' economic performance. The positive influence of women honey marketers' education on their profitability is an indication that their level of education increased the profitability (economic performance) of honey marketing enterprises. Thus, education is an enhancing factor in honey marketing profitability. This could be because education paves the way for the relevant information needed to boost revenue (Akanbi et al. 2022). A similar finding was reported by Arowolo et al. (2020), who found that years spent in school enhanced the marketing performance of honey. The positive influence of honey marketers' years of experience on honey marketing profitability is an indication that an increase in honey marketing experience increased the profitability of honey marketing enterprises. This is because years spent in an agribusiness enterprise determine the owner's skills and understanding of the business, which are needed to boost income. Thus, years of experience is an enhancing factor in honey marketing performance (profitability). This is in line with the findings of Arowolo et al. (2020) and Mshelia et al. (2013) that years of experience in honey marketing positively influenced the marketing profitability of honey.

The positive influence of access to credit (the amount borrowed) on the profitability of honey marketing implies that the profitability of honey marketing increases as the amount of credit borrowed and used in honey marketing increases. This is because credit serves as a means of capital used for investment (Falola et al. 2022b), which consequently increases the revenue generated from honey marketing. Thus, women honey marketers who could access credit had a higher net income than their counterparts who could not access credit facilities. The positive influence of association membership on the profitability of honey marketing is an indication that an increase in the probability of being a member of an association increases the profitability of honey marketing agribusiness enterprises. This could be because of the benefits derived from an association such as the pooling of resources to buy in large quantities (Mukaila et al. 2022; Musinguzi et al. 2018). This reduces the purchasing price and transportation cost which leads to the enjoyment of economies of scale among women (Mukaila et al. 2022). Therefore, honey marketers who belong to the association made a higher profit than their counterparts who did not.

The negative influence of age on women honey marketers' profitability is an indication that their economic performance (profitability) is reduced with an increase in their age. As a result, younger honey marketers profited more than older ones. This is because younger marketers are more educated, enlightened, and have access to relevant marketing information, which is needed for efficient marketing and consequently will result in an increase in the profitability of the ventures (Mukaila et al. 2021). The negative influence of purchasing costs on the profitability of honey marketing implies that an increase in purchasing costs will reduce the profitability of the honey marketing enterprise. This agrees with the a priori expectation, as purchasing costs are the most important variable costs (and have the highest share) in honey marketing. Thus, the lower the purchasing cost, the higher the profitability of honey marketing among women as long as the quality remains the same. The negative impact of transportation costs on the profitability of honey marketing implies that transportation costs reduced the profitability of honey marketing. This is because transportation and distribution are key marketing functions and an important variable cost. In addition, the cost of transportation increases the variable cost of the agricultural enterprise and

consequently decreases the profitability of the enterprise (Mukaila et al. 2022).

Regarding the constraints faced inefficient honey marketing among women, inadequate capital and a lack of credit were the most serious constraints faced by women in honey marketing. The women honey marketers complained of a lack of access to credit and low capital. This had an impact on their operations, limiting them to a small-scale level. This is because capital is an important aspect of agribusiness and contributes to farm investments (Falola et al. 2022b). Arowolo et al. (2020) and Yeserah et al. (2019) also reported that lack of credit access was a serious constraint in honey marketing. The second-most severe constraint was price fluctuation. Price fluctuation remains a serious barrier to off-farm agricultural activities among women as it comes with several uncertainties (Obetta et al. 2020b). The third-ranked constraint faced by women honey marketers was the high cost of honey. They lamented that the producers' price of honey has drastically increased in recent times, which forced them to also increase their price, and the consumers have not yet gotten used to the current increased price of honey in the study area. Yeserah et al. (2019) also reported that high producers' price was a severe constraint to honey marketing. Poor road infrastructure and high transportation costs were also cited as major impediments to women's honey marketing. This added to their variable cost of production and consequently reduced the net income from the enterprise. Poor enterprise development for honey marketing was also a severe constraint, as the women marketers have not been receiving special training on enterprise development either from the government or extension agents. Adulteration of honey by some producers was also a severe problem faced by honey marketers. This is in line with Arowolo et al. (2020), who reported that honey adulteration was a severe constraint in honey marketing.

Poor marketing information was another severe constraint hindering efficient honey marketing among women marketers. Information plays a critical role in agricultural marketing; some lamented that they do not receive information about the increase in the price of honey, especially from the producers, which was due to a poor network in the rural areas. They mostly learn about price increases when they get to the producers, which limits the amount they can buy because they have not planned for it. The lack of modern honey storage facilities also limits women's marketing activities. There are some seasons when the price of honey is relatively low; however, women marketers were unable to purchase large quantities during these times in order to store it for an extended period due to concerns about spoilage or a reduction in the quality of the honey. Poor consumer awareness of the importance of honey was also a severe constraint affecting efficient honey marketing among women. Some consumers were unaware of honey's numerous health benefits, such as the recently discovered key health benefits of honey in boosting the immune system during COVID-19 and mitigating COVID-19 risks, as reported by Al Naggar et al. (2021) and Lima et al. (2021). These low levels of awareness prevent consumers from being willing to pay more for honey during periods of price inflation. There was also a scarcity of skilled labor and those who are available charge women marketers a high premium. However, this was the least ranked constraint affecting female honey marketers.

Conclusion

This study reveals that honey marketing is a profitable venture and women honey marketers performed economically well. Education, experience in honey marketing, credit, membership in the association, age, purchasing costs, and transportation costs were responsible for their economic performance. The severe constraints faced in honey marketing were inadequate capital and lack of credit, price fluctuation, high cost of transportation, poor enterprise development, adulteration, and poor marketing information.

These findings call for financial assistance by government bodies and financial institutions to boost women marketers' capital, which is needed to expand their marketing businesses. Women marketers can form cooperative societies to get financial assistance and enjoy economies of scale. The provision of marketing and enterprise development training by extension agents to honey marketers is needed to boost their profit. Universities and research centers need to disseminate the benefits of using honey to treat a variety of ailments and strengthen body immunity in order to increase demand for honey. Future studies can focus on other honey bee hive products, which are also of great importance.

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Ethical issue: None.

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REFERENCES

Adedeji NK, Omoba OJ. An assessment of profitability of honey production in Edo State, Nigeria. *African Journal of Agricultural Economics and Rural Development,* 2016, *4*(6), 442 – 445.

Ajao, A.M., Oladimeji, Y.U. 2015. Structure, production and constraints of honey hunting and traditional beekeeping activities in Patigi, Kwara state, Nigeria. *Egyptian Academic Journal of Biological Sciences a Entomology*, 8(1), 41-52.

Akanbi SO, Mukaila R, Adebisi A. Analysis of rice production and the impacts of the usage of certified seeds on yield and income in Cote d'Ivoire. *Journal of Agribusiness in Developing and Emerging Economies,* 2022, https://doi.org/10.1108/JADEE-04-2022-0066

Akinade TG. Prospects and challenges of beekeeping in Potiskum Local Government Area of Yobe State, Nigeria. *International Journal of Innovative Agriculture & Biology Research,* 2019, *7*(2), 19-25.

Akinmulewo BO, Oladimeji YU, Abdulsalam Z. Assessment of the profitability of improved apiculture in Federal Capital Territory (FCT) Abuja, Nigeria. *Journal of Sustainable Development in Africa,* 2017, *19*(1), 23-35.

Al Naggar Y, Giesy JP, Abdel-Daim MM, Javed Ansari M, Al-Kahtani SN, Yahya G. Fighting against the second wave of COVID-19: Can honeybee products help protect against the pandemic? *Saudi Journal of Biological Sciences,* 2021, *28*(3), 1519–1527. doi.org/10.1016/j.sjbs.2020.12.031

Arowolo OV, Kabir GB, Fatoki OA, Oguntoye TO. Structure, conduct and performance analysis of honey markets in Oyo state, *Nigeria. Journal of Research in Forestry, Wildlife & Environment,* 2020, *12*(1), 167-177.

Asari MA, Sirajudeen KNS, Yusof NAM, Mohd Amin MSI. DHA-rich fish oil and Tualang honey reduce chronic stress-induced oxidative damage in the brain of rat model. *Journal of Traditional and Complementary Medicine,* 2022, 12, 361e366. doi.org/10.1016/J.JTCME.2021.10.001

Bannor RK, Oppong-Kyeremeh H, Aguah DA, Kyire SKC. An analysis of the effect of fall armyworm on the food security status of maize-producing households in Ghana. *International Journal of Social Economics*, 2022, 49(4), 562-580.

Belay A, Haki GD, Birringer M, Borck H, Lee YC, Kim KT, Melaku S. Enzyme activity, amino acid profiles and hydroxymethylfurfural content in Ethiopian monofloral honey. *Journal of Food Science and Technology,* 2017, *54*(9), 2769–2778.

Bhatta S, Baral S, Datta JP. Economic analysis of honey production in Chitwan District, Nepal. *American Journal of Agricultural and Biological Sciences,* 2020,*15*(1), 132.137.

Chiemela CJ, Mukaila R, Ukwuaba IC. Economics on the use of modern and traditional methods in honey production among farmers in Enugu State Nigeria. *Journal of Agriculture Faculty of Ege University,* 2022, *59*(4), 611-619. doi.org/10.20289/zfdergi.1162027

Cırık, VA, Aksoy B. Determination of pediatric nurses' knowledge, attitudes, and experiences on apitherapy: a cross-sectional multicenter study. *U.Arı.D.- Uludag Bee Journal,* 2020, 20(2), 157-171. doi.org/10.31467/uluaricilik.787299

DeGrandi-Hoffman G, Graham H, Ahumada F, Smart M, Ziolkowski N. The economics of honey bee (Hymenoptera: apidae) management and overwintering strategies for colonies used to pollinate almonds. *Journal of Economic Entomology,* 2019, 112(6), 2524–2533. doi.org/10.1093/jee/toz213

Demirkaya A, Sagdicoglu Celep AG. Effects of royal jelly on obesity. *Uludag Bee Journal,* 2022, 22(1), 87-95. doi.org/10.31467/uluaricilik.1058101

Elzaki E, Tian G. Economic evaluation of the honey yield from four forest tree species and the future prospect of the forest beekeeping in Sudan. *Agroforest System,* 2020, 94, 1037-1045. doi.org/10.1007/S10457-019-00478-1

Falola A, Mukaila R, Emmanuel JO. Economic analysis of small-scale fish farms and fund security in North-Central Nigeria. *Aquaculture International*, 2022a, *30*(6), 2937-2952. doi.org/10.1007/s10499-022-00944-1.

Falola A, Mukaila R, Abdulhamid K. Informal finance: its drivers and contributions to farm investment among rural farmers in Northcentral Nigeria. *Agricultural Finance Review,* 2022b, 82(5), 942-959. doi.org/10.1108/AFR-08-2021-0116

García NL. The current situation on the international honey market. *Bee World,* 2018, *95*, 89–94. doi.org/10.1080/0005772X.2018.1483814

Gebeyehu HR, Jalata D.D. Physicochemical and mineral contents of honey from Fitche and Addis Ababa districts in Ethiopia. *Food Chemistry Advances,* 2023, *2,* 100177. doi.org/10.1016/J.FOCHA.2022.100177

Gela A, Hora ZA, Kebebe D, Gebresilassie A. Physico-chemical characteristics of honey produced by stingless bees (Meliponula beccarii) from West Showa zone of Oromia Region, Ethiopia. *Heliyon,* 2021, *7*, e05875. doi.org/10.1016/J.HELIYON.2020.E05875

Likert R. A technique for the measurement of attitudes. *Archives of Psychology*, *1932*, 22(140), 1-55.

Lima WG, Brito JCM, da Cruz Nizer WS. Bee products as a source of promising therapeutic and chemoprophylaxis strategies against COVID-19 (SARS-CoV-2). *Phytotherapy Research,* 2021, *35*(2), 743–750. doi.org/10.1002/ptr.6872.

Machado, De-Melo AA, Almeida-Muradian LBD, Sancho MT, Pascual-Mate A. Composition and properties of Apis mellifera honey: a review. *Journal of Apiculture Research,* 2018, 57(1), 5–37.

Madas MN, Marghitas LA, Dezmirean DS, Bobis O, Abbas O, Danthine S, Nguyen BK. Labelling regulations and quality control of honey origin: a review. *Food Review International,* 2020, *36*(3), 215–240.

Masuku MB. Socioeconomic analysis of beekeeping in Swaziland: A case study of the Manzini Region, Swaziland. *Journal of Development and Agricultural Economics,* 2013, *5*(6), 236-241. doi.org/10.5897/JDAE2013.002

Mshelia SI, Dia YZ, Ahmed MA. Profitability analysis of honey marketing in Ganye and Toungo local government areas of Adamawa State, Nigeria. *Middle-East Journal of Scientific Research,* 2013, *13*(2), 207-212. doi.org/10.5829/idosi.mejsr.2013.13.2.1742

Mukaila R, Obetta AE, Awoyelu FE, Chiemela CJ, Ugwu AO. Marketing analysis of vegetables: The case of carrot and cucumber marketing in Enugu State, Nigeria. *Turkish Journal of Agriculture - Food Science and Technology*, 2021, *9*(2), 346–351. doi.org/10.24925/turjaf.V9I2.346-351.4000

Mukaila R, Obetta AE, Ogbu MC. Profitability of melon processing among women in Enugu State, Nigeria. *Journal of Tekirdag Agricultural Faculty,* 2022, 19(3), 620-631. doi.org/10.33462/jotaf.1049260

Mulugeta M, Belay A. Comb honey and processed honey of Croton macrostachyus and Schefflera

abyssinica honey differentiated by enzymes and antioxidant properties, and botanical origin. *Heliyon,* 2022, *8*, e09512. doi.org/10.1016/J.HELIYON.2022.E09512

Musinguzi P, Bosselmann AS, Pouliot M. Livelihoods-conservation initiatives: Evidence of socio-economic impacts from organic honey production in Mwingi, Eastern Kenya. *Forest Policy and Economics,* 2018, *97*, 132–145. doi.org/10.1016/j.forpol.2018.09.010

Obetta AE, Achike AI, Mukaila R, Taru B. Economic analysis of marketing margin of banana and plantain in Enugu state, Nigeria. *African Journal of Agriculture and Food Science*, 2020a, *3*(4), 52–62.

Obetta AE, Mukaila R, Onah OG, Onyia CC. Challenges of melon processing among women processors in Enugu-Ezike agricultural zone of Enugu State, Nigeria. *Turkish Journal of Agriculture - Food Science and Technology,* 2020b, *8*(11), 2421–2425. doi.org/10.24925/TURJAF.V8I11.2421-2425.3707

Ogunola AA, Agunbiade MO, Oluwalana EO. Profitability of honey production in Ogun State, Nigeria*. Direct Research Journal of Agriculture and Food Science,* 2019, 7(12), 345-349.

Onyekuru AN, Okorji EC, Machebe NS. Profitability analysis of honey production in Nsukka local government area of Enugu State, Nigeria. *Asian Journal of Experimental and Biological Sciences,* 2010, 1(*1)*, *166-169.*

Otim OS, Kajobe R, Kungu JM, Echodu R. The socio-economic factors influencing honey production in Uganda. *Global Journal of Agricultural Research,* 2019*, 6*(2), 1-9.

Ranneh Y, Akim AM, Hamid HA, Khazaai H, Fadel A, Za-karia ZA, Bakar MFA. Honey and its nutritional and anti-inflammatory value. *BMC Complementary Medicine and Therapies,* 2021, 21(1), 1–17. doi.org/10.1186/s12906-020-03170-5

Shrestha A. Study of Production economics and production problems of honey in Bardiya District, Nepal. *Sarhad Journal of Agriculture,* 2017*, 34*(2), 240-245. doi.org/10.17582/JOURNAL.SJA/2018/34.2.240.245

Stojanov DP, Dimitrov L, Danihlík J, Uzunov A, Golubovski M, Andonov S, Brodschneider R. Direct economic impact assessment of winter honeybee colony losses in three European Countries. *Agriculture,* 2021*, 11*(15), 1-15. doi.org/10.3390/AGRICULTURE11050398.

Vaziritabar S, Esmaeilzade SM. Profitability and socio-economic analysis of beekeeping and honey production in Karaj state, Iran. *Journal of Entomology and Zoology Studies,* 2016, 4(4), 1341-1350.

Verma TC, Meena KC, Aswal S, Singh DK. Socio-personal and economic analysis of apiculture enterprise in Hadauoti Region of Rajasthan. *Economic Affairs,* 2018, *63*(1), 261-268. doi.org/10.30954/0424-2513.2018.00150.32

Vrabcová P, Hájek M. The economic value of the ecosystem services of beekeeping in the Czech Republic. *Sustainability,* 2020*, 12*, 10179. doi.org/10.3390/su122310179

Yeserah S, Jenberie A, Begna D. Honey marketing, structure and conduct of honey market in Gozamen district, East Gojjam Zone, and Amhara Region. *Cogent Food & Agriculture,* 2019*, 5*,1, 1620153. doi.org/10.1080/23311932.2019.1620153.

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ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

MOLECULAR IDENTIFICATION OF MICROBIAL PATHOGENS IN HONEY BEES FROM AMASYA

Amasya Bal Arılarında Mikrobiyal Patojenlerin Moleküler Tanımlanması

Neşe Gül UTKAN, Gözde Büşra EROĞLU*

Department of Molecular Biology and Genetics, Faculty of Science, Erzurum Technical University, Erzurum, TÜRKİYE, E-posta: nese.utkann@gmail.com, ORCID No: 0000-0001-6405-3986, Yazışma Yazarı / Corresponding author: E-posta: gozdebusra.eroglu@erzurum.edu.tr, ORCID No: 0000-0001-8988-1315

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ABSTRACT

Honey bees, *Apis mellifera* are highly beneficial insects that constitute both the livelihood of the producers and the food source of the consumers. However, there are some diseases that affect the yield of bees and cause the collapse of almost the entire colony. Most of these diseases are caused by microbial pathogens originating from viruses, bacteria, and fungi. Beekeeping is an important source of livelihood both in the center of Amasya and in almost all its districts. In this study, microbial pathogens that cause mass bee deaths and epidemics in Amasya province were determined using molecular methods. The results showed that the most common honey bee pathogens in Amasya are the Deformed wing virus, Chronic bee paralysis virus, and *Aspergillus flavus* fungus. Thus, the profile of bee diseases in Amasya province was determined for the first time with this study. In addition, this study guides other studies planned for the prevention of bee diseases and healthy beekeeping.

Keywords: Honey bee, *Apis mellifera*, Honey bee pathology, Microbial pathogens, Amasya

ÖZ

Bal arıları, *Apis mellifera*, hem üreticilerin geçimini hem de tüketicilerin besin kaynağını oluşturan oldukça faydalı böceklerdir. Ancak arıların verimini etkileyen ve neredeyse tüm koloninin çökmesine neden olan bazı hastalıklar vardır. Bu hastalıkların çoğuna virüsler, bakteriler ve mantarlardan kaynaklanan mikrobiyal patojenler neden olur. Arıcılık gerek Amasya merkezde gerekse hemen hemen tüm ilçelerinde önemli bir geçim kaynağıdır. Bu çalışmada Amasya ilinde toplu arı ölümlerine ve salgın hastalıklara neden olan mikrobiyal patojenler moleküler yöntemler kullanılarak belirlenmiştir. Sonuçlar, Amasya'da en yaygın bal arısı patojenlerinin Deforme kanat virüsü, Kronik arı felci virüsü ve *Aspergillus flavus* mantarı olduğunu göstermiştir. Böylece Amasya ilindeki arı hastalıklarının profili ilk kez bu çalışma ile belirlenmiştir. Ayrıca bu çalışma, arı hastalıklarının önlenmesi ve sağlıklı arıcılık için planlanan diğer çalışmalara yol göstermektedir.

Anahtar Kelimeler: Bal arısı, *Apis mellifera*, Bal arısı patolojisi, Mikrobiyal patojenler, Amasya

GENİŞLETİLMİŞ ÖZET

Amaç: Bu çalışmanın amacı Amasya ilinde görülen toplu arı ölümlerine sebep olan mikrobiyal patojenlerin moleküler yöntemler kullanılarak araştırılmasıdır.

Giriş: Bal arıları, *Apis mellifera* (Hymenoptera: Apidea) tarımsal ürünlerin en önemli tozlaştırıcıları olup, polinasyonu sağlamaktadır. Özellikle bal arısı popülasyonunun büyük bir çoğunluğunu oluşturan işçi arılar bal, polen, propolis, arı sütü, arı zehri ve bal mumu gibi oldukça çeşitli ve ekonomik değeri

yüksek ürünler üretmektedir. Ancak bal arılarında görülen salgın hastalıklar arıcılık faaliyetlerinin gelişimini ve ilerlemesini oldukça olumsuz etkilemektedir. Arılarda salgın oluşturarak ani ölüm ve koloni kayıplarına yol açan hastalıkların büyük bir çoğunluğu mikrobiyal kaynaklıdır. Ancak Amasya ilinde görülen arı ölümlerinin hangi mikrobiyal patojenlerden kaynaklandığı şimdiye dek aydınlatılmamıştır.

Gereç ve yöntem: 2022 yılında Amasya il merkezi ve ilçelerinde bulunan arılıklardan hasta, uçamayan ve kovan önünde ölü olarak bulunan arılar toplanmıştır. Örneklerde bulunması muhtemel olan viral, fungal ve bakteriyal patojenlerin taranması için total nükleik asit izolasyonu (DNA/RNA) ekstrakte edilmiş ve spesifik primerlerin kullanılmasıyla polimeraz zincir reaksiyonu gerçekleştirilmiştir. DNA genomuna sahip olan bakteri ve mantar örnekleri için direkt polimeraz zincir reaksiyonu kurulurken, RNA genomuna sahip olan virüsler için ara bir basamak daha uygulanarak RNA komplementer DNA'ya (cDNA) çevrilmiştir. Bu aşamadan sonra tüm polimeraz zincir reaksiyonları sonucu elde edilen ürünler yatay jel elektroforezinde yürütülerek sonuçlar gözlenmiştir. Dizi sonuçları NCBI veri tabanında yer alan nükleotit Blast (Blastn) programı ile kıyaslanarak patojenlerin isimlendirilmesi yapılmıştır.

Bulgular ve tartışma: Çalışma sonucunda Amasya bölgesindeki bal arılarında iki çeşit virus (deforme kanat virüsü ve kronik arı felci virüsü), üç farklı bakteri (*Pseudomonas putida*, *Pseudomonas aeruginosa* ve *Pseudomonas fluorescens*) ve iki çeşit mantar (*Aspergillus flavus* ve *Ascosphaera apis*) tespit edilmiştir. Ek olarak bazı örneklerin birden fazla patojen ile enfekte olduğu çoklu enfeksiyonlar belirlenmiştir. Mikrobiyal etmenler kovan içinde hasta bireyden sağlıklı bireye çok kolay ve hızlı bir şekilde bulaşabilmektedir. Bu nedenle kovanların sık sık kontrol edilerek temizliğine dikkat edilmesi, hasta bireylerin kovandan uzaklaştırılması ve hastalık taşıyan vektörler (*Nosema* ve *Varroa*) ile mücadele edilmesi sağlanmalıdır.

Sonuç: Hastalık etmenlerinin prevalansı göz önüne alındığında Amasya ili bal arılarında en yaygın görülen patojenlerin arılarda kanat yapısının bozulmasına ve arıların uçamamasına sebep olan deforme kanat virüsü, arıların bacağında felce sebep olan ve arıların hareket edememesine neden olan kronik arı felci virüsü ve arılarda taş hastalığına sebep olan yani arının vücudundaki bütün nemi emerek sert bir hal almasını ve ileri aşamalarda arı bireyinin vücudunda mikozlanmanın görüldüğü *Aspergillus flavus* mantarı olduğu belirlenmiştir. Elde edilen veriler bölgede yaygın olan mikrobiyal hastalıkların önüne geçilerek arı kayıplarının önlenmesi ve verimin düşmemesi için yapılması planlanan çalışmalara yol gösterecektir.

INTRODUCTION

Honey bees are important pollinators of agricultural and horticultural plants (Ilyasov et al. 2020). For this reason, bee health has great economic importance worldwide (Antunez et al. 2006). Although Turkey has sufficient colonies in honey production, one of the main reasons for the low honey production efficiency is the diseases seen in bees (Dogaroglu 1999). In recent years, there has been an increase in honey bee diseases due to increasing global warming and changing environmental factors (Le Conte and Navajas 2008). Due to infections, honey, and brood production in bees decrease, hive deaths occur, and beekeeping in the country suffers significant economic losses. For this reason, pathogens that cause bee disease should be diagnosed quickly (Eroglu 2022a, Eroglu 2022b). Microbial pathogens originating from bacteria, viruses, and fungi are among the most important factors that cause disease in honey bees. Bacterial diseases in honey bees cause rotten odors in bees. Fungal factors cause stone disease [*Aspergillus flavus*, (Af)] and lime disease [*Ascosphaera apis* (Aa)] in honey bees (Şimşek 2005). The most common microbial agent in bees is viruses. To date, it has been determined that there are more than 30 viruses that cause infection in honey bees (Galbraith et al. 2018, McMenamin and Flenniken 2018, Schoonvaere et al. 2018). However, it has been reported that there are seven viruses that cause very serious diseases and colony collapses and threaten the world of beekeeping to a great extent. These viruses are: deformed wing virus (DWV), Israeli acute paralysis virus (IAPV), black queen cell virus (BQCV), acute bee paralysis virus (ABPV), Kashmir bee virus (KBV), Sacbrood virus (SBV), and chronic bee paralysis virus (CBPV) (Bailey et al. 1976, Chen et al. 2005, Baker and Schroeder 2008). The aim of this study is to identify microbial honey bee pathogens in Amasya province by using molecular methods and determine the distribution of microbial pathogens that adversely affect honey bee populations in Amasya province and its districts. As

a result of the data obtained, we describe the occurrence of 7 different microbial pathogens in individual bees, hives, apiaries, and regional scales by molecular methods.

MATERIAL AND METHODS

Collection of Samples:

In July-September 2022, mass bee deaths were observed in the vicinity of Amasya, Turkey. 192 worker bees and 16 queen bees that died spontaneously in front of 23 different hives in the districts of Amasya (11 worker bees and 4 queen bees of 4 hives from Göynücek, 40 worker bees and 2 queen bees of 3 hives from Gümüşhacıköy, 26 worker bees of 2 hives from Taşova, 34 worker bees and 4 queen bees of 5 hives from Hamamözü, 29 worker bees and 2 queen bees of 3 hives from Merzifon province and, 52 worker bees and 4 queen bees of 6 hives from the city center) were collected (Fig. 1). Honey bee samples could not be obtained from the Suluova district, where beekeeping is not carried out intensively. Dead bee individuals belonging to each hive were placed in separate falcon tubes and brought to the laboratory on ice. Samples were stored at -80°C until total nucleic acid isolation.

Figure 1. Field study location

Besides, 3 worker bees collected from the Gümüşhacıköy locality were found to be covered with fungi to a large extent and were taken into separate plastic tubes. To isolate this fungus, the fungus was taken with the help of a sterile round-tipped loop, and three-point inoculation was made on potato dextrose agar (PDA) medium. The petri dish was incubated a 28°C for 14 days and the growing fungal colonies were photographed. Afterward, PCR was performed using partial primers of the β-tubulin2a gene found in fungi, and the obtained bands were sent for sequence analysis.

Total Nucleic Acid Isolation

The samples to be studied were taken into 2 ml sterile homogenization tubes and 1 ml of phosphate buffer solution (PBS) was added. After the steel ball was added to it, it was disintegrated in the Tissue

lyser (Qiagen) device at a speed of 50 strokes for 7 minutes. Tissue samples were then centrifuged at 6000 rpm for 3 minutes at 4°C. 200 µl of the supernatant prepared for total nucleic acid isolation was transferred to a new 1,5 ml sterile tube. Total nucleic acid extraction of bee samples was performed according to the manufacturer's instructions using the Cador pathogen mini (Qiagen) kit. Total nucleic acids isolated in pure and clean form were stored at -20 °C until PCR processes.

Polymerase Chain Reactions:

After total nucleic acid isolation, the isolates were used directly in PCR reactions for bacterial and fungal screening. For the screening of RNA viruses, reverse transcription was performed using Maxime™ RT PreMix Kit (Random Primer, Intron). For cDNA synthesis, 4 µl of RNA from each sample was taken and 16 µl of cDNA Synthesis dissolved in dH_2O was added to the Premix solution. The samples were taken to the Thermal Cycler device and incubated for 60 minutes at 45 °C and then at 95 °C for 5 minutes. After this step, a PCR reaction was performed using the primers indicated in Table 1. For PCR, the reaction was established by adding 25 µl Ecotaq 2x PCR master mix, 2 µl forward primer (10 µM), 2 µl reverse primer (10 µM), 100 µg template DNA/cDNA and up to 50 µl dH_2O. The reaction conditions are as follows: 30 seconds at 98°C, 35 cycles of 10 seconds at 94°C, 15 seconds at 55-65°C, 15 seconds at 72°C, and a final extension of 1 minute at 72°C. After the PCR reaction was finished, all samples were run on a 1% agarose gel containing ethidium bromide at 75 Volts for 45 minutes and visualized under UV light. The samples with bands obtained as a result of PCR were sent to Sentebiolab (Ankara, Turkey) for sequence analysis. Sequence results obtained were corrected using the Clustal W multiple alignment program in Bioedit (7.2.5).

Table 1. Primer sequences

Primer name	Sequences	Bp and Tm	References
Chronic bee paralyses virus (RdRP)	Forward: GCAAACTGCCCACCAATAGT Reverse: TGGTACGGAAGGTGTGTCAA	500 bp, 55	Rüstemoglu and Sipahioglu 2019
Sacbrood bee virus (cp gene)	Forward: TATTCAGGGGGACGCTACAC Reverse: AGTGCTGCTTGAAACCCTGT	429 bp, 55	
Israeli acute paralyses virus (cp gene)	Forward: TTGGCGTGCAACTATGTGTT Reverse: TCTTCTGCCCACTTCCAAAC	402 bp, 55	
Black queen cell virus (cp gene)	Forward: GACAGCGTGCCAAAGAGAG Reverse: GCGAACCCGTCCAATACTTA	567 bp, 55	
Kashmir bee virus (cp gene)	Forward: CACATTCCGAACAATAA Reverse: GCGATAGGAATTTTGCGGTA	339 bp, 55	
Deformed wing virüs (Non-structural protein)	Forward: TTGGTATGCTCCGTTGACTG Reverse: ATTCCTCAGAAGTTGGTTTCG	488 bp, 55	
Acute bee paralyses virus (cp gene)	Forward: GTATGGAAGTGGGCTGAGGA Reverse: CGCGGTACTAAAAAGCTACGA	476 bp, 55	Rüstemoglu and Sipahioglu 2016
Bacteria Universal (16SrRNA)	Forward: ATTCTAGAGTTTGATCATGGCTCA Reverse: TGGTACCGTGTGACGGGCGGTGTGTA	1465 bp, 55	Weisburg et al. 1991
Ascosphaera apis (5.8srRNA ITS region)	Forward: GCACTCCCACCCTTGTCTA Reverse: GAWCACGACGCCGTCACT	550 bp, 62	James and Skinner 2005
Aspergillus flavus (β-tubulin2a)	Forward: GGTAACCAAATCGGTGCTGCTTTC Reverse: ACCCTCAGTGTAGTGACCCTTGGC	495 bp, 55	Glass and Donaldson 1995

*Bp: base pair, Tm: temperature melting

Data analysis

The prevalence graph of honey bee pathogens in Amasya and the pathogen prevalence graph according to localities were drawn using the GraphPad Prism 9.5.1 software program. The results were statistically analyzed in SPSS 24. The prevalence of pathogens in each locality was determined using Pearson's chi-square test at $p < 0.05$ by the use of the contingency table and two-way frequency table.

RESULTS

Detection of Microbial Pathogens

Microbial pathogen screening was performed with the primers specified in Table 1 for all samples. The band images obtained as a result of PCR are given in Fig. 2. Accordingly, as a result of the study, multiple microbial diseases were detected in dead bee samples taken from 6 different districts and the city center (Table 2).

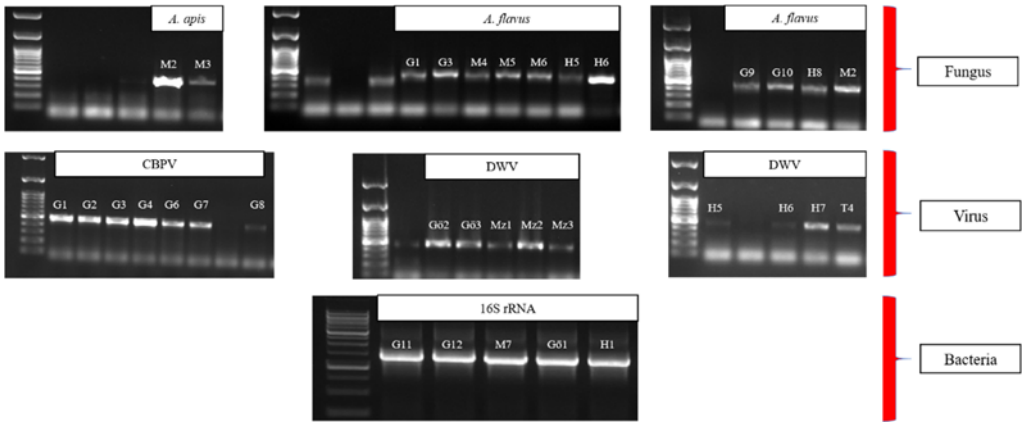

Figure 2. Agarose gel bands were obtained as a result of pathogen screening (M: Central, G: Gümüşhacıköy, H: Hamamözü, Gö: Göynücek, Mz: Merzifon, T: Taşova).

Sequences obtained as a result of analyses using the first-generation sequencing (Sanger-dideoxy) method have been sent to us. The nucleotide megablast application (https://blast.ncbi.nlm.nih.gov) of NCBI (The National Center for Biotechnology Information), Genbank in the database was used to identify the samples after cutting the poorly read parts from the beginning and end of the nucleotide sequences.

According to the results obtained, it was determined that DWV was the most common honey bee pathogen in Amasya province, and CBPV was the pathogen that caused the most deaths. In addition, while examining the bees brought to the laboratory after the fieldwork, it was morphologically observed that there was a fungal disease in the bodies of three worker bees collected from the Amasya Gümüşhacıköy district (Fig. 3).

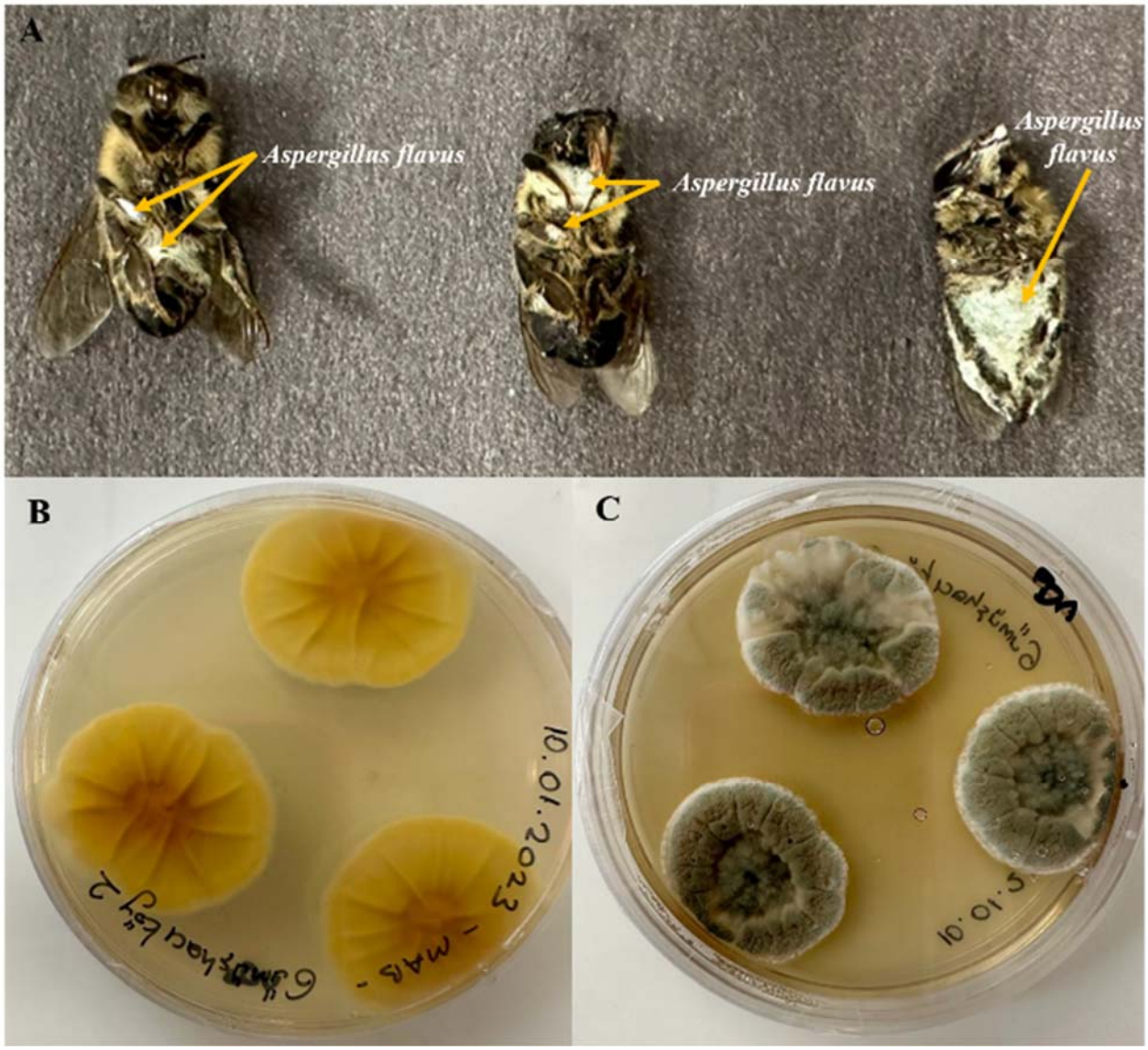

Figure 3. *Aspergillus flavus* infection in honey bees in Gümüşhacıköy. **A.** Morphological infection of bees with fungi, **B.** Top view of the fungus on PDA medium, **C.** Fungus viewed from below the petri dish.

After the blastn analyses, the samples were named according to the species with high similarity in the database. Accordingly, *A. flavus*, *A. apis*, and *P. putida* in dead honey bees in Amasya city center, DWV in dead bees in Taşova district, DWV, *A. flavus* and *P. aeruginosa* in Hamamözü district, CBPV, *A. flavus*, and *P. putida*, DWV, and *P. fluorescens* in Göynücek district, and DWV and *A. flavus* pathogens in Merzifon district (Table 2).

Table 2. Type, and name of infection, database and locality information of infected samples

Locality	Sample name	Sample type	Infection type	Pathogen name	Accession number	Base pair
Central	M2	Worker bee	Multiple	*A. apis*	OQ473574	372 bp
	M3		Single		OQ473575	372 bp
	M4		Single	*A. flavus*	OQ459690	495 bp
	M5		Single		OQ459691	495 bp
	M6		Single		OQ459692	495 bp
	M2		Multiple		OQ459693	495 bp
	M7		Single	*P. putida*	OQ472513	1022 bp
Hamamözü	H5		Multiple	*A. flavus*	OQ459694	495 bp
	H6		Multiple		OQ459695	495 bp
	H8		Single		OQ459696	495 bp
	H5		Multiple	DWV	OQ459684	414 bp
	H6		Multiple		OQ459685	414 bp
	H7	Queen bee	Single		OQ459686	414 bp
	H1	Worker bee	Single	*P.aeruginosa*	OQ472491	780 bp
Taşova	T4		Single	DWV	OQ459687	414 bp
Gümüşhacıköy	G1		Multiple	*A. flavus*	OQ459697	495 bp
	G3				OQ459698	495 bp
	G9		Single		OQ459699	495 bp
	G10				OQ459700	495 bp
	G1		Multiple	CBPV	OQ459671	462 bp
	G2	Queen bee	Single		OQ459672	471 bp
	G3	Worker bee	Multiple		OQ459673	462 bp
	G4	Queen bee	Single		OQ459674	471 bp
	G6	Worker bee			OQ459675	471 bp
	G7				OQ459676	462 bp
	G8				OQ459677	462 bp
	G11			*P. putida*	OQ472510	1025 bp
	G12				OQ472512	1022 bp
Göynücek	Gö2		Multiple	DWV	OQ459679	414 bp
	Gö3		Single		OQ459680	414 bp
	Gö1		Single	*P.fluorescens*	OQ472508	1428 bp
	Gö2		Multiple	*A. flavus*	OQ459689	495 bp
Merzifon	Mz1		Single	DWV	OQ459681	414 bp
	Mz2		Single		OQ459682	414 bp
	Mz3	Queen bee	Single		OQ459683	414 bp

According to the results obtained, it was determined that the most common honey bee pathogens in Amasya were of viral (DWV, CBPV) and fungal (*A. flavus*) origin (Fig. 4A). In addition, when the rates of microbial diseases by districts and city center were examined, the presence of pathogens was determined mostly in the samples taken from Gümüşhacıköy (p-value = 0.002, Chi square= 50.27), Amasya center (p-value = 0.002, Chi square= 47.88) and Hamamözü (p-value = 0.004, Chi square= 44.07) (Fig.4B). The three most common pathogens in Amasya were DWV, CBPV, and *A. flavus.*

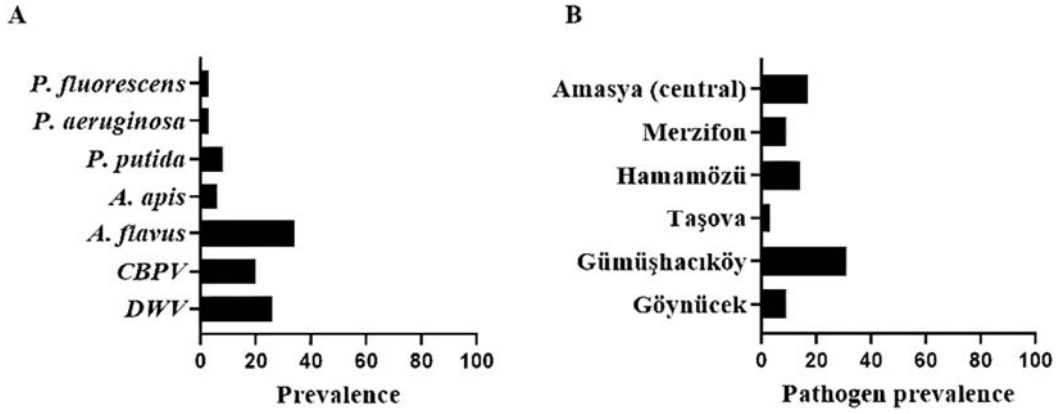

Figure 4. The prevalence of each of honey bee pathogens in Amasya province **(A)**, the prevalence of all honey bee pathogens in Amasya center and districts **(B)**

DISCUSSION

Honey bees, *Apis mellifera*, usually encounter many disease factors such as bacteria, fungi, parasites, and viruses during their developmental period. Significant economic losses occur in beekeeping due to the disease of honey bees in the world and in our country. Knowing, the early diagnosis and treatment of diseases in honey bees are very important to prevent economic losses in honey beekeeping. In studies carried out to date, pathogens causing disease in honey bees have been detected by PCR technique using universal or specific primers.

One of the biggest problems faced by the whole world is the rapid increase in bee deaths (Antunez et al. 2006). Among the honey bee pathogens, viruses, Bacteria, and fungi stand out (Glinski and Buczek 2003; Dolezal and Toth 2018). DWV, which is one of the most common bee viral pathogens all over the world, is known to be detected in both mobile and fixed beekeeping areas and has a high prevalence worldwide (Tentcheva et al. 2004; Welch et al. 2009). Berenyi et al. (2006), after examining 90 honey bee colonies in Austria, stated that the most common virus was DWV, which was found in 91% of the samples. Ghorani et al. (2017) reported that DWV was the most common pathogen in samples from 89 apiaries in four regions of Iran (Mazandaran, Hormozgan, Kurdistan, and Khorasan Razavi). According to Koziy et al. (2019) examined DWV-affected and newly hatched bees pathologically and reported that DWV-affected bees had a 2 times slower and 30% higher mortality rate compared to normal bees. In this study, it was determined that the most common virus in Amasya was DWV and it was found in several different localities throughout the city, not in a single locality like CBPV. Dittes et al. (2020) detected CBPV in samples from two *Apis mellifera carnica* colonies showing signs of paralysis and hairless black syndrome in 2019. They explained that the reason why the morphological symptoms caused by CBPV infection are so intense is that the weather situation in Germany was colder than normal in May 2019, and therefore, the duration of stay of the bees in the hive increased and the spread of the virus in the hive increased by increasing their contact with each other. In this study, CBPV infection was detected during PCR scanning in samples taken from asymptomatic worker bee individuals in Gümüşhacıköy, the westernmost district of Amasya. This situation reveals that the presence of CBPV usually progresses without symptoms, but it shows symptomatic findings in the presence of factors such as bad weather conditions or nectar deficiency (Ribiere et al. 2010; Dittis et al. 2020). Dias et al. (2023) determined that the most common pathogens were DWV, ABPV, and CBPV viruses in their study for the detection of honey bee pathogens in solitary and social bees in Brazil. However, while the presence of intense CBPV was observed in apiary areas, it reported the absence of CBPV in non-apiary areas. It is known that *A. flavus* propagated more than *A. apis* to produce infective ascospores and therefore releases higher titers of infective propagules into the environment but still

causes much fewer outbreaks than *A. apis* (Vojvodic et al. 2011; Foley et al. 2014). In this study, it was determined that the pathogen *A. flavus* was both more cosmopolitan and more prevalent in Amasya than *A. apis*.

Some of the studies on the diagnosis of microbial diseases in honey bees in Turkey are as follows: Gülmez et al. (2009) detected DWV for the first time in Turkey as a result of their study on honey bees in Ordu province. Muz and Muz (2009) identified DWV, *Nosema* sp., *Malpighamoeba mellificae*, and *Varroa destructor* as a result of their analysis of honey bees in Hatay province. Borum and Ülgen (2010) investigated the prevalence of fungal infections in beekeeping enterprises in Bursa province and its surroundings, as a result of their study, *A. apis* was found in 23.8% of the hives they examined and *Penicillium* sp. isolated fungi. Rüstemoglu and Sipahioglu (2016) defined ABPV from honey bees in Hakkari province. Muz and Muz (2018) detected BQCV in honey bees collected from different cities in Turkey. Kadirhan et al. (2019) detected *P. aeruginosa*, *Paenibacillus larvae*, and *Melisococcus pluton* bacteria in their study on the detection of bacterial diseases in honey bees in Kars and Ardahan provinces. Kalayci et al. (2019) detected SBV in honey bees from Muğla province. Rüstemoglu and Sipahioglu (2019) detected 6 viruses (BQCV, DWV, SBV, CBPV, KBV, IAPV) in honey bees in Hakkari province. Bog et al. (2020), as a result of the study they conducted on the investigation of the entomopathogenic bacterial flora of honey bees in Ordu province, 18 non-spore forming (*Staphylococcus lentus*, *Klebsiella oxytoca*, *Citrobacter freundii, Leuconostoc mesenteroides* ssp. *cremoris*, *Kocuria rosea*, *Kocuria kristinae*, *Sphingomonas paucimobilis slashline*, *Burkholderia cepacia*, *Leuconostoc mesenteroides* ssp. *dextranicum*, *Hafnia alvei*, *Escherichia coli*, *Aeromonas salmonicida*, *Citrobacter braakii*, *Pantoea agglomerans*, *Streptococcus equi* ssp. *zooepidemicus*, *Staphylococcus pseudintermedius*, *Staphylococcus lugdunensis*, and *Staphylococcus vitulinus*) and 2 spore-forming bacterias (*Bacillus licheniformis* and *Paenibacillus polymyxa*). Kalayci et al. (2020) reported that the DWV pathogen was the most common in honey bee samples from Adana, Aydın, Bursa, Izmir, Kütahya, Muğla, and Manisa, while the CBPV pathogen was less common. In addition, Eroglu (2023) determined that honey bee viruses (BQCV and KBV) were found in some wasps (*Vespula germanica*) found collectively dead in Erzurum. In this study, the molecular diagnosis of honey bee microbial diseases in Amasya province, where beekeeping is an important source of income, was made for the first time, and 2 different viruses (DWV, CBPV), 3 different bacteria (*P. putida*, *P. aeruginosa*, *P. fluorescens*) and 2 fungi. (*A. apis*, *A. flavus*) were detected.

Considering both the results of this study and the studies conducted in other provinces in the literature, it has been observed that honey bees in our country are frequently sickened by microbial pathogens and these diseases usually result in death. When the studies in the literature are examined, it has been determined that DWV is the most common honey bee pathogen in our country and it is common in Hakkari, Ordu, Hatay, and, with this study, Amasya. However, in this study, it was determined that CBPV and *A. flavus* pathogens, which are common pathogens in Amasya, are more limited in Turkey. It has been observed that these pathogens both cause the loss of honey bee colonies and the pathogenicity of *A. flavus*, especially containing aflatoxin, is widespread. Considering the risk of aflatoxin contamination in bee products, it is important for beekeepers to take the necessary precautions. One of the precautions to be taken in order to prevent this is that transported beekeeping should be done very carefully. Because, in the winter months, healthy beehives transported from cold provinces to different regions are infected with disease agents and these factors spread between cities. With the opening of the hives in spring, colony collapse is observed in many hives and pathogens can quickly infect other hives. Another consideration is the vectors that cause the spread of microbial pathogens. One of these vectors is the *varroa* mite. If the control of *varroa*, known as bee lice, is provided correctly (without stressing the bees and leaving no residue on bee products), the spread of diseases will also decrease. In this study, the microbial disease profile of honey bees in Amasya province was revealed. Thus, in order to prevent the most common viral (DWV, CBPV) and fungal (*A. flavus*) diseases in Amasya, the beekeepers were informed about the cleaning of the hive and the *Varroa* control to be done without stressing the bees.

Conclusion

In this study, microbial causes of mass mortality of honey bees in Amasya were investigated. The results showed that very dangerous and rapidly spreading microorganisms such as CBPV, DWV,

and *A. flavus* are common in honey bees in Amasya province. Microbial pathogens were detected relatively densely in honey bee samples taken from Amasya centre, Hamamözü, and Gümüşhacıköy compared to other localities. In this sense, it was given information about the beekeepers in this locality to clean the hive frequently, to be more careful about transported beekeeping in winter, and to carefully apply traditional methods used for the control of *Varroa* mite, which provides pathogen transfer from sick individuals to healthy individuals. Thus, it has been revealed that solutions should be sought against these factors in order to contribute to the country's economy and public health, especially in the province of Amasya.

Authors' contributions: GBE planned and designed the work. NGU did field work and collected data. GBE and NGU analyzed the data and wrote the manuscript. All authors read and approved the final manuscript.

Competing interests: The authors declare that they have no competing interests.

Ethical issue: Not applicable because this study is on dead bees.

Data availability: Data available on request from the authors.

Source of Finance: Not applicable because there is no funding source for this study.

REFERENCES

Antunez K, D'Alessandro, B, Corbella E, Ramallo G., Zunino P. Honey bee viruses in Uruguay. Journal of Invertebrate Pathology, 2006;93:67–70.

Bailey L, Ball BV, Woods RD. An iridovirus from bees. Journal of General Virolology, 1976;31:459-461.

Baker AC, Schroeder DC. The use of RNA-dependent RNA polymerase for the taxonomic assignment of Picorna-like viruses (order Picornavirales) infecting Apis mellifera L. populations. Virology Journal, 2008;5-10.

Berenyi, O, Bakonyi T, Derakhshifar I, oglberger H, and Nowotny N. Occurrence of six honeybee viruses in diseased Austrian apiaries. Appl. Environ. Microbiol. 2006;72:2414–2420.

Bog EŞ, Ertürk Ö, Yaman M. Pathogenicity of aerobic bacteria isolated from honey bees (Apis mellifera) in Ordu Province. Turkish Journal of Veterinary & Animal Sciences, 2020.;44(3):714-719.

Borum AE, Ülgen M. Güney Marmara Bölgesindeki Bal Arılarının Chalkbrood (Ascosphaera apis) İnfeksiyonunda Predispozisyon Faktörleri. Uludağ Arıcılık Dergisi, 2010;10(2):56-69.

Chen YP, Pettis JS, Feldlaufer MF. Detection of multiple viruses in queens of the honey bee Apis mellifera L. Journal of Invertebrate Pathology, 2005;90:118-121.

Dias CA, Taís Ferreira J, Weinstein Teixeira E, Pedro Lourenco A. Honey bee viruses in solitary bees in South America: simultaneous detection and prevalence. Journal of Apicultural Research, 2023;1-6.

Dittes J, Schafer MO, Aupperle-Lellbach H, Mulling CK, Emmerich IU. Overt infection with Chronic Bee Paralysis Virus (CBPV) in two honey bee colonies. Veterinary sciences, 2020;7(3):142.

Doğaroğlu M. Modern arıcılık. Anadolu Matbaa ve Ambalaj San. Tic. Ltd. Şti. İstanbul, 1999.

Dolezal AG, Toth AL. Feedbacks between nutrition and disease in honey bee health. Current opinion in insect science, 2018;26:114-119.

Eroglu GB. Phylogeographic Relationship of Honey Bee Dicistroviruses. Bee World, 2022a; 99(3):99-102.

Eroglu GB. RNA Viruses in Honey Bees. In: Distinguished Research from Different Disciplines. Gelenbevi Scientific Research Journal: Announcements. 2022b.;1:133-147.

Eroglu GB. Detection of honey bee viruses in *Vespula germanica*: Black queen cell virus and Kashmir bee virus. Biologia, 2023. doi:10.1007/s11756-023-01416-4.

Foley K, Fazio G, Jensen AB, Hughes WO. The distribution of Aspergillus spp. opportunistic parasites in hives and their pathogenicity to honey bees. Veterinary Microbiology, 2014;169:203-210.

Galbraith DA, Zachary LF, Allyson MR, Axel B, Maryann F, Mary W.G, J. Francisco IM. Investigating the Viral Ecology of Global Bee Communities with High-Throughput

Metagenomics. Scientific Reports, 2018;8:1-14.

Ghorani M, Madadgar O, Langeroudi AG, Rezapanah, M, Nabian S, Akbarein H, et al. The irst comprehensive molecular detection of six honey bee viruses in Iran in 2015-2016. Archives of virology, 2017;162: 2287-2291.

Glass NL, Donaldson GC. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Applied Environmental Microbiology, 1995;61:1323-30.

Gülmez Y, Bursalı A, Tekin S. First molecular detection and characterization of deformed wing virus (DWV) in honey bees (Apis mellifera L.) and mite (Varroa destructor) in Turkey. African Journal of Biotechnology, 2009;8(16).

Ilyasov RA, Lee MI, Takahashi JI, Kwon HW, Nikolenko, A.G. A revision of subspecies structure of western honey bee Apis mellifera. Saudi Journal of Biological Sciences, 2020;27(12):3615-3621.

James RR, Skinner JS. PCR diagnostic methods for Ascosphaera infections in bees. Journal of Invertebrate Pathology, 2005;90(2):98-103.

Kadirhan C, Kırpık MA, Öziç C, Gülen M. Kars ve Ardahan Yöresi Bal Arısında (Apis mellifera caucasica L.) Bakteriyel Hastalıkların Tespit Edilmesi. Kafkas Üniversitesi Fen Bilimleri Enstitüsü Dergisi, 2019;12(1):28-34.

Kalaycı G, Çağırgan AA, Pekmez K, Özkan B, Kaplan M. Molecular detection and phylogenetic analysis of the honey bee (Apis mellifera) sacbrood virus in Turkey. Turkish Journal of Veterinary & Animal Sciences, 2019;43(4):551-554.

Kalayci G, Cagirgan AA, Kaplan M, Pekmez K, Beyazit A, Ozkan B & Arslan F. The role of viral and parasitic pathogens affected by colony losses in Turkish apiaries. Kafkas Üniversitesi Veteriner Fakültesi Dergisi, 2020;26(5).

Koziy RV, Wood SC, Kozii I., van Rensburg CJ, Moshynskyy I, Dvylyuk I, Simko E. Deformed wing virus infection in honey bees (*Apis mellifera* L.). Veterinary Pathology, 2019;56(4): 636-641.

Le Conte Y, Navajas M. Climate change: impact on honey bee populations and diseases. Revue Scientifique et Technique-Office International des Epizooties, 2008;27:499-510.

McMenamin AJ, Flenniken ML. Recently identified bee viruses and their impact on three bee pollinators. Current Opinion Insect Science, 2018; 26:120–129.

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. Journal of apicultural research, 2009;48(3): 204-208.

Muz D, Muz MN. A molecular epidemiological study of black queen cell virus in honey bees (Apis mellifera) of Turkey: the first genetic characterization and phylogenetic analysis of field viruses. Apidologie, 2018;49(1):89-100.

Ribiere M, Olivier V, Blanchard P. Chronic bee paralysis: A disease and a virus like no other? Journal of Invertebrate Pathology, 2010;103:120–131.

Rüstemoğlu M, Sipahioğlu, HM. Occurrence and molecular characterization of acute bee paralysis virus (ABPV) in honey bee (Apis mellifera) colonies in Hakkari province. Yüzüncü Yıl Üniversitesi Tarım Bilimleri Dergisi, 2016.

Rüstemoğlu M, Sipahioğlu HM. Occurrence and prevalence of six honey bee viruses in Hakkari (Turkey) and their genomic divergence. Munis Entomology & Zoology, 2019;14(2):574-583.

Schoonvaere K, De Smet L, Smagghe G, Vierstraete A., Braeckman BP, de Graaf DC. Study of the Metatranscriptome of Eight Social and Solitary Wild Bee Species Reveals Novel Viruses and Bee Parasites. Published in Front. Microbiol. Biology, Medicine Frontiers in Microbiology, 2018;9:1-12.

Şimşek H. Elazığ yöresi bal arılarında bazı parazit ve mantar hastalıklarının araştırılması. Ankara Üniversitesi Veteriner Fakültesi Dergisi, 2005;52(2):123-126.

Tentcheva D, Gauthier L, Zappulla N, Dainat B, Cousserans F. Colin ME, et al. Prevalence and seasonal variations of six bee viruses in Apis mellifera L. And Varroa destructor mite

populations in France. Applied Environmental Microbiology, 2004;70:7185–7191.

Vojvodic S, Jensen AB, James RR, Boomsma JJ, Eilenberg J. Temperature dependent virulence of obligate and facultative fungal pathogens of honeybee brood. Veterinary microbiology, 2011;149:200-205.

Weisburg WG, Barns SM, Pelletier DA, Lane DJ. 16S ribosomal DNA amplification for phylogenetic study. Journal of Bacteriology, 1991;173:697–703.

Welch A, Drummond, F, Tewari S, Averill A, Burand, JP. Presence and prevalence of viruses in local and migratory honeybees (Apis mellifera) in Massachusetts. Applied Environmental Microbiology, 2009;75:7862–7865.

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ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

ISRAEL ACUTE BEE PARALYSIS VIRUS PREVALENCE IN APIARIES WITH COLONY LOSS IN TÜRKİYE

İsrail Akut Arı Felci Virüsü'nün Türkiye'de Koloni Kayıplı Arılıklardaki Yaygınlığı

Dilek MUZ[1*], Mustafa Necati MUZ[2]

[*1]Tekirdağ Namık Kemal Üniversitesi Veteriner Fakültesi Viroloji Anabilim Dalı, Tekirdağ, TÜRKİYE, Corresponding author/ Yazışma adresi: E-mail: dilekmuz@nku.edu.tr, ORCID: 0000-0001-9358-8103

[2]Tekirdağ Namık Kemal Üniversitesi Veteriner Fakültesi Parazitoloji Anabilim Dalı, Tekirdağ, TÜRKİYE, E-mail: mustafamuz@nku.edu.tr, ORCID: 0000-0002-1769-8498

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ABSTRACT

Honeybees are indispensable pollinator insects for vegetative pollination and biodiversity. Moreover, they serve medicinal importance with products such as honey, propolis, pollen, and royal jelly. Sudden bee deaths and colony collapse disorder (CCD) threaten the sustainability of colony health. Honeybee viruses, parasites, and pathogens trigger colony losses and CCD. This study investigated the presence and prevalence *of Israeli acute bee paralysis virus* (IAPV) in apiaries with sudden bee deaths, colony losses, and CCD-like problems in 16 provinces in different eco-geographic regions of Türkiye between 2011- 2021. Samples were tested for the coexistence of honeybee pathogens with IAPV. The sampled apiaries were evaluated for other bee pathogens such as *Acute bee paralysis virus, Black queen bee virus, Chronic bee paralysis virus, Deformed wing virus, Kashmir bee virus, Lake Sinai virus, Sacbrood virus,* Varroa mites, and *Nosema sp*. analyzed. Pathogen-specific RT-PCR assay was used for bee viruses. IAPV positivity was found to be 52.5% in apiaries. 97.5% of the sampled apiaries were positive for at least one pathogen. According to the results of this study, the presence of IAPV in apiaries suffering from colony loss and CCD-like problems was higher than in previous reports, and viruses of different species, *Nosema sp.,* and varroa infestation were found to be frequently encountered. The results suggest that the coexistence of IAPV and multiple pathogens may be effective in colony losses.

Keywords: IAPV, Honeybee Viruses, CCD, Colony Losses, Türkiye

ÖZ

Bal arıları bitkisel tozlaşma ve biyoçeşitliliğin devamında vazgeçilmez polinatör böceklerdir. Ayrıca bal, propolis, polen arısütü gibi ürünleriyle tıbbi öneme sahiptirler. Ani arı ölümleri ve koloni çöküş bozukluğu (CCD) koloni sağlığının sürdürülebilirliğini tehdit eder. Bal arısı virüsleri, parazit ve patojenleri koloni kayıplarını ve CCD-benzeri problemleri tetikler. Bu araştırmada 2011- 2021 yılları arasında Türkiye'de farklı eko-coğrafik bölgelerdeki 16 ilde ani arı ölümleri, koloni kayıpları ve CCD yaşanan arılıklarda *İsrail akut arı felci virüsünün* (IAPV) varlığı ve yaygınlığının tespiti amaçlanmıştır. Örnekler patojenlerinin IAPV ile bir arada bulunması açısından test edildi. Örneklenen arılıklar arı patojenlerinden *Akut arı felci virüsü, Siyah kraliçe arı virüsü, Kronik arı felci virüsü, Deforme kanat virüsü, Kaşmir arı virüsü, Lake Sinai virüsü, Sakbrood virüs*, Varroa akarları ve *Nosema sp.* yönünden analiz edildi. Arı virüsleri için patojen spesifik RT-PCR testleri kullanıldı. Örneklenen arılıkların %97,5'i en az bir patojen yönünden pozitif bulunurken, IAPV pozitifliği %52,5 olarak tespit edildi. Araştırma sonuçlarına göre, koloni kaybı ve CCD-benzeri problemler yaşanan arılıklarda IAPV'nin yaygınlığının önceki raporlara kıyasla daha yüksek olduğu belirlendi. IAPV pozitif arılıklarda farklı türlerdeki virüsler,

Nosema sp. **ve varroa enfestasyonuna rastlanıldığı tespit edildi. Sonuçlar IAPV ile birlikte çoklu patojen pozitifliğinin koloni kayıplarında etkili olabileceğini düşündürmektedir.**

Anahtar Kelimeler: IAPV, Bal Arısı Virüsleri, CCD, Koloni Kaybı, Türkiye

GENİŞLETİLMİŞ ÖZET

Amaç: Bu araştırma kapsamında Türkiye'de 2011-2021 yılları arasında koloni kaybı ve koloni çöküş bozukluğu şikayetleri olan arılıklarda İsrail akut arı felci virüsü (IAPV) varlığı ve yaygınlığını tespit etmek amaçlandı. Ayrıca örneklenen arılıklarda IAPV ile birlikte eş zamanlı bulunan bal arısı patojenleri araştırıldı.

Giriş: Bal arıları tarımsal biyoçeşitliliğinin ve üretimin devamlılığında kritik rolleri bulunan ekonomik yönden önemli polinatör böceklerdir. Çevresel faktörler, zirai ilaçlar, stres faktörleri, patojen ve parazitler arı sağlığı ve koloni sağlığının sürekliliğini tehdit ederler. 2006 yılından itibaren başta A.B.D. başta olmak üzere ülkemiz dahil dünya ülkelerinde görülen ani koloni kayıpları ve koloni çöküş bozukluğu (CCD) küresel bir sorun haline gelmiştir. "İyi arıcılık uygulamaları" kapsamında CCD ve kış kayıplarının nedenlerinin ve risk faktörlerinin araştırılması koloni sağlığının yönetilmesine katkı sağlayacaktır. Farklı viral enfeksiyonlar ile Varroa akarlarının bir arada bulunması CCD için yüksek risk olarak gösterilmektedir. *İsrail akut arı felci virüsü* (IAPV) ilk olarak 2004 yılında CCD'li arılıklarda izole edilmiş ve koloni kayıplarıyla ilişkilendirilmiştir. Yapılan çalışmalarda, IAPV tek başına CCD nedeni olarak gösterilmese de koloni kayıpları ve CCD vakalarında rol oynayabileceği ve diğer faktörlerin varlığında koloni sağlığını olumsuz etkileyeceği vurgulanmıştır.

Gereç ve Yöntem: Araştırmada 2011-2021 yılları arasında Türkiye'nin farklı eko-coğrafik bölgesinden 16 ilde koloni kayıpları ve CCD benzeri şikayetleri olan arılıklardan örneklemeler yapıldı. Bu amaçla toplam 120 arılıktan canlı arı örnekleri ve yavrulu petek örnekleri toplandı. Bu örnekler başta IAPV olmak üzere arı patojenlerinden *Akut arı felci virüsü* (ABPV), *Siyah kraliçe arı virüsü* (BQCV), *Kronik arı felci virüsü* (CBPV), *Deforme kanat virüsü* (DWV), *Kaşmir arı virüsü* (KBV), *Lake Sinai virüsü* (LSV), *Sakbrood virüs* (SBV), Varroa akarları ve *Nosema sp.* yönünden analiz edildi. Arı virüsleri patojen spesifik RT-PCR testleri kullanılarak araştırıldı. Arılıklardaki şikayetler kaydedildi.

Bulgular ve Sonuç: Yapılan RT-PCR sonuçlarına göre IAPV pozitifliği %52,5 (n=63) olarak tespit edildi. Test edilen arılıklar %97,5 oranında en az bir patojen yönünden pozitif bulundu. Örneklenen arılıklarda DWV ve BQCV sırasıyla %80 (n=96) ve %57,5 (n=69) oranlarıyla en yaygın arı virüsleri olarak belirlendi. Diğer arı virüslerinin pozitifliği LSV, CBPV, ABPV ve SBV, sırasıyla %30 (n=36), %15 (n=18), %10 (n=12) ve %7,5 (n=9) oranlarında olduğu tespit edildi. Örnekler KBV yönünden negatif bulundu. Arılıklarda *Nosema sp.* pozitifliği %32,5 (n=39) olarak belirlenirken Varroa enfestasyonu arılıkların %91,6'sında farklı seviyelerde kaydedildi. Koloni kayıplarının yaşandığı arılıkların patojen varlığı yönünden birden fazla virüs türü ile enfekte olduğu tespit edildi. IAPV varlığı diğer patojenlerle birlikte miks enfeksiyonlar içerisinde kaydedildi. Yapılan 10 yıllık araştırmada IAPV pozitifliği Türkiye'den bildirilen raporlara göre yüksek bulundu. Arılıklardaki şikayetlere göre ani gelişen koloni kaybı, yoğun kış kaybı yaşanan veya CCD şikayetleri bulunan arılıklardaki IAPV pozitifliği %44,4- 58,8 değişen oranlarda yüksek bulunurken, bu arılıklardaki pozitiflikler arasındaki farklılık istatistiki olarak anlamlı değildi. Sonuç olarak, kış kayıpları, koloni kayıpları yaşanan arılıklarda yüksek oranlarda IAPV varlığı tespit edildi. Arılıklarda koloni sağlığının sürekliliği sağlanması yönünde yapılacak iyi arıcılık uygulamaları kapsamında patojen varlığının araştırılması ve mücadelesi kapsamında stratejilerin geliştirilmesi önemli katkılar sunacaktır.

INTRODUCTION

Honeybees are among the most significant economically colonized pollinator insects, but various factors threaten the sustainability of honey bee colonies at ever-increasing levels (Mcmenamin and Genersch 2015). Colony Collapse Disorder (CCD) has caused serious significant global concerns since 2006 on colony health. CCD is a phenomenon that the disappearance of worker bees has characterized despite a queen, enough food, brood cells, and few nurse bees exist in a colony (VanEngelsdorp et al. 2007). Matching of variables like multiple pathogens, agricultural chemicals, and stress factors trigger colony losses and CCD.

Scientific results have reported that synergizing two or more factors may contribute to colony population declines (Cox-Foster et al. 2007). Within the scope of "good beekeeping practices," it has been accepted that investigating and revealing the causes of colony collapses and winter losses will contribute to easier management of colony health (Williams et al. 2010). Varroa mite is one of the majority factors causing the deterioration of bee health and they act as biological and mechanical vectors of many viruses. The coexistence of different viral infections and Varroa mites in colonies is considered a high risk for CCD (Muz 2008; De Miranda et al. 2011).

Honeybee viruses are known most common bee pathogens by their effects on colony health (Mcmenamin and Genersch 2015). They can show the covert infections specified with asymptomatic bees and overt infection with symptomatic bees in the colony (Hails et al. 2008). Some reports highlight the evidence that some bee viruses can trigger CCD (Cox-Foster et al.2007; Genersch et al. 2010; Corman et al. 2012). Many viruses have been reported from apiaries to date worldwide (Corman et al. 2012; Muz and Muz 2021). Israeli acute paralysis virus (IAPV) was first identified in CCD-affected colonies in 2004 (Maori et al. 2007). Since then, the association and interaction of IAPV with single or multiple variables remain an interesting research priority. Horizontal and vertical transmission routes were approved in IAPV transmission in honeybee colonies. IAPV can infect honeybees during separate biological stages, primarily in the digestive, nervous systems, and hypopharyngeal glands (De Miranda et al. 2010; Chen et al. 2014). Thus IAPV-infected colonies have asymptomatic or symptomatic bees suffering from trembling wings, paralysis, darkened body, and death (Maori et al. 2007). IAPV is an RNA virus classified in the Dicistroviridae subgroup in the Picornaviridae family.

Türkiye's suitable geographical location is so reasonable for beekeeping. Türkiye is remarkable in global ranking with its hive number and the produced honey amount. But colony losses seriously cause product losses to inconvenience beekeepers in most countries such as Türkiye (Çakmak 2012; 2016). Honeybee viruses have been reported in colony-lossed apiaries in Türkiye (Gülmez et al. 2009, Muz and Muz 2009; 2018, Kalaycı et al. 2020). Although the record of IAPV existence has been reported previously in Türkiye, its role and the inter-pathogen interrelationship on colony losses are not clear (Özkırım and Schiesser 2013; Rüstemoğlu and Sipahioğlu 2019; Çağırgan et al. 2020; Kalaycı et al. 2020; Çağırgan and Yazıcı 2021). In this study, the share of IAPV in pathogen distribution was investigated according to beekeeper complaints based on colony loss between 2011-2021, and some other pathogens were also tested.

MATERIAL AND METHODS

Sampling Area and Sample Collection:

The samples represent 16 provinces of different eco-geographic regions in Türkiye. The source points are Afyonkarahisar, Ağrı, Antalya, Balıkesir, Bursa, Düzce, Edirne, Erzurum, Giresun, Hatay, İstanbul, Mersin, Muğla, Sivas and Tekirdağ. The sampling period was between April and September of 2011-2021. In the study, the sample numbers were determined by considering the existence of colony loss and CCD-like complaints, the amount of hives in the sampled provinces. The sampling covered 120 apiaries with sudden bee deaths, brood deaths, unexpected high winter losses, severe colony losses, or CCD-like complaints. There were different symptoms in the sampled apiaries. The specimen from each colony per apiary included at least 20 alive nurser bees and brood comb (15 cm x 15 cm in size). Each apiary's worker bee, larva, and pupa samples were transferred to sterile tubes for molecular analysis. Bee samples for molecular tests were stored at -80 °C until analysis.

Homogenization, Nucleic Acid Extraction, and PCR Method

RNA was isolated from bee samples for use in molecular analyses. Briefly, sample pools with five bees were homogenized in 5 ml of PBS. Then, the obtained supernatant after centrifugation at 3000 rpm for 5 minutes was used in the extraction protocols. According to the kit's protocol, a commercial kit (GeneJET RNA Purification Kit, Thermo) was used for RNA extraction. The cDNA synthesis reaction was performed using the commercial kit (RevertAid First Strand cDNA Synthesis Kit) according to its protocol for cDNA synthesis. *Acute bee paralysis virus* (ABPV), *Black queen cell virus* (BQCV), *Chronic bee paralysis virus* (CBPV), *Deformed wing virus* (DWV), IAPV, *Kashmir bee virus* (KBV*), Lake Sinai virus* (LSV), *Sacbrood virus* (SBV) were analyzed in PCR tests. The PCR mixture was prepared as 5u Taq polymerase (Dream Taq polymerase, Thermo), 10x Taq buffer, 3mM MgCl2, 300 pmol dNTP mix, and

sterile water, in total 30 µl. ABPV, BQCV, CBPV, DWV, IAPV, KBV, LSV, SBV specific forward and reverse primers previously reported in the literature for each pathogen were added to the mixture (Stoltz et al., 1995; Chen et al., 2004; Berenyi et al., 2006; Palacious et al 2008) (Table 1). PCR reaction conditions included 5 min first denaturation at 95 °C, followed by 40 cycles of 30" denaturation at 95 °C, 30" annealing step between 48-60 ° C, and 30" elongation step at 72 ℃. The amplified DNA products were run on a 1% ethidium bromide gel using the agarose electrophoresis method. Positive samples were detected using a UV transmitter under UV light.

The nurse bees and sealed broods from each colony were used for varroa detection. The colonies infested more than seven mites were considered high, with 4-6 mites considered moderately, and those infested with 1-3 were considered mildly infested. To diagnose *Nosema sp* spores; the abdomen of ten nurse bees were crushed in a mortar with 10 ml of distilled water, and a drop of homogenate was examined under the light microscope between the lamellae.

To determine the colony losses, the answers given by the beekeepers were analyzed and the colonies were divided into three groups accordingly. It is the first group in which winter losses are at the lowest level, but symptomatic bees appear quickly at the beginning of spring. The symptoms of this group were recorded as trembling on the wings of the adult bees, deformity of the wings, paralysis, blackening, and brown/black spots in the larva and pupa samples. The second group had both high winter losses and severe colony losses. In the third group, there were only CCD-like complaints.

Table 1 The primer pairs used in RT-PCR protocols.

Virus	Amplified Region	Primer Sequence (5'-3')	Expected Length (bp)	Reference
ABPV	Capsid protein	F: GTGCTATCTTGGAATACTAC R: AAGGYTTAGGTTCTACTACT	618	Berenyi et al. 2006
BQCV	Nonstructural polyprotein	F: AGTAGTTGCGATGTACTTCC R: CTTAGTCTTACTCGCCACTT	472	Berenyi et al. 2006
CBPV	RdRp	F: TGTCGAACTGAGGATCTTAC R: GACCTGATTAACGACGTTAG	315	Berenyi et al. 2006
DWV	Helicase	F: ATCAGCGCTTAGTGGAGGAA R: TCGACAATTTTCGGACATCA	702	Chen et al. 2004
IAPV	IGR	F:GGTTGGCTGTGTGTCATCAT R:CGATGAACAACGGAAGGTTT	767	Palacious et al. 2008
KBV	Nonstructural polyprotein	F: GATGAACGTCGACCTATTGA R: TGTGGGTTGGCTATGAGTCA	415	Stoltz et al. 1995
SBV	Structural protein	F: ACCAACCGATTCCTCAGTAG R: CCTTGGAACTCTGCTGTGTA	487	Berenyi et al. 2006

Statistical Evaluation

The distribution of pathogen positivity in apiaries and the statistical evaluation of the relationship between this distribution were evaluated using the SPSS IBM (Version 25) program. For this purpose, independent Sample T-test, and correlation analysis were used. The apiaries were divided into three groups. Apiaries from which symptomatic bees were sampled constituted the first group. The second group sampled asymptomatic bees with colony losses and unexpectedly high winter losses. And apiaries with CCD-like complaints constituted the third group.

RESULTS

Sampling was performed in 16 provinces with a total of 120 apiaries in different years. Sampling was also repeated in additional times in provinces where colony loss complaints and beekeeping activities are intense (Figure 1). ABPV, BQCV, CBPV, DWV, IAPV, KBV, LSV, and Nosema sp. were analyzed in this research. Sampling was performed in 16 provinces of 120 apiaries over different years. Sampling was repeated in areas with intense colony loss complaints and beekeeping activities (Figure 1). The results showed 97.5 % positivity (n=117) of at least one pathogen in the apiaries. In terms of the bee virus's positivity, DWV was the prevalent pathogen with an 80% (n=96) rate, followed by BQCV and IAPV positivity with 57.5% (n=69) and 52.5% (n=63) rates, respectively (Figure 2). The LSV, CBPV, ABPV, and SBV positivity were detected at 30% (n=36), 15% (n=18), 10% (n=12), and 7.5% (n=9), respectively (Figure 2A). The samples were negative for KBV. Nosema sp. positivity was found to be 32.5% (n=39). Varroa infestation was determined at different levels at 91.6% of apiaries (n=110). IAPV-positive apiaries were all Varroa-positive. Varroa levels were at low, medium, and high levels determined in IAPV-positive apiaries (n=63) with 57.1 %, 28.6%, and 14.3 %, respectively. Compared to IAPV existence, the Varroa levels were statistically insignificant ($p<0.05$).

Only three apiaries were free of tested pathogens. The DWV has often been detected all years in the apiaries, although DWV occurrence is statistically ($p<0.05$) insignificant with the IAPV positivity over the years. Regarding the coexistence of the most prevalent pathogens in IAPV-positive apiaries were IAPV+DWV (n=51), IAPV+ BQCV (n=33) and IAPV+ Nosema sp. (n=24) combinations followed (Figure 2B). The coexistence of IAPV with SBV was not found. IAPV and other investigated pathogens were statistically insignificant ($p<0.001$, $p<0.05$) (Table 2). The existence of BQCV, LSV, CBPV, and *Nosema sp*. was found to be low-significant statistically in apiaries ($p<0.05$). Single pathogen positivity was in nine apiaries while multiple pathogens' existence was noted with dual (n=27, 23.1%), triple (n=45, 38.5%), and tetrad (n=36, 30.8%) in pathogen positive apiaries (Figure 2C). At least one pathogen positivity was also detected in all IAPV-positive apiaries. IAPV positivity was seen in 12 provinces and all sampled years (2011-2021).

Table 2. Correlations between tested variables

		Mn.	Sd.	1	2	3	4	5	6	7	8	9
1	IAPV	0,5250	0,50147	1								
2	DWV	0,8000	0,40168	0,150	1							
3	BQCV	0,5750	0,49642	-0,008	-0,177	1						
4	ABPV	0,1000	0,30126	0,150	-0,042	0,118	1					
5	SBV	0,0750	0,26450	-0,109	0,142	-0,139	-0,095	1				
6	LSV	0,3000	0,46018	-0,033	,191*	0,011	-,218*	-,186*	1			
7	CBPV	0,1500	0,35857	-0,021	-,315**	,220*	0,093	0,146	-0,122	1		
8	Nosema sp	0,3250	0,47034	0,126	-,320**	-0,051	-0,053	0,005	-,221*	-0,142	1	
9	Apiary status	1,8750	0,75105	-0,092	0,084	-0,008	0,056	-,206*	,255**	-0,023	-0,098	1

*: $p<0.05$, **: $p<0.01$

According to colony losses complaints, three groups was the first group consisted of 51 apiaries, the second group was 42 apiaries and the third included 27 apiaries. IAPV positivity was found as 58.8 % (n=30), 50 % (n=21), and 44.4 % (n=12) rates in the first, second, and third groups, respectively. Among groups, the positivity of IAPV was statistically insignificant ($p<0,05$). In the first group, darkened bees and brown/black spotted were noted in larvae and pupae brood samples in 27 (52.9%) apiaries. Fifteen (55.6%, 15/27) of these apiaries were positive for IAPV. IAPV tested positive in 36 (85.7%) of 42 apiaries in paralyzed bees were recorded.

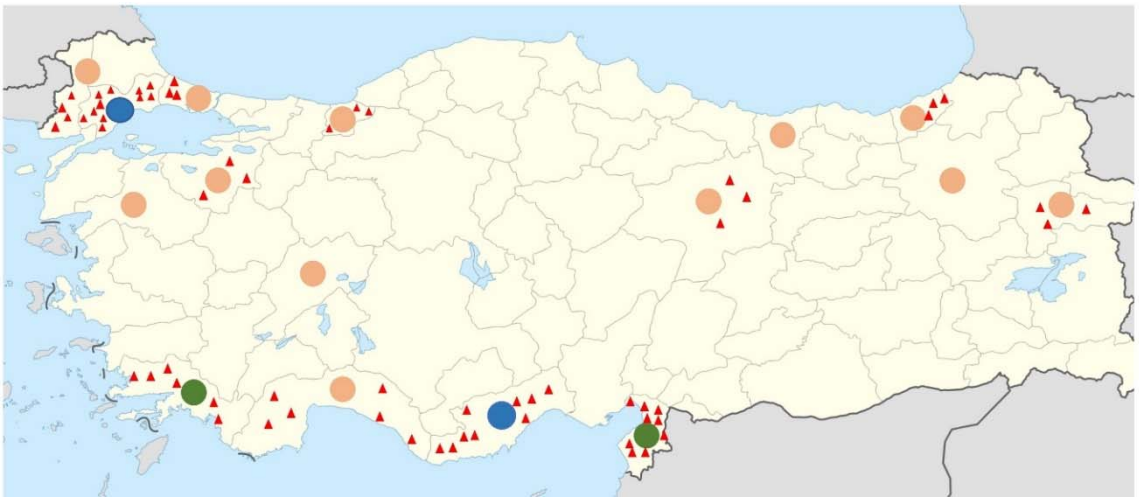

Figure 1: Sample area and sampling years are shown with different color circles. The orange circle: 1-3 years sampled, the green circle; 4-5 years sampled, blue circle; 6-8 years sampled. The IAPV-positive apiaries are marked with a red triangle in the sampled provinces.

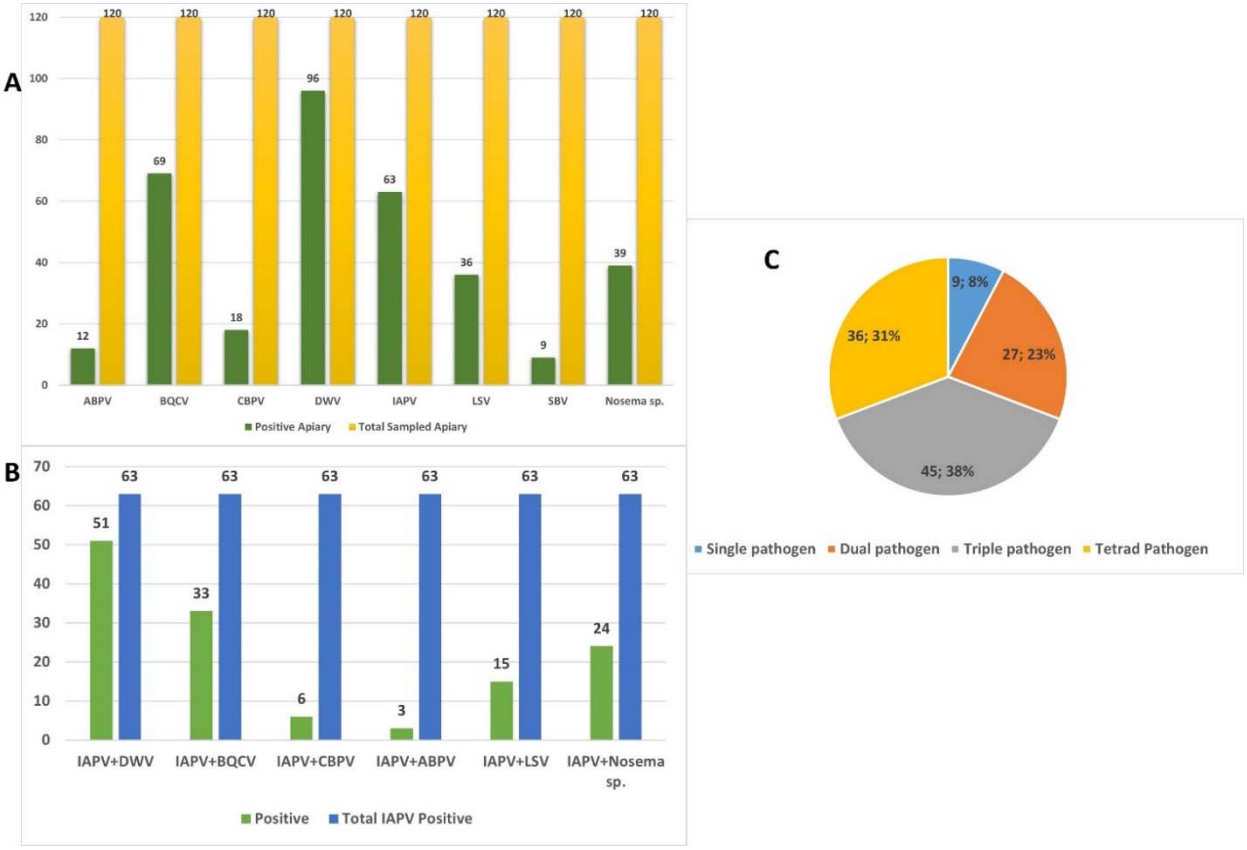

Figure 2: The results of pathogens' positivity of apiaries in this research. A. The distribution of pathogen positivity in sampled apiaries. B. The coexistence of pathogens and IAPV in positive apiaries. C.The distribution of single and multiple pathogens in positive apiaries.

DISCUSSION

Honeybee viruses may induce covert infections in worker and queen bees of healthy-looking colonies. As sick and clinically symptomatic bees may suffer from more severe problems depending on different factors. (Chen et al. 2005). Stress factors like increased mite loads and elongated overwintering periods trigger the viral multiply resulting in colony declines and collapse (Chen et al. 2014). Deformed wing virus (DWV) load increased significantly after Varroa infestation and caused colony collapses reported (Martin et al. 2012). IAPV, one of the Varroa-born viruses, can be transmitted through trophallaxis, fecal-oral route, robbing, and horizontal and vertical transmission (Amiri et al. 2019). All of these pose increased risks and severe threats to colony health. IAPV has been reported globally since it was first detected in symptomatic colonies with losses in Israel apiaries (Maori et al. 2007; Chen et al. 2014). Although initial results pointed to IAPV infection effects in CCD-like symptoms (Cox-Foster et al. 2007), the subsequent reports could not verify this relevance (Vanengelsdorp et al. 2009; McMenamin and Genersch 2015). Instead, IAPV infection in the colony is an effective factor in colony productivity and bee health (Hou et al. 2014). The IAPV, common in asymptomatic colonies, risks colony health and production. (De Miranda et al. 2010).

This study investigated some honeybee pathogens in 120 colonies in 16 provinces of five ecogeographic regions, where colony loss and CCD-like complaints were reported during 2011-2021. At least one pathogen was detected in 117 (97.5 %) of 120 problematic colonies with loss reported, while IAPV was detected in 63 (52.5%). Previously reported two IAPV records ranged from 6.5 to 21,12% in the Türkiye (Özkırım and Schiesser 2013; Kalaycı et al. 2020). The IAPV prevalence was higher than the results of previous reports spread over 10-year sampling. Results indicated that IAPV exists in different developmental stages of honeybees. IAPV was detected higher, especially in symptomatic adult bees with paralysis and brown/black darkened brood samples. The IAPV infection rate was also higher in larvae and pupae samples than in adult bees compared to previous reports (Maori et al. 2007; Chen et al. 2014). The rate of IAPV infection was reported to increase in weak colonies (Chen et al. 2014); IAPV does not restrict to cause only CCD but also an increase in bee deaths.

Experimental studies show that IAPV is also spread by close contact and virus particles are easily transmitted to bees topically (Amiri et al. 2019). It emphasized a positive association between IAPV infection, virus load, and mite infestation (Di Prisco et al. 2011). In this study, mite infestation was determined in all IAPV-positive apiaries. Although mite levels do not appear statistically significant for IAPV positivity, to possibly have negative effects on colony health. The mite-infested colonies under the effects of various stress factors, the virus multiplies rapidly, and the viral load increases, leading to possible bee death and colony collapse (Chen et al. 2004). High IAPV titer is highly lethal in worker bees and pupae, and may present with typical symptoms of trembling wings, progressive paralysis, and nerve dysfunction similar to experimental infections (Hou et al. 2014). In this study, the IAPV load was not analyzed but, high rates (85.7%) of IAPV positivity were noted in paralyzed bees sampled. It suggests that symptomatic overt viral infections would be an increasing risk for bee health and colony decline.

DWV has been reported to be linked to global honeybee colony losses. DWV and BQCV are two of the most prevalent viruses in apiaries in Türkiye and the world (Kalaycı et al 2020; Muz and Muz 2009; 2018; 2022). Although the prevalence of ABPV, CBPV, and SBV is mostly lower, it has also been reported from apiaries in different geographic regions in Türkiye (Gümüşova-Okursoy et al. 2010; Kalaycı et al. 2020; Çağırgan and Yazıcı 2021; Güller and Kurt 2022; Muz and Muz 2022). Current results revealed that multiple honeybee virus infections are common in colony losses and may play a critical role in colony health. Compatible with previous studies, DWV was determined the most prevalent virus followed by BQCV positivity in the second and IAPV in the third in sampled apiaries in this study. IAPV was described as the most common viral agent after DWV and BQCV in bee colonies worldwide. The status of IAPV infections is directly related to colony survival (Chen et al 2014). IAPV positivity in all three groups sampled (according to colony complaints) in the current study ranged from 44.4 to 58.8%. IAPV may be an influencing factor in intense winter losses, colony declines, and CCD cases.

Nosema ceranae prevalence was highly reported in local colony losses (Muz et al 2010; Ostroverkhova et al 2020). The threat posed by unexpected colony losses is compounded by the fact that bee colonies often suffer from several pathogens simultaneously

which negatively affect colony health (Martín-Hernández et al 2007; Berthoud et al 2010; Muz et al 2010; Botias et al 2013). Numerous studies have shown a correlation between *N.ceranae* infections and colony declines, but some do not confirm this relationship (William et al 2010; Ostroverkhova et al 2020). While the *Nosema sp* positivity is defined as 32.5% in our research results, it is similar to the previous epidemiological studies in Türkiye (Ivgın-Tunca et al .2016; Tosun and Yaman 2016; Ütük et al 2016; Muz and Muz 2022), while some local reports where beekeeping is intense (Muz et al 2010; Kartal et al. 2021) were reported at a lower rate. The results suggest that multiple pathogen positivity may be effective in colony losses. In conclusion, implementing the necessary control and treatment strategies to combat pathogens in colony losses is regularly essential. Complete and timely maintenance of the colonies in spring and winter can also contribute to the fight against pathogens.

Conclusion

In this study, IAPV investigated in apiaries with colony loss and CCD complaints in different eco-geographic regions in Türkiye by 10-year fieldwork. The IAPV positivity was determined at higher rates than other reports from Türkiye. The worker bee and brood samples were positive for IAPV, and more than one pathogen coexisted. Multiple viral infections, Varroa mite and *Nosema sp.* exist prevalent in apiaries. The results suggest that multiple pathogen positivity may be effective in colony losses. The presence of high varroa mites can trigger many viral infections to threaten colony health. The fight against pathogens must be carried out periodically to protect colony health.

Authors' contributions: Concept – DM planned the concept and designed the research. MNM worked on field study. DM and MNM worked on laboratory analysis, processing/interpretation of data, and writing the manuscript. All authors have read and approved the final manuscript.

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REFERENCES

Amiri E, Seddon G, Zuluaga Smith W, Strand MK, Tarpy DR, Rueppell O. Israeli Acute Paralysis Virus: Honey Bee Queen–Worker Interaction and Potential Virus Transmission Pathways. *Insects*. 2019; 10(1):9.

Berenyi O, Bakonyi T, Derakhshifar I, Koglberger H, Nowotny N. Occurrence of six honeybee viruses in diseased Austrian apiaries. Appl. Environ. Microbiol. 2006; 72 (4): 2414–20.

Berthoud H, Imdorf A, Haueter M, Radloff S, Neumann P. Virus infections and winter losses of honey bee colonies (Apis mellifera). J. Apicult. Res. 2010; 49, 60–65.

Botias C, Martín-Hernández R, Barrios L, Meana A, Higes M. Nosema spp. infection and its negative effects on honey bees (Apis mellifera iberiensis) at the colony level. Vet. Res. 2013; 44, 25.

Chen Y, Zhao Y, Hammond J, Hsu HT, Evans J, Feldlaufer M. Multiple virus infections in the honey bee and genome divergence of honey bee viruses. J. Invertebr Pathol. 2004 Oct-Nov;87(2-3):84-93.

Chen Y, Pettis JS, Feldlaufer MF. Detection of multiple viruses in queens of the honey bee Apis mellifera L. J. Invertebr. Pathol. 2005; 90: 118–121.

Chen YP, Pettis JS, Corona M, Chen WP, Li CJ, Spivak M, Visscher PK, DeGrandi-Hoffman G, Boncristiani H, Zhao Y, vanEngelsdorp D, Delaplane K, Solter L, Drummond F, Kramer M, Lipkin WI, Palacios G, Hamilton MC, Smith B, Huang SK, Zheng HQ, Li JL, Zhang X, Zhou AF, Wu LY, Zhou JZ, Lee ML, Teixeira EW, Li ZG, Evans JD. Israeli acute paralysis virus: epidemiology, pathogenesis and implications for honey bee health. PLoS Pathog. 2014;31;10(7): e1004261. doi: 10.1371/journal.ppat.1004261.

Cornman RS, Tarpy DR, Chen Y, Jeffreys L, Lopez D, Pettis JS, vanEngelsdorp D, Evans JD. Pathogen webs in collapsing honey bee colonies, PLoS ONE, 2012; 7: e43562

Cox-Foster DL, Conlan S, Holmes EC, Palacios G, Evans JD, Moran NA, Quan PL, Briese T, Hornig M, Geiser DM, Martinson V, vanEngelsdorp D, Kalkstein AL, Drysdale A,

Hui J, Zhai J, Cui L, Hutchison SK, Simons JF, Egholm M, Pettis JS, Lipkin WI. A metagenomic survey of microbes in honey bee colony collapse disorder. Science. 2007; 12;318(5848):283-7. doi: 10.1126/science.

Çagirgan AA, Yildirim Y, Usta A. The investigation of Israil acute bee paralysis virus, sacbrood virus, kashmir bee virus and chronic bee paralysis virus in honeybees (Apis mellifera). Eurasian J Vet Sci 2020; 36:2, 96-10. doi:10.15312/EurasianJVetSci.2020.265.

Çağirgan AA, Yazici Z. The prevalence of seven crucial honeybee viruses using multiplex RT-PCR and their phylogenetic analysis. Turkish Journal of Veterinary and Animal Sciences, 2021; 45, 44-55. https://doi.org/10.3906/vet-2004-139.

Çakmak İ. Bal arısı koloni kayıpları ve çözüm yolları. Ordu Arıcılık Araştırma Dergisi, 2012; 4(7):3-8.

Çakmak I. Türkiye'de Arıcılık ve Güncel Koloni Kayıpları. Uludağ Arıcılık Dergisi, 2016;16 (1),31-48. DOI:10.31467/uluaricilik379276.

De Miranda JR, Cordoni G, Budge G. The Acute bee paralysis virus–Kashmir bee virus–Israeli acute paralysis virus complex. J. Invertebr. Pathol. 2010; 103: 30–47.

De Miranda JR, Ribiere CY, Gauthier ML. Varroa and Viruses. In *Varroa*– still a problem in the 21st century? Carreck NL, editor. Cardiff: IBBA. 2011; 11–31.

Del Nozal MJ, Mayo R, Bernal JL. Honeybee colony collapse due to Nosema ceranae in Professional apiaries. Environ. Microbiol. Rep. 2009;1: 110–113.

Di Prisco G, Pennacchio F, Caprio E, Boncristiani HF, Evans JD, Chen Y. *Varroa destructor* is an effective vector of Israeli acute paralysis virus in the honeybee, *Apis mellifera*. J Gen Virol., 2011; 92: 151–155.

Genersch E, von der Ohe W, Kaatz H, Schroeder A, Otten C, Büchler R, Berg S, Ritter W, Mühlen W, Gisder S, Meixner M, Liebig G, Rosenkranz P. The German bee monitoring project: a long term study to understand periodically high winter losses of honey bee colonies. Apidologie, 2010; 41, 332–352. https://doi.org/10.1051/apido/2010014

Güller A, Kurt Z. Occurrence and Molecular Phylogeny of Economically Relevant Viruses Infecting Honey Bees (Apis mellifera L.) of Bingöl Province, Turkey. Journal of apicultural science, 2021; 66, 85-96. doi: 10.2478/jas-2022-0006

Gülmez Y, Bursalı A, Tekin S. Molecular detection and characterization of deformed wing virus (DWV) in honeybees (Apis mellifera L.) and mite (Varroa destructor) in Turkey. African Journal of Biotechnology, 2009; 8(16):3698-3702.

Gümüşova-Okursoy S, Albayrak H, Kurt M, Yazıcı Z. Prevalence of three honey bee viruses in Turkey. Veterınarskı Arhıv, 2010; 80 (6): 779-785.

Hails RS, Ball BV, Genersch E. Infection strategies of insect viruses M. Aubert, B.V. Ball, I. Fries, R. F. A. Moritz, N. Milani, I. Bernardinelli (Eds.), Virology and the Honey Bee, European Communities (2008), pp.255-275

Ivgin-Tunca R, Oskay D, Gosterit A, Tekin OK. Does Nosema ceranae Wipe Out Nosema apis in Turkey? Iran J Parasitol. 2016;11(2):259-264.

Kalaycı G, Cagirgan AA, Kaplan M, Pekmez K, Beyazit A, Ozkan B, Arslan F. The Role of Viral and Parasitic Pathogens Affected By Colony Losses in Turkish Apiaries. Journal of Kafkas University Faculty of Veterinary Medicine, 2020; 26(5), 671-677. https://doi.org/10.9775/kvfd.2020.24154.

Kartal S, Ivgin Tunca R, Özgül O, Karabağ K, koç H. Microscopic and Molecular Detection of *Nosema sp*. İn the Southwest Aegean Region. Uludağ Arıcılık Dergisi, 2021; 21 (1),8-20. DOI:10.31467/uluaricilik.880380.

Maori E, Lavi S, Mozes-Koch R, Gantman Y, Peretz Y, Edelbaum O, Tanne E, Sela I. Isolation and characterization of Israeli acute paralysis virus, a dicistrovirus affecting honeybees in Israel: Evidence for diversity due to intra- and inter-species recombination. J. Gen. Virol. 2007; 88: 3428–3438.

Martin SJ, Highfield AC, Brettell L, Villalobos EM, Budge GE, Powel M, Nikaido S, Schroeder DC. Global honey bee viral landscape altered by a parasitic mite. Science, 2012; 336: 1304–1306.

Martín-Hernández R, Meana A, Prieto L, Salvador AM, Garrido-Bailon E, Higes M. Outcome of colonization of Apis mellifera by Nosema ceranae. Appl. Environ. Microbiol. 2007; 73: 6331–6338.

McMenamin AJ, Genersch E. Honey bee colony losses and associated viruses. Curr Opin Insect Sci. 2015; 8:121-129. doi: 10.1016/j.cois.2015.01.015.

Muz MN. Bal arılarında ani koloni sönmesi. T Parazitol Derg. 2008; 32 (3): 271-275.

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. Apicult. Res., 2009; 48(3):204-208.

Muz MN. Girişgin AO, Muz D, Aydin L. Molecular detection of *Nosema ceranae* and *Nosema apis* infections in Turkish apiaries with collapsed colonies. J. Apicult. Res., 2010; 49(4): 342-344.

Muz D, Muz MN. A molecular epidemiological study of black queen cell virüs in honeybees (Apis mellifera) of Turkey: the first genetic characterization and phylogenetic analysis of field viruses. Apidologie, 2018; 49: 1-12. https://doi.org/10.1007/s13592-017-0531-5

Muz D, Muz MN. Bal Arılarının Viral Hastalıkları, Tanı ve Tedavisi Özdemir N, editör. Veteriner Arı Sağlığı ve Apiterapi, 2021; 1: 24-36

Muz D, Muz MN. Investigation of Honeybee Colony Losses in Thrace Region, 4th International Eurasian Conference on Science, Engineering and Technology (EurasianSciEnTech 2022) December 14-16, 2022.

Ostroverkhova NV, Konusova OL, Kucher AN, Kireeva TN, Rosseykina SA. Prevalence of the Microsporidian Nosema spp. in Honey Bee Populations (Apis mellifera) in Some Ecological Regions of North Asia. *Veterinary Sciences*. 2020; 7(3):111. https://doi.org/10.3390/vetsci7030111

Özkırım A, Schiesser A. Israeli acute paralysis virus (IAPV) in Turkish bees. J. Apicult. Res., 2013; 52(2): 56-57.

Palacios G, Hui J, Quan PL, Kalkstein A, Honkavuori KS, Bussetti AV, Conlan S, Evans J, Chen YP, vanEngelsdorp D, Efrat H, Pettis J, Cox-Foster D, Holmes EC, Briese T, Lipkin WI. Genetic analysis of Israel acute paralysis virus: distinct clusters are circulating in the United States. J Virol. 2008; 82(13):6209-17. doi: 10.1128/JVI.00251-08.

Rüstemoğlu M, Sipahioğlu HM. Occurrence and prevalence of six honey bee viruses in Hakkâri (Turkey) and their genomic divergence. Munis Entomology & Zoology, 2019; 14 (2): 574-583]

Stoltz D, Shen XR, Boggis C, Sisson G. Molecular diagnosis of Kashmir bee virus infection, Journal of Apicultural Research. 1995; 34:3, 153-160

Tosun O, Yaman M. The Effects of Temperature And Humidity Around The Beehives on The Distribution of Nosema ceranae, and also Geographical and Seasonal Activity of The Infection İn The Eastern Black Sea Region of Turkey. Journal of Environmental Science and Engineering B, 2016; 5(11): 513-522., Doi: 10.17265/2162-5263/2016.11.001

Ütük AE, Pişkin FC, Girişgin AO, Selçuk O, Aydın L. Microscopic and molecular detection of Nosema spp. in honeybees of Turkey. Apidologie, 2016; 47:267–271. DOI: 10.1007/s13592-015-0394-6.

VanEngelsdorp D, Underwood R, Caron D, Hayes JJ. An estimate of managed colony losses in the winter of 2006–2007: A report commissioned by the apiary inspectors of America. Am Bee J. 2007; 147: 599–603

Williams GR, Tarpy DR, Vanengelsdorp D, Chauzat MP, Cox-Foster DL. Colony collapse disorder in context. Bioessays, 2010; 32: 845–846.

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ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

PHENETIC VARIATION IN HONEY BEE (*Apis mellifera*) POPULATION OF THE TORATAU GEOPARK, THE REPUBLIC OF BASHKORTOSTAN

Başkortostan Cumhuriyeti Toratau Jeoparki Bal Arısı (*Apis mellifera*) Popülasyonunda Tergit Rengi Değişimi

Salavat T. SAGITOV[1, 3], Rustem A. ILYASOV*[2, 4, 6], Vener N. SATTAROV[3], Yuliya R. ABDRAKHIMOVA[1], Valery N. DANILENKO[4], Nailya R. GAZIZOVA[5], Amilya V. SATTAROVA[3], Dmitry V. BOGUSLAVSKY[6]

[1]Regional Branch of the Russian Geographical Society in the Republic of Bashkortostan, Ufa, RUSSIA, E-posta: salavatst@list.ru,ORCID No: 0000-0002-7211-1004, E-posta: abdrakhimova.rgo@internet.ru, ORCID No: 0009-0004-8690-5472.

[*2]Laboratory of Molecular Genetics, Scientific Educational Center in Bashkir State Agrarian University, Ufa, RUSSIA, Corresponding author / Yazışma Yazarı E-posta: apismell@mail.ru, ORCID No: 0000-0003-2445-4739.

[3]Department of Bioecology and Biological Education, Faculty of Natural Geography, Bashkir State Pedagogical University named after M. Akmulla, Ufa, RUSSIA, E-posta: wener5791@yandex.ru, ORCID No: 0000-0001-6331-4398, E-postal: amilywener@gmail.com, ORCID No: 0009-0004-0793-2414.

[4]Vavilov Institute of General Genetics, Russian Academy of Sciences, Moscow, RUSSIA, E-posta: valerid@vigg.ru, ORCID No: 0000-0001-5780-0621.

[5]Ufa Scientific Research Institute of Occupational Medicine and Human Ecology, Ufa, RUSSIA, E-posta: nelli.ga012@gmail.com, ORCID No: 0000-0003-4287-8594.

[6]Koltsov Institute of Developmental Biology, Russian Academy of Sciences, Moscow, RUSSIA, E-posta: boguslavsky@rambler.ru, ORCIDNo: 0000-0001-9601-640X.

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ABSTRACT

A phenetic analysis of the honey bee population of the Toratau Geopark (Russia) was performed. Over 1,000 worker and drone bee samples were collected from 250 colonies in 59 apiaries on the territory of the Toratau Geopark (Gafuriysky, Ishimbaysky, Meleuzovsky, and Sterlitamaksky districts of the Republic of Bashkortostan). Six phenes in worker bees and four phenes in drone bees were recognized. The phenes E, 1R, 2R, and 3R in workers and Is, I, and O-gray in drones were predominant in the honey bee population of the Toratau Geopark, which were associated with subspecies of the C-lineage. These phenes can be used as indicators of introgressive hybridization in the local dark European honey bee population. The phenes allow for quick evaluation of certain honey bee colonies hybridization states.

Keywords: Honey bee, phenes, Dark European bee, The Republic of Bashkortostan, Toratau Geopark

ÖZ

Toratau Jeoparkı'ndaki (Rusya) bal arısı popülasyonunun fenetik analizi yapılmıştır. Toratau Jeoparkı topraklarındaki (Başkurdistan Cumhuriyeti'nin Gafuriysky, Ishimbaysky, Meleuzovsky ve Sterlitamaksky bölgeleri) 59 arılıktaki 250 koloniden 1.000'den fazla işçi ve erkek arı örneği

toplanmıştır. İşçi arılarda altı fen ve erkek arılarda dört tergit rengi tespit edilmiştir. İşçilerde E, 1R, 2R ve 3R ve erkek arılarda Is, I ve O-gri fenleri Toratau Jeoparkı'ndaki bal arısı popülasyonunda baskındı ve bunlar C soyunun alt türleriyle ilişkiliydi. Bu tergit rengi, yerel koyu Avrupa bal arısı popülasyonunda içsel melezleşmenin göstergeleri olarak kullanılabilir. Bu tergit renkleri, belirli bal arısı kolonilerinin melezleşme durumlarının hızlı bir şekilde değerlendirilmesine olanak sağlamaktadır.

Anahtar Kelimeler: **Bal arısı, tergit renkleri, Kara Avrupa arısı, Başkurdistan Cumhuriyeti, Toratau Jeoparkı**

GENİŞLETİLMİŞ ÖZET

Çalışmanın amacı: Başkurdistan Cumhuriyeti Toratau Jeoparkı'ndaki işçi ve erkek arıların dağılımının değerlendirilmesidir**.**

Giriş: Batı Avrupa ülkelerinin topraklarında yaşayan bal arıları arasında en yaygın alt tür koyu Avrupa, koyu orman veya Orta Rus arısıdır (*Apis mellifera mellifera*). Şu anda, bu alt tür esas olarak Güney Urallar, Batı Sibirya ve orta Rusya'da hayatta kalmış ve yerel popülasyonlar oluşturmuştur. Tarihsel olarak, Urallar ve Başkurdistan Cumhuriyeti'nin (Rusya) doğal ve iklimsel bölgesi, şiddetli kışlar, bal bitkilerinin zengin bir tür bileşimi, ıhlamur ormanlarının bolluğu ile ayırt edildi ve bununla bağlantılı olarak cumhuriyetin arıları, koyu Avrupa arısının özel bir Başkurt popülasyonu olarak tanımlandı.

Gereç ve yöntem: 2018 yılında, Başkurdistan topraklarında, bölgesel olarak 4 belediye bölgesini kapsayan Toratau Jeoparkı oluşturuldu: Gafuriysky, Ishimbaysky, Meleuzovsky ve Sterlitamaksky. Jeoparkın gelişmiş düzenleyici çerçevesi, rezerv bölgesinin yakın konumu ve doğal ve iklimsel koşullar, Başkurt nüfusunun arılarına dayalı arıcılığın geliştirilmesi için ideal koşulları oluşturmuştur. Bal arısı popülasyonunun fenetik analizi gerçekleştirilmiştir. Toplam 2.000 arı toplanmıştır (1.000 işçi arı ve 1.000 erkek arı). Her bölgede 250 koloniden seçim yapılmıştır. Bu çalışmada Ruttner yöntemi kullanılmıştır.

Bulgular: Oda işleme, M. Akmulla'nın adını taşıyan Başkurt Devlet Pedagoji Üniversitesi Biyoekoloji ve Biyolojik Eğitim Bölümü temelinde gerçekleştirilmiştir. Yüzde ve oranların hesaplanması, grafik çizimi Statistica 12.0 (StatSoft Power Solutions, Inc. USA) programında yapılmıştır. İşçi arılarda altı tergit rengi, erkek arılarda ise dört tergit rengi bulunmuştur. İşçilerde E, 1R, 2R ve 3R ve erkek arılarda Is, I ve O-gri tergit renkleri Toratau Jeoparkı bal arısı popülasyonunda baskındır ve bunlar C-soyunun alt türleriyle ilişkilidir.

Tartışma ve sonuç: Bu tergit renkleri, yerel koyu Avrupa bal arısı popülasyonunda içsel melezleşmenin göstergeleri olarak kullanılabilir. Bu tergit renkleri, belirli bal arısı kolonilerinin melezleşme durumunu hızlı bir şekilde değerlendirmenizi sağlar. Safkan arıların varlığı, koyu Avrupa arısının Başkurt popülasyonunun korunması ve çoğaltılmasına yönelik önlemlerin uygulanması için fırsatlar sunmaktadır. Yerel koyu Avrupa arılarını melezleşmeden korumak acildir. Çünkü insan yardımı olmadan yerel koyu orman arısı gen havuzunun saflığını geri kazanılamaz.

INTRODUCTION

In the process of the evolution, about 30 subspecies of the honey bee (*Apis mellifera*) were formed in the Old World, extended in a wide range of climatic conditions (Ilyasov et al. 2020). The ability of honey bees to adapt well, the human consumption of bee products and the use of honey bees in plant pollination contributed to the wide anthropogenic spread of honey bee colonies to almost all around the world. However, despite the high adaptability and the large distribution area, the number of honey bee populations in the world is decreasing annually, which leads to a decrease of a genetic diversity, the adaptability of the populations and the biodiversity of biomes (Rua et al. 2009).

Among the honey bees inhabiting the territory of Western European countries, the dark European bee *Apis mellifera mellifera* is common. The dark forest, Central Russian, or dark European bee is an indigenous subspecies of the honey bee of the western and northern regions of Europe and Russia, evolutionarily formed in forest conditions. Currently, this honey bee subspecies has been preserved mainly in the Southern Urals, Western Siberia and the central part of Russia, forming local populations named by territorial affiliation: Altai, Arkhangelsk, Vladimir, Bashkir (Ilyasov et al. 2021; Petrov 1980; Petrov 2004). Historically, the natural and climatic

zone of the Ural and Bashkortostan has been distinguished by severe winters, a rich species composition of honey plants, and an abundance of linden forests, in connection with which the bees of the republic were singled out as a special Bashkir population of the dark European bee (Shafikov and Avetisyan 1976; Shafikov 1978; Abdulov and Shafikov 2004; Shafikov and Baimuratov 2002). Kozhevnikov (1931) wrote: “In the forests of Bashkiria and the Urals, we found the remains of a primal dark European bee, which currently is the greatest treasure in terms of genetics. It must be protected in every possible way from hybridization, and on its basis mass breeding of the basic dark European bee should be based, which, over thousands of years of natural selection in the harsh mountain climate, has developed endurance and vitality" (Kozhevnikov 1931). The Bashkir population of the dark European bee is characterized by the dark color of the body of individuals, only 1-2 phenes, the tergites of which do not have signs of yellowness, the sternites are colored from dark gray to dark brown without yellowness; the end of the abdomen in working individuals is more blunt than in bees of other breeds; the body shape of a sitting bee is squat (Krivtsov 1995; Krivtsov and Grankin 2004).

The Republic of Bashkortostan is located in the east of the European part of Russia, in the basin of the Belaya and Ural rivers, extends from north 56°31′N 54°31′E to south 51°34′N 57°12′E and from west 55°07′N 53°08′E to east 54°52′N 60°00′E. Apart from that the republic occupies a wide strip of the western Urals stretching from the north to the south (Figure 1). The Perm and Sverdlovsk regions are adjacent to the republic from the north, the Chelyabinsk Region from the east, the Orenburg Region from the south, the Republic of Tatarstan and the Udmurt Republic from the west.

Due to the presence of many natural areas (forest, mountain forest, forest-steppe, steppe), the Republic of Bashkortostan is a unique territory with a rich species composition of flora and fauna. In this regard, there are many conservation areas located here, for instance: The National Park «Bashkiria», the Nature Reserve «Altyn Solok», and the State Natural Biosphere Reserve «Shulgan-Tash». The latter was created for the purpose of preserving and breeding the Burzyan wild living honey bee (Bashkir honey bee ecotype), which is listed in all editions of the Red Book of the Republic of Bashkortostan (2004, 2014) and has the status of category 4 (population not defined by status). In addition, in 2018, not far from the Shulgan-Tash Nature Reserve, the Toratau Geopark was created, geographically covering 4 municipal districts: Gafuriysky, Ishimbaysky, Meleuzovsky and Sterlitamaksky. The main function of the Geopark is the preservation of the geological, biological, historical, and cultural heritage of the republic. At the same time, the developed regulatory framework of the Geopark, the close location of the reserve territory and natural and climatic conditions have created ideal conditions for honey bees breeding of the Bashkir population. However, at the beginning of the XXI century, due to the increase of the import of honey bees of the unknown origin into the Republic of Bashkortostan, the genetic pressure of other subspecies of honey bees began to pose a serious threat to both Bashkir and Burzyan populations. Therefore, if in the 80s of the last century the influx of other genetic material in the reserve did not exceed - 1% and 2-3% of the total number of honey bee colonies, then in recent years this marker is 10-12% (Abramova 2014; Koroleva et al. 2019; Mannapov et al. 2019; Ruttner 2006; Sabirdzhonova and Sattarov 2021). The result of these processes is that at present moment, the Burzyan bee population is surrounded by hybrid honey bee colonies and the structure of the Bashkir bee population is gradually and irrevocably being lost. In this case, of course, it is necessary to monitor the diversity of the honey bees’ breed in the buffer zone near the locality of honey bees kept in the conservation area.

When the honey bee breeds are being characterized, a different number of exterior indicators are used. Some of them are associated with the linear morphometric measurements, others express the ratio of some quantities to others, the third are characterized by area, the fourth proceed from the topographic incompatibility of the individual points and lines, the fifth are determined by mass, color, pubescence. Speaking of breed-defining features, the color of honey bees should be taken into account. Despite the fact that it plays an important role in determining the breeds of honey bees, scientists do not always pay attention to this feature. Only in some studies, the assessment of honey bees is given together with the honey bee coloring, although it has been proved long ago that the appearance of yellow coloration on the chitinous covers of the abdomen of the European dark bee indicates the processes of hybridization (Abramova et al. 2014; Koroleva et al. 2019; Mannapov et al.

2019; Ruttner 2006; Sabirdzhonova and Sattarov 2021; Sagitov et al. 2022a; Sagitov et al. 2022b).

Previously reported, the color variations of the cuticle of the insects are exclusively diverse and that this feature serves as a reliable sign for determining the species and various geographical forms of the studied animals (Chashchukhin and Lapteva 2011; Chashchukhin and Lapteva 2009). They also noted that the presence of yellow coloring on the tergites of the abdomen is a peculiarity differential of honey bees of many southern subspecies. The appearance of this colouring in the European dark bee indicates, first, the processes of hybridization. Taking into account the importance of maintaining the purity of the gene pool of honey bee subspecies populations (Sabirdzhonova and Sattarov 2021; Sagitov et al. 2022a; Sagitov et al. 2022b; Chashchukhin and Lapteva 2011), it was noted that the system of creating the areas of "pure" breeding should be based on principles that consider the contribution of drones and queens in maintaining the plasticity and stability of population structures. As it is known, the selection at the apiary must be carried out both on the maternal and paternal lines (Sagitov et al. 2022a; Chashchukhin and Lapteva 2011; Cherevko and Avetisyan 2007; Chibilev 2011; Sharygin and Krivtsova 2018). The purpose of the study is evaluation of worker and drone bee phenes distribution in the Toratau Geopark of the Republic of Bashkortostan.

MATERIALS AND METHODS

The collection of 2000 worker bees and drones from 59 apiaries that are part of the Gafuriysky (53°53'41"N. 56°28'07"E), Ishimbaysky (53°28'37"N. 56°30'43" E.), Meleuzovsky (52°57'00"N. 55°55'59" E) and Sterlitamak (53°37'59" N 55°57'00" E) districts, was used as the material for this study (Figure 1).

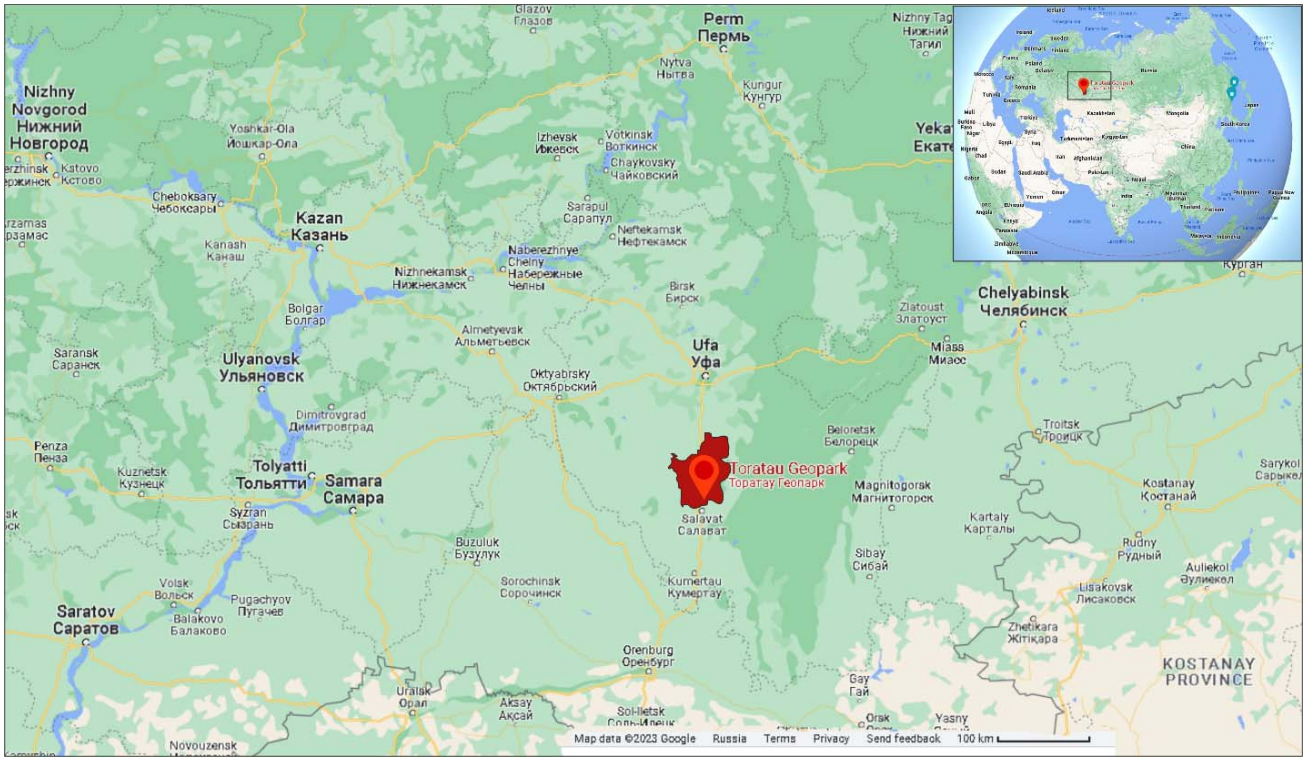

Figure 1. Geographical location of the Toratau Geopark in the Republic of Bashkortostan.

In total, 2,000 bees were collected (1,000 worker bees and 1,000 drones). In each district, the selection was carried out from 250 colonies. The Ruttner's method (Ruttner 2006) used in this work. The cameral treatment was carried out based on the department of Bioecology and Biological Education

of the Bashkir State Pedagogical University named after M. Akmulla. The calculation of percentages and proportions, and construction of graphs were performed with Statistica 12.0 (StatSoft Power Solutions, Inc. USA).

RESULTS

In the course of researches, 6 phenes of worker bees were identified: O, e, E, 1R, 2R and 3R (Figure 2). As we can see, the phenetic structure of the honey bees on this territory is characterized by high heterogeneity, since the aboriginal population would differ in the presence of only one (O) or two (O and e) phenes.

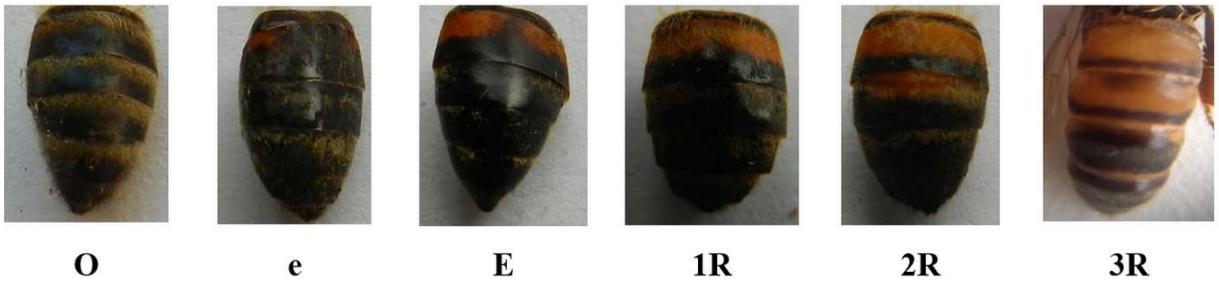

Figure 2. The detected phenes of the worker bees in the Toratau Geopark.

According to the obtained results, it can be seen that the honey bee colonies with phenes 2R and 3R predominate in almost all apiaries. At the same time, the occurrence (%) was: in the Gafuriysky district from 4.8 to 100 (2R) and from 50 to 100 (3R), in the Meleuzovsky district from 50 to 100 (2R) and from 14.3 to 100 (3R); in the Ishimbaysky district from 9.1 to 100 (2R) and from 6.7 to 100 (3R) and in Sterlitamaksky district from 5 to 92.3 (2R) and from 12.5 to 95 (3R) (Table 1).

Table 1. The phenes of worker bees in the Toratau Geopark

Locality, apiary	No. of colonies, pcs.	Worker bee phenes (pcs. / %)					
		O	e	E	1R	2R	3R
Gafuriysky district							
Kutluguza	15	-	-	-	-	2 (13.3)	13 (86.7)
Krasnousolsky	8	-	-	-	-		8 (100)
Uzbjakovo	16	-	-	-	-	-	16 (100)
Tolparovo	11	-	-	-	-	-	11 (100)
Tashly							
apiary 1	26	-	-	-	-	13 (50)	13 (50)
apiary 2	18	17 (94.4)	-	-	-	1 (5.6)	-
Tolparovo:							
apiary 1	17	-	-	-	-	3 (17.6)	14 (82.4)
apiary 2	11	-	-	-	-	11 (100)	-
Mendim:							
apiary 1	24	-	-	-	-	24 (100)	-
apiary 2	26	-	-	-	-	10 (38.5)	16 (61.5)
Tabynsk	21	20 (95.2)	-	-	-	1 (4.8)	-
Cowardy:							
apiary 1	17	-	-	-	-	1 (5.9)	16 (94.1)

apiary 2	12	-	-	-	-	12 (100)	-
Ibragimovo	13	-	-	-	-	-	-
Kyzyl Yar	15	-	-	-	-	4 (26.7)	-
Total	250	37 (14.8)	0	0	24 (9.6)	82 (32.8)	107 (42.8)
Meleuzovsky district							
Araslanovo	18	-	-	-	-	-	18 (100)
Alexandrovka:							
apiary 1	22	-	-	-	-	13 (59.1)	9 (40.9)
apiary 2	19	-	-	-	-	12 (63.2)	7 (36.8)
Meleuz:							
apiary 1	28	-	-	-	8 (28.6)	16 (57.1)	4 (14.3)
apiary 2	4	-	-	-	-	4 (100)	-
Aptrakovo	15	-	-	-	-	11 (73.3)	4 (26.7)
Pokrovka	15	-	-	-	-	10 (66.7)	5 (33.3)
Nugush	10	-	-	-	-	5 (50)	5 (50)
Zirikovo	30	-	-	-	10 (33.3)	20 (66.7)	-
Abitovo	30	-	-	-	-	20 (66.7)	10 (33.3)
Klenovaya gora	23	-	-	-	11 (47.8)	12 (52.2)	-
Kutlubulatovo	8	-	-	-	2 (25)	-	6 (75)
Smak	5	-	-	-	-	3 (60)	2 (40)
Sugar Factory Nursery	5	-	-	-	-	5 (100)	-
Voskresensko e	18	-	-	-	-	9 (50)	9 (50)
Total	250	0	0	0	31 (12.4)	140 (56)	79 (31.6)
Ishimbaysky district							
Makarovo:							-
apiary 1	5	-	3 (60)	-	-	2 (40)	
apiary 2	19	-	-	-	-	5 (26.3)	14 (73.7)
apiary 3	10	-	-	-	-	3 (30)	7 (70)
Gumerovo:							
apiary 1	8	-	-	-	-	1 (12.5)	7 (87.5)
apiary 2	8	-	-	-	-	8(100)	-
Sargaevo:							
apiary 1	17	-	-	-	-	4 (23.5)	13 (76.5)
apiary 2	5	-	-	-	-		5 (100)
Isyakaevo:							
apiary 1	35	-	-	10 (28.6)	18 (51.4)	4 (11.4)	3 (8.6)
apiary 2	22	-	-	-	-	10 (45.5)	12 (54.5)
Asiyalanovo	30	16 (53.3)	12 (40)	-	-	-	2 (6.7)
Verkhneitkulov o							
apiary 1	10	-	1 (10)	-	-	9 (90)	-
apiary 2	11	-	1 (9.1)	-	-	8 (72.7)	2 (18.2)
Armetovo	14	-	-	-	-	7 (50)	7 (50)
Asiyalan	11	-	-	-	-	1 (9.1)	10 (90.9)
Urazbaevo	14	-	-	-	-	7 (50)	7 (50)

Podgornaya	17	-	-	-	-	13 (76.5)	4 (23.5)
Kinzebulatovo	14	-	-	-	5 (35.7)	2 (14.3)	7 (50)
Total	250	16 (6.4)	17 (6.8)	10 (4)	23 (9.2)	84 (33.6)	100 (40)
Sterlitamaksky district							
Pokrovka	28	-	-	-	6 (21.4)	12 (42.9)	10 (35.7)
Berezovka	28	-	-	-	-	15 (53.6)	13 (46.4)
Karmaskaly	23	-	-	-	-	7 (30.4)	16 (69.6)
Spasskaya	26	-	-	-	-	12 (46.2)	14 (53.8)
Burikazganovo	25	-	-	-	-	16 (64)	9 (36)
Talachevo	15	-	-	-	-	5 (33.3)	10 (66.7)
Pokrovka	8	-	-	-	-	7 (87.5)	1 (12.5)
Aigulevo	15	7 (46.7)	-	-	1 (6.7)	5 (33.3)	2 (13.3)
Spasskaya	26	2 (7.7)	-	-	-	24 (92.3)	-
Kuganak	20	-	-	-	-	1 (5)	19 (95)
Kosyakovka	9	-	-	-	-	1 (11.1)	8 (88.9)
Strelkovka	27	-	-	-	-	9 (33.3)	18 (66.7)
Total	250	9 (3.6)	0	0	7 (2.8)	114 (45.6)	120 (48)
Total	1000	62 (6.2)	17 (1.7)	10 (1)	85 (8.5)	420 (42)	406 (40.6)

Honey bee colonies with the Dark European bee phenes - O and e were identified in all districts except Meleuzovsky. At the same time, only in the Ishimbaysky district there were honey bee colonies with both phenes. In the apiaries of the Gafuriysky and Sterlitamaksky districts, honey bees were found only with a phene - O: about: 14.8% and 3.6%, respectively. Purebred colonies were found in the apiaries of settlements: Tashly (94.4% or 17 colonies out of 18) and Tabynsk (95.2% or 20 out of 21 colonies) in the Gafuriysky district. Honey bee colonies with European dark bee phenes were noted in 4 apiaries: Makarovo (apiary 1) – 60% or 3 out of 5 colonies studied; Asiyalanovo – 28 colonies (93.3%) out of 30; Verkhneitkulovo (apiary 1 and 2) - 1 colonies out of 10 (10%) and 11 (9.1%) colonies in the Ishimbaysky district. In Sterlitamaksky district honey bees with a purebred phenes are registered in two localities: Aigulevo (colonies or 46.7% of 15 colonies) and Spasskaya (2 colonies – 7.7% of 27).

Honey bees with phenes E were found in apiaries of only 2 districts (%): Ishimbaysky - 4 and Sterlitamaksky district - 1. The situation with the phenes 1R was similar to honey bees with phenes 2R and 3R, i.e. 1R was noted in apiaries of all districts, but in minimal numbers compared to 2R and 3R: Gafuriysky – 9.6%, Meleuzovsky – 12.4%, Ishimbaysky – 9.2% and Sterlitamaksky – 2.8%. The occurrence of honey bee colonies with different phenes in apiaries is shown in Figure 3.

In quantitative terms, honey bee colonies with phenes 2R and 3R prevailed both: by districts and by the explored territory: Gafuriysky - 2R (32.8%) 3R (42.8%), Meleuzovsky - 2R (56%) 3R (31.6%), Ishimbaysky - 2R (33.6%), 3R (40%), Sterlitamaksky - 2R (45.6%), 3R (48%) and in the whole examined territory - 2R (42%), 3R (40.6%). The occurrence of phenes (O and e) related to the Dark European bee was 7.9%, which is significantly lower than the quantitative composition of colonies with phenes 1R (8.5%), 2R (42%), 3R (40.6%). The occurrence of colonies with phenes E was the lowest – 1% (10 colonies out of 1000).

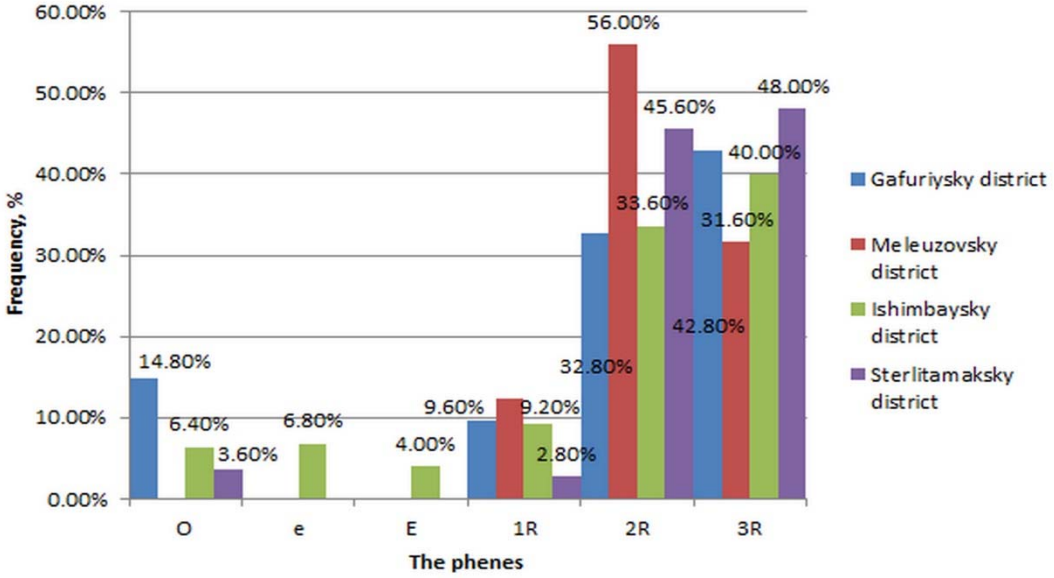

Figure 3. Distribution of the worker bee phenes in the Toratau Geopark.

The phenes of the drones that are found in the honey bee colonies in the apiaries of the Gafuriysky, Ishimbaysky, Meleuzovsky and Sterlitamaksky districts, which are part of the Toratau Geopark, are shown in Figure 4. As we can see, the phenetic structure of drones is also characterized by high heterogeneity (Is, I, O – dark and O- grey), as well as worker bees, because the purebred population of the Dark European bee is characterized only by the O-dark phene.

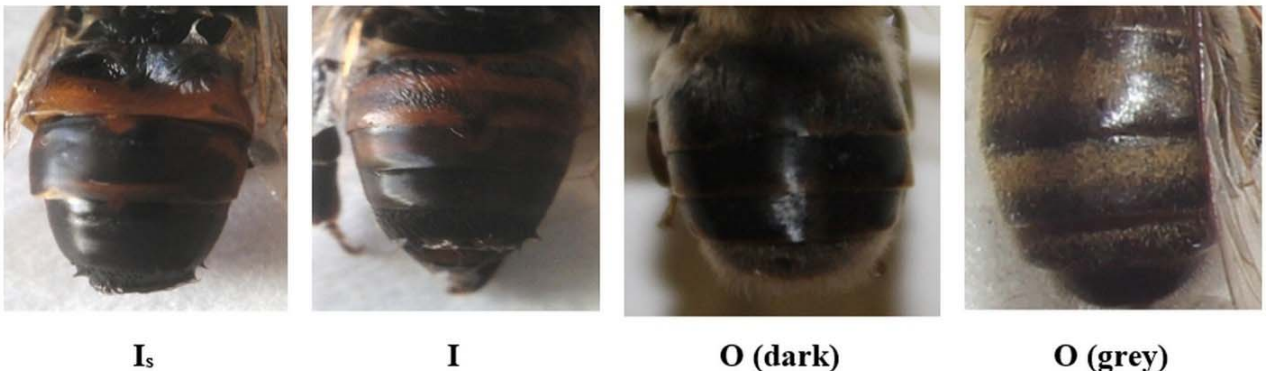

Figure 4. The detected phenes of the drone bees in the Toratau Geopark.

Drones with phenes Is and I prevailed in almost all apiaries, %: in the Gafuriysky district from 28.6 to 100 (I_s) and from 12.5 to 100 (I); in Meleuzovsky – from 33.3 to 100 (I_s) and from 28.6 to 60 (I); in Ishimbaysky – from 20 to 100 (I_s), 25 to 50 (I) and in the Sterlitamaksky district - from 37.1 to 100 (I_s) and from 40 to 57.1 (I) (Table 2).

Table 2. The phenes of the drone bees in the Toratau Geopark

Locality, apiary	No. of colonies, pcs	Drone bee phenes (pcs. / %)			
		I_s	I	O - dark	O - grey
Gafuriysky district					
Kutluguza	15	5 (33.3)	10 (66.7)	-	-
Krasnousolsky	8	-	8 (100)	-	-
Uzbjakovo	16	12 (75)	2 (12.5)	-	2 (12.5)
Tolparovo	11	11 (100)	-	-	-
Tashly					
apiary 1	26	13 (50)	13 (50)	-	-
apiary 2	18	-	-	16 (88.9)	2 (11.1)
Tolparovo:					
apiary 1	17	8 (47.1)	9 (52.9)	-	-
apiary 2	11	11 (100)	-	-	-
Mendim:					
apiary 1	24	12 (50)	10 (41.7)	-	2 (8.3)
apiary 2	26	10 (38.4)	8 (30.8)	-	8 (30.8)
Tabynsk	21	6 (28.6)	5 (23.8)	10 (47.6)	
Kowardy:					
apiary 1	17	5 (29.4)	-	6 (35.3)	6 (35.3)
apiary 2	12	12 (100)	-	-	-
Ibragimovo	13	6 (46.2)	7 (53.8)	-	-
Kyzyl Yar	15	5 (33.3)	10 (66.7)	-	-
Total	250	116 (46.4)	82 (32.8)	32 (12.8)	20 (8)
Meleuzovsky district					
Araslanovo	18	9 (50)	9 (50)	-	-
Alexandrovka:					
apiary 1	22	10 (45.5)	12 (54.5)	-	
apiary 2	19	11 (57.9)	8 (42.1)	-	-
Meleuz:					
apiary 1	28	12 (42.8)	8 (28.6)	-	8 (28.6)
apiary 2	4	4 (100)	-	-	-
Aptrakovo	15	5 (33.3)	7 (46.7)	-	3(20)
Pokrovka	15	10 (66.7)	5 (33.3)	-	-
Nugush	10	5 (50)	5 (50)	-	-
Zirikovo	30	10 (33.3)	15 (50)	-	5 (16.7)
Abitovo	30	12 (40)	18 (60)	-	-
Klenovaya gora	23	10 (43.5)	11 (47.8)	-	2 (8.7)
Kutlubulatovo	8	8 (100)	-	-	-
Smak	5	5 (100)	-	-	-
Sugar Factory Nursery	5	5 (100)	-	-	-
Voskresenskoe	18	9 (50)	9 (50)	-	-
Total	250	125 (50)	107 (42.8)	0	18 (7.2)
Ishimbaysky district					
Makarovo:					
apiary 1	5	-	-	5 (100)	-
apiary 2	19	6 (31.6)	6 (31.6)	6 (31.6)	1 (5.2)
apiary 3	10	2 (20)	-	7 (70)	1 (10)
Gumerovo:					

apiary 1	8	-	-	7 (87,5)	1 (12.5)
apiary 2	8	3 (37.5)	2 (25)	2 (25)	1 (12.5)
Sargaevo:					
apiary 1	17	12 (70.6)	5 (29.4)	-	-
apiary 2	5	5 (100)	-	-	-
Isyakaevo:					
apiary 1	35	15 (42.8)	10 (28.6)	5 (14.3)	5 (14.3)
apiary 2	22	11 (50)	11 (50)	-	-
Asiyalanovo	30	8 (26.6)	8 (26.6)	6 (20.2)	8 (26.6)
Verkhneitkulovo					
apiary 1	10	5 (50)	-	5 (50)	-
apiary 2	11	11 (100)	-	-	-
Armetovo	14	10 (71.4)	4 (28.6)	-	-
Asiyalan	11	11 (100)	-	-	-
Urazbaevo	14	7 (50)	5 (35.7)	2 (14.3)	-
Podgornaya	17	8 (47.1)	8 (47.1)	1 (5.8)	-
Kinzebulatovo	14	5 (35.7)	6 (42.9)	2 (14.3)	1 (7.1)
Total	250	119 (47.6)	65 (26)	48 (19.2)	18 (7.2)
Sterlitamaksky district					
Pokrovka	28	12 (42.9)	16 (57.1)	-	-
Berezovka	28	14 (50)	14 (50)	-	
Karmaskaly	23	11 (47.8)	10 (43.5)	-	2 (8.7)
Spasskaya	26	12 (46.2)	14 (53.8)	-	-
Burikazganovo	25	13 (52)	12 (48)	-	-
Talachevo	15	7 (46.7)	6 (40)	-	2 (13.3)
Pokrovka	8	8 (100)	-	-	-
Aigulevo	15	6 (40)	6 (40)	3 (20)	-
Spasskaya	26	10 (38.5)	12 (46.2)	4 (15.3)	-
Kuganak	20	10 (50)	10 (50)	-	-
Kosyakovka	9	5 (55.6)	4 (44.4)	-	-
Strelkovka	27	10 (37.1)	12 (44.4)	-	5 (18.5)
Total	250	118 (47.2)	116 (46.4)	7 (2.8)	9 (3.6)
Total	1000	478 (47.8)	370 (37)	87 (8.7)	65 (6.5)

Honey bee colonies with drone phenes of the European dark bees (O – dark) were found in all districts except Meleuzovsky. Similar situation was observed while evaluating worker bees in these areas. In Ishimbaysky district, drones with phenes O – dark were detected in the largest number of honey bee colonies, compared to other districts, %: 19.2 (Gafuriysky – 12.8; Sterlitamaksky – 2.8).

In the Gafuriysky district, purebred drones were registered in three apiaries: Tashly (apiary 2) – 88.9%, Tabynsk – 47.6% and Kowardy (apiary 2) – 35.3%. In Ishimbaysky district, drones with a phenes O – dark were found in 11 apiaries, %: Makarovo (three apiaries) – from 31.6 to 100; Gumerovo (2 apiaries) – from 25 to 87.5; Isyakaevo (1 apiary) – 14.4; Asiyalanovo – 20.2; Verkhneitkulovo (1 apiary) – 50; Urazbaevo and Kinzebulatovo by 14.3; Podgornaya – 5.8. Honey bees with a phene O - grey were found in apiaries of all districts, %: Gafuriysky - from 8.3 to 35.3; Meleuzovsky – from 8.7 to 28.6; Ishimbaysky – from 5.2 to 26.6, Sterlitamaksky – from 8.8 to 18.5.

In general, honey bee colonies with I_s phenes prevailed in the Meleuzovsky district – 50%, and honey bee colonies with I drones dominated in the Sterlitamaksky district – 46.4%. In the minimum number, the occurrence of I_s drones was 46.4% in the Gafuriysky district, and phene I in the Ishimbaysky district was 26% (Figure 5).

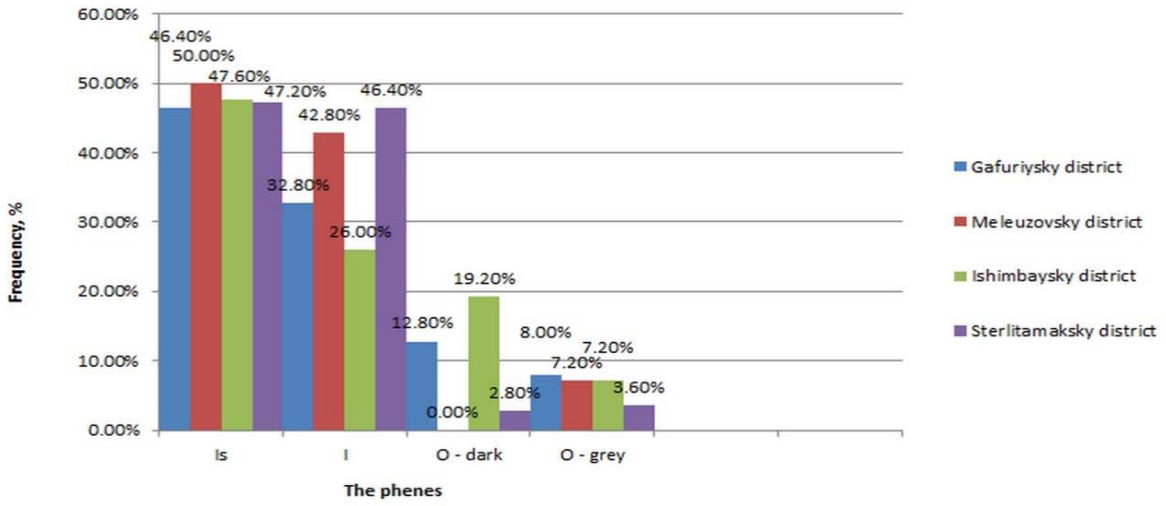

Figure 5. Distribution of the drone bee phenes in the Toratau Geopark.

According to the occurrence of colonies with drones O – grey, the Sterlitamaksky district was different, where they were represented at a minimum – 3.6%. In the Gafuriysky district, this indicator was the maximum compared to other districts – 8%. In the remaining two districts, the honey bee colonies with drones O – grey were represented in the same number – 7.2%.

DISCUSSION

The Bashkir dark European bees originally is characterized by the dark color of the body of individuals, only 1-2 phenes, the tergites of which do not have signs of yellowness, the sternites are colored from dark gray to dark brown without yellowness (Krivtsov 1995; Krivtsov and Grankin 2004). The conducted studies revealed a high phenetic heterogeneity of apiaries in the territory of the Toratau Geopark for worker bees (6 phenes) and drones (4 phenes). At the same time, the dominant content of worker bees' colouring-forming phenes (E, 1R, 2R, 3R) that do not meet the standards of the Dark European bee (*A. mellifera mellifera*) has been established, which, of course, indicates the processes of hybridization. Some occurrence of honey bee colonies with a European bark bee's phene in three districts (Gafuriysky – 14.8%, Ishimbaysky – 13.2%, Sterlitamaksky – 7.9%) is an indicator of the presence of a certain proportion of the gene pool of the local Bashkir bee population. However, maintaining the rate of hybridization in the near future may lead to a complete loss of the gene pool of purebred honey bees in this area. The honey bee colonies purebredness were checked using 9 microsatellite markers by Ilyasov R. A. in spring 2023 (unpublished yet).

The high occurrence of drones with phenes (Is, I, O – gray) that do not meet the requirements of the standard of the Dark European bee characterizes the processes of changing the structure of the population of native honey bees. It should also be noted that the results obtained in this research reveal the presence of southern subspecies in the colonies of queen bees. Unfortunately, purebred drones were not registered from the studied areas in Meleuzovsky district, however in Sterlitamaksky district their occurrence was only 8.7%. Of course, the presented facts require further analysis and annual monitoring of breed in apiaries. Though, in general, the occurrence of purebred drones on the explored territory shows the preservation of the biopotential of the Bashkir population of the European dark bees, which opens up opportunities for the implementation of measures aimed at the conservation and breeding of this population (Chashchukhin and Lapteva 2011; Chashchukhin and Lapteva 2009; Cherevko and Avetisyan 2007; Chibilev 2011; Sharygin and Krivtsova 2018). Taking into account the fact that the Toratau Geopark was created to preserve the geological, biological, historical, and cultural heritage of the republic, it is

necessary to create a network of breeding reproducers for breeding the European dark bee in the explored areas, as well as to provide state support to those apiaries where colonies of this subspecies are kept. It should also be mentioned that the most important areas of the sustainable ecosystem development strategy include the conservation of biodiversity in the context of control and management of the resources. At the same time, experts note that in order to implement scientific-based programs for the conservation of the biodiversity, it is necessary to apply various methods of its assessment both for the entire population of plants and animals, and for individual rare and vulnerable biological species. The assessment of biodiversity with geographical reference makes it possible to assess the uniqueness of regional biomes and ecosystems, their role and place, the conservation status, the boundaries of habitats and factors negatively affecting populations. Such assessments make it possible to plan optimal conservation strategies and develop necessary measures for the protection and restoration of endangered species of flora and fauna (Koroleva et al. 2019).

Conclusions

In the modern world, the most important direction of the strategy for sustainable development of ecosystems is the conservation of biodiversity in terms of control and management of resources. At the same time, experts note that in order to implement scientifically based biodiversity conservation programs, it is necessary to apply a set of assessment methods at different spatial scales. Biodiversity assessment makes it possible to determine the value and uniqueness of regional biomes and ecosystems, the role and place of conservation status, the boundaries of habitats, and factors that negatively affect their population. Such an assessment makes it possible to optimally plan environmental strategies and develop the necessary measures for the protection and restoration of endangered species of flora and fauna.

The results of the assessment of honeybee phenes on the territory of the Geopark Toratau (Republic of Bashkortostan, Russian Federation) indicate that the predominant content in the color of worker bees that form phenes (E, 1R, 2R, and 3R) that do not meet the standards of the dark European bees (*A. m. mellifera*) indicates hybridization processes. The presence of colonies with the Dark European bee phenotypes in the Toratau Geopark are an indicator of the presence of a certain proportion of the gene pool of the Bashkir bee population. The presence of purebred bees opens up opportunities for the implementation of measures aimed at the conservation and reproduction of the Bashkir population of the dark European bee. There is an urgent need to protect local dark European bees from hybridization, as without human help, the local dark forest bee cannot restore the purity of its gene pool.

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REFERENCES

Abdulov, T. F., & Shafikov, I. V. (2004). Restoration of purebred bees in Bashkortostan. *Russian Journal of Beekeeping*. 8: 13.

Abramova L.M. et al. (2014) The Red Book of the Republic of Bashkortostan: Vol. 2: Animals. — 2nd ed. — Ufa: Informreklama. 244 p.

Chashchukhin, V.A., Lapteva, I.S. (2011) Colored drones on apiaries of the Kirov region. *Russian Journal of Beekeeping*. 3: 18-19.

Chashchukhin, V.A., Lapteva, I.S. (2009) Morphological variability of drones on the northern border of the European range. *Russian Journal of Beekeeping*. 4: 4-5.

Cherevko, Yu.A., Avetisyan, G.A. (2007) Beekeeping. – M.: AST: Astrel. 367.

Chibilev, A.A. (2011) Ural: Natural Diversity and the Euro-Asian Border – Yekaterinburg: Ural Branch of the Russian Academy of Sciences. 160. ISBN 978-5-7691-1960-6.

Ilyasov R. A. et al. A revision of subspecies structure of western honey bee *Apis mellifera*. Saudi Journal of Biological Sciences. Volume 27, Issue 12, December 2020, 3615-3621

Ilyasov, R.A., Khan, G.Yu., Lee, M.L., Kim, K.V., Park, D.H., Takahashi, D.I., Kwon, H.V., Nikolenko, A.G. (2021) Evolutionary relationships of Caucasian and Carpathian honey bee populations. Russian journal of Beekeeping. 3: 16-19.

Krivtsov, N. I. (1995). Central Russian bees. 122.

Krivtsov, N. I., Grankin, N. N. (2004). Central Russian bees and their selection. 140.

Koroleva, E.G., Kashirina, E.S., Kazanjyan, I.M. (2019) Cartographic analysis of protected plants and animals of the Republic of Crimea. Ecosystems. 17: 3-14.

Kozhevnikov, G. A. (1931). The natural history of the bee. 118.

Mannapov, A.G., Sattarov, V.N., Ivancov, E.M. (2019) Assessment of morphobiological features of *Apis mellifera* under introgression conditions. Monography. Prospect LLC, Moscow, 144c. DOI: 10.31085/9785392241774-2019-144

Petrov, E. M. (1980). Bashkir bee. Ufa: Bashkir book publishing house. 235.

Petrov, E. M. (2004). On the origins of forest beekeeping in Bashkortostan. 152.

Rua P. et al. Biodiversity, conservation and current threats to European honeybees. Apidologie 40(3), May 2009, 263-284

Ruttner, F. (2006) Breeding technique and selection selection of bees: a practical guide...: per. M.: AST: Astrel. 175 p.

Sabirdzhonova, M.R., Sattarov, V.N. (2021) Phenotypic variability of *Apis mellifera* drones in apiaries of the northern part of Bashkortostan. *News of higher educational institutions. Volga region. Natural sciences*. 2(34): 74-83. DOI: 10.21685/2307-9150-2021-2-7

Sagitov, S.T., Sattarov, V.N., Abdrakhimova, Y.R., Zainullina, G.R., Sultanova, R.R. (2022a) Implementation of the project "Study of the Bashkir bee population in the territory of the Toratau Geopark". *Russian Journal of Beekeeping*. 8: 8-10.

Sagitov, S.T., Sattarov, V.N., Abdrakhimova, Y.R., Zainullina, G.R., Sultanova, R.R., Hannanova, L.F., Denisov, D.A., Nurkaeva, M.R., Nafikov, S.T., Iskhakov, Y.G., Ilyasov, R.A., Minnigulov, R.I. (2022b) The phenetic diversity of honey bees on the territory of the Toratau Geopark". *Russian journal of Beekeeping*. 10: 12-15.

Shafikov, I. V., & Avetisyan, G. A. (1976). Analytical selection of Burzyansky bees. *Russian Journal of Beekeeping*. 3: 10-12.

Shafikov, I. V. (1978). Study and selection of Burzyan wild bees of the Bashkir State Reserve. Abstract of the PhD thesis, 16.

Shafikov, I. V., Baimuratov, A. G. (2002). Bashkir bees. *Russian Journal of Beekeeping*. 4: 12-14.

Sharygin, A.M., Krivtsova, A.V. (2018) The concept of dark forest bee biotech. Beekeeping. 7: 4-6.

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ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

INVESTIGATION of MICROORGANISM CONTAMINATION POINTS in BEEKEEPING EQUIPMENTS WITH CLINICAL SIGNS of FOULBROOD in APIARIES

Yavru Çürüklüğü Klinik Bulguları olan Arılıklarda Arıcılık Ekipmanlarındaki Mikroorganizma Kontaminasyon Noktalarının Araştırılması

Ayşe Ebru BORUM[1*], İbrahim ÇAKMAK[2]

[*1]Balıkesir University Faculty of Veterinary Microbiology Departrment, 10000, Balıkesir TÜRKİYE, Yazışma Yazarı/Corresponding author: E-posta: ebruborum@balikesir.edu.tr, ORCID No: 0000-0002-6916-8982

[2]Bursa Uludag University, Beekeeping Development Application and Research Center-AGAM, Agricultural Faculty, Animal Sciences, Gorukle Campus, 16059, Bursa, TÜRKİYE, E-posta: icakmak@uludag.edu.tr, ORCID No: 0000-0002-8000-5770

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ABSTRACT

The goal of this study was to determine whether colonies with clinical signs of foulbrood in apiaries and hive tools, smokers, gloves, feeders and beekeeper's veils used in the same colonies were a reservoir source for microbial infections. For this purpose, samples were taken from colonies with clinical signs of foulbrood and collected from 29 different apiaries in the Southern Marmara region of Türkiye. The samples were brought to the laboratory under appropriate conditions, and agent isolation and identification were performed. Different microorganisms were isolated from the feeder, hive tool, beekeeper smoker, gloves and beekeeper suit samples collected from each apiary. Bacteria isolated from the samples taken from the hives with clinical signs of foulbrood and from the samples taken from the tools and equipment were isolated as the same species or as a mixture. As a result, an intense presence of microorganisms was detected in the hive tool, beekeeper suit, gloves, feeder, and beekeeper's smoker, used by beekeepers, and it was determined that these materials used in beekeeping were a source of microbial reservoirs.

Keywords: *Apis mellifera*, Microorganisms, Foulbrood, Contamination, Beekeeping equipments

ÖZ

Çalışmada, arılıklarda yavru çürüklüğü klinik bulguları bulunan koloniler ile aynı kolonilerde kullanılan el demiri, körük, eldiven, şerbetlik ve arıcı kıyafetlerinin mikrobiyal infeksiyonlar yönünden bir rezervuar kaynağı olup olmadıklarının belirlenmesi amaçlanmıştır. Bu amaçla Güney Marmara bölgesinde bulunan 29 farklı arılıktan örnekler toplanmıştır. Alınan örnekler uygun koşullarda laboratuvara getirilerek izolasyon ve identifikasyon yapılmıştır. Her arılıktan toplanan yemlik, el demiri, körük, eldiven ve arıcı kıyafetlerinde farklı mikroorganizmalar izole edilmiştir. Yavru çürüklüğü klinik bulguları görülen kovanlardan alınan örnekler ile alet ve ekipmandan alınan örneklerden izole edilen bakteriler aynı tür ya da karışık olarak izole edilmiştir. Sonuç olarak arıcıların kullandıkları yemlik, el demiri, körük, eldiven ve arıcı maske ve tulumlarında zengin bir mikroorganizma varlığı saptanmış ve arıcılıkta kullanılan bu malzemelerin mikrobiyal bir rezervuar kaynağı olduğu belirlenmiştir.

Anahtar Kelimeler: *Apis mellifera*, Mikroroganizma, Yavru çürüklüğü, Kontaminasyon, Arıcılık malzemeleri

GENİŞLETİLMİŞ ÖZET

Amaç: Bu çalışmanın amacı, çeşitli arılıklardaki yavru çürüklüğü klinik bulguları olan koloniler ile aynı arılıklarda kullanılan el demiri, körük, eldiven ve arıcı kıyafetlerinden etken izolasyon ve identifikasyonu yapılarak infeksiyon yönünden bir rezervuar kaynağı olup olmadıklarının tespit edilmesidir.

Gereç-Yöntem: Bu çalışmada Güney Marmara bölgesinde (Bursa, Balıkesir, Bilecik, Yalova ve Çanakkale) yavru çürüklüğü klinik bulguları olan arılıklardaki kolonilerden ölü ve şüpheli larva ve aynı arılıklarda kullanılan şerbetlik, el demiri, körük, eldiven ve arıcı kıyafetlerinden svap örnekleri alınarak mikroorganizma izolasyon ve identifikasyonu yapılmıştır. Yirmi dokuz farklı arılıktaki yavru çürüklüğü klinik bulguları bulunan 43 koloniden yavrulu petek, 43 şerbetlik, 32 el demiri, 29 körük, 30 eldiven ve 29 arıcı kıyafetinden örnekler alınmıştır. Toplanan örnekler uygun koşullarda laboratuvara getirilerek etken izolasyon ve identifikasyonu yapılmıştır. Düzensiz petek gözleri, kapalı yavru gözlerinde delik gibi yavru çürüklüğü infeksiyon bulguları olan kolonilerden yavrulu petekler alınmıştır.

Svap ve larva örnekleri 10 ml. NaCl %0,9 (w/v) içinde süspanse edilmiştir. Süspansiyon ikiye ayrılmıştır. Örneklerin ilk kısmı vejetatif bakterileri öldürmek için 80 °C'de 10 dakika ısıtılmıştır. Süspansiyonun ikinci kısmına ise herhangi bir işlem uygulanmamıştır. Herbir besiyerine süspansiyondan 200 µl inoküle edilmiştir. %5 koyun kanlı Columbia agar (Oxoid CM0331), thiaminli brain heart infüzyon agar (Oxoid CM1136), XLD agar (Oxoid CM0469), MacConkey agar (Oxoid CM0115) ve Nutrient agar (Oxoid CM0003) kullanılmıştır. *Paenibacillus larvae* ve *Melissococcus plutonius* izolasyonu için; MYPGP agara (maya özütü, Mueller-Hinton broth, glucose, K2HPO4, sodium pyruvate ve agar) ekimler yapılmıştır. Tüm besiyerleri 37 °C'de aerobik ve mikroaerofilik koşullarda 48-72 saat inkübe edilmiştir (Nordström ve Fries 1995, Kopcakova vd. 2022). Bütün besiyerlerinde günlük bakteriyel üreme kontrolleri yapılmıştır. İzolatlar, gram boyama ile mikroskopta incelenmiş, katalaz testi yapılmış BBL crystal system ile identifiye edilmiştir.

Bulgular ve Sonuç: Yavru çürüklüğü klinik bulguları görülen koloniler ve arıcılık malzemelerinden alınan örneklerden 69 mikroorganizma ve 28 farklı tür izole edilmiştir. *Bacillus subtilis* (%11,5) en fazla izole edilen tür olarak belirlenmiştir. Klinik bulgu görülen kolonilerden ise 43 yemlik, 32 el demiri, 29 körük, 30 eldiven ve 29 arıcı kıyafetinden svap ile örnekler alınmıştır. Eldiven ve arıcı kıyafetlerinden mikroorganizma izolasyon oranı %100'dür. En az mikroorganizma izolasyonu yapılan arıcılık malzemesi ise körük (%34,38) olmuştur. Örnek alınan kovanlardan ve malzemelerden aynı tür bakteriler izole edilmiştir. Kullanılan arıcılık malzemelerin yavru çürüklüğü görülen kovanlar arası infeksiyonun yayılmasına sebep olduğu bilinmektedir. Araştırmamızda kullanılan arıcılık malzemelerinden birçok mikroorganizma türü de izole edilmiştir. Sonuç olarak arıcılık malzemeleri hem koloniler arasında etkenlerin yayılmasına sebep olurken hem de mikrobiyal bir rezervuar kaynağı olabilmektedir. Bu nedenle arıcılar, arı hastalıklarının koloniler arasında yayılmasını engellemek için alet ve ekipmanın dezenfeksiyonuna önem vermelidir.

INTRODUCTION

Honey bee colonies, *Apis mellifera* produces honey, pollen, bee bread, apilarnil, propolis, royal jelly and bee venom and also has ecological importance in the reproduction of plants. Honey bee products are considered healthy food that provide benefits for people. Honey is known as antimicrobial and can be stored for long years. They also play an important role in the pollination of many economically cultivated plants for food and the economic value of pollination is about 153 billion dollars worldwide (Graham 1991, Gallai et al. 2009, Staveley et al. 2014).

Bees live in close-knit societies where each individual is responsible for the development and survival of the colony. The organization of a bee colony bears many similarities to a multicellular organism often referred to as a "superorganism" (Tautz 2008).

Microorganisms are a factor that negatively affects the health of the entire colony. Microorganisms that affect bees are bacteria, protists and fungi, which are important bee pathogens. Microorganisms generally spread rapidly by beekeeping activities. If left untreated, it causes serious bee deaths and colony losses. Controlling some microorganisms is economically very costly. Sometimes it may be necessary to destroy hives and entire colonies (Cunningham et al. 2022, Leska et al. 2021).

The aim of every beekeeper is to obtain quality and healthy products while avoiding colony losses and

infection problems. There are many different types of microorganisms in the environment. These microorganisms can be found everywhere in apiculture and beekeeping. It is also quite large in number. There are microorganisms that can cause infections under certain conditions, aggravate the course of another infection, cause deterioration in bee products and are harmful to consumer health (Bogdanov et al. 2003).

Sources of contamination can be environmental and beekeeping. Environmental resources can be divided into agricultural and non-agricultural resources (Devillers and Pham-Delègue 2002, Bogdanov et al. 2003). Bees usually fly in a range of 3 km. Therefore, bees and bee products can serve as biomarkers for contamination in this fly area. Contaminants in the flying area can be transmitted to the bee by air and water and carried to the colony with it. They can also be passed to plants through air, water and soil. From here, the plant can pass these contaminants to the bee with nectar and honeydew (Bogdanov et al. 2003).

The larvae are initially sterile, then fed nectar and pollen by worker bees. In this feeding process, their own microbiota is formed with nectar, pollen and worker microflora, or infectious agents are transmitted before the pupal stage (Snowdon and Cliver 1996).

Many microorganisms originate from certain foods or components of the ecosystem. *Actinetobacter*, *Bacillus, Clostridium, Corynebacterium, Pseudomonas, Psychrobacter* and *Vagococcus* are bacteria commonly found in soil. The most important sources of *Bacillus, Clostridium* and *Micrococcus* species are air and dust. *Bacillus* and *Clostridium* species are also bacterial pollutants of sugarcane and beet. *Saccharomyces* and *Torula* have been found in high humidity sugars and *Leuconostoc mesenteroides* sugar refineries. In plants and herbal products, *Brochothrix, Citrobacter, Enterobacter, Erwinia, Flavobacterium, Lactobacillus, Luctococcus, Leuconostoc, Listeria* and *Pediococcus* species are found. In bee intestines: 1% yeast, 29% gram-positive bacteria species (*Bacillus, Bifidobacterium, Streptococcus* and *Clostridium*) and 70% gram-negative bacteria (*Achromobacter, Citrobacter, Enterobacter*, *Erwinia, Escherichia coli, Flavobacterium, Klebsiella, Proteus* and *Pseudomonas*) found (Snowdon and Cliver 1996).

Honeybee diseases and pests, which cause colony losses in the beekeeping sector, cause the destruction of thousands of colonies every year. Especially American foulbrood (AFB) and European foulbrood (EFB) are common, important and dangerous bacterial diseases all over the world. Beekeeping equipment also plays an important role in the transmission of these infections between apiaries and colonies (vanEngelsdorp et al. 2013).

Paenibacillus larvae (American foulbrood), *Melissococcus plutonius* (European foulbrood), *Serratia marcescens*, *Aspergillus* spp. (Stonebrood), *Ascosphaera apis* (Chalkbrood) are important bacterial and fungal infections frequently seen in bees (Leska et al. 2021). However, apart from these infections, there is a common minor foulbrood infection, which is quite common and is confused with AFB and EFB by beekeepers. This disease shows the same clinical findings as AFB and EFB and causes concern in beekeepers. The causative agents of this infection are very diverse. *Bacillus* spp., *Corynebacterium* spp., *Staphylococcus* spp. and *Streptococcus* spp. are one of the most common factors. These factors are; human, animal and environmental origin. Beekeeping tools and equipments that are not sterilized and disinfected can infect the colonies and cause significant losses.

The aim of this study was to determine whether colonies with clinical signs of foulbrood in various apiaries with hive tools, smoker, glove and beekeeper suits-veils used in the same apiaries were a reservoir source in terms of infection by isolating and identifying the agents.

MATERIALS AND METHODS

In this study, microorganisms were isolated and identified by taking dead and suspicious larvae from colonies in apiaries with clinical signs of foulbrood and swab samples from the feeder, hive tool, beekeeper smoker, gloves and beekeeper suits used in the same apiaries. Samples of honeycomb with brood, 43 feeders, 32 hive tools, 29 beekeeper smokers, 30 gloves and 29 beekeeper suits were taken from 43 colonies with clinical signs of foulbrood in 29 different apiaries in Southern Marmara region of Türkiye. The collected samples were brought to the laboratory under appropriate conditions and agent isolation and identification were made. Honeycombs with brood were taken from colonies with irregular comb eyes, holes in

closed brood cells, and signs of foulbrood infection (Beekeeping equipments, Picture-1).

Picture 1. Hive tool, feeder, gloves, smoker and bee suit

Swab and larval samples were suspended in 10 ml of NaCl 0.9% (w/v). The suspension was divided into two. The first portion of the samples was heated at 80 °C for 10 minutes to kill vegetative bacteria. No treatment was applied to the second part of the suspension. 200 µl of the suspension was inoculated into each medium. 5% sheep blood Columbia agar (Oxoid CM0331), brain heart infusion agar with thiamine (Oxoid CM1136), XLD agar (Oxoid CM0469), MacConkey agar (Oxoid CM0115) and Nutrient agar (Oxoid CM0003) were used. For isolation of *Paenibacillus larvae* and *Melissococcus plutonius*; Inoculations were made on MYPGP agar (which contains yeast extract, Mueller-Hinton broth, glucose, K2HPO4, sodium pyruvate, and agar). All media were incubated at 37 °C under aerobic and microaerophilic conditions for 48-72 hours (Nordström and Fries 1995, Kopcakova et al. 2022). Bacterial growth controls of all plates were performed daily. The isolates were examined with light microscopy after gram staining and catalase test and were identified with the BBL crystal system (BBL Crystal Enteric/Nonfermenter ID and Gram Positive ID Kits -Becton Dickinson and Company, USA) (Özakın et al. 2003, Forsgren et al. 2013, De Graaf et al. 2013).

RESULTS

Samples were collected from 29 different apiaries in the Southern Marmara region. In the study, bacterial and fungal agents were isolated and identified by taking samples from feeders, hive tools, beekeeper smokers, gloves and beekeeper suits used in colonies with clinical signs of foulbrood in apiaries. Species isolated and identified from honeycomb and material samples collected from 29 different apiaries are given in Table 1.

Honeycomb samples with brood were taken from 43 colonies with clinical signs of foulbrood in 29 different apiaries in the study. The agents isolated from honeycomb samples are shown in Table 2. A total of 69 isolates were obtained from all samples. Twenty-eight different species were isolated from samples taken from colonies and beekeeping materials with clinical signs of foulbrood. *Bacillus subtilis* (11.5%) is the most isolated species.

Table 1. Samples isolated from different tool and equipment samples used in apiaries and from honeycomb samples in hives with clinical findings

Tablo 1. Arılıklarda kullanılan farklı alet ve ekipman örneklerinden ve kovanlardaki petek örneklerinden izole edilen örnekler

Apiary No	Sampled beekeeping equipment and isolated microorganism species					Microorganism species isolated from hive samples
	Feeder	Hive tool	Beekeeper smoker	Gloves	Beekeeper suit	
1	1 (-) 2 *Bacillus subtilis*	*Enterococcus faecalis*	*Enterococcus faecalis* *Bacillus brevis*	1 *Enterococcus faecalis* 2 (-)	*Bacillus licheniformis*	1 *Bacillus brevis* 2 *Enterococcus faecalis* *Bacillus subtilis*
2	1 (-) 2 *Bacillus subtilis*	*Enterococcus faecalis*	(-)	*Corynebacterium jeikum*	*Corynebacterium jeikum*	1 *Corynebacterium jeikeium* 2 *Bacillus subtilis* *Enterococcus faecalis*
3	*Bacillus subtilis*	*Staphylococcus epidermidis*	(-)	*Staphylococcus aureus*	*Bacillus circulans*	*Staphylococcus aureus* *Staphylococcus epidermidis* *Bacillus subtilis*
4	1 *Bacillus pumilus* 2 *Bacillus subtilis* 3 *Bacillus licheniformis*	*Staphylococcus epidermidis*	(-)	*Staphylococcus aureus*	*Corynebacterium jeikum* *Acinetobacter lwoffi*	1 *Bacillus pumilus* *Corynebacterium jeikum* 2 *Acinetobacter lwoffi* 3 *Bacillus licheniformis*
5	1 *Bacillus subtilis*	1 *Staphylococcus epidermidis* 2 *Bacillus subtilis*	*Bacillus brevis*	*Bacillus brevis* *Bacillus subtilis*	*Bacillus subtilis*	*Corynebacterium aquaticum* *Bacillus brevis* *Bacillus subtilis*
6	1 (-) 2 *Bacillus subtilis* 3 *Bacillus subtilis*	*Corynebacterium jeikum*	*Bacillus brevis*	*Corynebacterium aquaticum* *Corynebacterium jeikum*	*Corynebacterium jeikum*	1 *Corynebacterium pseudodiphteriticum* *Corynebacterium jeikum* 2 *Corynebacterium pseudodiphteriticum* *Corynebacterium jeikum* 3 *Corynebacterium aquaticum* *Aerococcus urinae*
7	1 (-) 2 *Bacillus brevis*	*Staphylococcus epidermidis* *Bacillus pumilus*	(-)	*Corynebacterium aquaticum* *Bacillus subtilis*	*Corynebacterium aquaticum*	1 *Corynebacterium aquaticum* *Bacillus pumilus* 2 *Lactococcus lactis ssp. Cremoris* *Micrococcus luteus*
8	(-)	*Bacillus brevis*	(-)	*Bacillus brevis* *Staphylococcus aureus*	*Staphylococcus aureus*	*Bacillus licheniformis* *Bacillus brevis*
9	*Enterococcus faecalis*	*Staphylococcus saprophyticus* *Enterococcus faecalis*	*Staphylococcus saprophyticus*	*Enterococcus faecalis*	*Staphylococcus saprophyticus*	*Staphylococcus saprophyticus* *Enterococcus faecalis*
10	(-)	*Bacillus cereus*	(-)	*Corynebacterium bovis* *Bacillus subtilis*	*Bacillus subtilis*	*Corynebacterium bovis* *Bacillus cereus*
11	*Bacillus subtilis*	1 *Bacillus cereus* *Staphylococcus epidermidis* 2 *Staphylococcus epidermidis*	*Bacillus subtilis*	*Staphylococcus epidermidis*	*Bacillus subtilis*	*Staphylococcus epidermidis* *Bacillus subtilis*
12	1 (-) 2 (-)	*Corynebacterium pseudotuberculosis*	*Corynebacterium*	*Corynebacterium*	*Corynebacterium*	1 *Corynebacterium pseudotuberculosis*

			pseudotuberculosis	*pseudotuberculosis*	*pseudotuberculosis*	2 *Corynebacterium pseudotuberculosis*
13	1 *Bacillus subtilis* 2 (-)	*Corynebacterium renale*	(-)	*Corynebacterium renale*	*Bacillus subtilis*	1 *Rhodococcus equis* *Corynebacterium renale* 2 *Bacillus subtilis*
14	1 *Bacillus subtilis*	*Staphylococcus aureus*	(-)	*Staphylococcus aureus* *Staphylococcus epidermidis*	*Staphylococcus epidermidis* *Bacillus subtilis*	*Staphylococcus aureus* *Staphylococcus epidermidis* *Bacillus subtilis*
15	1 (-)	*Staphylococcus aureus* *E.coli*	(-)	*E.coli*	*Staphylococcus aureus* *E.coli*	*E.coli* *Morganella morgani*
16	(-)	*Corynebacterium jeikum*	*Staphylococcus epidermidis*	*Corynebacterium jeikum*	*Corynebacterium jeikum* *Staphylococcus epidermidis*	*Corynebacterium jeikum*
17	1 *Bacillus subtilis* 2 *Bacillus subtilis*	*Bacillus subtilis*	*Corynebacterium bovis*	*Corynebacterium bovis* *Bacillus subtilis*	*Bacillus subtilis*	1 *Corynebacterium bovis* *Bacillus subtilis* 2 *Corynebacterium bovis*
18	1 *Staphylococcus simulans* 2 *Bacillus subtilis*	*Staphylococcus simulans* *Staphlococcus warneri*	(-)	*Staphylococcus simulans* *Bacillus subtilis*	*Staphylococcus warneri*	1 *Staphylococcus simulans* *Staphylococcus warneri* 2 *Providencia stuartii*
19	*Bacillus subtilis*	*Bacillus cereus*	*Bacillus cereus*	*Staphylococcus epidermidis* *Bacillus cereus*	*Bacillus cereus*	*Bacillus cereus*
20	(-)	*Escherichia coli* *Enterococcus faecalis*	(-)	*Enterococcus faecalis*	*Escherichia coli*	*Enterococcus faecalis*
21	1 (-) 2 (-)	*Bacillus pumilus*	*Bacillus licheniformis*	*Enterococcus faecalis*	*Escherichia coli*	1 *Bacillus licheniformis* *Bacillus pumilus* 2 *Bacillus pumilus*
22	1 (-) 2 *Bacillus brevis*	*Bacillus brevis* *Enterococcus faecalis*	(-)	*Enterococcus faecalis*	*Enterococcus faecalis*	1 *Bacillus brevis* 2 *Enterococcus faecalis*
23	(-)	*Bacillus brevis* *Bacillus cereus*	(-)	*Klebiella oxytoca*	*Klebiella oxytoca*	*Bacillus brevis* *Klebiella oxytoca*
24	(-)	1 *Staphylococcus epidermidis* *Bacillus cereus* 2 *Staphylococcus epidermidis*	(-)	*Staphylococcus epidermidis*	*Staphylococcus epidermidis*	*Staphylococcus epidermidis* *Sphingomonas paucimobilis*
25	(-)	*Corynebacterium bovis* *Bacillus cereus*	(-)	*Corynebacterium bovis*	*Bacillus cereus*	*Corynebacterium bovis*
26	*Bacillus subtilis*	*Corynebacterium striatum*	(-)	*Corynebacterium striatum*	*Corynebacterium striatum* *Bacillus cereus*	*Corynebacterium striatum*
27	1 (-) 2 (-)	*Corynebacterium pseudodiphtheriticum*	(-)	*Corynebacterium jeikum* *Enterococcus faecalis*	*Corynebacterium jeikum*	1 *Corynebacterium pseudodiphtheriticum* 2 *Corynebacterium jeikum*
28	*Bacillus pumilus*	*Staphylococcus saprophticus*	(-)	*Staphylococcus saprophticus*	*Staphylococcus saprophticus*	*Bacillus pumilus* *Staphylococcus saprophyticus*
29	(-)	*Staphylococcus epidermidis* *Staphylococcus saprophticus*	(-)	*Staphylococcus epidermidis*	*Staphylococcus epidermidis*	*Staphylococcus epidermidis*

Table 2. Bacterial species isolated from honeycomb samples taken from hives with foulbrood clinical signs

Tablo 2. Yavru çürüklüğü klinik bulguları görülen kovanlardan alınan petek örneklerinden izole edilen bakteri türleri

Isolated microorganisms	Positivity rate
Bacillus subtilis	8 (11.5%)
Bacillus brevis	5 (7.24%)
Bacillus pumilus	5 (7.24%)
Bacillus cereus	2 (2.89%)
Bacillus licheniformis	3 (4.34%)
Staphlococcus aureus	2 (2.89%)
Staphylococcus epidermidis	5 (7.24%)
Staphylococcus saprophyticus	2 (2.89%)
Staphylococcus simulans	1 (1.44%)
Staphylococcus warneri	1 (1.44%)
Corynebacterium jeikum	6 (8.69%)
Corynebacterium aquaticum	3 (4.34%)
Corynebacterium pseudodiphteriticum	3 (4.34%)
Corynebacterium striatum	1 (1.44%)
Corynebacterium bovis	4 (5.79%)
Corynebacterium renale	1 (1.44%)
Corynebacterium pseudotuberculosis	2 (2.89%)
Klebiella oxytoca	1 (1.44%)
Sphingomonas paucimobilis	1 (1.44%)
Enterococcus faecalis	5 (7.24%)
Escherichia coli	1 (1.44%)
Acinetobacter lwoffi	1 (1.44%)
Morganella morgani	1 (1.44%)
Providencia stuartii	1 (1.44%)
Rhodococcus equi	1 (1.44%)
Lactococcus lactis ssp. Cremoris	1 (1.44%)
Micrococcus luteus	1 (1.44%)
Aerococcus urinae	1 (1.44%)
Total	69 (100%)

From the colonies with clinical signs, samples were taken from 43 feeders, 32 hand irons, 29 smokers, 30 gloves and 29 beekeeper suits by swab. Microorganism isolation rates from equipment used by beekeepers are shown in Table 3. The microorganism isolation rate from gloves and beekeeper suits was 100%. Beekeeper smoker was determined as the beekeeping material with the lowest microorganism isolated. (34.38%).

Table 3. Beekeeping equipment and microorganism isolation rates

Tablo 3. Arıcılık ekipmanları ve mikroorganizma izole edilme oranları

Beekeeping Equipments	Number of samples	Microorganism isolation rates
Feeder	43	22 (51.16%)
Hive tool	30	30 (100%)
Beekeeper smoker	29	10 (34.48%)
Gloves	30	29 (96.66%)
Beekeeper suit	29	29 (100%)

DISCUSSION

Microorganisms were investigated predominantly on honey bees, and partly on nectar, pollen and have been reported in research and review studies. Particulary pathogenic microorganisms were intensively studied and reported in a number of research papers around the world since they cause colony losses in honey bees (Snowdon and Cliver 1996, Gillliam 1997).

In a recent study by Bayrakal et al. (2020) honey, bee and bee larva were examined from 900 samples in 300 colonies by molecular method for bacterial, fungal, viral and parasitic factors. They reported a number of bacterial, fungal and parasitic agents from those samples. Another study by Cunninham et al. (2022) reports bees as bioindicators of the environment and analyzed plant pathogens carried by honey bees in the environment.

On the other side, contamination of microorganisms in beekeeping pieces of equipment has not been studied and it is difficult to compare these data to other studies and assess the rate of contamination by those materials used in beekeeping activities. Hive tools, beekeeper suits, gloves, feeders, and beekeeper smoker were determined as the source of microorganisms in this study as 100%, 100%, 96%, 51% and 34% respectively. This explains the reason for the fast and high rate of microorganism contamination in apiaries. These results also provide a good dataset to demonstrate the source of microbial reservoirs of apiaries in beekeeping.

In this study, a total of 69 microorganisms and 28 different bacterial species were isolated from samples taken from colonies and beekeeping materials showing clinical signs of foulbrood as a result of isolation and identification. The same species of bacteria were isolated from the sampled hives and materials. THe high number of microorganisms particularly bacteria underlines the importance of hive materials for the source of contamination and this should be considered in beekeeping practices.

Honeybees can be affected by a variety of bacteria, fungi, viruses and parasites and disease management is an important part of beekeeping activities. Good beekeeping and biosecurity practices are very important to control bee pathogens (Arbia and Babbay 2011, Al-Waili et al. 2012, Borum 2022, Rasovic 2021). Pathogenic microorganisms often spread rapidly due to beekeeping activities and some of them can be fatal to bees if left untreated. In addition, some infections such as American foulbrood are very hard to treat or expensive to treat. Sometimes, it may require the destruction of infected hives or even entire colonies (Leska et al. 2021). Some practices by beekeepers can be a source of pathogen contamination. Especially foulbrood agents can be transmitted by beekeeping tools and pieces of equipments (Fries and Camazine 2001, CFIA 2013).

The bacterial species isolated from the materials were the same as the agents isolated from the brood combs taken from the hives with clinical signs of foulbrood and bacteria grew at different rates at samples taken from the feeders, hive tools, gloves, beekeeper smokers and beekeeper suits. This gives an idea of the route of contamination in apiaries. The continuous use of these materials without disinfection will cause contamination and this should be avoided in apiaries (Locke et al. 2019, Tomljanović et al. 2020). Hygiene is of great importance for maintaining the health of bees and bee products (vanEngelsdorp et al. 2013, Rasovic 2021). In particular foulbrood diseases such as American foulbrood (no effective treatment available) and European foulbrood cases will

increase among colonies and apiaries without disinfection and this will cause economic losses.

In conclusion, the data provided here may help to improve disease management and hygienic applications to avoid pathogenic infections in honey bee colonies or apiaries. Beekeepers should be informed about contamination routes of pieces of beekeeping equipments and apply disinfection procedures during beekeeping applications to avoid pathogenic infections. *Since beekeeper smoker has less infection compared to other beekeeping equipments due to high temperature in burning smoker beekeepers are advised to disinfect the hive tool with smoker before the beekeeping practices in the field to reduce infection rates of other colonies.* More research is needed in this area to reduce or avoid contamination of bee colonies with equipments and use less medications in beekeeping.

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Data avaliability: Research data can be supplied if requested poperly in a certain time period.

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REFERENCES

Al-Waili N, Salom K, Al-Ghamdi A, Ansari MJ. Antibiotic, pesticide, and microbial contaminants of honey: human health hazards. Sci World J. 2012;930849, DOI:10.1100/2012/930849.

Arbia A, Babbay B.. Management Strategies of Honey Bee Diseases. J Entomol. 2011;8(1): 1-15. DOI:10.3923/je.2011.1.15.

Bayrakal GM, Ekici G, Akkaya H, Sezgin FH, Dümen E. Detection and molecular examination of pathogens in honey and bees in the northern Marmara region, Turkey. Kaskad Univ vet Fak Derg 2020;26(3).313-319. DOI: 10.9775/kvfd.2019.22845.

Bogdanov S., Imdorf A., Fluri P., Kilchenmann V. The contaminants of the bee colony. Bulg J Vet Med. 2003; 6(2):59-70.

Borum AE. Biosecurity and good beekeeping practices in beekeeping (Arıcılıkta biyogüvenlik ve iyi arıcılık uygulamaları). U. Arı D./U. Bee J. 2022;22(2):246-275. DOI: 10.31467/uluaricilik.1175874.

CFIA. Section 1: Bee Health Management. Honey Bee Producer Guide to the National Bee Farm-level Biosecurity Standard: Government of Canada; 2013.

Cunningham MM, Tran L, McKee C, Newman T, Gladish DW, Lofano AK, Bilodeau GJ, Rott M, Guarna MM. Honey bees as environmental biomonitors of pathogens and contaminants. Ecological Indicators. 2022;108457.

De Graaf DC, Alippi AM, Antúnez K, Aronstein KA, Budge G, De Koker D, De Smet L, Dingman DW, Evans JD, Foster LJ, Fünfhaus A, Garcia-Gonzalez E, Gregorc A, Human H, Murray KD, Neguyen BK, 99 Poppinga L, Spivak M, Vanengelsdorp D, Wilkins S, Genersch E: Standard methods for American foulbrood research. J Apic Res. 2013;51(3):1-27.

Devillers J, Pham-Delègue MH. Honey Bees: Estimating the environmental impact of chemicals, Taylor & Francis, London and New York, 2002.

Forsgren E, Budge GE, Charriere JD, Hornitzky MAZ: Standard methods for European foulbrood research. J Apic Res. 2013;52(1):1-14.

Fries I, Camazine S. Implications of horizontal and vertical pathogen transmission for honey bee epidemiology. Apidologie. 2001;32(3):199-214.

Gallai N, Salles JM, Settele J, Vaissière B E. Economic valuation of the vulnerability of world agriculture confronted with pollinator decline, Ecol Econ. 2009;68(3):810-82. https://doi.org/10.1016/j.ecolecon.2008.06.014.

Gilliam M. Identification and roles of non-pathogenic microflora associated with honey bees. FEMS Microbiol Lett. 1997;155(1):1-10.

Graham J. The hive and The honey bee. Dadant and Sons.1991.

Kopcakova A, Salamunova S, Javorsky P, et al. The Application of MALDI-TOF MS for a Variability Study of *Paenibacillus larvae*. Vet Sci. 2022;9(10):521. DOI:10.3390/vetsci9100521.

Leska A, Nowak A, Nowak I, Górczyńska A. Effects of Insecticides and Microbiological Contaminants on *Apis mellifera* Health. Molecules. 2021;26(16):5080. DOI:10.3390/molecules26165080.

Locke B, Low M, Forsgren E. An integrated management strategy to prevent outbreaks and eliminate infection pressure of American foulbrood disease in a commercial beekeeping operation. Prev Vet Med. 2019; 167:48-52.

Nordström S, Fries I. A comparison of media and cultural conditions for identification of *Bacillus larvae* in honey. J. Apic. Res. 1995;34:97-103.

Özakın C, Aydın L, Çakmak I, Güleğen E. Hazır ve eski peteklerin bakteriyolojik ve mikolojik yönden incelenmesi. U. Arı D./ U. Bee J. 2003;3(3):26-30.

Rašović MB. The most important methods of disınfection in beekeeping. Poljoprivreda Sumarstvo. 2021;67(3):167-176. https://doi.org/10.17707/AgricultForest.67.3.14.

Staveley JP, Law SA, Fairbrother A, Menzie CA. A Causal Analysis of Observed Declines in Managed Honey Bees (*Apis mellifera*). Hum Ecol Risk Assess. 2014;20(2):566-591. DOI:10.1080/10807039.2013.831263.

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.

Tautz, J. The Buzz about bees: Biology of a superorganism. Springer, 2008.

Tomljanović Z, Cvitković D, Pašić S, Volarević B, Tlak Gajger I. Production, practices and attitudes of beekeepers in Croatia. Vet Arh. 2020; 90:413-427.

vanEngelsdorp D, Tarpy DR, Lengerich EJ, Pettis JS. Idiopathic brood disease syndrome and queen events as precursors of colony mortality in migratory beekeeping operations in the eastern United States. Prev Vet Med. 2013;108(2-3):225-233. DOI:10.1016/j.prevetmed.2012.08.004.

Williams M. Identification and roles of non-pathogenic microflora associated wit honey bees. FEMS Microbiology Letters 1997, 155:1-10.

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DERLEME / REVIEW

FONKSİYONEL BİR ARICILIK ÜRÜNÜ OLAN ARI SÜTÜNÜN BAZI ÖZELLİKLERİ ve SAĞLIK ÜZERİNE ETKİLERİ

Some Properties of Royal Jelly a Functional Beekeeping Product and Its Health Effects

Gülizar MUTLU, Doğa AKBULUT, Nurten Seha AYDIN, Ceren MUTLU*

Balıkesir Üniversitesi Mühendislik Fakültesi Gıda Mühendisliği Bölümü, Balıkesir, TÜRKİYE, E-posta: glzrmutlu2808@icloud.com, ORCID No: 0009-0004-4757-5693 E-posta: dogakbulut@gmail.com, ORCID No: 0009-0006-9248-2239, E-posta: sehaaydin1522@gmail.com, ORCID No: 0009-0008-8938-0295, Yazışma Yazarı / Corresponding author: ceren.mutlu@balikesir.edu.tr, ORCID No: 0000-0003-4943-2798

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ÖZ

İşçi bal arılarının hipofaringeal ve mandibular bezlerinden salgılanan arı sütü beyazımsı renkte, kendine özgü kokuda, ekşimsi tatlı bir tatta ve viskoz yapıda olan doğal bir arıcılık ürünüdür. Yapısında çeşitli karbonhidratlar, proteinler, esansiyel aminoasitler, lipitler, yağ asitleri, B grubu vitaminleri ile A, C, D ve E vitaminleri, potasyum, kalsiyum, sodyum, magnezyum gibi mineraller ve fenolik bileşikler bulunması nedeniyle arı sütünün besin değeri yüksektir. Arı sütünün sahip olduğu bu zengin biyoaktif bileşik içeriği sayesinde antimikrobiyal, antioksidan, antiinflamatuar, antidiyabetik, antikanser ve antihipertansif etkiler ile bağışıklık, sinir ve sindirim sistemleri üzerine birçok olumlu etkileri bulunmaktadır. Bu nedenle toplumun farklı kesimleri tarafından doğrudan arı sütü şeklinde veya bal, polen veya propolis karışımları halinde takviye gıda olarak tüketimi tercih edilmektedir. Bu çalışma arı sütünün bazı fiziksel, duyusal ve kimyasal özellikleri, sağlık üzerine etkileri ve gıda olarak tüketimi ile ilgili bilgilerin derlenmesi amacıyla gerçekleştirilmiştir.

Anahtar kelimeler: Arı sütü, royalisin, 10-HDA, antimikrobiyal, antidiyabetik

ABSTRACT

Royal jelly, secreted from the hypopharyngeal and mandibular glands of worker honeybees, is a natural beekeeping product with a whitish colour, distinctive odour, sour-sweet taste, and viscous structure. It has a high nutritional value because it contains various carbohydrates, proteins, essential amino acids, lipids, fatty acids, B group vitamins, vitamins A, C, D, and E, and minerals such as potassium, calcium, sodium, magnesium, and phenolic compounds. It has antimicrobial, antioxidant, antiinflammatory, antidiabetic, anticancer, and antihypertensive effects and many positive effects on the immune, nervous, and digestive systems because of its rich bioactive contents. For this reason, its consumption is preferred by society as a supplementary food and can be consumed directly in the form of royal jelly or honey, pollen, or propolis mixtures. This study was carried out to review information about some physical, sensorial, and chemical properties of royal jelly, its effects on health, and its consumption as a food.

Keywords: Royal jelly, royalisin, 10-HDA, antimicrobial, antidiabetic

EXTENDED ABSTRACT

Purpose: Royal jelly, produced in the hypopharyngeal and mandibular glands of worker honeybees, is the only food source of queen bees throughout their lives, and the bee larvae are also fed with royal jelly in the first few days of development. It is a nutritious beekeeping product for humans same as for honeybees. Additionally, since the economic value of royal jelly is higher than other beekeeping products such as honey, pollen, and propolis, its commercial production has gained importance and become an important source of income for beekeepers around the world. Therefore, it was aimed to summarise the production, some physical and chemical properties, health effects, usage as food, and some side effects of consumption of this valuable product based on the literature.

Discussion: Royal jelly has a whitish colour, a distinctive strong odour, a sourish-sweet taste, and gelatinous consistency. It includes carbohydrates such as fructose, glucose, sucrose, trehalose, maltose, erlose, melibiose, ribose, gentiobiose, isomaltose, and raffinose; main royal jelly proteins, royalicin, jelleines, apisimin, royalactin and essential amino acids such as arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan; waxes, steroids, phospholipids and fatty acids such as 8-hydroxyoctanoic, 3-hydroxydecanoic, 9-hydroxydecanoic, 9-hydroxy-2-decanoic, 10-hydroxydecanoic, 10-hydroxy-2-decenoic, 3,10-dihydroxydecanoic, 2-octene-1,8-dioic and 2-decene-1,10-dioic acid; B group vitamins such as thiamine, riboflavin, niacin, pantothenic acid, pyridoxine, biotin, folic acid and cobalamin, and vitamins A, C, D and E; minerals such as potassium, calcium, sodium, magnesium, zinc, iron, copper and manganese; phenolic compounds such as *p*-coumaric acid, benzoic acid, rosmarinic acid, kaempferol, gallic acid, *p*-hydroxybenzoic acid, ferulic acid, quercetin, myricetin, pyrogallol, syringic acid, ellagic acid and cinnamic acid. It has antimicrobial, antioxidant, antiinflammatory, antidiabetic, anticancer, and antihypertensive effects and many positive effects on the immune, nervous, and digestive systems due to these compounds.

Conclusion: Royal jelly is an important food source for honeybees due to its rich bioactive compounds, but it is also a potential natural nutritive product for humans. Royal jelly is commercially available in natural form or found as a mixture of other products such as honey, pollen, and propolis. It can be consumed daily in different amounts by babies, children, and adults according to their physiological status. However, royal jelly may cause allergic symptoms in some people, and it is recommended that people who are sensitive to allergic reactions, pregnant and lactating women, and young children should be careful in the consumption of royal jelly therefore allergy tests should be done before the consumption of royal jelly.

GİRİŞ

Fonksiyonel gıdalar, besleyici değerlerinin yanı sıra bireyler üzerinde fizyolojik veya psikolojik bir etkiyi olumlu ve özel olarak teşvik edecek şekilde sağlığın korunmasına katkıda bulunan gıdalar olarak nitelendirilmektedir. Bal, propolis ve arı sütü gibi arıcılık ürünlerinin fonksiyonel gıda özelliği taşıyan ürünler oldukları belirtilmektedir (Viuda-Martos vd. 2008).

Arı sütü işçi bal arılarının hipofaringeal ve mandibular bezlerinden salgılanmaktadır (Özkök vd. 2021). Arı sütü kraliçe arıların tüm yaşamları boyunca tek besin kaynağıdır ve aynı zamanda larvaların gelişiminin ilk birkaç gününde larvalar arı sütü ile beslenmektedir (Damico vd. 2021). Arı sütü yapısında su, proteinler, aminoasitler, karbonhidratlar, yağ asitleri, lipitler, mineraller, vitaminler ve uçucu bileşikler bulunan karmaşık bileşime sahip bir üründür (Collazo vd. 2021). İçerdiği doğal biyoaktif bileşenler sayesinde antikanser, antioksidan, antimikrobiyal, antiinflamatuar etkiler gibi farmakolojik özellikler göstermekte ve bu özellikler arı sütünü sağlığı koruyucu ve geliştirici kriterlere sahip bir besin takviyesi haline getirmektedir. Geleneksel tıpta özellikle Asya ve Eski Mısır'da uzun süredir kullanılan arı sütü son yıllarda doğal ürünlere olan ilginin büyük ölçüde artmasının bir sonucu olarak ilaç, gıda ve kozmetik endüstrileri gibi birçok farklı alanda değerlendirilmektedir (Salama vd. 2022). Arı sütü ile ilgili gerçekleştirilen araştırmalardan hareketle bu çalışmada arı sütünün bazı fiziksel, duyusal ve kimyasal özellikleri, sağlık üzerine etkileri ve gıda olarak kullanımı ile ilgili bilgilerin derlenmesi amaçlanmıştır.

Arı Sütü Üretimi

Arı sütünün ekonomik değeri bal, polen ve propolis gibi diğer arı ürünlerinden daha yüksek olduğu için

ticari olarak üretimi önem kazanmıştır ve dünya çapında arıcıların önemli bir gelir kaynağı haline gelmiştir. Çin yılda yaklaşık 4000 ton arı sütü üretimi ile dünyadaki toplam üretimin %90 kadarını gerçekleştirmektedir. Ayrıca Vietnam, Tayvan, Kore, Japonya, Yunanistan, İspanya, Fransa, İtalya ve Meksika gibi ülkelerde de arı sütü üretimi yapılmaktadır (Khan vd. 2021). Arı sütü üretim aşamaları Şekil 1'de gösterilmiştir.

İşçi bal arılarının hipofaringeal ve mandibular bezlerinden salgılanan arı sütünün yüksek verimde üretimi için üretim sezonu boyunca iyi bir kraliçe arı, büyük ve güçlü bir koloni, yeterli besin kaynağı, uygun sıcaklık (20-30 °C), etkin ve uygun üretim malzemeleri ve deneyimli arıcılar gerekmektedir. Tüm bu gerekliliklerin arasında en önemli faktör ise arı sütü üretim performansını önemli düzeyde etkilemesi bakımından iyi bir kraliçe arının varlığıdır (Altaye vd. 2019). Arı sütünün standart üretimi yapay larvaların aşılanması ile yapılmaktadır. İşçi arı larvaları yumurtadan çıktıktan 12-18 saat sonra aşılama kalemi ile yapay kraliçe arı hücrelerine aşılanır ve koloni, işçi arıları larvalarını beslemek için arı sütü üretmeye teşvik edilir. Larvalar 68-72 saat sonra cımbızla aşılandıkları yerden çıkarılarak üretilen arı sütü toplanır. Larva aşılama ve arı sütü üretimi zaman alıcı, çok emek isteyen, larvaların varlığına ve üreticinin yeteneklerine bağlı bir işlem olduğundan, arı sütü üretiminde larva aşılaması gerektirmeyen yeni bir yöntem geliştirilmiştir (Hu vd. 2019, Altaye vd. 2019). Bu yeni yöntemde, aşılama gerektirmediğinden verimli olduğu kadar kullanışlı da olan bir cihaz kullanılmaktadır. Kullanılan cihaz; düzenli delikleri olan bir plastik temelden, delikleri doldurmak için peteğe sokulabilen bir çubuğa monte edilmiş plastik hücre tabanlarından ve arı sütü üretim çubukları üzerindeki dipsiz plastik kraliçe kaplarından oluşmaktadır. Ayrıca arı sütünü toplama makineleri geliştirilmesiyle arı sütü üretiminde işçilik ihtiyacı azalmış ve hasat verimliliği artmıştır. Çin'de geliştirilen bir arı sütü toplama makinası ile 40 dakikada 12 kg kadar arı sütünün toplanabildiği bildirilmiştir (Altaye vd. 2019).

Şekil 1. Arı sütü üretim aşamaları

Arı Sütünün Fiziksel ve Duyusal Özellikleri

Arı sütü beyazımsı bir renk ile karakterize edilmekte olup bu renk depolama sırasında sarıya dönme eğilimi göstermektedir (Miguel ve El-Guendouz 2017, Kausar ve More 2019, El-Guendouz vd. 2020a). Chen vd. (2023) tarafından yapılan bir çalışmada bir hafta depolanmış farklı arı sütü örneklerinin *L**, *a** ve *b** renk değerlerinin sırasıyla 48.99, -3.70 ve 16.19 olarak tespit edildiği

bildirilmiştir. Ayrıca liyofilizasyon ile kurutulmuş arı sütü örneğinin *L**, *a** ve *b** renk değerlerinin de sırasıyla 89.80, −0.53 ve 20.54 olduğu rapor edilmiştir (Li vd. 2022).

Arı sütü ayırt edilebilir keskin bir kokuda, ekşimsi tatlı bir tatta ve yüksek asitliğe sahip (pH 3,4-4,5) bir üründür. Ayrıca suda kısmen çözünür özellik gösteren arı sütünün yoğunluğunun 1,1 g/mL olduğu bildirilmiştir (Sabatini 2009, El-Guendouz vd. 2020a).

Arı sütü jelatinimsi kıvamı ile karakteristik bir yapıdadır ve viskozitesi su içeriğine ve tazeliğine bağlı olarak değişiklik göstermektedir. Ayrıca arı sütünün yapısının depolama koşullarına bağlı olarak da etkilendiği ve arı sütünün oda sıcaklığında depolanmasının renk ve viskozite gibi organoleptik özelliklerini değiştirdiği rapor edilmiştir (Ramanathan vd. 2018). Uygun olmayan koşullarda depolanan arı sütünün viskozitesinin arttığı, renginin koyulaştığı ve arı sütünde ransit tat oluştuğu bildirilmiş olup, kalitesinin korunması için donmuş halde saklanması önerilmektedir (Kausar ve More 2019, Ramanathan vd. 2018). Yapılan bir çalışmada oda sıcaklığında bir hafta depolanan arı sütü örneklerinin viskozite değerinin 7.05 Pa.s olduğu ve bu değerin 30 günlük depolama sonunda 8.74 Pa.s değerine yükseldiği bildirilmiştir (Chen vd. 2023).

ARI SÜTÜNÜN KİMYASAL KOMPOZİSYONU

Su içeriği

Arı sütü yüksek miktarda (>%60) su içeriğine sahiptir ve su içeriği arı sütünün kalitesi üzerinde etkilidir (Kausar ve More 2019). ISO 12824 arı sütü standardına göre saf arı sütünün su içeriğinin %62,00-68,50 aralığında (Anonymous 2016) ve TS 6666 arı sütü standardına göre ise %60,00-70,00 aralığında olması gerekmektedir (Anonim 2010). Arı sütünün su içeriği üretim mevsimi, üretim şekli ve saklama koşullarına bağlı olarak değişiklik gösterebilmektedir (Kanelis vd. 2015, Bagameri vd. 2022). ISO 12824 Standardı'na göre arı sütünün taşıması gereken kimyasal bileşim özellikleri Tablo 1'de verilmiştir.

Tablo 1. ISO 12824 Standardı'na göre arı sütünün taşıması gereken kimyasal bileşim özellikleri (Anonymous 2016)

Özellikler		Standart Değerleri	
		Tip 1	Tip 2
Nem içeriği	En az	%62,00	%62,00
	En çok	%68,50	%68,50
10-hidroksi-2-dekenoik asit (10-HDA)	En az	%1,40	%1,40
Protein	En az	%11,00	%11,00
	En çok	%18,00	%18,00
Toplam şeker	En az	%7,00	%7,00
	En çok	%18,00	%18,00
Fruktoz		%2,00-9,00	%2,00-9,00
Glikoz		%2,00-9,00	%2,00-9,00
Sakkaroz		<%3,00	-
Erloz		<%0,50	-
Maltoz		<%1,50	-
Maltotrioz		<%0,50	-
Toplam asitlik [(1 mol/L NaOH) mL/100 g]	En az	30,00	30,00
	En çok	53,00	53,00
Toplam lipit		%2,00-8,00	%2,00-8,00
C_{13}/C_{12} oranı (δ ‰)		−29 ila −20	−29 ila −14

Bazeyad vd. (2022) tarafından yapılan bir çalışmada, Suudi Arabistan'da toplanan 12 farklı taze arı sütü örneğinin nem içeriklerinin %61,70-76,80 aralıklarında olduğu bildirilmiştir. Türkiye'de yapılan çalışmalarda arı sütü örneklerinin nem içeriklerinin %62,50-68,50 (Kanelis vd. 2015) ve %61,60-73,00 (Kolayli vd. 2016) değerlerinde olduğu rapor edilmiştir. Yunanistan'dan toplanan arı sütü örnekleri ile gerçekleştirilen bir araştırmada ise arı sütü örneklerinin nem içeriklerinin %46,70-73,20 aralığında bulunduğu bildirilmiştir (Kanelis vd. 2015).

Karbonhidrat içeriği

Arı sütü bileşiminin %7,50-15,00 kadarını karbonhidratlar oluşturmaktadır. ISO 12824 arı sütü standardına göre saf arı sütünün toplam şeker içeriğinin %7,00-18,00 aralığında olması gerekmektedir (Anonymous 2016). Arı sütünün karbonhidrat içeriğinin mevsime, coğrafik bölgeye, botanik kaynağına, arı türüne ve üretim metoduna göre değişiklik gösterebildiği rapor edilmiştir (Kunugi ve Mohammed Ali 2019). Ancak majör karbonhidratların türlerinin çoğunlukla aynı olduğu, sadece miktarlarında farklılık olabildiği belirtilmiştir (Maghsoudlou vd. 2019).

Arı sütünün karbonhidrat içeriğinin %90 kadarı fruktoz ve glikozdur ve bu karbonhidratlar arı sütünde ortalama %2,30-8,10 oranında bulunmaktadır (Collazo vd. 2021). Ayrıca arı sütünün yapısında %0,80-3,60 oranda sakkaroz da bulunmaktadır (Kunugi ve Mohammed Ali 2019). ISO 12824 arı sütü standardına göre saf arı sütünün fruktoz ve glikoz içeriklerinin %2,00-9,00 aralığında ve sakkaroz içeriğinin ise %3,00 değerinin altında olması gerekmektedir (Anonymous 2016). Bu karbonhidratların yanı sıra az miktarda trehaloz, maltoz, erloz, melibiyoz, riboz, gentiobiyoz, izomaltoz, rafinoz ve melezitozun da arı sütünün yapısında bulunduğu ve bu bileşenlerin ürünün saflığının kontrolünde önemli olduğu rapor edilmiştir (Bagameri vd. 2022).

Protein ve aminoasit içeriği

Arı sütünün protein içeriği %9,00-18,00 değerleri arasında değişmektedir (El-Guendouz vd. 2020a). ISO 12824 arı sütü standardına göre saf arı sütünün toplam protein içeriğinin %11,00-18,00 aralığında (Anonymous 2016) ve TS 6666 arı sütü standardına göre ise %9,00-18,00 aralığında olması gerekmektedir (Anonim 2010). Farklı ülkelerde üretilen arı sütleri ile yapılan çalışmalarda ise arı sütü örneklerinin protein içeriklerinin %10,20-19,60 olduğu rapor edilmiştir (El-Guendouz vd. 2020a). Protein içeriğinin %80 kadarını ise molekül ağırlıkları 49-87 kDa arasında değişen arı sütünün ana proteinleri (MRJP) oluşturmaktadır (Maghsoudlou vd. 2019, Melliou ve Chinou 2014). Bu proteinler birçok esansiyel aminoasidi yapısında bulundurduğu için kraliçe arıların gelişiminde önemli rol oynamaktadır (Maghsoudlou vd. 2019). Arı sütünün ana proteinlerinde bulunan esansiyel aminoasitlerin arginin, histidin, izolösin, lösin, lisin, metiyonin, fenilalanin, treonin, triptofan ve valin olduğu ve ana proteinlerin MRJP1, MRJP2, MRJP3, MRJP4, MRJP5, MRJP6, MRJP7, MRJP8 ve MRJP9 olmak üzere dokuz farklı alt üyesinin bulunduğu rapor edilmiştir (Ramanathan vd. 2018). Ancak son yıllarda filogenetik olarak eski bir bal arısı türü olan *Apis florae* kaynaklı ve MRJP10 olarak adlandırılan yeni bir üyenin de olduğu belirtilmiştir (Helbing vd. 2017). MRJP1, 2 ve 4 üyelerinde lösin ve valin; MRJP3 üyesinde arginin ve lisin; MRJP5 üyesinde arginin ve metiyonin; MRJP6, 7 ve 8 üyelerinde lösin ve MRJP9 üyesinde ise izolösin aminoasitlerinin miktarlarının daha yüksek olduğu bildirilmiştir (Ramanathan vd. 2018).

MRJP1, arı sütü ana proteinlerinin yaklaşık %45 kadarını oluşturan ve moleküler ağırlığı 350-420 kDa arasında olan zayıf asidik bir glikoproteindir (Maghsoudlou vd. 2019). Bu proteinin degradasyonu arı sütünün depolama sıcaklığı ve süresi ile pozitif korelasyon gösterdiğinden MRJP1 proteini arı sütü kalitesi ve tazeliğini değerlendirmede önemli bir indikatör olarak kabul edilmektedir (Guo vd. 2021). MRJP2, 3, 4 ve 5 proteinleri sırasıyla 49,60-70,60 ve 80 kDa moleküler ağırlıklara sahip bazik özellik gösteren glikoproteinlerdir (Maghsoudlou vd. 2019). MRJP6 ve MRJP7 arıların hipofaringeal bezlerinden salgılanan proteinlerdir. Ayrıca MRJP7'nin bakıcı arıların beyinlerinde de bulunduğu belirtilmiştir. MRJP 8 ve 9 proteinleri arı sütü ana protein ailesinin en eski üyeleri olarak ifade edilmektedir ve MRJP8 proteini diğer proteinlere göre arı sütünde daha az miktarlarda bulunmaktadır (Ramanathan vd. 2018).

Arı sütü ana proteinlerinden daha düşük miktarda bulunan diğer arı sütü proteinleri ise royalisin, jelleinler, apisimin ve royalaktindir. Royalisinin kökeni bilinmemekle beraber doğrudan bal arısı kaynaklı olabileceği varsayılmaktadır. Royalisin 51 aminoasit kalıntısından oluşan amfifilik bir proteindir ve yapısında bulunan disülfit bağları sayesinde yüksek sıcaklık ve düşük pH değerlerinde oldukça stabildir. Jelleinler, MRJP1 proteinlerinin spesifik proteazlar ile parçalanması sonucu oluşan ve

hidrofobik kalıntılar içeren bir protein fraksiyonudur (Fratini vd. 2016). Apisimin proteininin bal arısının kafasında yüksek oranda var olduğu ve MRJP 1 proteinini güçlü bir şekilde bağlama kapasitesine sahip olduğu rapor edilmiştir. Arı sütü proteininde bulunan ve 54 aminoasit kalıntısından oluşan bir polipeptit olan apisiminin valin (%18,5) ve serin (%16,7) aminoasitleri bakımından zengin olduğu, yapısında sistein içermediği ve aromatik aminoasitlerden sadece fenilalanini bulundurduğu rapor edilmiştir. Ayrıca içerdiği 54 aminoasit kalıntısında metiyonin, prolin, arginin, histidin, tirozin ve triptofan da bulunmadığı bildirilmiştir (Bărnuţiu vd. 2011). Royalaktin proteininin ise kraliçe arının farklılaşmasında rol oynadığı rapor edilmiştir (Fratini vd. 2016).

Lipit ve yağ asidi içeriği

Arı sütündeki lipit fraksiyonu %3,00-6,00 kadar olup lipit içeriğinin koloninin gelişmesi için önemli biyolojik aktivitelerden sorumlu olduğu belirtilmiştir (Collazo vd. 2021). Arı sütündeki lipitlerin özellikle kolesterol seviyelerinin düşürülmesinde ve sağlıklı nöronları kaplayan miyelinin yalıtımını sağlayan glial hücrelerinin uyarılmasında rol oynadıkları belirtilmiştir (Bărnuţiu vd. 2011).

Arı sütünde bulunan lipitlerin %80-90 kadarını ise serbest yağ asitleri oluşturmaktadır (Collazo vd. 2021). Arı sütünde ayrıca mumlar (%5,0-6,0), steroidler (%3,0-4,0) ve fosfolipitlerin (%0,4-0,8) de bulunduğu rapor edilmiştir (Ahmad vd. 2020, Li vd. 2013).

Yağ asidi fraksiyonunun yaklaşık %80-90 kadarı zincirde 8 ve 10 karbon atomu içeren mono- ve dihidroksi asitler ve dikarboksilik asitlerden oluşmaktadır (Kocot vd. 2018, Collazo vd. 2021). Arı sütünde 8-hidroksioktanoik, 3-hidroksidekanoik, 9-hidroksidekanoik, 9-hidroksi-2-dekenoik, 10-hidroksidekanoik, 10-hidroksi-2-dekenoik (10-HDA), 3,10-dihidroksidekanoik, 2-okten-1,8-dioik ve 2-deken-1,10-dioik asitlerin bulunduğu rapor edilmiştir (Kolayli vd. 2016). Arı sütünün ana yağ asidi ise 10-HDA olarak bildirilmiş olup bu yağ asidinin varlığı başka bir arıcılık ürününde veya doğal ham bir kaynakta rapor edilmediğinden arı sütünün kalitesi ve doğallığı hakkında bilgi vermektedir (El-Guendouz vd. 2020a). Arı sütündeki 10-HDA miktarının %0,75-3,39 aralığında değiştiği bildirilmiştir (Kocot vd. 2018). Ayrıca 10-HDA bileşiği, 10-hidroksidekanoik asit ve sebasik asit ile birlikte arı sütünün toplam organik asit içeriğinin %80,00-90,00 kadarından fazlasını oluşturmaktadır (Çelik vd. 2022). Yapılan bir çalışmada, 18 farklı arı sütü örneğinin toplam yağ asidi içeriğinin %2,30-7,30 ve 10-HDA içeriğinin ise %1,00-3,90 aralığında olduğu rapor edilmiştir (Kolayli vd. 2016).

Vitamin içeriği

Arı sütünün vitamin içeriği arı sütündeki vitaminlerin temel kaynağı olan çiçek polenlerini etkileyen mevsimsel değişimlere bağlı olarak farklılık göstermekle birlikte, arı sütünde B grubu vitaminlerin ve özellikle de pantotenik asit miktarının yüksek olduğu belirtilmiştir (Fratini vd. 2016).

Arı sütünün yapısında tiamin (B1), riboflavin (B2), niasin (B3), pantotenik asit (B5), piridoksin (B6), biyotin (B7), folik asit (B9) ve kobalamin (B12) bulunduğu rapor edilmiştir (Collazo vd. 2021, Guo vd. 2021). Ayrıca bu vitaminlerin yanı sıra arı sütünde A, C, D ve E vitaminlerinin de olduğu bildirilmiştir (Collazo vd. 2021). Arı sütünde bulunan A, C, D, E, B1, B2, B3, B5, B6, B9 ve B12 vitaminlerinin miktarlarının sırasıyla 1,10; 2,00; 0,20; 5,00; 2,06; 2,77; 42,42; 52,80; 11,90; 0,40 ve 0,15 mg/100 g olduğu rapor edilmiştir (Bărnuţiu vd. 2011, Xue vd. 2017).

Mineral içeriği

Arı sütünün mineral içeriğinin kuru maddede %2,00-5,00 aralığında olduğu rapor edilmiştir (Collazo vd. 2021). TS 6666 arı sütü standardına göre saf arı sütünün kül içeriğinin %0,80-3,00 aralığında olması gerekmektedir (Anonim 2010). Arı sütünde bulunan temel mineraller bulunma miktarlarına göre sırasıyla potasyum, kalsiyum, sodyum, magnezyum, çinko, demir, bakır ve mangan olarak bildirilmiştir. Ayrıca arı sütünde iz miktarda kobalt, baryum, tungsten, krom, nikel, vanadyum, kurşun ve molibden gibi minerallerin de olduğu rapor edilmiştir (Bălan vd. 2020). Yapılan bir çalışmada, 30 farklı arı sütü örneğinin potasyum, fosfor, magnezyum, kalsiyum, sodyum, çinko ve demir içerikleri ortalamalarının sırasıyla 2031, 2009, 259, 153, 95, 21 ve 17 mg/kg olduğu; bakır, alüminyum, mangan, baryum, krom, selenyum, nikel, vanadyum ve molibden gibi minerallerin miktarının ise 5 mg/kg değerinin altında bulunduğu rapor edilmiştir (Balkanska vd. 2017).

Bal ve polen gibi diğer arı ürünlerinin mineral içerikleri botanik kaynağına ve üretildiği bölgenin toprak özelliklerine göre geniş bir aralıkta farklılık gösterirken, arı sütlerinin mineral madde içeriklerinin farklı botanik kaynaklardan veya coğrafi bölgelerde üretilseler dahi önemli derecede farklılık göstermedikleri raporlanmıştır. Bu durumun anne

sütüne benzer olarak bakıcı arılar tarafından arı sütündeki temel ve iz mineral maddelerin homeostatik olarak ayarlanması ile ilişkili olduğu bildirilmiştir (Collazo vd. 2021).

Fenolik bileşik miktarı ve kompozisyonu

Fenolik bileşikler sahip oldukları çeşitli terapötik ve biyolojik özelliklerinden dolayı en önemli kimyasal bileşik sınıflarından birisi olarak kabul edilmektedir. Fenolik bileşikler, biyoaktif yağ asitleri ve proteinler ile birlikte arı sütünün biyolojik özelliklerinin kaynağını oluşturmaktadır (El-Guendouz vd. 2020a). Arı sütü genç işçi arılar tarafından salgılandığı ve sınırlı miktarlarda diğer arı ürünleri ile karıştırıldığı için bal, polen ve propolis gibi diğer arıcılık ürünlerine göre daha düşük miktarlarda fenolik madde içermektedir (Kolayli vd. 2016).

Türkiye'de beş farklı arı sütü örneği ile gerçekleştirilen bir çalışmada örneklerin ortalama toplam fenolik madde miktarının 59,16 mg GAE/100 g olduğu rapor edilmiştir (Özkök ve Silici 2017). Bir diğer araştırmada ise farklı üretim zamanlarında temin edilen arı sütü örneklerinin toplam fenolik madde miktarlarının 27,09-36,48 mg GAE/100 g olduğu ve fenolik bileşik miktarlarında örnekler ve toplanma zamanları açısından farklılık gözlendiği bildirilmiştir (Emir 2020). Arı sütünün fenolik bileşik kompozisyonunun incelendiği bir araştırmada ise arı sütünde *p*-kumarik asit, benzoik asit, rozmarinik asit, kaempferol, gallik asit, *p*-hidroksi benzoik asit, ferulik asit, *o*-kumarik asit, resveratrol, kuersetin, mirisetin, pirogallol, siringik asit, ellajik asit ve sinamik asidin bulunduğu rapor edilmiştir (Hassan vd. 2022). Ayrıca arı sütünde çeşitli flavonların (apigenin ve glikozitleri, luteolin, krizin ve akasetin), flavanonların (naringenin, hesperetin ve izosakuranetin), flavonollerin (kaempferol ve izoramnetin glikozitleri) ve izoflovonoidlerin (genistein ve formononetin) bulunduğu da bildirilmiştir (Ahmad vd. 2020).

ARI SÜTÜNÜN SAĞLIK ÜZERİNE ETKİLERİ

Antimikrobiyal etki

Arı sütü eski çağlardan beri antimikrobiyal ajan olarak kullanılmaktadır (Khazaei vd. 2018, Civelek 2022). Arı sütünün gram pozitif ve gram negatif bakterilere karşı antimikrobiyal özelliğe sahip olduğu ilk kez 1939 yılında McCleskey ve Melampy tarafından keşfedilmiş ve daha sonra yapılan çeşitli araştırmalarda arı sütünün *Bacillus cereus*, *B. subtilis*, *Enterococcus faecium*, *E. faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella infantis*, *S. typhi*, *Staphylococcus aureus* ve *S. epidermidis* gibi mikroorganizmalar üzerinde inhibe edici etkisinin olduğu rapor edilmiştir (Fratini vd. 2016).

Arı sütünün bileşimindeki 8-9 aminoasit kalıntısı içeren peptitler, apalbumin, royalisin, jelleinler, royalaktin, apisimin ve 10-HDA gibi biyoaktif bileşenlerin antimikrobiyal aktivite üzerinde etkili olduğu belirtilmiştir (Šedivá vd. 2018, Nader vd. 2021, Collazo vd. 2021). Antimikrobiyal peptitlerin pozitif yükü, negatif yüklü hücre zarlarıyla etkileşime girerek bakteri hücrelerini denatüre etmekte ve böylelikle bakterilerin gelişimlerini inhibe etmektedir (Collazo vd. 2021).

Yapılan bir çalışma ile arı sütü proteinlerinden apalbumin2'nin *Paenibacillus larvae*, *B. subtilis* ve *E. coli* üzerinde antimikrobiyal etkisinin olduğu ve bu etkinin apalbumin2 bileşiğinin yapısında bulunan yüksek mannoz içeriğinden kaynaklanabileceği rapor edilmiştir (Bíliková vd. 2009). Arı sütünün protein bileşenlerinden biri olan royalisinin toplam 51 aminoasit ve bunlar arasında ise 6 sistein kalıntısı içerdiği ve mantarlara ve bakterilere karşı antimikrobiyal etki gösterdiği bildirilmiştir. Rekombinant olarak üretilen royalisinin de gram pozitif bakterilerin hücre duvarları ve zarlarını parçalayarak işlev bozukluğuna neden olduğu rapor edilmiştir (Kim vd. 2019). Yapılan bir çalışmada, üretilen rekombinant royalisinin gram pozitif bakteriler olan *B. subtilis*, *Micrococcus flavus* ve *S. aureus* mikroorganizmalarının hücre zarları ve duvarlarına zarar vererek inhibe ettiği, ancak gram negatif bakteriler olan *E. coli, S. typhimurium* ve *Proteus vulgaris* ile *Aspergillus oryzae*, *Penicillium viridicatum* ve *Pichia pastoris* üzerinde antimikrobiyal bir etki göstermediği bildirilmiştir (Shen vd. 2012). Arı sütünde jelleinler olarak bilinen antimikrobiyal peptitlerin ise *B. subtilis*, *E. coli*, *K. pneumoniae*, *Listeria monocytogenes* ve *S. aureus* mikroorganizmaları üzerine inhibe edici etki gösterdiği belirtilmiştir (Fratini vd. 2016).

Antimikrobiyal peptitler dışında arı sütünün bileşiminde bulunan 10-HDA bileşiğinin de bal arısı larvalarında Amerikan yavru çürüklüğüne neden olan *Paenibacillus larvae* mikroorganizmasının farklı suşları üzerinde antimikrobiyal etki gösterdiği rapor edilmiştir (Šedivá vd. 2018).

Antioksidan etki

Arı sütünün antioksidan özelliğe sahip olmasını sağlayan maddelerin bileşiminde bulunan proteinler (MRJP1-9) ve peptitler olduğu belirtilmiştir (Collazo

vd. 2021, Civelek 2022). Yapılan bir çalışma ile arı sütünde antioksidan etki içeren 29 peptit izole edilmiş olup bu peptitler arasından 2-4 aminoasit kalıntısı olan (alanin-lösin, fenilalanin-lisin, fenilalanin-arginin, izolösin-arginin, lisin-lösin, lösin-aspartik asit-arginin, lisin-asparajin-tirozin-prolin) ve özellikle tirozin kalıntıları içeren küçük peptitlerin antioksidan özellik taşıdığı bildirilmiştir (Guo vd. 2009). Ayrıca protein ve peptitlerin yanı sıra arı sütünde bulunan fenolik bileşiklerin de antioksidan aktiviteye katkı sağladığı rapor edilmiştir (Ecem Bayram vd. 2021). Fas, Portekiz ve İspanya'dan temin edilen farklı arı sütü örnekleri ile yapılan bir araştırmada örneklerin ABTS, DPPH, süperoksit ve nitrik oksit radikalleri ile hidrojen peroksit üzerine antioksidan aktivite gösterdiği bildirilmiştir. Bu çalışmada arı sütündeki fenolik bileşiklerin tüm radikaller ve hidrojen peroksit üzerinde etkili olduğu, ancak proteinlerin ise sadece DPPH radikali ile hidrojen peroksit üzerinde antioksidan etki gösterdiği belirtilmiştir (El-Guendouz vd. 2020b). Fareler üzerinde yapılan bir çalışmada ise kısıtlama ve soğuk stres uygulanan farelere arı sütü takviye edilerek arı sütünün farelerin glisemi, plazma enzimleri, kortikosteron seviyesi ve hepatik antioksidan sistemi üzerine etkisi incelenmiştir. Deney sonucunda arı sütü takviyesinin kortikosteron seviyesini düşürdüğü, toplam antioksidan kapasiteyi iyileştirdiği ve stres ortamında glisemi kontrolüne yardımcı olduğu rapor edilmiştir (Caixeta vd. 2018).

Antiinflamatuar etki

İnflamasyon fiziksel, kimyasal veya mikrobiyal etkiler ile oluşan lokal tahribe karşı vücudun gösterdiği tepki olup kızarıklık, ısınma, acı ve şişme şeklinde göstergeleri bulunmaktadır. Arı sütü antiinflamatuar etkisi nedeniyle ilaçlara karşı alternatif doğal bir ürün olarak önerilmektedir (El-Guendouz vd. 2020a). Arı sütündeki antiinflamatuar etkiye sahip önemli bileşenlerden birisinin 10-HDA olduğu belirtilmiştir (Yang vd. 2018). Ayrıca yapılan araştırmalarda MRJP1, MRJP2 ve MRJP3 proteinlerinin de antiinflamatuar aktivite gösterdiği rapor edilmiştir (Mureşan vd. 2022).

Yapılan bir araştırmada arı sütünün farelerde proinflamatuar sitokinlerin (TNF-α, IL-6 ve IL-1) sekresyonunu baskılaması nedeniyle antiinflamatuar aktivite gösterdiği bildirilmiş olup arı sütünün inflamatuar bağırsak hastalıkları gibi otoimmün hastalıklarda yaşam kalitesinin iyileştirilmesi için etkili bir besin takviyesi olabileceği belirtilmiştir (Kohno vd. 2004). Yapılan bir diğer araştırmada arı sütünden izole edilen 10-HDA bileşiğinin proinflamatuar sitokinler olan TNF-α, IL-1β ve IL-8'in üretimini inhibe ettiği ve bu nedenle arı sütündeki 10-HDA bileşiğinin antiinflamatuar aktivitesi yoluyla gastrointestinal sisteme fayda sağlayabileceği değerlendirilmiştir (Yang vd. 2018). Fareler üzerinde yapılan bir araştırmada ise 2,4,6-trinitrobenzensülfonik asit ile indüklenen kolitte arı sütü ile ağızdan uygulanan tedavi sonucunda arı sütünün bağırsak mukozasını korumada etkili olduğu bildirilmiştir (Manzo vd. 2015).

Bağışıklık düzenleyici etki

Arı sütünün vücuttaki hücrelerin yenilenmesi, üretimi ve metabolizması üzerinde etkili olarak organizmanın tüm dokularında canlılık, sağlık, enerji ve yüksek bağışıklık sağladığı bildirilmektedir. Arı sütünde bulunan Ɣ-globülin, enfeksiyonu engellemekte ve bağışıklık sistemini güçlendirmektedir (Strant vd. 2019). Ayrıca yapılan *in vivo* ve *in vitro* çalışmalarda MJRP3 proteininin güçlü bağışıklık düzenleyici aktiviteye sahip olduğu rapor edilmiştir. Arı sütünde bulunan 10-HDA ve 3,10-DDA (3,10-dihidroksidekanoik asit) gibi yağ asitlerinin de allojenik T-hücre proliferasyonunu ve IL-2 üretimini azaltarak güçlü immünomodülatör aktivite gösterdikleri belirtilmiştir (Ahmad vd. 2020).

Çocuklarda sistemik lupus eritematozus ile ilgili yapılan bir çalışmada, üç aylık arı sütü uygulamasından sonra iyileşme görüldüğü bildirilmiştir (Zahran vd. 2016). Yapılan bir diğer çalışmada β-laktoglobuline (β-Lg) alerjisi olan farelere arı sütü uygulaması ile serumdaki anti β-Lg, IgE, IgG ve plazmadaki histamin seviyelerinin düştüğü, alerjik semptomların hafiflediği ve bağırsak disfonksiyonunun önemli ölçüde azaldığı rapor edilmiştir (Guendouz vd. 2017). Fareler üzerinde yapılan başka bir araştırmada ise siklofosfamid ile indüklenen farelerde vücut, timus ve dalak ağırlığının 10-HDA etkisi ile geri kazanıldığı ve buradan hareketle 10-HDA bileşiğinin immüno-organ korumada potansiyel rolünün bulunduğu bildirilmiş olup, arı sütünün hipoimmünite tedavisinde doğal bir ürün olarak kullanılabileceği belirtilmiştir (Fan vd. 2020).

Sinir sistemi üzerine etki

Arı sütünün hafızayı geliştirme, yaşlılığı önleme, enerjiyi artırma, kaygıyı azaltma ve hiperaktif bireyleri sakinleştirme gibi etkilerinin olduğu bildirilmiştir (Pavel vd. 2011). Yapılan araştırmalarda arı sütünün bal arılarının öğrenmelerini ve hafıza

yeteneklerini geliştirdiği (Shi vd. 2018) ve farelerde ise bilişsel eksiklikleri iyileştirdiği (You vd. 2018) belirtilmiştir.

Arı sütü hem periferik hem de merkezi sinir sistemlerinde bir nörotransmitter ve somatik sinir sisteminin motor bölümünde kullanılan tek nöromodülatör olan asetilkolini içermektedir (Pavel vd. 2011). Arı sütünde bulunan 10-HDA bileşiğinin de nöronların oluşumunu artırdığı ifade edilmiştir (Mohamed vd. 2015). Yapılan bir çalışmada arı sütünün beyin hücresi farklılaşmasında önemli bir rol oynadığı, oral olarak uygulanan arı sütü tedavisinin bilişsel süreçte kritik rol oynayan hipokampal granül hücrelerinin yenilenmesi nedeniyle nöral işlevi iyileştirdiği ve ayrıca beyni oksidatif hasardan koruduğu bildirilmiştir (Ahmad vd. 2020). Bu etkilerin yanı sıra arı sütü alımının menopoza bağlı nörolojik bozuklukların hafifletilmesinde etkili olduğu ve kolesterol ve beta-amiloid seviyelerinin düşürülmesi, östrojen seviyelerinin yükseltilmesi ve kan-beyin bariyerinin iyileştirilmesi etkileri arı sütünün nöroprotektif rolünü gösteren mekanizmalar olarak rapor edilmektedir (Bălan vd. 2020).

Sindirim sistemi üzerine etki

Arı sütünde bulunan 10-HDA bileşiğinin *S. aureus*, *Streptococcus alactolyticus*, *S. intermedius* B, *S. xylosus*, *Salmonella cholearasuis*, *Vibro parahaemolyticus* ve *E. coli* gibi mikroorganizmalara karşı antimikrobiyal etkisi ve kolon adenokarsinomu olan WiDr hücrelerine karşı gösterdiği sitotoksik aktivite nedeniyle insan gastrointestinal sistemi üzerine olumlu etki sağlama potansiyelinin bulunduğu rapor edilmiştir (Yang vd. 2018). Yapılan bir araştırmada asetik asitle indüklenen kolit farelerde oral takviye ile arı sütü uygulamasının daha önce bozulmuş olan bağışıklık fonksiyonunu eski haline getirmede yararlı olabileceği belirtilmiştir (Karaca vd. 2012). Bir diğer çalışmada ise arı sütünün, diklofenakın neden olduğu gastrointestinal hasara karşı koruma sağladığı ve inflamatuar yanıtı azalttığı rapor edilmiştir (Mostafa vd. 2020). Ayrıca arı sütünün farelerden izole edilen ileum üzerinde gastrointestinal motilite etkisinin incelendiği bir araştırmada ise arı sütü tüketiminin bağırsak hareketliliğini artırmadığı ve normal şartlar altında ishal gibi ciddi semptomlara neden olmayacağı değerlendirilmiştir (Miyauchi-Wakuda vd. 2019).

Antidiyabetik etki

Diyabet günümüzde insan sağlığı için tehlike oluşturan önemli bir sorundur ve dünyada diyabetli kişi sayısının 2030 yılına kadar 439 milyon kişiye ulaşacağı tahmin edilmektedir. Bu nedenle diyabeti önlemek için çeşitli araştırmalar yapılmaktadır. Arı sütünde bulunan proteinlerin ve 10-HDA bileşiğinin de diyabete karşı olumlu etkilerinin bulunduğu bildirilmiştir (Maleki vd. 2019).

Yapılan bir araştırmada arı sütünün streptozotosin ile indüklenen diyabeti önemli ölçüde azalttığı ve karaciğerdeki malondialdehit seviyesi, glutatyon içeriği, süperoksit dismutaz, katalaz ve glutatyon peroksidaz aktivitelerinde önemli artışlar sağladığı bildirilmiştir (Abdelsalam vd. 2023). Bir diğer çalışmada ise bal ve arı sütü karışımı (%98:2) ile yapılan uygulamanın streptozotosin kaynaklı Tip 1 diyabetli sıçanların kan şekerini etkili bir şekilde kontrol edebildiği, çok düşük yoğunluklu lipoprotein kolesterolünü ve trigliseritleri azalttığı ve böylece bu karışımın umut verici bir alternatif antidiyabetik takviye olabileceği rapor edilmiştir (Nohair 2021). Ayrıca yapılan bir çalışmada da 60 erkek bireyde yoğun egzersiz ile birlikte arı sütü takviyesinin kardiyovasküler hastalığa ve çeşitli diyabet türlerine eğilimli obez veya aşırı kilolu bireylerde kan şekeri ve insülin seviyelerini iyileştirdiği ve insülin direnç indeksini azalttığı rapor edilmiştir (Gohari vd. 2022). Bir diğer araştırmada ise Tip 2 diyabetli 50 kadın ile yapılan 8 hafta boyunca günlük 1000 mg arı sütü uygulamasının açlık kan şekerini 163,05 mg/dL seviyesinden 149,68 mg/dL seviyesine azalttığı, ayrıca eritrosit süperoksidaz dismutaz ve glutatyon peroksidaz aktivitelerini önemli ölçüde artırdığı ve arı sütü takviyesinin diyabetin etkilerini kontrol etmede faydalı olabileceği bildirilmiştir (Pourmoradian vd. 2014).

Antikanser etki

Arı sütünün antikanser etki gösteren doğal maddelerden biri olduğu ve antioksidan aktivitesi sayesinde kanser tedavisinde kemoterapi ve diğer tedaviler ile birlikte kullanım için alternatif bir ürün olabileceği bildirilmiştir (Shakib Khoob vd. 2022). Arı sütünün antikanser özellikleri yapısında bulunan hesperetin, naringenin, izosakuranetin, krizin, akasetin, luteolin, apigenin, kaempferol ve izoramnetin glikozitleri ve çeşitli proteinler ile ilişkilendirilmektedir (Salama vd. 2022). Polifenol bileşiklerin kanser önleyici aktivitelerini sinyal yolu değişikliği ile hücreleri ortadan kaldırması, hücre döngüsünü baskılaması, apoptozu indüklemesi ve reaktif oksijen türlerini temizleyici enzim aktivitelerini kontrol etmesi gibi mekanizmalar aracılığıyla gösterdiği belirtilmiştir (Alnomasy ve Al Shehri

2022). Ayrıca yapılan çalışmalar doğrultusunda arı sütünde bulunan MRJP2 proteininin, 10-HDA, HPO-DAEE, (10-HDA'nın türevi), AMP-N1 oksit bileşikleri ve analoglarının da antikanser etki gösteren bileşikler olduğu rapor edilmiştir (Guo vd. 2021).

Yapılan bir çalışmada arı sütünden izole edilen MRJP2 ve onun izoformu olduğu tahmin edilen X1 proteinlerinin karbon tetraklorür ile indüklenen hepatotoksisite ve Hep-G2 hücrelerinin gelişimine karşı önemli düzeyde inhibe edici etkisinin olduğu bildirilmiştir (Abu-Serie ve Habashy 2019). Bir diğer çalışmada ise arı sütünde bulunan 10-HDA bileşiğinin tirozinaz enzimi ile tirozinaz ile ilişkili TRP-1 ve TRP-2 melanojenik enzim ekspresyonlarını inhibe etmesine bağlı olarak melanomaya karşı etkili potansiyel bir bileşik olabileceği rapor edilmiştir (Peng vd. 2017). Arı sütünün PC-3 prostat (Abandansari vd. 2018), MCF-7 meme (Nakaya vd. 2007) ve HT-29 kolon (Ayna vd. 2021) kanseri hücrelerinin inhibisyonu üzerinde de etkili olduğu bildirilmiştir.

Antihipertansif aktivite

Hipertansiyonun yaşlı bireylerde en yaygın rastlanan kardiyovasküler risk faktörlerinden biri olduğu bilinmektedir (Bălan vd. 2020). Arı sütünün bileşimindeki MRJP1 proteinlerinin antihipertansif etkisinin olduğu bildirilmiştir (Collazo vd. 2021). Ayrıca arı sütünde bulunan triptofan-valin-lösin, tirozin-tirozin-serin-prolin, izolösin-tirozin, valin-tirozin, izolösin-valin-tirozin, tirozin-tirozin, izolösin-fenilalanin, lisin-serin, aspartik asit-glisin-lösin ve lösin-tirozin-fenilalanin biyoaktif peptitlerinin de antihipertansif etkiye sahip oldukları rapor edilmiştir (Escamilla vd. 2022).

Yapılan bir çalışma doğrultusunda arı sütünde bulunan üç peptidin (izolösin-valin-tirozin, valin-tirozin ve izolösin-tirozin), sadece 28 günlük tedaviden sonra hipertansif sıçanlarda sistolik kan basıncını normalleştirdiği rapor edilmiştir (Bălan vd. 2020). Arı sütünün fareler üzerindeki antihipertansif etkisinin araştırıldığı bir diğer çalışmada ise sürekli arı sütü tüketiminin kan basıncı değerlerinde yükselmeyi engellediği rapor edilmiştir (Escamilla vd. 2022). Pan vd. (2019) tarafından yapılan bir çalışmada ise arı sütünün, nitrik oksidin azalması ve serbest oksijen radikallerinin artmasıyla bağlantılı olan hipertansiyonu, nitrik oksit üretimini artırarak engelleyebileceği ve bu nedenle antihipertansif özelliklere sahip olduğu bildirilmiştir.

Arı Sütü Tüketimi

Genel olarak arı ürünleri, vücudumuzu sağlıklı tutmak için tüketilen besin kaynakları arasındadır. Çevre kirliliği ve kimyasalların aşırı kullanımı sonucunda ortamın toksik yükü arttıkça sağlığımızı korumamız zorlaşmakta olup hem besin kaynakları hem de diyetler çoğu zaman beslenme açısından yetersiz kalmaktadır. Bal, taze arı poleni, arı sütü, arı ekmeği, kraliçe arı larvası, apilarnil ve propolis gibi arı ürünleri sağlığımızı desteklemek ve güçlendirmek için günlük beslenmemizde kullanabileceğimiz fonksiyonel ürünlerdir (Strant vd. 2019).

Arı sütü beslenmemiz açısından çok önemli olan fonksiyonel bir gıdadır. Arı sütü içerisinde bol miktarda bulunan 10-HDA, B grubu vitaminler ve folat gibi bileşenler sayesinde yüksek düzeyde beslenme sağlamaktadır (Strant vd. 2019). Arı sütünün vücuttaki en önemli işlevleri detoksifikasyon ve yenilenmeye dayalı hücre işlevlerini dengelemek ve normalleştirmektir (Menkovska 2013). Ayrıca arı sütünün hipotansif aktivite, büyüme hızında artış, yaşlanma karşıtı etki, dezenfektan etki, antiinflamatuar ve antitümör etki dahil olmak üzere birçok farmakolojik aktiviteye sahip olduğu bildirilmiştir (Ramadan ve Al-Ghamdi 2012). Arı sütü kendisine atfedilen bu biyolojik özellikleri nedeniyle ilaç, gıda ve kozmetik sektörlerine kadar birçok alanda kullanılmaktadır (Strant vd. 2019).

Arı sütü ticari olarak saf halde veya bal, polen ve propolis gibi diğer ürünlerle karışım halinde satışa sunulmaktadır (Genç ve Aslan 1999). Arı sütünün doğrudan tüketiminde sıvı, toz kapsül veya tablet şeklinde formlarının olduğu rapor edilmiştir (Collazo vd. 2021). Ayrıca yapılan farklı çalışmalarda inek sütün arı sütü ile zenginleştirildiği ve böylece elde edilen ürünlerde antioksidan aktivitede artış olduğu, biyoaktif peptitlerin birikimi ile antihipertansif etki gözlendiği ve bazı gram-pozitif ve gram-negatif bakterin gelişimlerinin engellendiği bildirilmiştir (Alu'datt vd. 2015; Collazo vd. 2021). Bir diğer araştırmada ise arı sütü, bal, propolis, ekinezya ve çivanperçemi karışımı hazırlanarak elde edilen fonksiyonel ürünün antioksidan aktivitesinin, prolin aminoasidi içeriğinin, vitamin ve mineral miktarlarının yüksek olduğu rapor edilmiştir (Soylu ve Bayram 2020).

Bebekler, çocuklar ve yetişkinler tarafından fizyolojik durumlarına göre farklı miktarlarda günlük olarak tüketilebilen arı sütünün hastalara önerilmesi sırasında hastaların özel ihtiyaçları, sağlık durumları

ve yaşları kadar araştırmacıların laboratuvarda gözlemlediği sonuçlar da göz önünde bulundurulmalıdır. Bazı durumlarda çok düşük dozlar bireyler üzerinde çok etkili olabilirken bazen ise daha yüksek dozlar gerekebilmektedir (Strant vd. 2019).

Arı Sütünün Tüketimi ile İlgili Potansiyel Yan Etkiler

Alerjik reaksiyonlar arı sütünün en sık görülen yan etkisi olmakla birlikte oluşum sıklıkları çok bilinmemektedir. Arı sütü alerjisinin oluşum riski, başka alerjileri olan veya diğer arıcılık ürünlerine de alerjisi bulunan bireylerde daha yüksektir (Pavel vd. 2011).

Arı sütünün tüketimi alerjik reaksiyonlar, gastrointestinal problemler, oral alerji sendromu ve atopi gibi bazı küçük yan etkiler ile anafilaktik şok, akut astım, bağırsak kanaması ve hatta ölüm gibi ciddi etkilere de neden olabilmektedir (Torrijos vd. 2016, Ahmad vd. 2020). Arı sütünün merhemler halinde veya saf olarak topikal uygulamalarının ise deri döküntülerine ve egzamaya yol açabileceği bildirilmiştir (Pavel vd. 2011).

Arı sokması ve bal alerjisi veya astım sorunları olan kişiler ile hamile ve emziren kadınlar ve küçük çocukların arı sütü tüketiminde dikkatli olmaları ve bu nedenle arı sütü tüketiminden önce *in vivo* deri delme ve histamin salınımı gibi alerji testlerinin yapılması önerilmektedir (Ahmad vd. 2020).

Sonuç

Fonksiyonel bir gıda özelliği taşıyan arı sütünün yapısında bulunan farklı biyoaktif bileşikleri sayesinde antimikrobiyal, antioksidan, antiinflamatuar, antidiyabetik, antikanser ve antihipertansif etkiler göstermesi nedeniyle sağlığı koruyucu ve geliştirici bir gıda takviyesi olarak insanlar tarafından tüketimi tercih edilmektedir. Toplumda fonksiyonel gıdalara ve sağlıklı beslenmeye karşı artan ilgiye bağlı olarak arı sütüne olan ilgi de giderek artmaktadır. Bu bakımdan arı sütünün hem gıda bilimi hem de endüstrisi bakımından önemli bir potansiyelinin olduğu değerlendirilmiştir.

Çıkar Çatışması: Yazarların çalışma ile ilgili olarak bir çıkar çatışması yoktur.

Etik Belgesi: Bu çalışma için etik belgesi gerekmemektedir.

Mali Kaynak: Bu çalışmada herhangi bir mali kaynak kullanılmamıştır.

Veri Sağlama Durumu: Çalışmada bulunan bilgi ve veriler akademik etik kurallarına uygun bir şekilde verilmiştir. Derleme bir çalışma olduğu için kullanılan veriler literatüre atıf yapılarak kullanılmıştır.

Yazar Katkıları: Bu çalışmanın gerçekleştirilmesine yazarlar eşit olarak katkı sağlamıştır.

KAYNAKLAR

Abandansari RM, Parsian H, Kazerouni F, Porbagher R, Zabihi E, Rahimipour A. Effect of simultaneous treatment with royal jelly and doxorubicin on the survival of the prostate cancer cell line (PC3): an in vitro study. Int J Cancer Manag, 2018, 11: e13780.

Abdelsalam HM, Diab AA, Hussien AG, Helmi SM, Aziz JA. Ginseng and royal jelly diminish diabetic hepato-and nephrotoxicity in STZ-induced diabetic male rats. Nutrire, 2023, 48(1): 8.

Abu-Serie MM, Habashy NH. Two purified proteins from royal jelly with in vitro dual anti-hepatic damage potency: Major royal jelly protein 2 and its novel isoform X1. Int J Biol Macromol, 2019, 128: 782-795.

Ahmad S, Campos MG, Fratini F, Altaye SZ, Li J. New insights into the biological and pharmaceutical properties of royal jelly. Int J Mol Sci, 2020, 21(2): 382.

Alnomasy SF, Al Shehri ZS. Anti-cancer and cell toxicity effects of royal jelly and its cellular mechanisms against human hepatoma cells. Pharmacogn Mag, 2022, 18(79): 635-640.

Anonim. TS 6666/2000 ve T1/2010 Arı sütü standardı. Türk Standardları Enstitüsü, 24 Haziran 2010 kabul tarihli standart, Ankara.

Anonymous. ISO 12824 international royal jelly standard. 15/09/2016, https://standards.iteh.ai/catalog/standards/sist/59729cd3-3d97-41c6-b828-32d63f914334/iso-12824-2016.

Altaye SZ, Meng L, Li J. Molecular insights into the enhanced performance of royal jelly secretion by a stock of honeybee (*Apis mellifera ligustica*) selected for increasing royal jelly production. Apidologie, 2019, 50: 436-453.

Alu'datt MH, Rababah T, Obaidat MM, Ereifej K, Alhamad MN, Mhaidat N, Andrade JE, Johargy A, Ayadi W. Probiotics in milk as functional food: characterization and nutraceutical properties of extracted phenolics and peptides from fermented skimmed milk inoculated with royal jelly. J Food Saf, 2015, 35(4): 509-522.

Ayna A, Tunc A, Özbolat SN, Bengü AŞ, Aykutoğlu G, Canli D, Polat R, Çiftci M, Darendelioğlu E. Anticancer, and antioxidant activities of royal jelly on HT-29 colon cancer cells andmelissopalynological analysis. Turk J Bot, 2021, 45(8): 809-819.

Bagameri L, Baci GM, Dezmirean DS. Royal jelly as a nutraceutical natural product with a focus on its antibacterial activity. Pharmaceutics, 2022, 14(6): 1142.

Bălan A, Moga MA, Dima L, Toma S, Elena Neculau A, Anastasiu, CV. Royal jelly-A traditional and natural remedy for postmenopausal symptoms and aging-related pathologies. Molecules, 2020, 25(14): 3291.

Balkanska R, Mladenova E, Karadjova I. Quantification of selected trace and mineral elements in royal jelly from Bulgaria by ICP-OES and etaas. J Apic Sci, 2017, 61(2): 223-232.

Bărnuţiu LI, Mărghitaş LA, Dezmirean DS, Mihai CM, Bobiş O. Chemical composition and antimicrobial activity of Royal Jelly-Review. Sci P Anim Sci Biotechnol, 2011, 44(2): 67-72.

Bazeyad AY, Al-Ghamdi AA, Alattal YZ. Physicochemical characteristics of local royal jelly produced in Al-Baha region, Saudi Arabia. World J Adv Res Rev, 2022, 14: 284-292.

Bíliková K, Mirgorodskaya E, Bukovská G, Gobom J, Lehrach H, Šimúth J. Towards functional proteomics of minority component of honeybee royal jelly: The effect of post-translational modifications on the antimicrobial activity of apalbumin2. Proteomics, 2009, 9(8): 2131-2138.

Caixeta DC, Teixeira RR, Peixoto LG, Machado HL, Baptista NB, de Souza AV, Vilela DD, Franci CR, Salmen Espindola, F. Adaptogenic potential of royal jelly in liver of rats exposed to chronic stress. PloS One, 2018, 13(1): e0191889.

Chen D, Guo C, Lu W, Zhang C, Xiao C. Rapid quantification of royal jelly quality by mid-infrared spectroscopy coupled with backpropagation neural network. Food Chem, 2023, 418: 135996.

Civelek İ. Biological activities of royal jelly: a mini-review. Anatol J Biol, 2022, 3(1): 1-8.

Collazo N, Carpena M, Nuñez-Estevez B, Otero P, Simal-Gandara J, Prieto MA. Health promoting properties of bee royal jelly: Food of the queens. Nutrients, 2021, 13(2): 543.

Çelik S, Gerçek YC, Özkök A, Ecem Bayram N. Organic acids and their derivatives: minor components of bee pollen, bee bread, royal jelly and bee venom. Eur Food Res Technol, 2022, 248(12): 3037-3057.

Damico ME, Rueppell O, Shaffer Z, Han B, Raymann K. High royal jelly production does not impact the gut microbiome of honeybees. Animal Microbiome, 2021, 3(1): 1-11.

Ecem Bayram N, Cebi N, Celik S, Gercek YC, Bayram S, Tanuğur Samancı AE, Sağdıç O, Özkök A. Turkish royal jelly: amino acid, physicochemical, antioxidant, multi-elemental, antibacterial and fingerprint profiles by analytical techniques combined with chemometrics. J Apicult Res, 2021, 60(5): 751-764.

El-Guendouz S, Lyoussi B, Miguel MG. Insight into the chemical composition and biological properties of Mediterranean royal jelly. J Apic Res, 2020a, 59(5), 890-909.

El-Guendouz S, Machado AM, Aazza S, Lyoussi B, Miguel MG, Mateus MC, Figueiredo, AC. Chemical characterization and biological properties of royal jelly samples from the Mediterranean area. Nat Prod Commun, 2020b, 15(2): 1934578X20908080.

Emir M. Effect of harvesting period on chemical and bioactive properties of royal jelly from Turkey. Eur Food Sci Eng, 2020, 1(1): 9-12.

Escamilla KIA, Ordóñez YBM, Sandoval-Peraza VM, Fernández JJA, Ancona DAB. Anti-hypertensive activity in vitro and in vivo on royal jelly produced by different diets. Emir J Food Agr, 2022, 34(1): 9-15.

Fan P, Han B, Hu H, Wei Q, Zhang X, Meng L, Nie J, Tang X, Tian X, Zhang L, Wang L, Li J. Proteome of thymus and spleen reveals that 10-hydroxydec-2-enoic acid could enhance immunity in mice. Expert Opin Ther Targets, 2020, 24(3): 267-279.

Fratini F, Cilia G, Mancini S, Felicioli A. Royal Jelly: An ancient remedy with remarkable antibacterial properties. Microbiol Res, 2016, 192: 130-141.

Genç M, Aslan A. Determination of trans-10-hydroxy-2-decenoic acid content in pure royal jelly and royal jelly products by column liquid chromatography. J Chromatogr A, 1999, 839(1-2): 265-268.

Gohari I, Khajehlandi A, Mohammadi, A. Effect of high-intensity interval training with royal jelly consumption on serum levels of glucose, insulin, and insulin resistance index of overweight and obese middle-aged men: A quasi-experimental study. Jundishapur J Chronic Dis Care, 2022, 11(4): e123363.

Guendouz M, Haddi A, Grar H, Kheroua O, Saidi D, Kaddouri, H. Preventive effects of royal jelly against anaphylactic response in a murine model of cow's milk allergy. Pharm Biol, 2017, 55(1): 2145-2152.

Guo H, Kouzuma Y, Yonekura M. Structures and properties of antioxidative peptides derived from royal jelly protein. Food Chem, 2009, 113(1): 238-245.

Guo J, Wang Z, Chen Y, Cao J, Tian W, Ma B, Dong, Y. Active components and biological functions of royal jelly. J Funct Foods, 2021, 82: 104514.

Hassan AAM, Elenany YE, Nassrallah A, Cheng W, Abd El-Maksoud AA. Royal jelly improves the physicochemical properties and biological activities of fermented milk with enhanced probiotic viability. LWT, 2022, 155: 112912.

Helbing S, Lattorff HMG, Moritz RF, Buttstedt A. Comparative analyses of the major royal jelly protein gene cluster in three *Apis* species with long amplicon sequencing. DNA Res, 2017, 24(3): 279-287.

Hu H, Bezabih G, Feng M, Wei Q, Zhang X, Wu F, Meng L, Fang Y, Han B, Ma C, Li J. In-depth proteome of the hypopharyngeal glands of honeybee workers reveals highly activated protein and energy metabolism in priming the Secretion of Royal Jelly*[S]. Mol Cell Proteomics, 2019, 18(4): 606-621.

Kanelis D, Tananaki C, Liolios V, Dimou M, Goras G, Rodopoulou A, Karazafiri, E, Thrasyvoulou AA. Suggestion for royal jelly specifications. Arh Hig Rada Toksikol, 2015, 66: 275–284.

Karaca T, Şimşek N, Uslu S, Kalkan Y, Can I, Kara A, Yörük M. The effect of royal jelly on CD3+, CD5+, CD45+ T-cell and CD68+ cell distribution in the colon of rats with acetic acid-induced colitis. Allergol Immunopathol, 2012, 40(6): 357-361.

Kausar SH, More VR. Royal jelly: Organoleptic characteristics and physicochemical properties. Pharm Chem J, 2019, 6: 20-24.

Khan KA, Ghramh HA, Ahmad Z, El-Niweiri MA, Ahamed Mohammed ME. Queen cells acceptance rate and royal jelly production in worker honey bees of two *Apis mellifera* races. PloS One, 2021,16(4): e0248593.

Khazaei M, Ansarian A, Ghanbari E. New findings on biological actions and clinical applications of royal jelly: a review. J Diet Suppl, 2018, 15(5): 757-775.

Kim BY, Lee KS, Jung B, Choi YS, Kim HK, Yoon HJ, Gui ZZ, Lee J, Jin BR. Honeybee (*Apis cerana*) major royal jelly protein 4 exhibits antimicrobial activity. J Asia-Pac Entomol, 2019, 22(1): 175-182.

Kocot J, Kiełczykowska M, Luchowska-Kocot D, Kurzepa J, Musik I. Antioxidant potential of propolis, bee pollen, and royal jelly: possible medical application. Oxi Med Cell Longev, 2018: 7074209.

Kohno K, Okamoto I, Sano O, Arai N, Iwaki K, Ikeda M, Kurimoto M. Royal jelly inhibits the production of proinflammatory cytokines by activated macrophages. Biosci Biotechnol Biochem, 2004, 68(1): 138-145.

Kolayli S, Sahin H, Can Z, Yildiz O, Malkoc M, Asadov A. A member of complementary medicinal food: anatolian royal jellies, their chemical compositions, and antioxidant properties. J Evid-Based Complement Altern Med, 2016, 21(4): NP43-NP48.

Kunugi H, Mohammed Ali A. Royal jelly and its components promote healthy aging and

longevity: from animal models to humans. Int J Mol Sci, 2019, 20(19): 4662.

Li L, Wang P, Xu,Y, Wu X, Liu X. Effect of trehalose on the physicochemical properties of freeze-dried powder of royal jelly of northeastern black bee. Coatings, 2022, 12(2): 173.

Li XA, Huang C, Xue, Y. Contribution of lipids in honeybee (*Apis mellifera*) royal jelly to health. J Med Food, 2013, 16(2): 96-102.

Maghsoudlou A, Mahoonak AS, Mohebodini H, Toldra F. Royal jelly: chemistry, storage and bioactivities. J Apic Sci, 2019, 63(1): 17-40.

Maleki V, Jafari-Vayghan H, Saleh-Ghadimi S, Adibian M, Kheirouri S, Alizadeh M. Effects of royal jelly on metabolic variables in diabetes mellitus: A systematic review. Complement Ther Med, 2019, 43: 20-27.

Manzo LP, de-Faria FM, Dunder RJ, Rabelo-Socca EA, Consonni SR, de Almeida ACA, Regina A, Souza-Brito ARM, Luiz-Ferreira, A. Royal jelly and its dual role in TNBS colitis in mice. Sci World J, 2015: 956235.

Melliou E, Chinou I. Chemistry and bioactivities of royal jelly. Stud Nat Prod Chem, 2014, 43: 261-290.

Menkovska M. The newest experience with effervescent tablets containing royal jelly as functional food on packing, dosage and synergistic action in prevention, prophylaxis and healing. J Food Process Technol, 2013, 4(10): 272.

Miguel MG, El-Guendouz S. Volatile compounds of royal jelly. Bee Products-Chemical and Biological Properties, Springer, 2017, 191-197.

Miyauchi-Wakuda S, Kagota S, Maruyama-Fumoto K, Wakuda H, Yamada S, Shinozuka K. Effect of royal jelly on mouse isolated ileum and gastrointestinal motility. J Med Food, 2019, 22(8): 789-796.

Mohamed AAR, Galal AA, Elewa YH. Comparative protective effects of royal jelly and cod liver oil against neurotoxic impact of tartrazine on male rat pups brain. Acta Histochem, 2015, 117(7): 649-658.

Mostafa RE, El-Marasy SA, Jaleel GAA, Bakeer RM. Protective effect of royal jelly against diclofenac-induced hepato-renal damage and gastrointestinal ulcerations in rats. Heliyon, 2020, 6(2): e03330.

Mureşan CI, Dezmirean DS, Marc BD, Suharoschi R, Pop OL, Buttstedt A. Biological properties and activities of major royal jelly proteins and their derived peptides. J Funct Foods, 2022, 98: 105286.

Nader RA, Mackieh R, Wehbe R, El Obeid D, Sabatier JM, Fajloun, Z. Beehive products as antibacterial agents: a review. Antibiotics, 2021, 10(6): 717.

Nakaya M, Onda H, Sasaki K, Yukiyoshi A, Tachibana H, Yamada K. Effect of royal jelly on bisphenol A-induced proliferation of human breast cancer cells. Biosci Biotechnol Biochem, 2007, 71(1): 253-255.

Nohair SFA. Antidiabetic efficacy of a honey-royal jelly mixture: Biochemical study in rats. International J Health Sci, 2021, 15(4): 4-9.

Özkök D, Silici S. Antioxidant activities of honeybee products and their mixtures. Food Sci Biotechnol, 2017, 26: 201-206.

Özkök A, Akbay E, Samancı A, Mayda N, Onur M. Evaluation of bioactive compounds and proliferation properties of different royal jelly samples. Prog Nutr, 2021, 23(1): e2021029.

Pan Y, Rong Y, You M, Ma Q, Chen M, Hu F. Royal jelly causes hypotension and vasodilation induced by increasing nitric oxide production. Food Sci Nutr, 2019, 7(4): 1361-1370.

Pavel CI, Mărghitaş LA, Bobiş O, Dezmirean DS, Şapcaliu A, Radoi I, Mădaş MN. Biological activities of royal jelly-review. Sci Pap Anim Sci Biotechnol, 2011, 44(2): 108-118.

Peng CC, Sun HT, Lin I, Kuo PC, Li JC. The functional property of royal jelly 10-hydroxy-2-decenoic acid as a melanogenesis inhibitor. BMC Complement Altern Med, 2017, 17(1): 1-8.

Pourmoradian S, Mahdavi R, Mobasseri M, Faramarzi E, Mobasseri M. Effects of royal jelly supplementation on glycemic control and oxidative stress factors in type 2 diabetic female: a randomized clinical trial. Chin J Integr Med, 2014, 20: 347-352.

Ramadan MF, Al-Ghamdi A. Bioactive compounds and health-promoting properties of royal jelly: A review. J Funct Foods, 2012, 4(1): 39-52.

Ramanathan ANKG, Nair AJ, Sugunan VS. A review on Royal Jelly proteins and peptides. J Funct Foods, 2018, 44: 255-264.

Sabatini AG, Marcazzan GL, Caboni MF, Bogdanov S, Almeida-Muradian LBD. Quality and standardisation of royal jelly. J Apiprod Apimed Sci, 2009, 1(1): 1-6.

Salama S, Shou Q, Abd El-Wahed AA, Elias N, Xiao J, Swillam A, Umair M, Guo Z, Dagila M, Wang K, Khalifa SAM, El-Seedi HR. Royal jelly: Beneficial properties and synergistic effects with chemotherapeutic drugs with particular emphasis in anticancer strategies. Nutrients, 2022, 14(19): 4166.

Šedivá M, Laho M, Kohútová L, Mojžišová A, Majtán J, Klaudiny J. 10-HDA, a major fatty acid of royal jelly, exhibits pH dependent growth-inhibitory activity against different strains of Paenibacillus larvae. Molecules, 2018, 23(12): 3236.

Shakib Khoob M, Hosseini SM, Kazemi S. In vitro and in vivo antioxidant and anticancer potentials of royal jelly for dimethylhydrazine-induced colorectal cancer in wistar rats. Oxi Med Cell Longev, 2022, 9506026.

Shen L, Liu D, Li M, Jin F, Din M, Parnell LD, Lai CQ. Mechanism of action of recombinant Acc-royalisin from royal jelly of Asian honeybee against gram-positive bacteria. PloS One, 2012, 7(10): e47194.

Shi JL, Liao CH, Wang ZL, Wu XB. Effect of royal jelly on longevity and memory-related traits of *Apis mellifera* workers. J Asia-Pac Entomol, 2018, 21(4). 1430-1433.

Soylu P, Bayram B. Bal, propolis, arı sütü, çivanperçemi (*Achillea millefolium*) ve ekinezya (*Echinacea paradoxa*) karışımından fonksiyonel gıda üretimi, ürünün fizikokimyasal ve biyokimyasal özelliklerinin incelenmesi. Bahri Dağdaş Hayvancılık Araştırma Dergisi, 2020, 9(1): 25-38.

Strant M, Yücel B, Topal E, Puscasu AM, Margaoan R, Varadi A. Use of royal jelly as functional food on human and animal health. Hayvansal Üretim, 2019, 60(2): 131-144.

Torrijos EG, Diaz YM, Segade JMB, Brito JFF, Arias TA, Bonilla PAG, Fernandez AL, Rodríguez RG. Occupational allergic respiratory disease due to royal jelly. Ann Allergy Asthma Immunol, 2016, 117(1): 102-103.

Viuda-Martos M, Ruiz-Navajas Y, Fernández-López J, Pérez-Álvarez JA. Functional properties of honey, propolis, and royal jelly. J Food Sci, 2008, 73(9): R117-R124.

Xue X, Wu L, Wang K. (2017). Chemical composition of royal jelly. Bee products-chemical and biological properties. Springer, 2017, 181-190.

Yang YC, Chou WM, Widowati DA, Lin IP, Peng CC. 10-hydroxy-2-decenoic acid of royal jelly exhibits bactericide and anti-inflammatory activity in human colon cancer cells. BMC Complement Altern Med, 2018, 18: 1-7.

You MM, Chen YF, Pan YM, Liu YC, Tu J, Wang K, Hu FL. Royal jelly attenuates LPS-induced inflammation in BV-2 microglial cells through modulating NF-κB and p38/JNK signaling pathways. Mediators Inflamm, 2018, 7834381.

Zahran AM, Elsayh KI, Saad K, Eloseily EM, Osman NS, Alblihed MA, Badr G, Mahmoud MH. Effects of royal jelly supplementation on regulatory T cells in children with SLE. Food Nutr Res, 2016, 60(1): 32963.