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RESEARCH PAPER

Pollen Preference of Honeybees Depending on Protein Contents

Şeref Cınbırtoğlu[1,*], Fazıl Güney[1]

[1] Apiculture Research Institute Directorate, Ordu, Turkey

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***Corresponding Author**
Tel.: +905383003110
E-mail: seref.cinbirtoglu@tarimorman.gov.tr

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Trifolium repens L.

Abstract

This study was carried out in the spring period of 2013 and 2015 in Turkey. Pollen traps were attached to the colonies when the flight activity starts, in the flowering period of March, April, and May. The reference pollen slides were prepared, and pollens collected by bees were determined using reference pollen slides by microscopic examination. The protein contents of the bee pollens were also evaluated. Protein contents of pollens varied between 7.27%-24.90% in 2013 and 7.47%-21.82% in 2015. The highest protein content was determined in *Lamium purpureum* L., while the lowest protein was found in *Carex sp.* Honeybees preferred mostly the pollens of *Laurocerasus officinalis* R., *Ornithogalum* sp. and *Taraxacum officinale* W. in March, *Juglans regia* L., and *Bellis perennis* L. in April, *Diospyros lotus* L., and *Trifolium repens* L. in May.

Introduction

The pollen is the primary natural protein sources of honeybees. It is crucial for honeybees, and they need pollen mostly in spring and the beginning of the summer for breeding, beeswax producing, brooding, and population increasing activities (Guler, 2017).

Pollen gathering and storage at adequate levels are crucial for entering the main nectar flow with healthy worker bees, maintaining broods' reproduction, and consequently providing desired honey production (Erdogan & Dodologlu, 2005). A bee colony can collect about 18-50 kg of pollen per year, and a honeybee consumes 120-145 mg pollen from the egg stage to adulthood (Sammataro & Avitabile, 1998).

200 substances are identified in pollen, including proteins, amino acids, carbohydrates, lipids and fatty acids, phenolic compounds, enzymes, vitamins and bio elements at different proportions according to the floral source (Komosinska-Vassev, Olczyk, Kaźmierczak, Mencner, & Olczyk, 2015). It is not precisely explained whether honeybees choose pollen according to the quality, odour, or other visual properties (Lunau, 2000).

Every flora and geographic region has some specific or endemic plants as a nectar source. Beekeeping is not possible without flowers. For that reason, beekeepers have to move their hives to fields, which have a long flowering period and pollen source plants (Öztürk, Yalçın, & Tutkun, 2001). It is necessary to discover the species of pollen plants that honeybees visit extensively (Tutkun, 2011). In the studies related to this topic, plants in different floras preferred by honeybees (*Apis mellifera* L.) as pollen sources, were detected.

The plants preferred by honeybees for pollen source are not well known in the study field. Thus, this study aimed to identify essential plants that honeybees use as pollen sources and measuring their protein contents.

Materials and Methods

This research was carried out in the area of 20000 decares in Dedeli Village, which represents the flora of Ordu City. Plant species, pollen grains and protein content were determined in March, April and May of 2013-2015. No data was collected due to unfavourable seasonal and floral conditions in 2014. Pollen samples were collected with traps placed in beehives. Pollen traps were set between 07:00-15:00 o'clock on days when flowers bloom and seasonal conditions are favourable for bees to forage. According to Sawyer (1988) method, 200 pollen pellets were randomly collected from 3 colonies separately and daily. The fresh pollen pellets collected from the hives were classified according to their colours and then microscopically examined.

Preparation of Reference Pollen Slides

The collected plants were identified with the aid of literature and then labelled. (Anonymous, 2008; Anonymous, 2013; Davis, 1965-1985; Gungor, Atatoprak, Ozer, Akdag, & Kandemir, 2007). Firstly, reference pollen slides were prepared according to Louveaux, Maurizio and Vorwohl (1978). Briefly, pollen taken from anthers was placed on a clean slide and 2-3 drops of 96% alcohol were dripped on it. The slides were kept on the heater until the alcohol evaporated, then basic fuchsine was added. Some glycerin-gelatin was put on the pollens and melted. It was mixed with a clean needle to distribute the pollen, and a coverslip was covered on it. Slides were left to dry by turning them upside down and sealed with paraffin. These slides were used for later comparison with the pollen types.

Figure 1. *Taraxacum officinale* W. plant collected for reference pollen slides

Figure 2. *Trifolium repens* L. plant collected for reference pollen slides

Identification of pollens

Pollen grains were prepared and identified according to Louveaux et al. (1978). The pollen slides were researched with Zeiss Axio Scope A1 microscope and identified with the aid of prepared reference pollen slides and with the use of microphotographs from the literature (Sorkun, 2008; PalDat, 2015).

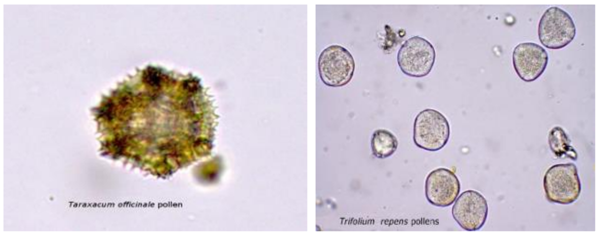

Figure 3. Some view of identified pollens in a microscope

Protein Analyses of Pollens

The protein analyses were performed with a protein-nitrogen analyzer (LECO FP-528, USA). According to Dumas principle, samples were heated to destruction in a combustion tube at high temperatures (900-1200 °C) in an oxygen atmosphere (AOAC, 2002).

Results

Protein contents of pollens preferred by honeybees are given in Table 1. Protein amount, a crucial quality property of pollens, showed a significant variation in plant species. It was found that *Lamium purpureum* L. pollens have the most considerable protein amount and *Carex sp.* pollens contain the least amount of protein. On the other hand, *Trifolium repens* L., *Laurocerasus officinalis* R. and *Diospyros lotus* L. pollens also had more protein than the rest of the plants.

Table 1. Protein contents of pollens collected by honeybees in 2013 and 2015

Species	Protein (%) (Av. ± SD)*	
	2013	2015
Lamium purpureum L.	24.90 ± 2.02	21.82 ± 0.39
Diospyros lotus L.	23.13 ± 0.98	20.99 ± 0.10
Laurocerasus officinalis R.	22.85 ± 0.60	18.91 ± 1.39
Trifolium repens L.	22.39 ± 1.46	19.31 ± 0.54
Ornithogalum sp.	17.26 ± 1.61	18.84 ± 0.40
Taraxacum officinale W.	16.23 ± 3.52	15.91 ± 1.83
Salix sp.	15.92 ± 1.04	16.85 ± 0.34
Veronica sp.	14.57 ± 0.34	14.09 ± 0.67
Bellis perennis L.	13.65 ± 0.59	12.19 ± 0.27
Geranium asphodeloides B.	13.45 ± 0.57	13.49 ± 0.41
Juglans regia L.	12.86 ± 0.68	15.92 ± 0.26
Carex sp.	7.27 ± 0.65	7.47 ± 0.48

*Av. ± SD: Average ± Standard Deviation

Discussion

Protein contents of pollens varied between 7.27% -24.90% in 2013 and 7.47% -21.82% in 2015. Previous studies support variation of protein amounts, found in our study. It was reported that the protein content of pollen was in a wide range of 2.5%–61% (Roulston, Cane, & Buchman, 2000). Taha, Al-Kahtani, and Taha (2019) also stated that the protein content of bee pollens may vary between 2.90% to 33.51%, depending on the botanical origin. In a study conducted in six regions of Turkey, Başdoğan, Sağdıç, Daştan, Düz, and Acar (2019) found the protein values between 16.6% and 20.2%.

Although protein amounts varied in years, *Lamium purpureum* L. pollens had the most abundant protein amount while the lowest protein amount was found in *Carex sp*. *Diospyros lotus* L., *Laurocerasus officinalis* R. and *Trifolium repens* L. pollens were also rich in protein content. The rest of the other plants' protein amount differed between 12.19% and 18.84%. Teleria, Salgado-Laurenti, Marinozzi, Apóstolo, and Pérez (2019) stated protein amounts of *Taraxacum officinale* W. pollens between 13.25% and 14.0%. Radev (2018) reported that protein amounts were 24.1% and 26.0% for *Trifolium repens* L. and *Lamium purpureum* L., respectively. These findings are in accordance with our results.

In our study, it was also observed that honeybees preferred pollens with more protein content. *Laurocerasus officinalis* R. was preferred by honey bees as a pollen source in March, while *Diospyros lotus* L. and *Trifolium repens* L. were preferred in May. Ghosh, Jeon, and Jung (2020) indicated honey bee colonies collected pollens from *Trifolium repens* L. first, which had the highest total protein content in their study. Özkök and Sorkun (2016) reported that may be due to the high protein content of poppy pollen, bees preferred the poppy flower at a rate of 84%. Andrada and Telleria (2005) also have stated that honeybees preferred plant species containing high protein levels (*Condalia microphylla*, *Chuquiraga erinacea, Discaria americana, Grindelia tehuelches, Larrea divaricata, Prosopis sp., Prosopidastrum globosum*, and *Vicia epampicola*) in spring. In contrast to our findings; Çelemli, Barkan, Özenirler, Demiralp, and Sorkun (2017) reported that honeybees may not always prefer the pollen types that had higher protein content. These researchers found that the highly preferred pollen had a lower protein content, while the least preferred pollen sample had a relatively higher protein content level.

When the pollen storage was sufficient in the hives in the spring, honeybees primarily preferred protein-rich pollens for their colony activities. However, in the case of protein-rich pollens insufficiency in the flora, honey bees started to collect pollens with lower protein content. It was observed honeybees collected *Juglans regia* L. pollens and *Bellis perennis* L. pollens. Even though the protein amounts of these pollens were moderate, they were preferred by honey bees extensively.

Conclusion

The pollen preferences of honey bees may change depending on the flora. The ecological and climatic conditions and also the colony's requirements are responsible for these differences. Honey bees mostly need pollen during breeding and feeding activities, especially in the spring months. It is seen once again in this study, honey bees prefer protein-rich pollens. If the protein-rich pollens are not adequate, honey bees prefer other plants with lower protein content. Within this study's scope, plant species are sufficient and meet the protein requirement for breeding and colony activities in the spring season.

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RESEARCH PAPER

Growth Characteristics of Commercial Bumblebee Colonies in Open Field Conditions May Be Evidence for Their Invasion Potential

Ayhan Gosterit[1,*], Cengiz Erkan[2]

[1] Isparta University of Applied Science, Faculty of Agriculture, Department of Animal Science, Isparta, Turkey
[2] Van Yuzuncu Yıl University, Faculty of Agriculture, Department of Animal Science, Van, Turkey

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***Corresponding Author**
Tel.: +905337323985
E-mail: ayhangosterit@isparta.edu.tr

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Abstract

Colony parameters of laboratory reared *Bombus terrestris* L. colonies that placed to the field at the beginning of the social phase were observed. Founder queen of these field colonies was reared in commercially produced colonies. When the colony population reached about ten workers, they were transferred to the open field. No sugar syrup or pollen was supplied to colonies and was checked twice a week. The time of the first young queen and male observing, switch point, competition point and number of individuals produced in colonies were recorded during the colony controls. The first male and young queen emerging time after the end of the diapause were calculated as 85.00±3.21 and 62.33±2.67 days respectively. Competition point time was 40.50±1.32 days, while switch point time was 16.00±3.70 days in the social phase of colonies. Colonies produced 9.67±5.93 young queens (gynes) and 39.50±14.20 males during their life cycle. Results of this study revealed that commercially produced *B. terrestris* colonies can survive and produce sexuals in the native habitat.

Introduction

Bees which include more than twenty thousand species are the most valuable insect group and have about 8 or 9 families according to morphological traits (Donovan, 1980). Honeybees (*Apis mellifera* L.) are the most effective pollinator of natural flora due to their extensive rearing all over the world. Bumblebees, of which about 250 species have been reported, are other important pollinators of many flowering plants. In many countries, bumblebees are used as pollinators of many cultivated plants (Velthuis & Van Doorn, 1996; Williams, 1998). Because their rearing is easy and the worker population is crowded than the other species, *Bombus terrestris* L. is the most year-round reared, according to other bumblebee species. This species includes nine subspecies and is used mainly for tomato pollination in the greenhouse. They improve the quantity and quality of crops and decrease pollination labour costs. *B. terrestris* colonies which reared commercially are used in many countries, including some outside of their native range (Velthuis & Van Doorn, 1996; Rasmont, Coppee, Michez, & De Meulemeester, 2008).

Bumblebees are valuable and indispensable pollinators. However, it is also known that *B. terrestris* has an invasion potential into new areas where they are non-native. Early seasonal emergence, generalist or polylectic foraging strategies, high adaptability under adverse environmental conditions in a range of habitats, and their great phenological flexibility are the most critical invasive features. After its commercial introduction, it was determined that this species is invasive, can spread into new areas, and may disturb local ecology (Goulson, 2003; Dafni, Kevan, Gross, & Goka, 2010). Competition with native organisms for nest sites and floral resources (Hingston & McQuillan, 1998), the transportation of pathogens and parasites (Goka, Okabe, Yoneda, & Niwa, 2001), hybridization with wild species (Ings, Ings, Chittka, & Rasmont, 2010) and change of natural pollination systems (Hanley & Goulson, 2003) are major problems that caused by the invasion and the increase in the population of introduced bumblebee in the new locations. A single colony can produce about a hundred new queens. These young queens could escape from greenhouses and found nests in the native habitat. Therefore, these environmental risks should be taken into account seriously (Gosterit & Baskar, 2016).

Many studies have been carried out to determine the growth characteristics of commercial *B. terrestris* colonies under controlled conditions. Certainly, the results of the colony development patterns that have obtained from these studies belong to the colonies grown in the laboratory where the conditions in terms of climate and nutrients are optimum. However, it is expected that the colony growth characteristics, the number of queen and males produced, and the colony life span of these laboratory colonies are different from those colonies in field conditions where climatic conditions are variable and food resources are limited. In the field conditions, there are many factors that adversely affect the colony development such as rapid climatic changes, parasites, natural enemies, diseases and pests, agricultural and non-agricultural practices that limit nest areas and food resources. Despite the many characteristics of the *B. terrestris* species stated regarding the rapid spread, the local populations of this species live in a certain balance in accordance with the habitat and ecology they live in their natural range under the influence of these negative factors. It is estimated that this balance continues with a slow change for a long time unless there is an external intervention (Velthuis & Van Doorn, 2006; Goka, 2010). Despite the possible risks to the natural ecosystem, the number of young queens and males produced in commercial *B. terrestris* colonies, whether these young queens and males produced in colonies can mate in the natural environment, whether they can establish a nest by surviving the diapause period, or the developmental characteristics of the nests in the open field are not fully known. This study, examining the development characteristics of commercial colonies under field conditions, was carried out to obtain scientific data on the spreading potential of *B. terrestris*.

Materials and Methods

Bombus terrestris colonies founded by queens reared in commercial colonies were used as bee material of the study. Standard rearing methods were applied for rear the colonies (Tuna & Gosterit, 2017). According to the study aim, artificially hibernated queens were placed into starting boxes and allowed to found colonies under laboratory conditions (27–28^{o}C and 50% RH). In this stage, fresh pollen and sugar solution (50 Brix degree) were used to fed queens and their colonies. When the population reached about 10 workers in the social phase, the colonies were transferred to rearing boxes and placed in the field without pollen and sugar solution. There was at least 25 meters distance between colonies placed on the field. The study was performed in Isparta (Turkey) between May and June, and the colonies were brought to the laboratory in the evenings twice a week and checked under red light. After the checking process, the colonies were taken back to their same location. Some growth characteristics were obtained for colonies: first male emergence time, first gyne emergence time, competition point (observation of some clues such as worker oviposition, oophagy, and egg-cup destruction), switch point (conversion time from worker production to queen and/or male production), and the total number of individuals (workers, males, and gynes) produced by each colony (Duchateau & Velthuis, 1988). While the first male emergence time, the young queen emergence time and queen longevity were calculated from the date when the queens put into starting boxes, switch and competition point were calculated from the date when the first worker emerge (beginning of social phase). During the two months, the flowering plants which bumblebees forage and their flowering period were also determined. Minitab Statistical Software (Version 16.2.4) was used for data analysis and descriptive statistics of each colony growth traits were calculated.

Results and Discussion

Bombus terrestris colonies were placed in to the field at the beginning of the social phase without pollen and sugar solution and no extra feeding was provided to them until the end of their life cycle. Flowering plants used by bumblebees as nectar and pollen sources during the experiment and their flowering period are given in Table 1.

Table 1. Major flowering plants and their flowering period in study area

Family	Species	Flowering period
Rosaceae	*Malus communis* L.	15 April - 5 May
Ranunculaceae	*Adonis spp.*	20 April - 20 May
Brassicaceae	*Sinapis arvensis* L.	25 April -15 June
Fabaceae	*Onobrychis sativa* L.	1 May - 25 May
Rosaceae	*Rosa damascena* Mill.	20 May – 20 June
Apiaceae	*Pimpinella spp.*	25 May - 15 June

Some development traits of *B. terrestris* colonies under field conditions are given in Table 2. Young queens and males are needed to establish a nest in native fauna in the next generation. Therefore, the most critical characteristics related to the invasion of bumblebees are the total number of males and young queens produced in the colonies. According to results, colonies produced 39.50±14.2 young queens and 9.67±5.93 males under field conditions.

Table 2. Some growth characteristics of bumblebee colonies in open field conditions

Growth characteristic	N	Mean ± SEM	Min.	Max.
First male emergence time (days)	7	85.00±3.21	70.00	91.00
First gyne emergence time (days)	3	62.33±2.67	57.00	65.00
Switch point (days)	7	16.00±3.70	12.00	26.00
Competition point (days)	8	40.50±1.32	37.00	44.00
Total number of workers	10	55.70±9.78	26.00	110.00
Total number of young queens	6	39.50±14.20	13.00	95.00
Total number of males	3	9.67±5.93	1.00	21.00
Queen longevity (days)	10	89.90±4.59	80.00	112.00

In order to compare the results obtained in this study, the data obtained from the control groups of the other studies conducted by different researchers are given in Table 3. It is seen that fewer gynes and males are produced in colonies under field conditions compared to colonies that reared under laboratory conditions.

Table 3. Colony traits of captive *B. terrestris* colonies reported by different researchers

	Reported literatures			
Colony traits	Tuna, 2016	Gosterit and Oytun Cicek, 2017	Bulus, 2019	Ozansoy Aksoy and Gosterit, 2020
First male emergence time (days)	-	-	74.64±3.01	-
Switch point (days)	11.52±3.86	-	25.03±1.98	18.35±3.48
Competition point (days)	31.54±2.01	26.55±1.12	30.95±1.06	35.05±1.62
Total number of workers	147.8±12.50	108.73±6.51	118.08±7.28	203.40±11.90
Total number of young queens	42.61±5.33	29.44±4.55	108.40±10.50	162.20±13.70
Total number of males	42.61±5.33	34.94±7.57	75.05±9.10	27.53±6.75

Figure 1. Male and young queen production success of colonies in field conditions

Conclusion

The rearing of *Bombus terrestris* under controlled conditions is completely independent of nature. Both founder queens and colonies are kept under controlled environmental conditions and nutrition is provided to them unlimitedly. It is expected that colonies reared in the laboratory have some advantages over natural colonies in terms of their developmental characteristics and reproductive power due to optimum conditions in terms of nutrient source and physical environments. However, as before domestication, this species survived for years in the wild and still continues to live in nature. Our results showed that commercial bumblebee colonies can survive in natural fauna, maintain colony development and produce enough males and gynes to produce next generation or spread in nature. The results of the research have the quality to contribute scientifically to future studies about the invasive potential of bumblebees. In addition, it is also important to investigate some issues such as whether the young queens and males produced in colonies can mate, whether they can survive in diapause and found colony in following generations.

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RESEARCH PAPER

A Research on the Breeding Activities of Organic Beekeeping Enterprises

Tunay Kılıçin[1,*], Gökhan Akdeniz[1], Salim Aktürk[1], Dilek Kabakcı[2], Ümit Karataş[1], Soner Çankaya[3]

[1] Apiculture Research Institute Directorate, Ordu, Turkey
[2] Muş Alparslan University, Department of Animal Production and Technologies, Faculty of Applied Sciences, Muş, Turkey
[3] Ondokuz Mayıs University, Yaşar Doğu Sport Science Faculty, Samsun, Turkey

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***Corresponding Author**
Tel.: +905434222058
E-mail:
tunay.kilicin@tarimorman.gov.tr

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Abstract

With its low investment cost, beekeeping is an important agricultural activity that can be done without being connected to the soil and allows beekeepers to increase their income levels in a short time. On the other hand, organic beekeeping is a production model made only with allowed inputs without using drugs that are harmful to human health and whose every stage is controlled and certified from production to consumption. This study, it was aimed to examine the structure and production activities of organic beekeeping enterprises. The primary material of the study was data collected from 118 organic beekeeping enterprises. It was determined that 50.70% of the enterprises obtained the queen bee by natural methods, 23.4% by larva transfer, 26.6% from commercial queen bee enterprises, and 64.52% used pure Caucasian or Caucasian hybrid bee races in their colonies. The annual colony loss of the enterprises was 19.44%, and it was observed that colony loss was 50% higher in enterprises that obtained the queen bee commercially and bred it by natural means compared to enterprises that transferred larvae. It was determined that enterprises that considered organic beekeeping as the first profession had a higher number of colonies (P=0.007) and 25% fewer colony losses (P=0.088) than enterprises that saw it in the second and third orders. Finally, it was found that 53.69% of enterprises made additional feeding only with honey they produced, and the production of other organic bee products other than honey in enterprises was very limited.

Introduction

Organic beekeeping is a production model made only with allowed inputs without using drugs that are harmful to human health and whose every stage is controlled and certified from production to consumption (Korkmaz, 2001). Organic beekeeping can only be done in organic farming areas or pastures and plateaus where the natural structure is intact. Within a radius of 5-7 km, that is a bee flight distance or at least in a 3 km area that bees use very intensively, industrial facilities and their waste and treatment centers, highways, and waste incineration plants should not be present (Emsen & Genç, 2004). It is possible to perform organic honey and pollen production, especially in vast pasture areas, pine forests, and areas containing nectar-blooming plants, such as acacia, chestnut, and linden (Gökçe, 2002). One of the mandatory requirements of the transition from conventional beekeeping to organic beekeeping is to control the infrastructure residues released and honeycombs in the colony and meet the needs of beekeepers transitioning to organic beekeeping, such as organic beeswax (Gül, Şahinler, Akyol, & Şahin, 2005).

Table 1 shows that 70385 colonies, 387 organic beekeepers produced 1028 tons of organic honey in Turkey, according to the 2020 statistics of the General Directorate of Plant Production of the Ministry of Agriculture and Forestry (Anonymous, 2021).

In recent years, one of the most important actors of organic agriculture aimed at restoring the natural balance that has deteriorated and disappeared in the ecosystem is honey bees which are away from all kinds of inputs and elements that can be harmful to human health and whose every stage from production to consumption is controlled and certified. In addition, honey bees play a vital role in the ability of most plants to continue their generation and in the enrichment of the natural plant vegetation. The honeybee is considered an indispensable element of agriculture and used effectively in pollination is mandatory in Turkey. Thus, on the one hand, natural pasture areas with appropriate ecology and genetic richness are evaluated for beekeeping, while on the other hand, the quality and quantity in plant production are being increased (Cengiz, 2013). It is observed that producers who had started organic beekeeping returned to conventional beekeeping, especially during the transition process. Therefore, to meet certain conditions and overcome difficulties in the transition to organic beekeeping, scientific research is needed (Saner et al., 2011). This study, it was aimed to examine the structure and production activities of organic beekeeping enterprises.

Table 1. Data on organic beekeeping between 2010 and 2020

	Organic Beekeeping Data			Transition Period	
Year	Number of Producers	Colony Presence	Production (tons)	Number of Producers	Colony Presence
2020	387	70385	1028.39	107	18743
2019	249	50100	576.76	170	21484
2018	334	51742	493.89	121	17313
2017	304	45848	391.10	273	41014
2016	276	40731	349.00	364	35871
2015	322	38296	421.49	238	32680
2014	321	36391	280.00	213	22634
2013	279	32342	344.00	471	62836
2012	355	47065	516.83	395	45077
2011	205	19105	221.31	549	53554
2010	191	14699	208.15	225	13258

Materials and Methods

The main material of the study was data collected from the organic beekeeping enterprises, which were determined by the simple random sampling method, through conducting face-to-face interviews with the help of a survey. The population of the research was 696 organic beekeeping enterprises operating in Turkey. During the sampling process, the presence of colonies owned by organic beekeeping enterprises was used as a sampling criterion, and it was studied on a 10% margin of error and 95% confidence interval. The number of beekeepers to be surveyed was determined by using the following formula calculated by taking the number of hives as criteria and using the "simple random sampling" method (Yamane, 1967).

$$n = \frac{N(zC)^2}{Nd^2 + (zC)^2}$$

Where N is the number of beekeepers in the population, z is the standard normal distribution value corresponding to the desired degree of confidence (1.95), C is the coefficient of variation, d is the margin of error accepted in the study (±10%), and n is the number of beekeepers to be surveyed. Accordingly, the number of beekeepers to be surveyed was 192 with a 95% confidence degree and a 10% margin of error. It was found that 61.46% of enterprises (118) were engaged in organic production activities, 13.02% (25 enterprises) were in the transition period, 16.67% (32 enterprises) had left the transition period, and 8.85% (17 enterprises) had given up organic production. In this study, we used the surveyed data collecting from 118 actively carried out organic beekeeping enterprises.

In the evaluations of the data obtained from the enterprises participating in the survey study, some descriptive statistics such as arithmetic and weighted mean, n, and percentage (%) were used. Primarily, normality and variance homogeneity tests were applied to the continuous data of the study (P>0.05). Differences among colony numbers or loss of the enterprises by professional priority order variables were determined by one-way ANOVA and the Tukey's Multiple Comparison test. On the other hand, the Pearson correlation coefficient revealed the relationship between the properties studied in the study. In addition, the structure of the relationship between variables showing categorical properties was evaluated by chi-square (χ2) analysis (Fisher's exact test).

Findings and Discussion

The survey was conducted with 118 beekeepers and 97.46% of the interviewees were male and 2.54% were female. The mean age of beekeepers was 51.60, and while the oldest beekeeper was 74 years old, the youngest beekeeper was 28 years old. In terms of the educational level of the beekeepers, 1.69% were literate, 29.66% were primary school, 16.95% were secondary school, 27.12% were high school, 11.02% were college, and 13.56% were undergraduate graduates.

It was found that 61.46% of enterprises (118) were engaged in organic production activities, 13.02% (25 enterprises) were in the transition period, 16.67% (32 enterprises) had left the transition period, and 8.85% (17 enterprises) had given up organic production. 42.37% of the enterprises carrying out organic production were engaged in migratory beekeeping, and 57.63% were engaged in stationary beekeeping. It was revealed that enterprises had an average of 27 years of experience in beekeeping activities, and 1.65 years of this experience had been gained in the transition period and 5.64 years in organic beekeeping cultivation. In a study conducted in Gökçeada district of Çanakkale province, when agricultural activity, beekeeping, and organic beekeeping experiences of organic beekeeping enterprises were examined, it was determined that agricultural activity experiences were 16.1 years, beekeeping experiences were 8.9 years, and organic beekeeping experiences were 6.4 years on average (Özsayın, Tan, & Everest, 2018). In another study conducted in Kemalpaşa district of Izmir province, it was found that the experiences of beekeepers were 26 years in agricultural activities and were 14.6 years in organic beekeeping activities (Saner et al., 2011).

In this study, 75.21% of the enterprises said they kept records, while 24.79% said they did not keep any records. 19.47% of these enterprises stated that they kept records related to queen bee exchange, 19.47% related to diseases and pests,19.47% related to input amounts and application times of breeding, 6.84% related to information belonging to organic companies or other organizations, 7.37% related to prices, expenditures and costs, and 6.84% related to all stages of production. In the study, it was found that the record-keeping status in organic beekeeping enterprises did not change depending on the training status of beekeepers (χ^2 = 4.852; P=0.434).

59.83% of organic beekeepers found the services of the certification bodies adequate. The average number of visits of certification bodies to the organic beekeeping enterprises was determined as 2.21 days/year.

Factors that were effective on enterprises to decide to start organic beekeeping activities are given in Table 2. It was determined that in making this decision, their own experience had been effective at a rate of 27%, the officials of the Ministry of Agriculture and Forestry at a rate of 24%, and the foundations and chambers at a rate of 17%.

Table 2. Effective factors on the decision of the enterprises to start organic beekeeping activities

Preferences	Number of Enterprises (n)	Frequency (%)
My Own Experience	32	27
Officials of the Ministry of Agriculture and Forestry	28	24
Foundations and chambers	20	17
Neighbor Friend	19	16
Union Officials	10	8
Certification Firm Officials	7	6
Agricultural Consultants	2	2
Total	118	100

Sources of knowledge obtained by enterprises engaged in organic beekeeping related to the methods used in organic production are given in Table 3. In Table 3, it is seen that 18.47% of the enterprises obtained knowledge from the certification bodies which they worked together, 16.67% from the training, seminars and course programs they attended, 13.06% from books and brochures, 12.16% from leading beekeepers, 12.16% from the technical personnel of the Ministry, 9.91% via the internet, 7.66% from agricultural consultants, 5.41% from Union staff, and 4.50% from foundation and chamber representatives. A study conducted in Gökçeada District of Çanakkale province showed Provincial-District directorates of agriculture as the most important source of knowledge for organic beekeeping enterprises (Özsayın et al., 2018). In a study conducted in Kemalpaşa District of Izmir province, on the other hand, it was determined that most of the beekeepers received knowledge from the Beekeepers Association, universities, and district directorates of agriculture (Saner et al., 2011).

The production quantities of bee products and their rates are given in Table 4 below. It was found that the most produced bee products in the enterprises after honey are pollen, wax, propolis (bee glue), and royal jelly, respectively. In enterprises, other organic bee products other than honey were observed to be carried out at extremely limited levels.

Regarding of collected data of the field study, 118 enterprises produce 1395.86 kg honey and 21 enterprises have 185.48 kg pollen production. Moreover, other 16 enterprises have 17.40 kg propolis (Bee glue) production and 8 enterprises have been producing royal jelly an amount of 2.7 kg. Furthermore, 6 organic honey bee enterprises have 126 kg of wax production.

In the study, it was determined that 50.70% of enterprises obtained the queen bee by natural methods, 23.4% by larva transfer, and 26.6% from commercial queen bee enterprises. 13.68% of organic production enterprises stated that they changed the queen bee every year, 65.81% every two years, 17.95% every three years, and 0.85% every four years. 1.71% of enterprises, on the other hand, said that the colony had changed its queen. Races and genotypes of queen bees used by the organic beekeeping enterprises in their colonies are given in Table 5. 64.52% of the enterprises were determined to use a pure Caucasian or Caucasian hybrid queen bee race in their colonies.

8.39% of the enterprises reported using one of the Yigilca, Aegean, Mugla, Carpathian, Italian and Syrian queen bee races (Table 5). Additional feeding practices of the organic beekeeping enterprises are given in Table 6. 53.69% of the beekeepers stated that they made additional feeding only with honey they produced. It is observed that organic beekeeping enterprises use syrup and cake (41%) intensively in feeding activities.

Table 3. Professional knowledge sources of organic beekeeping enterprises

Knowledge Sources	Number of Enterprises (n)*	Frequency (%)
Certification Bodies	41	18.47
Training, seminars and courses	37	16.67
Books and brochures	29	13.06
Leading Beekeepers	27	12.16
Technical Personnel of the Ministry	27	12.16
Internet	22	9.91
Agricultural Consultants	17	7.66
Union Personnel	12	5.41
Foundation and Chambers	10	4.50
Total	222	100.00

* Multiple responses were given by the enterprises

Table 4. The producers of organic bee products and their rates

Organic Bee Products	Number of Enterprises (n)	Frequency (%)
Honey	92	77.97
Honey+Pollen	7	5.93
Honey+Propolis	3	2.54
Honey+Bee wax	1	0.85
Honey+Pollen+Propolis	5	4.24
Honey+Propolis+Royal jelly	1	0.85
Honey+Pollen+Bee wax	1	0.85
Honey+Pollen+Bee wax+Propolis	1	0.85
Honey+Pollen+Royal jelly+Bee wax	1	0.85
Honey+Pollen+Royal jelly+Propolis	4	3.39
Honey+Pollen+Royal jelly+Propolis+Bee wax	2	1.69
Total	118	100.00

Table 5. Races and genotypes of queen bees used in the organic beekeeping enterprises

Race and Genotypes	Number of Enterprises (n)*	Frequency (%)
Caucasian Hybrid	77	49.68
Pure Caucasian	23	14.84
Carniolan	17	10.97
Anatolian Bee	14	9.03
Other	13	8.39
Gokceada Bee	7	4.52
Buckfast Bee	4	2.58
Total	155	100.00

* Multiple responses were given by businesses.

Table 6. Additional feeding materials used in the organic beekeeping enterprises

Additional Feeding Type	Number of Enterprises (n)*	Frequency (%)
Honey	80	53.69
Cake + Syrup	36	24.16
Syrup	14	9.40
Cake	11	7.38
Not	8	5.37
Total	149	100.00

* Multiple responses were given by businesses.

To the question "What are the diseases and pests seen in your colony", most of the enterprises answered of "varroa parasite". The fight times of the enterprises with the varroa parasite, which leads to severe colony losses worldwide, are given in Table 7. When Table 7 is examined, it is seen that almost all of the enterprises are fighting against the parasite at the right time. In addition, colony loss in the studied organic beekeeping enterprises was determined as 19.44%.

Among the beekeepers participating in the study, the number of colonies of those who saw organic beekeeping as the first profession was found to be relatively high compared to those who saw it in the second, and third orders (*P*=0.007) (Table 8). It was determined that colony numbers of enterprises that consider organic beekeeping as the first profession were higher (*P*=0.007), and their colony losses were 25% less (*P*=0.088) compared to enterprises that see it in the second and third orders (Table 9). In terms of honey production, no significant difference was determined by the professional priorities (*P*=0.789).

33% of the enterprises that saw beekeeping as the first of the professional priority order started to look for alternatives in terms of product diversity. 18% of the enterprises increased their product diversity, while about 15% reduced their product diversity (*P*=0.05).

Table 7. Time for the organic beekeeping enterprises to against varroa

Fight Time	Number of Enterprises (n)	Frequency (%)
Early Spring - Late Autumn	65	55.08
Spring-Autumn	50	42.37
Not against varroa	2	1.69
When varroa mite is appear	1	0.85
Total	118	100.00

Table 8. Colony numbers of the enterprises by professional priority order

	n	Min	Max	Mean	Standard Deviation
First priority	77	9	500	136.13	117.113
Second priority	33	10	300	77.7	74.221
Third priority	6	30	70	43.67	17.224
No response	2	-	-	-	-

(*P*=0.007)

Table 9. Colony loss of the enterprises by professional priority order

	n	Min(%)	Max(%)	Mean(%)	Standard Deviation
First priority	72	2	80	16.90	15.097
Second priority	31	1	90	25.06	21.787
Third priority	5	10	30	21.00	8.944
No response	10	-	-	-	-

(*P*=0.088)

It was observed that enterprises that increased product diversity were stationary beekeepers generally (Table 10).

It was found that in organic beekeeping enterprises, while the number of colonies of enterprises using both larval transfer and commercial queen bee as queen bee obtaining method was maximum (289 colonies/enterprises), the numbers of colonies of enterprises obtaining queen bee only natural ways (81 colonies/enterprises) and only commercial way (87 colonies/enterprises) were minimum (P<0.001) (Table 11).

It was found that organic beekeeping enterprises that produce the queen bee by larval transfer had a higher number of colonies than those that did not. It was determined that although there were no statistically significant differences, the production of honey per colony in enterprises using only commercial queen bees (average 8 kg) was 50% less compared to enterprises producing queen bees by larval transfer (average 12 kg). Similarly, it was observed that colony loss was 50% higher in enterprises that obtained the queen bee via commercial ways and bred the queen bee by natural means compared to enterprises that performed the larval transfer. It can be seen in Table 12, honey production increases as the number of colonies increases in the studied organic beekeeping enterprises (r=0.255; P=0.004), on the other hand, honey production decreases in enterprises with more colony losses (r=-0.311; P<0.001). There appears to be any research exploring about organic beekeeping in Turkey to compare this study.

Table 10. Product diversity status by professional priority order

Priority Order		Product Diversity			Total
		Unchanged	Increased	Decreased	
First Priority	n	51	14	11	76
	%	67.10%	18.40%	14.50%	100.00%
Second Priority	n	26	1	1	28
	%	92.90%	3.60%	3.60%	100.00%
Third Priority	n	6	0	0	6
	%	100.00%	0.00%	0.00%	100.00%
No response	n	-	-	-	8

(P=0.05)

Table 11. Number of colonies by the method of obtaining the queen bee

Queen Bee Obtaining Method	n	Mean	Standard Deviation
Natural	51	80.94	99.455
Larval Grafting	26	168.77	118.316
Natural + Commercial Queen Bee	17	117.06	75.789
Commercial Queen Bee	16	86.63	42.594
Larval Grafting + Commercial Queen Bee	3	289.33	218.177
Natural + Larval Grafting	3	182.67	99.806
No response	2	-	-

(P<0.001)

Table 12. Relationship between colony number, colony loss, and honey production

		Colony Number	Colony Loss	Honey Production
Colony Number	r	1		
	P			
	N	142		
Colony Loss	r	-0.13	1	
	P	0.149		
	N	124	136	
Honey Production	r	.255**	-.311**	1
	P	0.004	0	
	N	124	128	138

Conclusion

In Turkey, organic beekeeping activities are carried out under the "Organic Farming Law" numbered 5262 (Anonymous, 2004), and the "Regulation on Principles and Implementation of Organic Agriculture" came into force with the Official Gazette dated 18 August 2010 and numbered 27676 (Anonymous, 2010). Organic beekeeping is a production model made only with allowed inputs without using drugs that are harmful to human health and whose every stage is controlled and certified from production to consumption. In the regulation of organic beekeeping, conditions for performing many activities such as colony development, additional feeding, disease and pest control, location selection, characteristics of colonies and queen bees, use of honeycomb frame, and harvesting are clearly stated. In this study, it was observed that the use of syrup and cake (41%) is performed intensely in the additional feeding activities of the enterprises. Concerning additional feeding, the regulation includes the following statement: "*Organic honey is used in feeding bee colonies during the spring period. In cases where climatic conditions accelerate the crystallization of honey, the use of sugar syrup or organic sugar molasses, produced by organic methods may be allowed by the authorized organization, instead of honey produced by an organic method in feeding.*" It was determined that the enterprises had difficulty accessing organic sugar and derivative products when they needed them. It was determined that about 65% of the enterprises used the Caucasian or Caucasian hybrid queen bee race in their colonies, while 15% of them used the queen bee races of foreign origin. It is considered that in terms of preserving and ensuring the sustainability of our native honey bee races and genotypes, our local bees should be kept at the forefront of production activities.

The organic beekeeping enterprises reported that the biggest threat was the *Varroa destructor* parasite regarding the diseases and pests' issue. It was observed that a large proportion of the enterprises correctly planned the time of spraying against varroa. It was also determined that some enterprises resorted to chemical methods in fighting this parasite. It is recommended that organic acids, which do not leave residues in bee products, should be used more widely in fighting against parasites by enterprises and that the dose, method of use, and location differences of the effective control organic acids should be supported by scientific studies. In this study, it was determined that record-keeping operation, one of the most important factors affecting success in beekeeping, achieving at a high level by the organic beekeeping enterprises. Annual colony losses of enterprises were found to be greater in enterprises that obtained the queen bee commercially and bred the queen bee by natural means than in enterprises that transfer larvae. It was determined that enterprises that considered organic beekeeping as the first profession had a high number of colonies and fewer colony losses compared to enterprises that saw it in the second and third orders. It was also observed the production of organic bee products other than honey was carried out at a very limited level that in the enterprises. It is believed that the popularization of organic production of bee products with high nutrient content, such as royal jelly imported from abroad, pollen, propolis, bee bread, and apilarnil, will make a significant contribution to the Turkish economy.

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REVIEW PAPER

The Effects of Propolis on Human Health

Özge Yılmaz Tekiner[1,*], Burcu Yeşilkaya[2]

[1]Istanbul Health Sciences University (SBU) Kartal Koşuyolu High Speciality Educational and Research Hospital, Istanbul, Turkey
[2] Okan University, Institute of Health Sciences, Department of Nutrition and Dietetics, Istanbul, Turkey

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***Corresponding Author**
Tel.: +905069804308
E-mail: ozgeyilmaz188@gmail.com

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Abstract

Propolis is a natural substance that honey bees collect from various plants such as poplar, palm, pine, conifer secretion, gum, resin, mucilage and leaf buds. Propolis gets its antioxidant and antimicrobial properties from its phytochemical content. The health-promoting mechanism of propolis appears to be related to its antioxidant and anti-inflammatory activity. The antioxidant activity of propolis is one of the most well-known properties. Flavonoids in propolis scavenge free radicals and protect the cell against lipid peroxidation. Propolis and its components prevent oxidative stress by increasing antioxidant enzyme activity and reducing lipid peroxidation. The first of the antibacterial effects of propolis is related to the direct action on the microorganism, the other to the stimulation of the immune system, which leads to the activation of the organism's natural defence system. The main components of propolis that offer anti-tumour potential are caffeic acid, phenethyl ester, chrysin, artepillin C, nemoroson, galangin, cardanol. These compounds are implicated in various genetic and biochemical pathways in cancer progression. At the same time, propolis has been thought to improve the inflammatory response in Covid-19 infection. Caffeic acid phenethyl ester (CAPE), a propolis component, is also known as an immunomodulating agent and helps reduce the excessive inflammatory response.

Introduction

Propolis is an apitherapeutic natural product, which is produced by honey bees in the hive after they are collected from the bark of trees, buds and sprouts of plants, does not dissolve in water, the colour changes according to the source and the age of the propolis, and is usually yellow, green and brown. Basically, it is an antiseptic that protects the beehive from microbial infections and prevents invaders from decomposing. In addition, propolis has been used in traditional medicine for centuries (Anjum et al., 2019; Gavanji & Larki, 2015). Many records show that propolis was used by the ancient Egyptians, Persians and Romans. S.C. 466-377 Hippocrates used propolis to heal external and internal wounds and ulcers (Kuropatnicki, Szliszka, & Krol, 2013).

Chemical Composition

Much work has been done on the chemical composition of propolis and more than 300 chemical compounds have been identified (Huang, Zhang, Wang, Li, & Hu, 2014). The chemical composition of propolis varies depending on the plants from which the resin, which is its main source, was collected, the climate, the harvesting season and the time elapsed after it was collected (Toreti, Sato, Pastore, & Park, 2013). Propolis contains resin, wax, pollen, organic compounds (Aliyazicioglu, Sahin, Erturk, Ulusoy, & Kolayli, 2013). Propolis gets its antioxidant and antimicrobial properties from the phytochemical content. Phenolic acids, flavonoids, esters, aldehydes, amino acids, vitamins, and minerals are some of the compounds (Batista et al., 2012). Propolis contains beneficial minerals as well as vitamins B1, B2, B6, C and E.

Propolis also contains several enzymes (Gomes-Caravaca, Gomez-Romero, Arraez-Roman, Carretero, & Gutierrez, 2006; Lotfy, 2006). CAPE is the main component of temperate propolis with extensive biological activity (Ahn et al., 2007). This variety of chemical composition provides an antibacterial function to propolis (Pamplona-Zomenhan, Pamplona, Silva, Marcucci, & Mimica, 2011).

Effects on Health

Propolis has many applications in the treatment of many diseases due to its phenolic compounds and various enzymes (Biesalski et al., 2009). The health-promoting mechanism of propolis appears to be related to its antioxidant and inflammatory activity. Clinical studies in animals and humans reported that propolis and its components have been generally well tolerated and non-toxic unless administered in very large amounts (Cornara, Biagi, Xiao, & Burlando, 2017). However, due to the limited number of clinical studies of propolis in humans, it has been reported that attention should be paid to the dosage. There are no adequate studies on the acute and chronic toxicity of propolis. The administration of propolis at doses of 200 and 5000 mg/g body weight/day did not cause toxic deaths in experimental animals, and it was reported that the safe dose not toxic to humans was 1.4 mg/kg body weight after necessary calculations were made (Alkis et al., 2015; Burdock, 1998). However, propolis consumption is not recommended for people who are allergic to bee venom or those with bronchial asthma (Menniti-Ippolito, Mazzanti, Vitalone, Firenzuoli, & Santuccio, 2008).

Antioxidant Effect

The antioxidant activity of propolis is one of the most well-known function. The majority of the results showed a reduction in oxidative stress markers. To reduce tissue damage caused by oxidative stress, antioxidant components have developed a protective mechanism (Bazmandegan et al., 2017; Kwon et al., 2004). Cellular metabolism produces reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), superoxide anion (O_2-), and reactive nitrogen species (RNS) (especially nitric oxide, NO) hydroxyl ion. While these reactive oxygen species are vital, they must be neutralized at the end of their activity. Phenolic compounds found in propolis inhibit ROS production and protect the cell against lipid peroxidation and DNA damage (Weaver et al., 2008). Propolis is developed a mechanism to prevent oxidative stress by reducing lipid peroxidation (Alkis et al., 2015). The main studies on the antioxidant properties of propolis were done in cell cultures or animals (Alkis et al., 2015; Mujica et al., 2017). There are few studies in the literature investigating the antioxidant effect of propolis on humans.

One study assessed the effect of oral administration of propolis solution (twice daily, 15 drops each time, 90 days) on oxidative stress and it was seen that the use of propolis increased the antioxidant capacity as well as improving oxidative stress (Mujica et al., 2017). Propolis has been reported to increase antioxidant capacity in diabetic rats (Zhang et al., 2014). In another study, 900 mg/day propolis administration for 18 weeks in people with Type 2 diabetes showed an increase in total polyphenol levels. However, propolis application did not change serum glucose. Based on these results, propolis was thought to affect oxidative stress in type 2 diabetics, but not diabetes parameters (Zhao et al., 2016). In studies conducted for the effect of propolis on Parkinson's disease, it has been emphasized that propolis protects neurons against oxidative stress and realizes this mechanism (Amira & Kunugi, 2020).

Antibacterial Effect

The antibacterial effect of propolis is characterized by its flavones content and the main flavones are pinocembrin, caffeic acid, phenethyl ester. The amount of these compounds is of great importance for antibacterial properties (Biesalski et al., 2009). The antibacterial effect occurs in two ways. Direct effect on microorganism is the first of these. The second is the activation of the organism's natural defence by stimulating the immune system (Sforcin & Bankova, 2011). Assuming that propolis and other bee products have antibacterial activity, their combination enhances this effect. This relationship to the combination of propolis and honey collected in Egypt has been studied and it was concluded that the combination of honey containing propolis increases the antimicrobial effect for *E. coli and S. aureus* (Al-Waili, Al-Ghamdi, Ansari, Al-Attal, & Salom, 2012). In another study, the effect of propolis on trophozoites was evaluated in vitro. It has been reported that it prevents the growth of Giardia duodenalis trophozoites from adhering (Freitas, Shinohara, Sforcin, & Guimarães, 2006).

Moreover, it was reported that propolis extract had an antimicrobial effect against gram-positive bacteria, but no effect against gram-negative bacteria (Arıkan & Solak, 2019). In another study, Turkish propolis showed antibacterial effects against gram positive (*Staphylococcus aureus, Bacillus cereus*) and gram negative bacteria (*Pseudomonas aeruginosa and Salmonella enteritidis*) (Baskan, Kilic, & Siriken, 2019). In a study conducted by Ceylan and Alic (2020), Bodrum Milas propolis region highly inhibited the *S.mutans* and *S.typhimurium* biofilm formation, especially at minimum inhibitory concentrations. In another study of propolis from different regions of Turkey's 11 was analyzed. It was found that propolis originated from the Marmara Region had a higher phenolic content and antioxidant capacity. This confirms that propolis differs according to the region, similar to other studies (Ozdal et al., 2019).

In a study conducted by Baltaş et al., propolis extract, one of the alternative approaches, was used to inhibit *Helicobacter pylori (H. pylori)* and hence treat many stomach diseases (Baltas, Yildiz, & Kolayli, 2016). It has been reported that regular consumption of propolis extract may contribute to a reduction in various types of diseases associated with *H. Pylori* (Sforcin & Bankova, 2011). Oral mucositis is one of the most common complications after chemotherapy or local radiotherapy or a combination of both. Propolis has been reported to be effective in the prevention and treatment of oral mucositis (Ozdal et al., 2019).

Antitumor Effect

Researchers have reported that propolis has an antitumor effect in vivo and in vitro (Sforcin, 2007). Some of the components of propolis that provide antitumor potential are caffeic acid, phenethyl ester, and chrysin. These components are involved in various genetic and biochemical mechanisms to prevent cancer progression (Patel, 2016). One study reported that propolis has potential for the treatment of breast cancer due to its anti-tumour activity in human breast cancer cells. Again, in the same study, it was reported that its use in high doses may have pro-oxidant effects, not antioxidants (Xuan et al., 2014). In another study, propolis has been shown to have a cytotoxic effect on lung cancer cells (Demir et al., 2015). In addition, studies have shown that raw, water-soluble propolis combined with chemotherapeutic agents potentially minimizes postchemotherapeutic reactions, maximizes enhanced immunity and increases treatment efficacy without drug interaction (Patel, 2016). In another study, it was reported that whether propolis interacts with chemotherapeutic drugs is an issue that needs to be investigated yet and its use during chemotherapy is contradictory (Münstedt & Männle, 2019).

In an in vitro study, the antiproliferative effects of propolis extract for human colon leukemia, prostate carcinoma and glioblastoma were evaluated and it was found that propolis extract provided a significant decrease in tumour cell concentrations (Oliveira Reis et al., 2019).

Effects on Asthma

One study showed that propolis, taken 75 mg/day for one month, can suppress the main clinical signs of asthma, including cough, shortness of breath, airway hyperresponsiveness and fever symptoms, and improve asthma control (Mirsadraee, Azmoon, Ghaffari, Abdolsamadi, & Khazdair, 2020). Physical examination revealed significant improvement in wheezing and the frequency of acute asthma attacks requiring emergency department or medications.

Although there is strong evidence of the potential effect of propolis on asthma in traditional medicine, no documented clinical experience of the effect of honey and propolis has been reported. In addition, the proposed mechanism of action of propolis on asthma has not yet been defined (Mirsadraee et al., 2020; Wanget al., 2009).

Studies on Covid-19

It binds to angiotensin converting enzyme 2 (ACE2), SARS-CoV-2, which is used as a resupper for invasion and replication in the host cell. This causes damage to the cell and also increases interpersonal transmission (Berretta, Silveira, Capcha, & Jong, 2020). Therefore, ACE2 inhibitors have been seen as useful drug alternatives for Covid-19. In the study by Güler, Tatar, Yıldız, Belduz and Kolaylı (2020), showed propolis extract inhibitory effects by binding to ACE2. Inhibition of PAK1 is one of the goals to prevent the damage of Covid-19. CAPE, the main component of propolis, is one of the PAK1 inhibitors. CAPE can prevent coronavirus-induced lung damage by inhibiting PAK1 (Maruta & He, 2020). One study aimed to develop a formulation to increase the antiviral effect of propolis against Covid-19. The results showed that the optimized formula of Propolis has a significant inhibitory effect against Covid-3CL protease compared to other propolis extracts. These findings have defined propolis as a promising treatment approach against Covid-19 (Refaat, Mady, Sarhan, Rateb, & Alaaeldin, 2020). Immunomodulation is desirable because the coronavirus dysregulates the immune response in the early stages of infection and facilitates virus replication. However, in the later stages of Covid-19, the body can develop an inflammatory response that can cause severe damage to the lungs and other organs, preventing that response through its CAPE-inhibiting mechanism of action and showing a protective effect against possible damage. Further research is needed on this subject (Berretta et al., 2020).

Conclusion

The health-promoting mechanism of propolis appears to be related to its antioxidant and anti-inflammatory activity. Flavonoid and phenolic contents in propolis add many biological activities to propolis. These properties of propolis differ according to the region and the hive. High phenolic content gives propolis powerful antioxidant and anti-inflammatory effect. It reduces intracellular oxidative stress by scavenging free radicals and prevents tissue damage and DNA mutations caused by free radicals. In addition, it is considered as an alternative method to reduce the inflammatory response and interpersonal contagion in Covid-19. The health effects of propolis have been shown mainly in animal studies. Therefore human studies are needed.

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REVIEW PAPER

Microbiota and Its Importance in Honey Bees

Ayse Ebru BORUM[1,*]

[1] Balıkesir University, Veterinary Medicine Faculty, Department of Microbiology, Balıkesir, Turkey

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***Corresponding Author**
Tel.: +905436780123
E-mail: ebruborum@balikesir.edu.tr

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Abstract

In recent years, research on human and animal health has emerged as a very important microbiota and microbiome. Microbiota; has important functions in metabolism, immune system, growth and development. In recent years, it has been understood that the microbiota is effective in the protection of bee health in colony so prevent losses in honey bees. Season, flora, food sources, age of the individual, duties in the hive, chemicals used in the fight against parasites and pathogens, and many other factors can be effective on the microbiome of honey bees.

Introduction

A larger organism or microbial community living in an intensive environment is called the "microbiota". Animals that live as a community and have social relationships often use a characteristic microbiota (Pascale et al., 2018). Microbiota is defined as a mixed ecosystem of microorganisms that are critical in various metabolic functions such as regulation of glucose and lipid homeostasis, energy management and vitamin production, and regulation of satiety (Greiner & Bäckhed, 2011; LeBlanc et al., 2013). It is effective in the production of metabolites and other substances that regulate various biochemical and physiological mechanisms. It has important functions in the functioning of the immune system and in stimulating immune responses, producing anticarcinogenic and anti-inflammatory activities also regulates induction of protective responses of the host against pathogens and harmless antigen tolerance (Molloy, Bouladoux, & Belkaid, 2012; Belkaid & Harrison, 2017; Agus, Planchais, & Sokol, 2018). The composition of the microbiota varies considerably, both between different species and within the same species. Microbiota diversity is affected by short-term changes in microbial communities and topographic differences. Specific microorganisms settle in specific environments during different growth and developmental stages of the host (Nicholson et al., 2012).

Characteristics

Insects are the most diverse animal species in terms of number of species, ecological habitats, and overall biomass. (Engel & Moran, 2013a). *Apis mellifera* has a high host adaptive microbial community. The honey bee microbiota has some similarities with mammals. But in reality, it is of a simpler composition (Zheng, Steele, Leonard, Motta, & Moran, 2018).

Young worker bees in a honeybee colony have few or no core gut microbiota (Dong et al., 2020). Microbial communities begin to colonize as they are fed orally by nurse bees (Martinson, Moy, & Moran, 2012; Motta, Raymann, & Moran, 2018). In the pupal stage, bacteria in the gut microbiome are excreted together with the intestinal epithelium. The next colonization is formed by contact with other bees in the hive and by trophallaxis. (Bleau, Bouslama, Giovenazzo, & Derome, 2020). Bacterial diversity in the gut microbiome reaches its maximum in 3-5 days after the bee matures into adulthood. Taxonomic changes occur after 3-8 days (Li et al., 2017). The rectal microbiome completes its development three days after the adult stage. The ileum is more variable and its final structure appears after 8 days. Prevalence of core species, associated alteration of ileum environment and host immune response are factors affecting this process (Anderson & Ricigliano, 2017).

Worker bees perform different tasks depending on age. New adult bees are generally responsible for hive cleaning and maintenance. Interactions between adult bees take place through social contact, cleaning and feeding behaviors. As a result, the microbiome of the species is formed (Powell, Martinson, Urban-Mead, & Moran, 2014; Li et al., 2017). The intestinal microbiota of *A. mellifera* worker bees were investigated within 0-40 days after hatching. It was determined that *Snodgrassella, Gilliamella* and *Frischella* species were colonized in the honey bee intestine from the 1st day. *Bifidobacterium, Commensalibacter,* and *Lactobacillus* colonize within 3 days, while *Gilliamella* is reduced simultaneously *Lactobacillus kunkeei* and *Bartonella* sp., colonize significantly in 12 days. *Shigella sp., Escherichia sp., Bacteroides sp.,* and *Porphyromonadaceae* 19 to 25 days, *Commensalibacter sp. and Bifidobacterium sp.* decreased at 25 days (Dong et al., 2020).

The microbiota of honey bees consists of microbial communities in different intestinal sections, also called the stomach, which is located between the esophagus and the ventriculus, which is used to store and transport nectar to the hive. Different microbial communities in the hindgut, ileum, lumen, and in the distal rectum form the honeybee microbiome (Vásquez et al., 2012; Engel & Moran, 2013a). *Parasaccharibacter* sp, which is located in the microbiome, is relatively abundant in worker hypopharyngeal glands (Corby-Harris et al. 2014). Adult worker bees are thought to have about nine bacterial species in their guts (Jones et al., 2017).

Similar to humans, the microbial communities of honey bees also contain anaerobic microorganisms. The shaping of bacterial species in the microbiota occurs through social interactions between colony individuals (Alberoni, Gaggìa, Baffoni, & Di Gioia, 2016). All bacterial species that make up the microbiota in honey bees can be grown under laboratory conditions, unlike the mammalian gut microbiome (Kwong & Moran, 2016a). As a result of 16S rDNA research, nine different bacterial species that make up 95-99.9% of the microbiota have been identified in the intestines of almost all worker bees (Jones et al., 2017; Moran, Hansen, Powell, & Sabree, 2012; Tola, Waweru, Hurst, Slippers, & Paredes, 2020). Two gram negative bacterial species that are members of the *Protobacteria* phylum have been identified. *Snodgrasella alvi*, a member of the *Neisseriaceae* family, is a type of bacteria that does not ferment sugars and forms films directly on the intestinal wall. The other, *Gilliamella apicola*, lives in the central areas of the lumen and has the ability to ferment sugar. It belongs to the *Orbaceae* family (Jones et al., 2017; Zheng, Powell, Steele, Dietrich, & Moran, 2017; Tola et al., 2020). Gram-positive bacteria in the *Firmicutes* phylum are abundant in many environments. *Lactobacillus* Firm-4 and Firm-5 living in the distal rectum are among these bacterial species. (Jones et al., 2017; Zheng et al., 2017). Most adult workers also have much smaller amounts of *Bifidobacterium asteroides*. (Kwong and Moran, 2016a; Bleau et al., 2020). These bacteria are the most important microorganisms in the honey bee gut and are called "core bacteria" (Kešnerova et al., 2020). Less dense species than *Proteobacteria* are; *Commensalibacter* sp. (Alpha 2.1) and *Bartonella apis* (Alpha 1); *Parasaccharibacter apium, Bombella apis , Bombella mellum, Bombella favorum* (*Acetobacteraceae* family, Alpha 2.2), *Apibacter adventoris*, *Apibacter mensalis* and *Frischella perrara* (Orbaceae family) (Kešnerová, Moritz, & Engel, 2016; Kwong & Moran, 2016b; Jones et al., 2017; Kwong, Steele, & Moran, 2018; Bleau et al., 2020; Dong et al., 2020; Tola et al., 2020; Hilgarth, Redwitz, Ehrmann, Vogel, & Jakob, 2021). Four *Lactobacillus* species, 2 *Gilliamella* species, 1 *Bifidobacterium* species and 1 *Snodgrassella species*, which constitute the intestinal core microbiome of honey bees, were determined. *Bartonella* sp. and *Frischella* sp. are non-nuclear members of the honeybee gut microbiome that may vary depending on the environment. *Parasaccharibacter apium* is a sporadic species in honey bees. This species is often isolated from both the worker's and queen's gut, and from environments such as honey and bee bread (Martinson et al., 2011; Anderson et al., 2013; Anderson, Rodrigues, Mott, Maes, & Corby-Harris, 2016). *Lactobacillus* Firm-4 and Firm-5 are actual symbionts of the gut and are rarely isolated outside of bee guts. Other *Lactobacillus* species, such as *Lactobacillus kunkeei*, can be found inside and in hive materials (Olofsson, Alsterfjord, Nilson, Butler, & Vásquez, 2014; Milani et al., 2018; Raymann et al., 2018a).

In ileum; *Lactobacillus* Firm-4 and Firm-5, *Snodgrasella alvi*, *Bifidobacterium* sp. *Gilliamella apicola*, typical core bacteria, while *Parasaccharibacter apium*, *Frischella perrara*, *Bombella apis*, *Bartonella apis*, *Apibacter advantoris* and *Apibacter mensalis* are less common. In the honey crop part, there are fewer environmental bacteria species with *Apilactobacillus kunkei* predominant. The midgut microbiome is unstable. In the rectum, *Bifidobacterium* sp., *Lactobacillus* Firm-4 and Firm-5 are the dominant species (Subotic et al., 2019; Kešnerová et al., 2020).

Lactic acid bacteria (LAB) are an important part of the microbiom in honeybees as in other animals (Piccart, Vásquez, Piepers, De Vliegher, & Olofsson, 2016). The microaerophilic environment of the honey bee digestive system is an ideal environment for sugars from nectar and lactic acid bacteria with a temperature of 35°C (Iorizzo et al., 2020a). LAB plays a role in many different functions that have positive effects on the host. LAB in the microbiota prevents the colonization and invasion of the intestine by competing with the pathogens for the food in the environment (Iorizzo et al., 2020b). Metabolism products such as carbon dioxide, organic acids, hydrogen peroxide or ethanol produced by the microbiota play an important role in defense against pathogens (Serna-Cock et al., 2019). LAB also produces bacteriocins. They can biosynthesize many different types of antagonistic molecules (Alvarez-Sieiro, Montalbán-López, Mu, D., & Kuipers 2016). As a component of the microbiota, LAB participates in important interactions in immunomodulation (Foligne et al., 2007). LAB increases anti-inflammatory and pro-inflammatory cytokines. LAB components can directly induce the immune system. It has been determined that LAB also affects lipid metabolism (Kishino et al., 2013). LAB effectively protects both human and animal intestinal epithelial cells from enteric viral infections. *Lactobacillus rhamnosus* and *Lactobacillus casei* Shirota are the most effective species for protection. It has significant antiviral effects on *Lactobacillus fermentum, Lactobacillus plantarum, Lactobacillus pentosus* and *Enterococcus faecium* (Maragkoudakis, Chingwaru, Gradisnik, Tsakalidou, & Cencic, 2010).

Thirteen bacterial species belonging to *Lactobacillus* and *Bifidobacterium* genera were determined in honey bees. *Lactobacillus kunkei* was found to be the most dominant species (Vásquez et al. 2012). The honey bee microbiota and bacteria isolated under hive environmental conditions are presented in Table 1.

Functions

The gut microbiome is involved in the growth, development, and reproduction of insects. It has important contributions to their metabolism. These microorganisms enhance effective digestion, promote the absorption of food and synthesize essential nutritional compounds. (Pernice, Simpson, & Ponton, 2014). The insect gut microbiome is often limited due to the lack of interaction between individuals. However, in social insects such as honey bees, the microbiome is more various. Social interactions ensure the diversity of gut microorganisms. Thus, social insects such as honey bees have a gut microbiome that has important functions in nutrition and protection (Engel & Moran, 2013b).

Metabolic Functions

The gut microbiota of honey bees is as important as the mammalian microbiome. Intestinal microbiota has very important functions especially in nutrient biosynthesis and biomass degradation. In nutritional function, the insect microbiome has been proven to help produce nutrients such as vitamins and amino acids that are not found in food. In biomass degradation and digestion, it was determined that the release of cellulolytic enzymes responsible for hydrolysis and the activity of microorganisms increase (Shi, Syrenne, Sun, & Yuan, 2010; Anderson & Ricigliano, 2017; Belkaid & Harrison, 2017; Zheng et al., 2017).

The gut microbiota has important functions in the digestion of lipids and proteins, detoxification of secondary plant compounds. It also has positive effects on insect resistance to insecticides, while positively affecting survival, development and egg production (Jing, Qi, & Wang, 2020).

Compared to the gut microbiota of other animals, the honeybee microbiota has effective functions in specific adaptations to a sugar-rich diet. It provides sugar uptake pathways of various phosphotransferase systems to the honeybee. Most carriers are classified in the mannose family. Nectar contains traces of mannose, but becomes highly toxic when ingested at higher concentrations. Therefore, this feature of bacteria is very important (Engel & Moran, 2013b). The bee microbiome enriches the host with arabinose flow permeases. This family of carriers plays a role in the transport of different compounds such as sugars, antimicrobial proteins, and amino acids. Various carriers protect against various pesticides applied in agriculture (Engel & Moran, 2013a). In addition, the intestinal microbiome plays an important role in the conversion of nectar into honey and bee secretions into propolis with its fermentation properties. It is also responsible for the freshness of honey (Pachila, Ptaszynska, Wicha, Olenska, & Małek, 2017; Silva et al., 2017).

LAB, which constitutes an important group of bee microbiota, contributes to the nutrition of honey bees. It has been suggested that bacteria of the genus *Bifidobacterium*, *Simonsiella* and *Lactobacillus* can produce acetic acid, a waste product of carbohydrate metabolism. The microbiota supports the diet of bees by aiding the digestion of these compounds. After consumption, food is stored in the rectum for quite a long time. Thus, extra nutrients are obtained from rectal bacteria during wintering (Martinson et al., 2011).

This effect of the microbiota on body weight gain causes an increase in the level of vitellogenin, which is responsible for the regulation of nutrition in honey bees, by making insulin and insulin-like signal changes. This is associated with changes in gene and endocrine signal expression (Ihle, Baker, & Amdam, 2014; Zheng et al., 2017). In a study, it was determined that *Bifidobacterium asteroides* stimulated the production of juvenile hormone and host-derived prostaglandin derivatives, which are effective in bee growth (Kešnerová et al., 2017).

Intestinal microorganisms can also prolong the lifespan of insects. It was determined that the life span of *Drosophila melanogaster* increased significantly after probiotic and symbiotic applications (Westfall, Lomis, & Prakash, 2018). The microbiome synthesizes enzymes such as glycosidases and proteases. These enzymes synthesize the vitamins necessary for the host, metabolize indigestible polysaccharides, and are responsible for xenobiotic metabolism. With these features, the microbiota contributes significantly to the biochemical activities of the host (Sommer & Bäckhed, 2013).

Carbohydrates and oligosaccharides that cannot be digested by the host are fermented by microbiome bacteria of the genus *Bifidobacterium*, *Bacteroides*, *Faecalibacterium*, and *Roseburia* to form short-chain fatty acids (SCFAs) such as propionate, acetate, and butyrate (Jandhyala et al., 2015; Wang et al., 2020). These SCFAs formed provide rich energy sources to the host. The resulting butyrate prevents the accumulation of toxic by-products for metabolism (Jandhyala et al., 2015). Pollen is an important bee food. It is the most important source of vitamins, minerals, amino acids, and fats. Most of these are absorbed by the host midgut. Compounds that are the most difficult to digest, such as cellulose, emicellulose, pectin, remain. These compounds are degraded by the hindgut microbiota (Mollet, Leroux, Dardelle, & Lehner, 2013).

Immune System Functions

Honeybee microbiota has important functions in regulating and stimulating the immune system of honey bees and in the formation of an effective immune response. Microbiota species are very effective in the development and morphogenesis of the immune system (Egert & Simmering, 2016; Schroeder & Bäckhed, 2016; Kwong, Mancenido, & Moran, 2017). Microorganisms alter the intestinal environment, limiting the growth of insect parasites and stimulating the host immune system. Additionally, species in the microbiota produce antimicrobial peptides that play an important role in control and defense against bacterial pathogens and parasites. The overall microbiome or *S. alvi* colonization alone increases the regulation of the antimicrobial peptides apidaesin and hymenoptaesin in intestinal epithelial cells (Azambuja, Garcia, & Ratcliffe, 2005; Kwong et al., 2017). Antimicrobial peptides are important innate immune components that play a role in the destruction of pathogenic microorganisms. A high increase in these antimicrobial peptides was detected in honey bees treated with probiotics (Kwong et al., 2017). *F. perrara* is a member of the microbiota in the majority of honey bees. It was determined that the host immune response increased considerably by colonizing the pylorus, which is the midgut ileum transition region (Powell et al., 2014).

The largest surface area in contact with foreign antigens originating from the external environment is the intestinal microbiota. It therefore plays a primary role in mucosal immunity (Sekirov, Russell, Antunes, & Finlay, 2010). The microbiome controls the overgrowth and migration of microorganisms in mucosal immunity, while preventing the induction of harmful systemic immune response (Purchiaroni et al., 2013). It can affect the host by altering the composition of the gut microbiome. *Ascosphaera apis*, *Nosema* sp., *Paenibacillus larvae*, *Melissococcus plutonius*, and *Serratia marcescens*, such pathogens cause significant infections in honey bees. LAB, an important member of the microbiota, is highly effective in protecting against these pathogens. (Wu et al., 2014; Arredondo et al., 2018; Iorizzi et al., 2020b; Peghaire et al., 2020).

Bacteria constituting the microbiome control intestinal homeostasis through various mechanisms including lipopolysaccharide, peptidoglycans, and flagellin. These structures interact with cell receptors in the toll like pathway. This mechanism activates intracellular signaling pathways associated with cell survival, inflammatory response, replication, and apoptosis (Evans et al., 2006; Valentini et al., 2014; Yiu, Dorweiler, & Woo, 2017).

The gut microbiome is involved in xenobiotic metabolism. This ability ensures the preservation of the microbiome, which is a necessary condition for drugs used in the treatment of various diseases to be effective (Bäckhed et al., 2004; Vergnolle, 2016).

Honey bees are frequently exposed to a wide variety of pesticides. The microbiota also plays an important role in the detoxification of xenobiotics, particularly neonicotinoid insecticides. Microbiota microorganisms stimulate the expression of detoxification enzymes. Thus, an effective endogenous detoxification occurs in the host, increasing thiacloprid and fluvalinate resistance (Wu et al., 2020).

Social and Individual Behavior Functions

It has been proven by various studies that gut microbiota and brain compatibility. This means that gut microorganisms cause changes in host neurophysiology and insect behavior (Westfall et al., 2018; Leger & McFrederick, 2020).

Microbiota species can alter both the volatile profiles and olfactory behaviors of the host. As a result, they regulate the way individuals make decisions regarding interacting, gathering in groups, foraging, and mating through chemical communication. In addition, intestinal microorganisms increase the host's memorization and learning capacity. Thus, it also affects the neurophysiological development of the host by supporting memory (Liberti & Engel, 2020). It has been determined that the gut microbiome of honey bees influences the neurophysiology and behavior of their hosts. Levels of biogenic amines such as octopamine, dopamine, and serotonin can be altered by microbiota species, affecting host behavior. The levels of these compounds in the brain of worker bees change seasonally. Levels of the compounds increase during the summer months, when foraging activity is at its highest. The brain activity of newly developed bees, whose microbiome is not yet fully formed, is significantly lower than that of older adults (Harris & Woodring, 1992). Microbiota plays an important role in the regulation of social behavior in honey bees (Vernier et al., 2020).

Factors affecting honey bee microbiota

Many factors affect the microbiome, such as the host species, feeding preference, host habitat, and the host life stage. Especially the high diversity of plants causes changes in the honey bee microbiome, especially *Proteobacteria* and *Firmicutes* species (Jones et al., 2017).

Many pesticides such as imidacloprid, coumaphos and chlorothalonil have serious adverse effects on bee health. It also causes significant problems by negatively affecting the structure and function of the microbiome. It also increases susceptibility to opportunistic pathogens. Honey bees are exposed to pesticides through contaminated water, nectar and pollen. It was determined that the amount of *Lactobacillales* decreased significantly in honey bees exposed to chlorothalonil. Non-lethal doses of insecticides such as thiamethoxam, fipronil, imidacloprid and coumaphos caused *Bifidobacterium* sp. and *Lactobacillus* sp. has been found to greatly reduce the number of (Rouzé, Moné, Delbac, Belzunces, & Blot, 2019). The gut microbiome of honey bees exposed to glyphosate was adversely affected, affecting the total bacterial count in the gut and *Bifidobacterium* sp., *Lactobacillus* (Firm-4 and Firm-5), and decreased in *S. alvi* (Motta et al., 2018). Nitenpyram, a neonicotinoid insecticide, has caused significant changes in the microbiome community. Due to the insecticide effect, important metabolic changes have occurred in the host and the effectiveness of the immune system has decreased (Zhu, Qi, Xue, Niu, & Wu, 2020). Pesticides such as imidacloprid and thiacloprid, *Bifidobacterium* sp., *Frischella* sp. and *Lactobacillus* (Firm-4 and Firm-5), produce numerous adverse changes in various species, causing intestinal dysbiosis (Daisley et al., 2017; Diaz, Del-Val, Ayala, & Larsen, 2019).

Honeybee gut core microbiota is also affected by global seasonal changes (Rouzé et al., 2019). While the amount of Enterobacteriaceae decreased in autumn, Neisseriaceae increased (Bleau et al., 2020). The lowest diversity in the microbiome was detected in winter (Purchiaroni et al., 2013).

As a result of the high-fat honey bee diet containing palm oil, the amount of *Gilliamella* sp. in the microbiome increased significantly, while the rate of *Bartonella* sp. decreased (Wang et al., 2021).

Antibiotics can affect the host by causing changes in the species that form the gut microbiota. (Daisley et al., 2020). Antibiotic applications cause a decrease in bacterial species in the honeybee microbiota. This weakens the immune system of bees and increases their susceptibility to infections (Raymann, Bobay, & Moran, 2018b). In one study, the microbiome was destroyed as a result of antibiotic administration. As a result, the expression of genes encoding antimicrobial peptides decreased and honey bees became more susceptible to *Nosema ceranae* infection (Li et al., 2017). In another study, disruption of the gut microbiota by tetracycline was caused by the opportunistic pathogen *Serratia* sp. increased infections and shortened the life span of bees (Bonilla-Rosso & Engel, 2018).

Air pollution, microplastics and heavy metals also negatively affect the honeybee gut microbiome composition (Mutlu et al., 2018; Costa et al., 2019; Rothman, Leger, Kirkwood, & McFrederick, 2019).

Conclusion

Honey bee microbiota and microbiome are very important for growth, development, metabolism and immune system. It is especially effective in inhibiting pathogens in bee health. Misfeeding, mistakes in colony management and beekeeping practices, unnecessary and continuous antibiotic applications affect the microbiota negatively. Feeding and practices that support and strengthen the microbiota are extremely important for healthy, strong and highly productive beekeeping.

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