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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

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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).

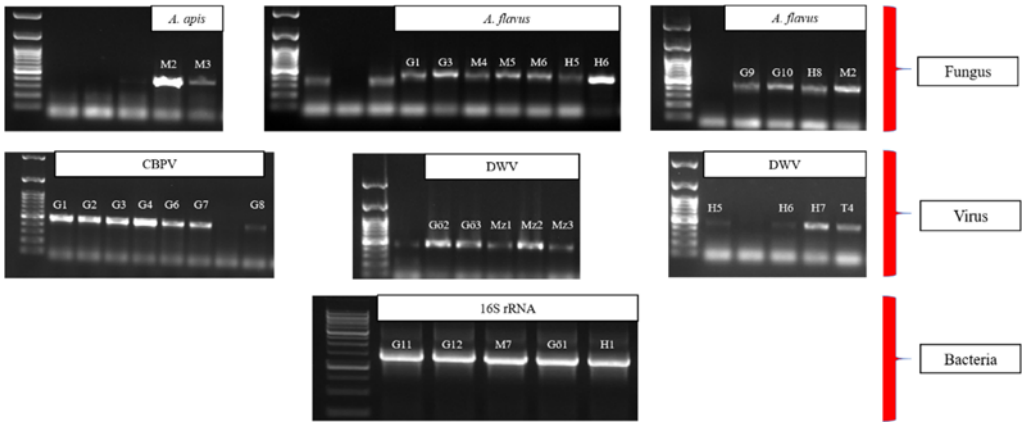

Figure 2. Agarose gel bands were obtained as a result of pathogen screening (M: Central, G: Gümüşhacıköy, H: Hamamözü, Gö: Göynücek, Mz: Merzifon, T: Taşova).

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).

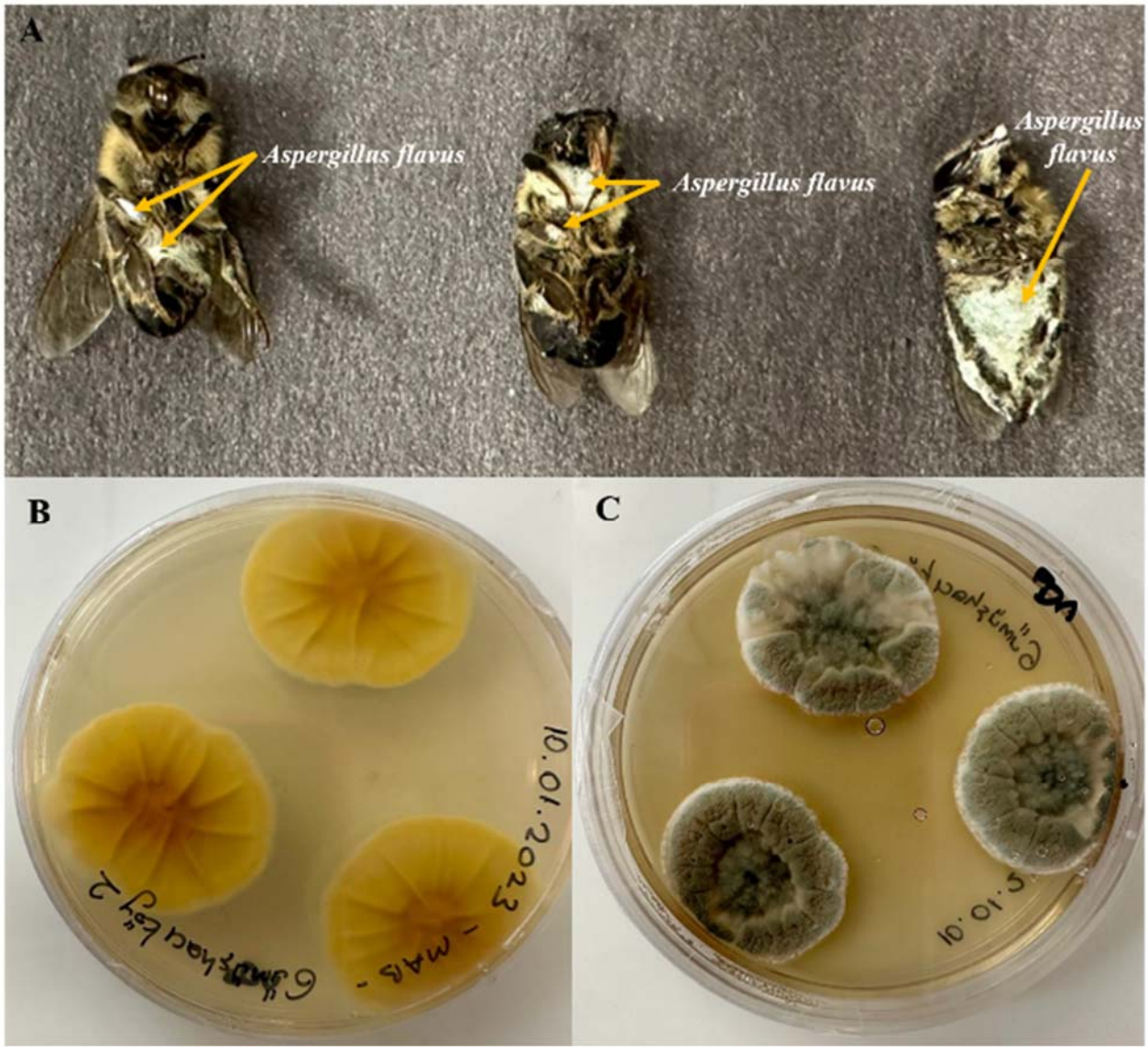

Figure 3. *Aspergillus flavus* infection in honey bees in Gümüşhacıköy. **A.** Morphological infection of bees with fungi, **B.** Top view of the fungus on PDA medium, **C.** Fungus viewed from below the petri dish.

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.*

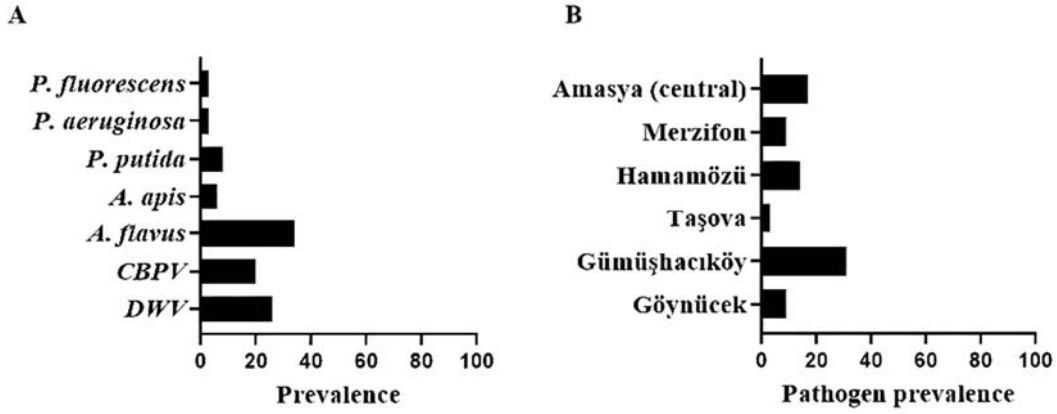

Figure 4. The prevalence of each of honey bee pathogens in Amasya province **(A)**, the prevalence of all honey bee pathogens in Amasya center and districts **(B)**

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.

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