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ARAŞTIRMA MAKALESI / RESEARCH ARTICLE

EUROPEAN PAPER BEES, *POLISTES DOMINULA* AND *POLISTES NIMPHA* (CHRIST, 1791) (HYMENOPTERA: VESPIDAE) PATHOGENS PRESENCE AND THEIR POTENTIAL INSECTICIDAL EFFECTS ON HONEYBEES ADULTS OF *APIS MELLIFERA CAUCASIA* (POLLMANN, 1889)

Avrupa Kağıt arıları, *Polistes dominula* ve *Polistes nimpha* (Christ, 1791) (Hymenoptera: Vespidae) Patojenlerinin Varlığı ve bu Patojenlerin *Apis mellifera caucasia* (Pollmann, 1889) Bal Arısı Erginleri Üzerindeki Potansiyel Insektisidal Etkileri

Ömer ERTÜRK[1], Didem SAĞIRKAYA[1], Gönül ALGI[1*], Selim GÖRGÜN[2]

[1]Ordu University, Faculty of Arts and Science, Department of Molecular Biology and Genetics, Ordu, TÜRKİYE, E-mail: oseerturk@hotmail.com, ORCID No: 0000-0001-5837-6893, E-mail: didemegriboz@gmail.com, ORCID No: 0000-0002-1101-7583, Corresponding author / Yazışma yazarı E-mail: gonul_algi@hotmail.com, ORCID No: 0000-0003-3946-629X

[2]Samsun Training and Research Hospital, Department of Microbiology and Clinical Microbiology, 55090 Samsun, TÜRKİYE, E-mail: selimgorgun55@gmail.com, ORCID No: 0000-0001-5841-591X

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ABSTRACT

Honeybee (*Apis mellifera*) is an important element of biodiversity and terrestrial ecosystems. Any pathogenic infection in this beneficial insect can lead to major undesirable disasters. This study investigated the pathogenic bacteria and fungi from *Polistes dominula* and *Polistes nimpha* wasps and their potential insecticidal effects on *Apis mellifera caucasia*. For this purpose, bacteria and fungi were isolated from dead and diseased bees collected from Terme district of Samsun province in Türkiye in May and June 2020. In the study, *Granulicatella adiacens*, *Staphylococcus xylosus*, *Sphingomonas paucimobilis* bacteria and *Cryptococcus laurentii* and *Candida famata* fungi were obtained from the internal tissues and organs of *Polistes dominula* paper wasp adults. *Staphylococcus xylosus* and *Sphingomonas paucimobilis* were found to be common bacteria in both bee species. *Serratia marcescens* and *Enterococcus faecalis* bacterial species were found to have a very lethal effect on honeybees. Bioassay experiments were performed on the detected fungi, and it was observed that *Cryptococcus laurentii* and *Candida famata* fungi species also had lethal effects on honeybees. It has been revealed that entomopathogenic bacteria, which are known to be very effective in biological control against harmful insects, can cause unwanted infections in honeybees.

Keywords: ***Apis mellifera, Polistes dominula*, *Polistes nimpha*, Bacterial flora**

ÖZ

Bal arısı (*Apis mellifera*), biyolojik çeşitliliğin ve karasal ekosistemlerin önemli bir unsurudur. Bu faydalı böcekteki herhangi bir patojenik enfeksiyon, büyük istenmeyen felaketlere yol açabilir. Bu çalışmada *Polistes dominula* ve *Polistes nimpha* eşekarısı kaynaklı patojen bakteri ve mantarlar ile bunların *Apis mellifera caucasia* üzerindeki potansiyel böcek öldürücü etkileri araştırılmıştır. Bu

amaçla Mayıs ve Haziran 2020 aylarında Türkiye'de Samsun ili Terme ilçesinden toplanan ölü ve hastalıklı arılardan bakteri ve mantarlar izole edilmiştir. Çalışmada, *Polistes dominula* kağıt yaban arısı erginlerinin iç doku ve organlarından *Granulicatella adiacens*, *Staphylococcus xylosus*, *Sphingomonas paucimobilis* bakterileri ile *Cryptococcus laurentii* ve *Candida famata* mantarları elde edildi. *Staphylococcus xylosus* ve *Sphingomonas paucimobilis*'in her iki arı türünde de görülen yaygın bakteriler olduğu belirlendi. *Serratia marcescens* ve *Enterococcus faecalis* bakteri türlerinin bal arıları üzerinde oldukça öldürücü etkiye sahip olduğu tespit edildi. Tespit edilen mantarlar üzerinde bioassay deneyleri yapıldı ve *Cryptococcus laurentii* ve *Candida famata* mantar türlerinin de bal arıları üzerinde öldürücü etkileri olduğu görüldü. Zararlı böceklere karşı biyolojik mücadelede oldukça etkili olduğu bilinen entomopatojen bakterilerin, bal arılarında istenmeyen enfeksiyonlara neden olabileceği ortaya çıktı.

Anahtar Kelimeler: *Apis mellifera*, *Polistes dominula*, *Polistes nimpha*, Bakteriyel flora

GENİŞLETİLMİŞ ÖZET

Amaç: Arılar, tozlaşma ve bal üretimindeki rolleriyle bilinen eşekarısı ve karıncalarla yakından akraba olan uçan böceklerdir. Arılar, Apoidea ailesi içinde tek tip bir soydur ve bugün Anthophila sınıfı olarak kabul edilmektedir. Yaban arıları, dünya çapında yüz binden fazla tanımlanmış türe sahip olduğu tahmin edilen ve henüz tanımlanacak çok daha fazlasının olduğu düşünülen çeşitli bir gruptur. Yaban arılarının büyük çoğunluğu tozlaşmada etkili değildir, bazı türler polen taşıyabilir ve çeşitli bitki türlerinin tozlaşmasını sağlayabilir. Avrupa kağıt eşek arısı (*Polistes dominula*), Polistes cinsindeki en yaygın ve iyi bilinen sosyal eşek arısı türlerinden biridir özellikle Avrupa'da geniş yayılıma sahiptir. *Polistes nimpha* ise Avrupa genelinde Türkiye, Finlandiya, Estonya ve Letonya'da bulunmaktadır. Bu arılar, ayrıca Kuzey Afrika, Pakistan, İran, Hindistan (özellikle Jammu, Keşmir ve Himaşal Pradeş'in kuzey eyaletlerinde), Kazakistan, Moğolistan ve Çin'de de görülmektedir.

Bal arıları (şu anda sekiz türü tanınan *Apis* cinsi) oldukça sosyaldir ve en bilinen böcekler arasındadır. Bu türlerden biri olan *Apis mellifera*'nın Avrupa, Ortadoğu ve Afrika'ya özgü 31 alt türü bulunmaktadır. *Apis mellifera*'nın en önemli faydalarından biri tarımsal üretimde verimi arttırmak için çiçekli bitkilerin tozlaşmasını sağlamak ve değerli bir ürün olan bal üretimidir. Böcekler de diğer hayvanlar ve bitkiler gibi hastalığa neden olan mikroorganizmalar tarafından enfekte edilir. Böceklerin hastalık yapmasına ve ölümüne neden olan bu mikroorganizmalara genel olarak entomopatojenler adı verilmektedir. Ayrıca Bal arılarında hastalığa neden olan mikroorganizmaların araştırılması, sağlıklı arı kolonilerinin elde edilmesi, kovan başına bal veriminin arttırılması, daha aktif ve etkili işçi arıların sağlanması açısından büyük önem taşımaktadır. Zararlı böceklere karşı biyolojik mücadelede oldukça etkili olduğu bilinen entomopatojenler, bal arılarında istenmeyen enfeksiyonlara neden olabilmektedir. Bu çalışmanın amacı, eşekarısı populasyonun taşıdıkları bakteri, mantar patojenleri ve protistlerin varlığını tespit etmektir. Çalışmanın bu alanda entomopatojenlerin tespitinde ve yeni entomopatojen türlerinin tanımlanmasına önemli katkılar sağlayacağı düşünülmektedir.

Gereç ve Yöntem: Çalışmalarda kullanılan *Polistes dominula* ve *Polistes nimpha* örnekleri Mayıs ve Haziran 2020 aylarında Türkiye'nin Samsun ili Terme ilçesinden toplanmıştır. İnsektisidal çalışmalarda kullanılan sağlıklı *Apis mellifera* örnekleri ise Ordu Arıcılık Enstitüsü tarafından toplanarak temin edildi. Entomopatojenlerin türlerinin belirlenmesi amacıyla Samsun Eğitim ve Araştırma Hastanesi Mikrobiyoloji Laboratuvarı'nda kurulu VITEK® 2 (Biomerieux, Fransa) tam otomatik bakteri tanımlama sistemi kullanıldı, protistler için ise ışık ve elektron mikroskobu kullanıldı. Elde edilen entomopatojenlerin insektisidal etkileri ise biyoassay deney düzenekleri hazırlanarak tespit edildi.

Bulgular ve sonuç: Yapılan çalışmalar sonucunda toplam 18 adet saf kültür elde edildi. Kültür çalışmalarında, 3'ü gram negatif, 3'ü gram pozitif bakteri olmak üzere 6 cinse ait 6 bakteri ve 3 mantar türünden oluşan toplam 9 farklı izolat elde edildi. İzolatlar kodlandı ve numaralandırıldı. *Polistes dominula* ve *Polistes nimpha*'dan izole edilen bakteri ve mantarların morfolojik özellikleri belirlendi. Işık mikroskobu çalışmaları sonrasında microsporidium enfeksiyonunun böceğin bağırsaklarını, malpighi tüplerini ve hemolenfini enfekte ettiği belirlendi. Özel kamera ve resim sistemlerine sahip bir mikroskop kullanılarak, daha önce ışık mikroskobu altında tespit edilen mikrosporların enfekte olduğu dokuların fotoğrafı çekildi. Işık mikroskobu ve elektron mikroskobu çalışmalarında Coccidia patojeni ilk kez eşekarısı içinde gözlendi. Arı dokusunda görüntülenen bu yapılar hem Işık Mikroskobu hem de SEM altında görüntülendi. VITEK® 2 sonuçları değerlendirilerek insektisit etkisi olduğu düşünülen 9 izolatla ilgili biyoanaliz çalışması yapıldı. Her izolat için 250-300 adet sağlıklı *Apis mellifera* kullanıldı. Biyoanaliz çalışmasının sonuçları, Abbott formülü kullanılarak düzenlendi.

INTRODUCTION

Wasps are winged insects belonging to the Vespidae family, which are neither bees nor ants, from the suborder Apocrita in the order Hymenoptera. Yellow jackets and hornets which most widely known wasps are from the Vespidae family and live together in a nest with an egg-laying queen and non-breeding workers. (Ertürk and Sarıkaya, 2020). Polistinae is the most diverse subfamily of Vespidae and has a fairly wide distribution and spread. *Polistes* Latreille, 1802, are a populous genus and live in North America up to Eurasia (Carpenter 1996, Pekkarinen 1999, Bağrıaçık, 2013, Mielczarek et al. 2021).

European paper wasp, *Polistes dominula* (Christ, 1791) (Hymenoptera: Vespidae), is a species of wasp belonging to the Vespidae family, most common in Europe, and native to the Palearctic Region around the, North Africa, the Middle East, Mediterranean, Southern Europe, and eastern China. It originated in the Near Polar Region of North America in the 1970s and 1980s (Madden 2010). Even though originally found on the east coast of the United States, then they spread to Midwest and currently to the Western and Southwestern United States. *Polistes dominula* was introduced in the 1990s to Canada, including Ontario, Nova Scotia, and British Columbia, as well as to Chile and Argentina in the Neotropical Region, and Western Australia in the Australian Region (Cervo and Turillazzi 1985). *Polistes dominula* habitats are temperate and terrestrial habitats, including forest and grassland ecosystems. They also live in urban and agricultural areas. Because they nest in human structures, they tend to live closely with humans. They also live in forests, on plants where they can feed and build nests (Cervo and Turilazzi 1985). *Polistes nimpha* is all social research found throughout Europe, especially in Türkiye, Finland, Estonia, and Latvia (Pekkarinen 1999, Mielczarek et al. 2021). It is also seen in China, Mongolia, Kazakhstan and Iran, Pakistan, Northern Africa, India (especially in the northern states of Jammu, Kashmir, and Himachal Pradesh). These regions with steppe vegetation and summers are usually hot climate and dry, in winter is relatively cold and snowy (Carpenter 1996).

Bees, closely related to wasps and ants, are flying insects that play an important part in pollination and honey production, and are one of the most valuable factors for agricultural productivity and are also very important in terms of the terrestrial ecosystem and sustainability. Bee pollination is important both ecologically and commercially, and the decrease in wild bees is increasing the value of pollinating hives of commercially controlled honey bees (Canale and Benelli 2021). Unlike honeybees, the main function of the majority of wasps is not to carry pollen, but some wasp species can carry pollen and contribute to the pollination of various plants (Sühs et al. 2009). It is a uniform lineage within the Apoidea family and today they are considered to be the class of Anthophila. There are more than 16,000 known bee species belong to the seven families (Michener 2000, Danforth et al. 2006, Grosch et al. 2021). Wasps are a diverse group estimated to have more than one hundred thousand described species worldwide and likely many more that are undescribed. Stings from these bees can cause both local and systemic allergic reactions and even life-threatening anaphylaxis in humans (Sahiner and Durham 2019).

Honeybees, the genus *Apis* Linnaeus, 1758, of which eight species are now recognized, are highly social andare among the best-known insects. One of these species, *Apis mellifera* Linnaeus, 1758 (Hymenoptera: Apidae), has 31 subspecies native to Europe, the Middle East, Asia, and Africa. The most important benefits of *Apis mellifera* are the pollination of flowering plants in agricultural production and the production of honey. With rapid population growth, people's demands for food are increasing rapidly. To meet theserequirements, bees are of high importance in terms of increasing production and ensuring continuity (McGregor 1976).

Insects, like other animals and plants, are infected by many microorganisms. These microorganisms, which cause disease and death of insects, are generally called entomopathogens. Entomopathogens that cause disease or direct death in insects are viruses, bacteria, rickettsias, protists, fungi, and nematodes, and can act on insects by reducing their feeding and growth rates. They can also act by slowing, inhibiting, or killing their reproduction. Areas of ecological overlap of wasps and honeybees, such as orchards and hives can enable harmful pathogens to be transferred between species. These organisms cause diseases by multiplying and spreading naturally in the insect population under certain conditions and when the insect density is high (Sharma 2020).

Coccidia (Coccidiasina) is a subclass belonging to the apicomplexan class, Conoidasida, are single-celled, microscopic, spore-forming and obligate intracellular parasites (Urquhart 1996a). Since they are obligate intracellular parasites, their reproduction and survival depend on living host cells. Coccidia parasites are the largest group of apicomplexan protozoa and can infect mammals, birds, fish, reptiles and some amphibians, and infect the intestinal tract. Additionally, most Coccidia species are host selective, meaning they are specific to their host (Yatoo 2013).

Microsporidia are obligate intracellular parasites that form spores and infect a wide range of vertebrates and invertebrates (Pan et al. 2018). Microspores, which prefer a single host in nature, do not cause the death of the pest when they infect but cause a shortened life span, loss of appetite, weight loss, and reduced reproductive availability. They are pathogens suitable foruse in biological control because they only affect the target organism and are specific to only one host. As a result of studies on microsporidia, these parasites have been isolated from mammals, reptiles, fish, amphibians, and insects (Tamim et al. 2020).

Studying microorganisms that cause disease in honeybees is of great importance in terms of obtaining healthy bee colonies, increasing honey yield per hive, and providing more active and effective worker bees (McGregor 1976, Sharma 2020). Entomopathogenic bacteria are the most valuable and effective organisms known in biological control against harmful insects, but they can cause undesirable infections in honeybees. In this study, we determined bacterial flora and presence fungal pathogens of *Polistes dominula* and *Polistes nimpha*. It is thought that this study will make significant contributions to the identification of new entomopathogenic bacterial and fungal species on European paper wasps.

MATERIAL AND METHODS

Obtaining the bees

The *Polistes dominula* and *Polistes nimpha* samples used in the studies were collected from the Terme district of Samsun province in Türkiye in May and June 2020. Healthy *Apis mellifera* samples used in insecticidal studies were collected and provided by Ordu Apiculture Institute.

Bacteria and fungi isolation from bee

Two methods were used for bacterial isolation. While it was made by taking samples directly from the hemolymph from bee samples with intact internal tissues, the samples were crushed in a sterile environment for bees with damaged internal tissues. To isolate bacteria from the collected wasp samples, 20 adult insects from each species were selected and placed in separate sterile tubes. Surface sterilization of wasps placed in tubes was done with 70% alcohol (Poinarand Thomas, 1978). After this procedure, the samples were washed three times with sterile distilled water under aseptic conditions. In the first method, some liquid was taken from the cuticle of the bee to reach the hemolymph with a fine-tipped injector, diluted, and spread directly on Nutrient Agar (Merck) medium. In the second method, 10 mL of sterile distilled water was added to the tubes and the samples were crushed in distilled water until they became homogeneous. After this process, 100 µL was added to Nutrient Agar, and spread plate cultivation was performed.

Determination of insecticidal efficacy of isolated bacteria and fungi

To determine the effect of the isolates obtained in this study on honeybees, 250-300 healthy honey bees were selected for each isolate from Ordu Apiculture Research Institute and an experimental group was formed. Rectangular queen bee breeding hives with a side length of 40 cm and a width of 20 cm were used for the experimental groups. In this stage, glucose-water solution (1:1) was prepared for feeding the experimental groups placed in the hives. 5 mL of the prepared solution was taken and the density was adjusted to 0.5 Mcfarland separately for each of the bacterial isolates. Small 25 mL spray cups were used to contaminate the bacteria (Fig. 3, 4). Five mL of the adjusted bacteria-solution mixture was sprayed into the feeding cups of the bees, allowing the bees to take the bacteria through food. Organisms whose density was adjusted with Mcfarland were added to the prepared solution under aseptic conditions and observed for 7 days. The test was completed after 7 days, paying attention to the number of dead and surviving honey bees (Kämpfer 1995). Experimental groups created during the test were checked daily. Insects that died during the control period were removed from the containers with forceps (Ombui et al. 1996, Swiecicka 2001). Average mortality rates were determined by determining the number of insects that died each day. This application was repeated three times for each isolate. Control groups were also used for the application.

To detect microspore and Coccidian pathogens, which are the subject of the study, wasp adults were dissected in the prepared Ringer solution. Ringer's solution was obtained by dissolving 8.0 g Sodium chloride (NaCl), 0.25 g Calcium chloride ($CaCl_2$), 0.25 g Potassium chloride (KCl), and 0.25 g Sodium bicarbonate ($NaHCO_3$) in 1000 ml distilled water for bee tissues. It isused in dissection processes in terms of creating the most ideal isotonic environment. Dissection was performed by carefully removing all tissues and organs from the abdomen and thorax to determine in which tissues the pathogen was active. The preparation was examined under a light microscope (Olympus CX41) with magnifications from 40x to 1000x. Infection-detected preparations were reexamined with a DP-25 digital camera and an Olympus BX51 microscope equipped with a DP2-BSW picture system, photographs of the pathogen were taken and necessary measurements were made for its characterization.

Then, to provide further imaging, bacterial cell suspensions of M17 broth and RSM were centrifuged at 3000 rpm for 1 min for scanning electron microscopy for more detailed imaging. To stabilize the protein, they were fixed in 3% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2 for 2 hours, followed by 15 min intervals in 0.1M phosphate buffer several times. The samples were exposed to serial ethyl alcohol solutions of 25%, 50%, 75%, 95% and 100% for 5 minutes each and dried. Critical Point Dryer (Polaron, Waterford, England) mounted on aluminum SEM studs, sputter coated with gold (Spi module spray coater, spi structure probe section materials). The samples were examined by SEM at 10-25 KV. Since the reliability of the measurements is based on the accuracy of the method, great care was taken.

Determining the contact of *Polistes dominula* and *Polistes nimpha* wasps with honeybees *Apis mellifera* in their natural habitats

This experiment was designed under natural conditions. A beehive was made by hollowing out the inside of a 30 cm diameter pine tree trunk. There were 5 honeycombs left in this hive, containing approximately 50-60 worker bees, including 1 queen. We specifically placed our hive where there were fruit trees. We placed the beehive 2-3 meters away from a fig tree where fig fruits are abundant. We observed it for approximately 15 days.

RESULTS

Bacteria and fungi isolation

A total of 18 pure cultures were obtained in the studies carried out.A total of 9 different isolates of 6 bacteria and 3 fungal species were obtained from 6 genera, 3 of which were gram-negative and 3 of which were gram-positive bacteria. All isolates obtained were numbered and coded (Tables 1, 2) and no spore-forming bacteria were detected. VITEK® 2 fully automatic bacterial identification system was used to determine the species of the isolates in Samsun Training and Research Hospital Microbiology Laboratory. According to the VITEK® 2 result, 9 of the isolates were identified at the species level. In the study, bacteria from the inner tissues and organs of diseased and dead *Polistes dominula* paper wasp adults *Serratia marcescens* (no.1), *Enterococcus faecalis* (no.6), *Staphylococcus xylosus* (no.9), *Sphingomonas paucimobilis* (no.11), *Staphylococcus lentus* (no.13) and *Candida ciferrii* (M2) fungus was isolated. From the internal tissues and organs of *Polistes dominula* paper wasp adults, the bacteria *Granulicatella adiacens* (No.2), *Staphylococcus xylosus* (No.10), *Sphingomonas paucimobilis* (No.15), and *Cryptococcus laurentii* (M1) and *Candida famata* (M3) (as *Debaryomyces*, *Hansensis,* and *Torulopsis candida* also known) mushrooms were obtained. It was observed that *Staphylococcus xylosus* and *Sphingomonas paucimobilis* were common bacteria in both bee species. The isolates, test codes, percentages and content obtained from *Polistes dominula* and *Polistes nimpha* in the study are given in Table 1. The morphological characteristics of bacteria and fungi isolated from *Polistes dominula* and *Polistes nimpha* are shown in Table 2.

Table 1. The isolates, test codes and percentages obtained from *Polistes dominula* and *Polistes nimpha*.

Wasp species	Isolate no	isolates	Percentage rates
Polistes dominula	1	*Serretia marcescens*	99%
	6	*Enterococcus faecalis*	99%
	9	*Staphylococcus xylosus*	86%
	11	*Sphingomonas paucimobilis*	97%
	13	*Staphylococcus lentus*	88%
	M2	*Candida ciferrii*	95%
Polistes nimpha	2	*Granulicatella adiacens*	98%
	10	*Staphylococcus xylosus*	89%
	15	*Sphingomonas paucimobilis*	93%
	M1	*Cryptococcus laurentii*	89%
	M4	*Candida famata*	98%

Table 2. Morphological characteristics of bacteria and fungi isolated from *Polistes dominula* and *Polistes nimpha*.

Species Name	Locality	Gram	Spor	Bacteria shape	Colony shape	Colony color
Staphylococcus lentus	feces	+	-	Coccus	Round	**White**
Sphingomonas paucimobilis	feces	-	-	Bacil	Convex	**Yellow**
Enterococcus faecalis	wasp internal tissue	-	-	Coccus	Round	**Off-white**
Serratia marcescens	wasp internal tissue	-	-	Rod	Round	**Red**
Staphylococcus xylosus	wasp internal tissue	+	-	Coccus	İrregular structure	**Yellowish**
Granulicatella adiacens	feces	+	-	Coccus	İrregular structure	**Off-white**
Cryptococcus laurentii	feces					
Candida ciferrii	wasp internal					
	tissue					
Candida famata	**feces**					

Bioassay studies for determination of insecticidal effects

VITEK® 2 results were evaluated, and a bioassay study was conducted with 9 isolates thought to have an insecticidal effect. For each isolate, 250-300 healthy *Apis mellifera* were used. Comparisons of death rates of bees in terms of isolated microorganism species was shown in Table 3. The results of the bioassay study were arranged using the Abbott formula (Table 4). The death rates of bacteria were shown in Fig. 1 and the mortality rates of fungi were shown in Fig. 2. At the end of the calculation, it was observed that the bacteria *Serratia marcescens* and *Enterococcus faecalis* and the fungal isolates of *Cryptococcus laurentii* and *Candida famata* showed the highest insecticidal effect. The pathogenicity of the isolated and identified bacteria and fungi on *Apis mellifera* was also tested. For this, the insecticidal activity of isolates at doses of 1.8 × 10 bacteria/mL within 7 days of application to healthy *Apis mellifera* adults was tested in several bioassay experiments. Especially *Serratia marcescens* and *Enterococcus faecalis* of our isolates caused mortality in 81.01% and 89.1% of *Apis mellifera* with large percentage. Most of the dead bees had diarrhea. The insecticidal activity of our other bacteria, which is the study material, was not very high and the values were close to each other. *Granulicatella adiacens*, *Sphingomonas paucimobilis*, *Staphylococcus xylosus,* and *Staphylococcus lentus* have a mortality rate of 10.18%, 18.9%, 24.32%, and 27%, respectively (Table 4). Some of these bacteria were previously isolated from *Apis mellifera*. In our study, fungi isolated from *Polistes dominula* and *Polistes nimpha* caused more and more effective mass deaths than bacteria. Almost all of the dead bees died within 5-7 days, and they were piled up in one place and their bodies became very soft and they had diarrhea. As a result of the calculations, mortality rates were found as M1 numbered *Cryptococcus laurentii* 83.78%, M2 number *Candida ciferrii* 41.51%, and M3 number *Candida famata* 94.59% (Fig. 1, 2). These fungi, especially *Cryptococcus laurentii* and *Candida famata*,caused mass mortality in honey bees with a high percentage of 83.78% and 94.59%. Fungi had created a higher rate against bacteria and great disinformation of the dead bees both physiologically and morphologically. In the observations we made in the beehives, it was determined that the bees were dying together in clusters.

Table 3. Comparisons of mortality rates of bees in terms of isolated microorganism species.

		Mortality		Survive		Total	p
		n	%	n	%	n	
n		940	100,0	860	100,0	1800	
Staphylococcus lentus							**<0.001**
	Yes	54	27,0	**146**	**73,0**	200	
	Others	886	55,4	714	44,6	1600	
Sphingomonas paucimobilis							**<0.001**
	Yes	38	19,0	**162**	**81,0**	200	
	Others	902	56,4	698	43,6	1600	
Enterococcus faecalis							**<0.001**
	Yes	**178**	**89,0**	22	11,0	200	
	Others	762	47,6	838	52,4	1600	
Serratia marcescens							**<0.001**
	Yes	**162**	**81,0**	38	19,0	200	
	Others	778	48,6	822	51,4	1600	
Staphylococcus xylosus							**<0.001**
	Yes	49	24,5	**151**	**75,5**	200	
	Others	891	55,7	709	44,3	1600	
Granulicatella adiacens							**<0.001**
	Yes	20	10,0	**180**	**90,0**	200	
	Others	920	57,5	680	42,5	1600	
Cryptococcus laurentii							**<0.001**
	Yes	**167**	**83,5**	33	16,5	200	
	Others	773	48,3	827	51,7	1600	
Candida ciferrii							**0.001**
	Yes	83	41,5	**117**	**58,5**	200	
	Others	857	53,6	743	46,4	1600	
Candida famata							**<0.001**
	Yes	**189**	**94,5**	11	5,5	200	
	Others	751	46,9	849	53,1	1600	

Those marked with a dark color indicate cells with statistically significant high levels compared to other cells. The survival rate in bees from which *Staphylococcus lentus* was isolated was found to be significantly higher than in bees from which other microorganisms were isolated (73.0% vs. 44.6%; p<0.001) (Table 3). The mortality rate in bees from which *Enterococcus faecalis* was isolated was significantly higher than in bees from which other microorganisms were isolated (89.0% vs. 47.6%; p<0.001). In other words, while the mortality rate in bees from which some microorganisms were isolated was significantly higher than in other isolate types, it was found to be significantly lower for some species. This finding shows that the microorganism types with significant high levels are directly related to mortality in these bees (Table 3).

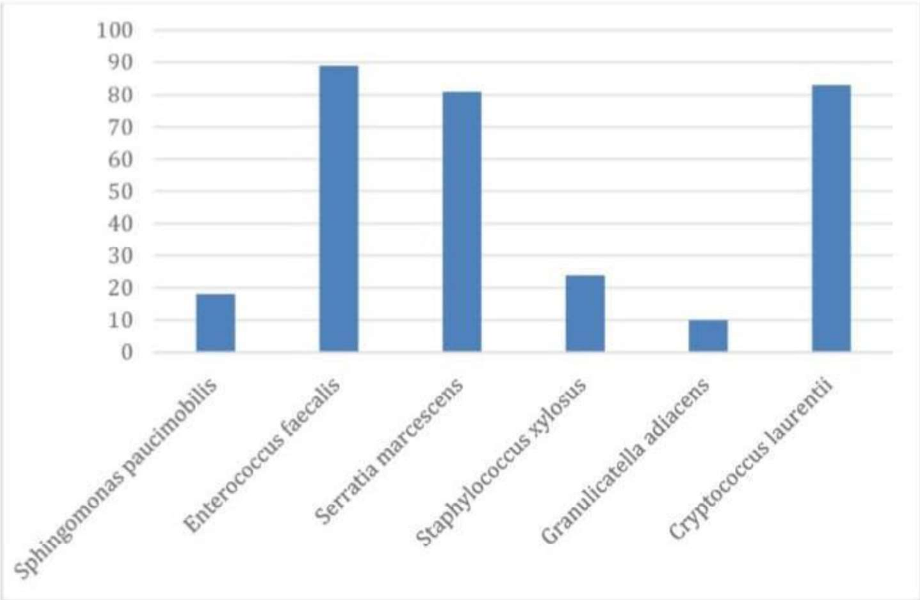

Figure 1. Graphic showing the mortality rates of bacteria.

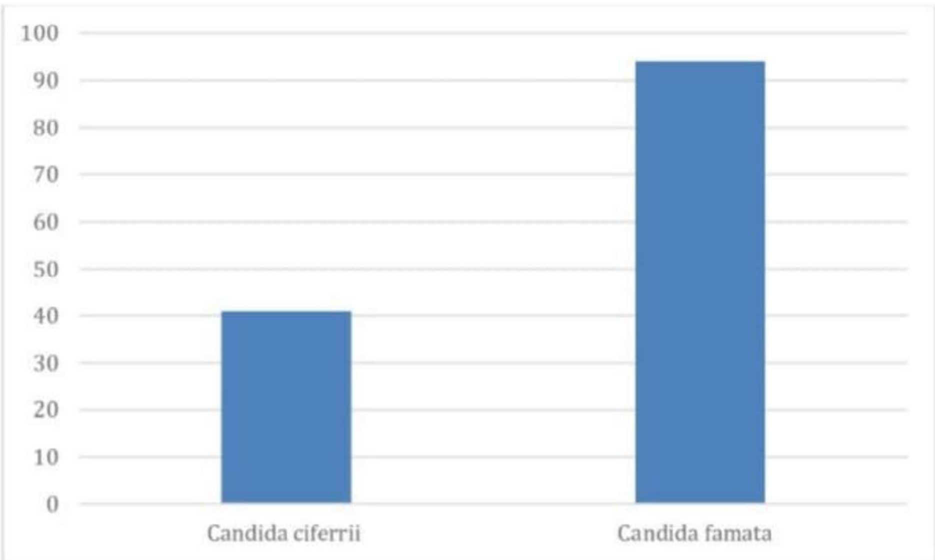

Figure 2. Graphic showing the mortality rates of fungi.

Table 4. Abbott's analysis results of insecticidal effects of bacteria and fungi isolated from *Polistes dominula* and *Polistes nimpha* on *Apis mellifera*.

Microorganisms	Bee Numbers	Mortality Percentage(Abbott)
Staphylococcus lentus	200±10	**%27±1.76**
Sphingomonas paucimobilis	200±10	**%18.9±2.753**
Enterococcus faecalis	200±10	**%89.1±3.33**
Serratia marcescens	200±10	**%81.01±4.64**
Staphylococcus xylosus	200±10	**%24.32±349**
Granulicatella adiacens	200±10	**%10.18±5.32**
Cryptococcus laurentii	200±10	**%83.78±5.53**
Candida ciferrii	200±10	**%41.51±2.11**
Candida famata	**200±10**	**%94.59±3.85**

Microscope studies

Important life stages of the microsporidium pathogen were tried to be determined carefully in wasp samples dissected for examination in light microscopy studies. During the direct examination of fresh tissues, the presence of infection was determined by comparing the morphological differences in the tissues with microsporidium infection with those of normal tissues. Damage to the tissues of the host was observed in fresh preparations. After light microscopy studies, it was determined that microsporidium infection infects the intestine, Malpighi tubes, and hemolymph of the insect. Using a microscope with a special camera and picture systems, the tissues infected by the microspores previously detected under the light microscope were photographed (Fig. 3). In light microscopy and electron microscopy studies, the Coccidia pathogen was observed for the first time in wasps (Fig. 3, 4). These structures, which were visualized in the bee tissue, were visualized both under the Light Microscope and SEM (Fig. 5). Some biochemical test results for gram positive and gram negative bacteria obtained from both types of paper bees are shown in (Table 5). As a result of the study, coccidian, and microsporidia pathogens, which we detected from *Polistes dominula* and *Polistes nimpha* wasps, could not be reproduced, so no bioassay study was performed on *Apis mellifera*. In addition, coccidian and microsporidia pathogens, which we detected from *Polistes dominula* and *Polistes nimpha* wasps, could not be reproduced, so no bioassay study was performed on *Apis mellifera*.

Figure 3. The appearance of microspores in the light microscope.

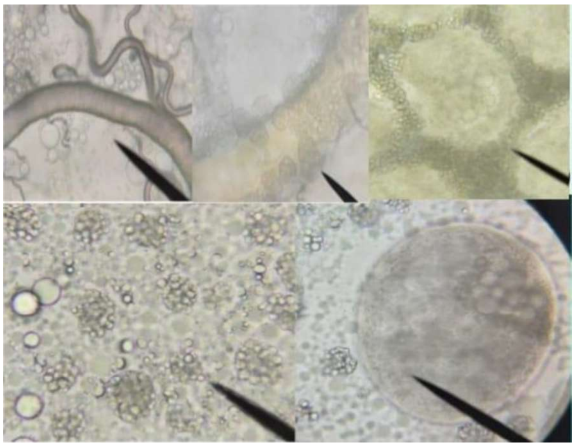

Figure 4. The coccidian pathogen in the light microscope.

Table 5. Physiological and biochemical properties of bacteria isolated from *Polistes dominula* and *Polistes nimpha*.

EXPERİMENTS	*Serratia marcescens*	*Sphingomonas paucimobilis*	*Enterococcus faecalis*	*Staphylococcus xylosus*	*Staphylococ cus lentus*	*Granulicatella adiacens*
Gram stain	-	-	+	+	+	+
Growth at pH 3	-	-	-	-	-	-
Growth at pH 5	+	+-	+	-	+	+
Growth at pH 7	+++	+++	+	+	++	+
Growth at pH 9	+	+	+++	++	++	-
Growth at pH 10	+	+	+	-	-	-
Control (NB)	++	++	+++	+++	+++	+++
NB+ growth at 2% NaCl	+++	++	+	+	+	+
NB+ growth at 3% NaCl	++	++	+	+	+	+
NB+ growth at 4% NaCl	+	+	+	++	++	+
NB+ growth at 5% NaCl	+	+-	+++	+	+	+
NB+ growth at 7% NaCl	+	-	+	+	-	-
NB+ growth at 10% NaCl	-	-	+	+	-	-
Growth at 25˚C	+	++	+	+	+	
Growth at 36 ˚C	++	++	+++	++	++	++
Growth at 40˚C	-	Dormant at 37°C.	+++	+++	+++	+++
Morphology	Rod	Bacil	Cocci	Cocci	Cocci	Cocci
Gas	+	+	-	-	-	-
Indole	-	+	-	-	-	-
Methyl Red	-	+	+	+	+	+
Voges-Proskauer	+	-	+	-	+	+
Citrate	+	+	-	-	-	-
Glucose	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+
Lactose	-	-	+	+	+	-
H_2S	-	+	+		+	-
Galactose	+	+	+	+	-	-
Urease	-	+	-		-	-
MacConkey growth morphology	++	++	-	-	-	-
Length (µm)	0.8 ± 23 µm	1.4 ± 08 µm	1.48 ± 0.31 µm	09 ± 04 µm	08 ± 05 µm	09 ± 02 µm
Diameter (µm)	0.5 ± 45 µm	0,13 ± 07 µm	1.38 ± 0.31 µm	09 ± 04 µm	07 ± 02 µm	06 ± 06 µm

Observation of the contact between honeybee and wasps

As a result of our observation, we noticed that bees especially attacked the sugar of figs and all three types of bees settled on the same fruit. These wild paper bees attack fruit sugars a lot in nature, and as a result, they come into contact with honeybees, which may be the reason for the transmission of microorganisms between these species. In addition, many studies have found that there are common microorganisms in all three species. However, because of this study, bee farms should not be placed too close to orchards both for wintering and for making honey. In addition, we observed that the hornets started to fight with the honeybees and eventually most of the honeybees died and some of them left the hive.

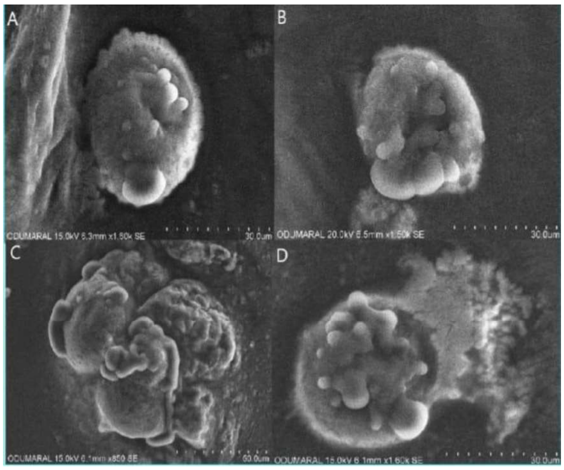

Figure 5. Coccidian pathogen in scanning electron microscopy (SEM).

DISCUSSION

European honeybees have been the most researched insects in Türkiye and the world due to their important role in agriculture and the ecosystem and their high economic value. In addition to producing honey, propolis and beeswax, which are the most valuable roles of honeybees, they are also important for agriculture and sustainability. A highly adaptable species, the bees' habitat extends throughout Asia and Africa, from the southern parts of Scandinavia to the Central (Sheppard and Meixner 2003, Avci et al. 2022).

Many various microorganisms found in bees are associated with honey bees. In recent years, studies on bees by scientists have reported serious bee losses from beehives and a decrease in bee populations (Stathers 2016, Potts 2020). Bees are now making a great contribution to the food chain, directly or indirectly, on earth. Honeybees have been faced with mass deaths in some places in recent years. There are different causes of these deaths. One of them is microorganisms. Our aim in this study was to seek answers to the questions of whether bacteria and fungi that we will isolate from wasps have negative infections on honeybees or do they cause mass deaths if any.

Wasps encounter honeybees directly or indirectly at some times of their lives, sometimes in theform of a flower, sometimes fruit, sometimes a nest raid, or a direct encounter with the honey bee itself. Individuals of these wasp populations can infect honeybees with some bacterial and fungal species they carry in their bodies.

When aerobic and facultative anaerobic bacteria isolated from the intestines of worker bees were identified according to biochemical and 16S rDNA sequence analyses; *Firmicutes*, *Proteobacteria* and *Actinobacteria* were detected at higher rates. Additionally, opportunistic commensal bacteria such as members of *Staphylococcus haemolyticus* and *Sphingomonas paucimobilis* were also identified in the hive environment of these bees (Anjum et al. 2018). The eastern honey bee bird feeds mainly on bees and wasps. The gut microbiome of this bird of prey was found to be rich in *Firmicutes* and *Bacteroidetes*. This information provides clues that bee populations carry common bacterial species (Nagai et al. 2018).

Some bacterial species isolated in our study were similar to the species detected in a different study on the bacterial flora of honeybees previously (Przybyłek and Karpiński 2019, Bog et al. 2020). As an example, *Staphylococcus lentus* and *Sphingomonas paucimobilis* bacterial species were detected in the study conducted in honeybees collected from Ordu and 9 districts (Bog et al. 2020), while the same bacterial species were found in wasps collected from the Terme district of Samsun in our study. This similarity may suggest that the bacteria are common to both species or that they were formed after an infection.

As a result of this study, *Serratia marcescens* bacteria, one of the bacteria obtained from the digestive system (midgut and hindgut) of *Polistes dominula* and *Polistes nimpha* wasps, is a great potential source of Gram-negative bacterial entomopathogens, new toxins, and metabolites that can be used in insect control programs. However, this bacterium is a member developed as a biocontrol product (Ferreira et al. 2021). *S. marcescens* infects both invertebrates and vertebrates but is considered an important pathogen of insects, most commonly causing bacteremia (presence of bacteria in the bloodstream) and rapid insecticide (Grimont and Grimont 2006, Ishii et al. 2014).

In a study, the pathogenicity of *S. marcescens* isolates against these pests, which was isolated and characterized using bioassays from the larvae of *Plodia interpunctella* and *Ephestia kuehniella*, was evaluated. These bacterial cells were injected into the hemolymph or added to the insect's diets. Compared to the control group, the survival rates of insects, especially larvae, exposed to different *S. marcescens* concentrations were significantly reduced. The results of our study showed that this *S. marcescens* isolate has valuable potential for use in biological control of harmful insects. In addition, these results can be considered as a source of bioactive molecules and genes useful in the development of insect-resistant plants, biological control and biotechnology applications. (Bidari et al. 2018). In this study, we obtained, *Granulicatella adiacens*, compared to other streptococci, is rarely involved in infections because of isolation but part of the oral, gastrointestinal and urogenital commensal flora. These have been reported to cause mostly bacteremia and endocarditis and, less frequently, device-related infections such as meningitis, breast implant infections, and peritoneal dialysis-associated peritonitis (Christensen and Facklam 2001).

Another isolate of our study, *Sphingomonas paucimobilis*, has been isolated from insects and bees in many previous studies. In a study conducted in Ordu, bacteria were obtained from sick and dead honey bees collected from 9 regions. Eighteen of the pathogenic bacteria were isolated from non-spore-forming bacteria. One of these isolates is *Sphingomonas paucimobilis* (Bog et al. 2020). However, *Sphingomonas paucimobilis* has been found to be an opportunistic pathogen in honey bees that have been found to multiply in the hive environment (Anjum et al. 2018). In another study, these bacteria, which are frequently seen among bacteria isolated from some Tabanidae species that fall into insect traps, are also bacteria found in foods, various water sources, wastes, feces, and everywhere (Fukui et al. 1999, Köseoğlu et al. 2019). In addition, some strains of this bacterium were isolated and identified from the guttation fluid of anthuriums by Fukui et al. (Fukui et al. 1999). In our study, it is thought that *S. paucimobilis* probably infect honeybees when they collect nectar from flowers.

Staphylococcus xylosus, which is present in honeybees and domestic waste water, was obtained by characterizing and identifying proteolytic bacteria in the gut of the velvet bean caterpillar (*Anticarsia gemmatalis*) (Visotto et al. 2009). However, this isolate was also isolated in the study with bees (Christensen and Facklam 2001, Essa and El-Gayar 2018) characterized bacteria isolated from two domestic waste water treatment plants in Jazan, KSA. Domestic wastewater can be considered a suitable environment for the survival of a wide variety of microorganisms. In the identification and diagnosis of bacterial isolates, researchers were use morphological and biochemical tests. As a result of the study, both Gram-positive and Gram-negative bacterial strains were isolated, including the *Staphylococcus xylosus* species. In many studies, the bacteria we obtained from wasps were also isolated from honeybees, which shows us that there is microorganism transmission between these species. In addition, many studies have found that there are common microorganisms in all three species (Anjum et al. 2018, Essa and El-Gayar 2018).

Enterococcus faecalis is an important bacterial species among the isolates we obtained in our study. This isolate has been isolated from different insects by many researchers. In a study, *Enterococcus faecalis* was isolated from different insect guts. Microbial colonies with different criteria were selected for identification based on their size, shape, color and other visual characteristics and investigated using physical and biochemical methods for their identification. Fifteen isolates representing twelve different genera were identified from the 19 colonies obtained. Among the isolates obtained from *Ocymyrmex velox*, *Enterococcus faecalis* and *Serratia marcescens* bacteria are also present. In spite of different bacterial species were detected in the guts of different insect species in this study, this does not stated that they contain common bacteria. (Shil et al. 2014). Another study found a bacterial disease in the beet caterpillar *Spodoptera exigua* (Hübner). Epizootic disease has been observed in the blackened body of infected larvae, especially in intersegmental areas. It was identified as *Enterococcus faecalis*, a gram-positive bacteria isolated from the hemolymph of infected 5th instar larvae (Park et al. 2002).

Fungi are widespread in nature and have been isolated from plant and animal surfaces, air, food, and sugary substrates such as flower nectar and fruit juices. (Ingram 1955, Last and Price 1969). The relationship fungi with insect guts is well known. Just as flower nectar is a rich source of fungi, pollinator bees, contain fungi, especially in their nectar sacs. The connection between some fungi, flowers and insects is that and also transfer fungi to flower nectars during their visits to entomophilous flowers (Buchner 1965, Batra et al. 1973).

In this study, species belonging to *Candida*, *Debaryomyces*, *Hansenula,* and *Torulopsis* fungal genera obtained from wasps were determined. In other studies, similar species and species of fungi have been identified from the honey stomachs of pollinating bees. *Cryptococcus laurentii* and *Candida ciferrii* fungi were obtained in our study. These two fungi were isolated from the surface of healthy apple fruits collected from organic production orchards in the south of Uruguay with biocontrol agents (Vero et al. 2022).

In another study, a small amount of *Candida famata* was detected in isolates obtained from mastitic Anatolian buffalo district milk samples. In another study, biological control of *Candida famata* isolates obtained from fig leaves on orange fruits was reported in Sardinia. In terms of host-antagonist-pathogen interaction, the fungus, alone or artificially inoculated, has been found to induce the fruit to produce associated phytoalexins (scoparone and scopoletin) in significantly varying concentrations (Arras 1996). In this study, microsporidium was detected in dissection samples from wasps. In the study, the spore stage, which is the most basic stage in the detection of the pathogen under the microscope, was observed in many dissections. Spores with the characteristic features of the microsporidium pathogen are distinguished from other tissues of the host in that they refract light differently and have the same size and shape.

Coccidia pathogen was observed for the first time in wasps used in our thesis study. In this way, it has the distinction of being the first. Coccidia is a subclass of Apicomplexan class and microscopic, unicellular, spore-forming, obligate intracellular parasites belonging to the Conoidasida (Urquhart et al. 2001). This infection with parasites is known as Coccidiosis. Depending on the Coccidia species, infection in bees can cause nervous system effects, vomiting, fever,muscle pain, diarrhea, behavioral changes and death. Diagnosis of Coccidiosis is made by finding oocysts in stool smears. In the early stages of the disease, very few oocysts may be shed and a negative test does not exclude the disease. Treatment of coccidiosis is usually with Coccidiostats, a group of medications that block the growth of Coccidia. The most administered Coccidiostat in dogs and cats are sulfate-based antibiotics. Once reproduction has stopped, recovery can occur on its own, a process that depends on several factors, including the animal's immune system and the severity of the infection, and can take several weeks (Chapman 2013).

In conclusion, the fact that similar bacteria and fungi obtained from honeybees in previous studies with the wasps in our study are common in both species may indicate that this is a result of contamination between bees. It is thought that bumblebees and honeybees sometimes meet indirectly or directly when they visit flowers (collect nectar), sometimes at fruits, and sometimes when raiding nests. This may indicate that bumble bee communities can transmit some types of bacteria and fungi that they carry in their bodies to honeybees, causing infections in bee colonies and negatively affecting them. In this case, it can be thought that some of the bacteria we obtain from wasps can be used as biocontrol products, in microbial control of insect pests, in insect control by developing insect-resistant plants, and in biotechnology applications. Honey bees are economically very valuable as they pollinate a significant portion of the world's crops. Additionally, honeybees' hive products, such as residues, are used to feed cattle. The amount of pollination worldwide is €153 billion, representing 9.5% of world human food agricultural production in 2005 (Gallai et al. 2009). However, in recent years, increasing colony losses have been reported in the USA, Europe and elsewhere (Gallai et al. 2009, Biesmeijer et. al. 2006, Ghazoul 2005).

Losses of honeybees have been particularly evident in Colony Collapse, a serious disease that caused the loss of 50-90% of colonies in beekeeping operations across the United States in 2007. An opportunistic pathogen, *Serratia marcescens* has a lethal effect not only on animals and plants but also on honey bees and poses a threat to the generation of bees. (Raymann et al. 2018). In another study conducted in Sudan, twenty-three strains of *Serratia marcescens* were isolated in pure culture from diseased honeybee larvae (El Sanousi et al. 1987).

Honeybees are major pollinators of crops and flowers worldwide, and pathogens and parasites that threaten the health of bees are of utmost importance. To determine the presence of bacteria, fungi and different entomopathogenic organisms in the bodies of two wild bees, *Polistes dominula* and *Polistes nimpha*, in the honeybee *Apis mellifera* is important. Another important issue is the determination of the occurrence and prevalence of these pathogens and parasites and their infections on honeybees, especially in regions where beekeeping is at the forefront in Türkiye. We conducted a nationwide survey. In our study, we found evidence of very high rates of infections caused by certain bacteria and fungi isolated from two species of wasps, *Polistes dominula and Polistes nimpha;* We found these to be linked to declines in the population of honeybees. Wild bees are bees that do not make honey but feed on ready-made food. In our observations, we observed that the bees doing this were found in hives with honeybees, especially those located close to orchards. For the purpose of testing, we put worker bees in a hive in an environment with fig fruit, and they landed on the same fruit, especially the sugary liquid that the fig fruit leaks, and the fruits both on the tree and on the ground were invaded by these two different bees. Approximately 20-25 days later, wild bees entered the hive containing honeybees and killed and dispersed the honeybees in the hive. The honeybees struggling in this hive may have definitely been contaminated with parasites or microorganisms hosted by wild bees and may have met with other honeybees on some occasion. This situation may turn into an undesirable mass death of bees. An infection similar to the Covid-19 pandemic that our world has experienced in recent years is in honeybee populations. It has been reported that Trophallaxis (food exchange) increases in young adult honey bees infected with *Nosema ceranae* microsporodian (Lecocq et al, 2016).

This change in behavior provides evidence that the spread of infection may occur more frequently in honeybee and wasp populations that share common feeding grounds. Beekeepers in areas where *Polistes dominula* and *Polistes nimpha* are common may install alternative water sources and dummy hives to limit competition and interaction between bee populations. As the coccidian and microsporidia pathogens that we detected because of the study could not be reproduced, it could not be determined whether they have insecticidal effects on honeybees.

Our results show that *Polistes dominula* and *Polistes nimpha* are microorganisms isolated from wasp, bacterial and fungal infections occurring in a region throughout Türkiye, and that wild honey growers come from different regions of Türkiye in spring and summer to meet with other honey growers and share their experiences. This current study highlights a number of processes that are potentially of interest in terms of pathogen-host interaction and biological control. Although the results of this study provide a foundation, further research, including empirical studies, is needed to better understand the effects and outcomes of these processes. These processes will have significant impacts on the health of the native honey bee Apis mellifera and the continuity of native bee species in beekeeping in Türkiye.

Conclusion: In this study, the fact that both species carry common pathogens may indicate that it is a result of contamination between bees. It is thought that wasps and honeybees sometimes meet indirectly or directly when they visit flowers (collect nectar), sometimes at fruits, and sometimes when raiding nests. This may indicate that wasp communities may cause infections in bee colonies and negatively affect them by infecting honey bees with some types of bacteria and fungi that they carry in their bodies. In this case, it can be thought that some of the bacteria we obtained from wasps can be used as a biocontrol product, in the microbial control of insect pests, in insect control and biotechnology applications by developing insect-resistant plants.

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