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RESEARCH PAPER

Palynological, Antioxidant and Physicochemical Properties of Pollen Loads from Eastern Anatolia

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Abstract

Bee pollen is a very important bee product with its wide biological content as well as being a protein source in the nutrition of larvae and young bees. In our study, palynological, antioxidant and some physicochemical analyses were performed on bee pollen loads taken from some provinces of the Eastern Anatolia Region of Turkey. According to the palynological analysis, pollen grains belonging to 62 plant taxa were observed. The highest and the lowest value of total phenolic content were determined as 15.05±0.46 and 9.81±0.59 µg GAE/ mg, respectively. Antioxidant analysis of DPPH radical scavenging activity was performed. As a result, the best activity was found at 16.93±0.92 µg TE/mg. The pH, electrical conductivity and L, a b values of the samples were found to be 4.62±0.015-4.86±0.005, 2371.0±22.6-3008.0±22.9 mS/cm, 56.6±0.02-59.9±0.01, 11.5±0.01-12.2±0.02, 50.9±0.01-53.1±0.03, respectively. In this context, it can be said that the physicochemical and biological activities of bee pollen samples vary depending on the plant and geographical origin.

Introduction

Interest in the biological activity of foods is increasing day by day. Considering the polyphenol, vitamin and mineral content, the importance of the biological activities of bee products has been known from past to present (Bobiş et al., 2010). In addition, the value of them increased because of the viral pandemic that society faced after 2019. The immune-enhancing effect of bee products is emphasized (Lima et al., 2021).

Pollen load is one of the crucial bee products for young worker bees and larvae because of its high protein content and as a food supplement for human beings (LeBlanc et al., 2009). The honey bee (*Apis mellifera* L.) collects pollen from flowers' stamens of seed plants and combines this pollen with their oral secretions into pellets. Each pollen load has characteristic features such as color, size, morphology and aroma specific to the flower type (Yang et al., 2013). The botanical source and chemical composition of pollen load have a significant impact on its color. Bee pollen is named unifloral when it's derived from a single botanical source and its biochemical and organoleptic features are similar to those of the plant where it was collected. When bee pollen consists of several plants' pollen, it is called multifloral (Modro et al., 2009). Palynological analysis is the best method for determining the plants used by honey bees as pollen sources. The results of palynological evaluation also make it possible to determine the plant taxa that are used by bees as pollen forage in the regions (Atanassova & Lazarova, 2010).

Bee pollen content varies depending on geographical origin, botanical source and beekeeping practices. In addition, storage conditions significantly affect this. Bee pollen contains carbohydrates, lipids, fatty acids, polyphenols, carotenoids, vitamins and minerals (Genc & Dodologlu, 2002). Considering the chemical content, pollen loads are accepted as a complete food source in terms of nutrition. Pollen loads have a variety of activities, including antioxidants, antimicrobial, anti-inflammatory, and anticancer properties. Biological activities of pollen loads effects from phenolic compounds, flavonoids, vitamins and carotenoids (Kostić et al., 2020). Studies have found that bee pollen contains polyphenol groups such as quercetin, myricetin, rutin, tricetin, luteolin, selagin, isorhamnetin (Carpes et al., 2008; Freire et al., 2012).

In vivo studies the positive effects of pollen loads on health have increased. It was determined that they support the antioxidant system by increasing the levels of superoxide dismutase and glutathione in the blood and brain (Khalil & El-sheikh, 2010). Considering the chemical content that changes depending on the geographical and floral source, it is important to determine the biochemical activities and physicochemical properties of bee pollen.

With oxygen respiration, molecules known as free radicals are formed in the body. Free radicals carry unpaired electrons in their outer orbitals. Therefore, they tend to bond easily. Antioxidants can prevent many possible damage in the body by reacting with free radicals. For example, antioxidants; It is effective in preventing oxidative stress, which is one of the leading causes of important diseases such as cancer, cardiovascular problems, diabetes, gastrointestinal diseases (Tsao & Deng, 2004)

In this study, it was aimed to determine the palynological, antioxidant and some physicochemical properties of bee pollen samples taken from the provinces of Bitlis, Muş and Tunceli in the Eastern Anatolia Region of Turkey.

Materials and Methods

Bee pollen samples that were used in this study were obtained from the provinces of Bitlis, Muş and Tunceli from Eastern Anatolia Region of Turkey in 2021. Pollen loads were ground into powder before analysis. Bee pollen powders were stored at -20˚C before analysis.

Palynological Analysis of Bee Pollen

Palynological analysis of pollen loads was performed with minor revisions to the method proposed by Barth et al. (2010). 2 g pollen load was dissolved in 13 mL 70% ethanol solution. After resting the solution for 30 minutes to homogenize the pollen loads it was kept in the sonicator for 5 minutes. Pollen loads containing substantial oil, were subjected to ethanol extraction twice after centrifugation. The sediment was diluted in a 1: 1 water / glycerin mixture. A drop was taken from the well mixed pollen suspension and that was applied to the microscope slide by glycerin gelatin with basic fuchsin. Microscope slides were examined using a Leica DM 2500 brand microscope and pollen grains were determined by 60x and immersion objectives (100x). More than 500 pollen grains were counted on each slide in order to provide an evaluation of the relative abundance for every pollen type (number of pollen grains from species /total number of pollen grains). Pollen types were classified as predominant pollen (PP) (≥45%), secondary pollen (SP) (>16–44%), important pollen (IP) (3–15%), and minor pollen (MP) (<3%) based on relative abundance (Barth et al., 2010).

pH and Electrical Conductivity of Bee Pollen

2.5 g of bee pollen powder dissolved in 10 mL of distilled water. The solution was homogenized by mixing well and left it for 30 minutes. Then, pH and electrical conductivity values were recorded with a digital pH meter (Adaškevičiūtė et al., 2019).

Color Analysis of Bee Pollen Samples

Color analysis of bee pollen samples was carried out using the Minolta color measurement device. The powder form of pollen samples was placed in the device chamber and the results were recorded. Color results are given with L, a, b values (Adaškevičiūtė et al., 2019).

Extraction of Bee Pollen Samples

Extraction of bee pollen samples was carried out for antioxidant and total phenolic analyses. 2 g of powdered pollen sample was weighed into falcon tubes and dissolved in 20 mL of 70% ethanol. The prepared solutions were incubated at 40˚C for 1 hour in an ultrasound device. After 1 hour, the solutions were centrifuged at 3600 rpm for 15 minutes. The supernatant was removed and passed through filter paper. Filtered extracts were stored at -80˚C until analysis (Almeida et al., 2017).

Determination of Total Phenolic Content

The total phenolic content of bee pollen samples was determined spectrophotometrically based on the Folin-Ciocalteu method. It was prepared using 0.2 N folin reagent and 7.5% Na_2CO_3 distilled water. The solutions of bee pollen samples obtained as a result of extraction were diluted and 25 mg/mL concentration of pollen samples was used. Gallic acid was used to prepare the standard curve. Dilutions in the range of 7.8 µg/mL-500 µg/mL were prepared using 1 mg/mL gallic acid stock solution. After the solutions were pipetted into the falcon tubes, they were kept in the dark for 2 hours, and absorbance values were recorded at 760 nm wavelength. The procedure was repeated three times. The gallic acid equivalent of bee pollen samples was calculated using the gallic acid curve (Saroglu, 2018).

DPPH Analysis

DPPH radical scavenging assay was used to determine the antioxidant activity of bee pollen samples. A 0.1 mM DPPH solution was prepared with ethyl alcohol. The dilution process was applied to the solution obtained by bee pollen extraction. Pollen solutions with a concentration of 5 mg/mL were used in the analysis by dilution. According to different trolox concentrations, standard curve was generated. The prepared solutions were kept in the dark for 45 minutes after pipetting. Finally, absorbance values were recorded with a spectrophotometer at a wavelength of 517 nm. The procedure was carried out in 3 repetitions. Results are given as trolox equivalent (Freire et al., 2012).

Statistical Analysis

Each bee pollen sample was analysed in triplicate. Results are shown as arithmetic mean values ± standard deviation. Statistical analyses were carried out using R studio (R Core Team, 4.1 Version, 2020).

Results and Discussion

Palynological analysis was performed to determine the plant sources of pollen loads. As a result of the analysis bee pollen loads were classified into 4 groups according to the relative abundance (RA): predominant pollens (PP) (≥45%), secondary pollens (SP) (16–44%), important pollens (IP) (>3–15%) and minor pollens (MP) (<3) (Table 1). If pollen grains RA ≥80%, this indicates the botanical origin of bee pollen and it is named unifloral. In this study all pollen samples were accepted as heterofloral. In total, 64 pollen taxa distributed into 30 families were found in the pollen samples (Table 1).

Table 1. Pollen taxa identified from pollen loads and their RA

	Muş	Tunceli	Bitlis 1	Bitlis 2
Adoxaceae				
Sambucus	1.02 (MP)	1.41 (MP)		
Asparagaceae				
Muscari			2.28 (MP)	5.95 (IP)
Amaryllidaceae				
Allium				0.37 (MP)
Apiaceae	1.27 (MP)			1.12 (MP)
Chaerophyllum	3.81 (IP)	0.20 (MP)		
Daucus			2.54 (MP)	
Eryngium			0.51 (MP)	
Ferula		6.02 (IP)		1.12 (MP)
Heracleum			0.25 (MP)	0.37 (MP)
Pimpinella	0.25 (MP)		0.25 (MP)	
Asteraceae				
Achillea				1.86 (MP)
Anthemis	0.25 (MP)			
Artemisia				0.37 (MP)
Centaurea			0.76 (MP)	
Centaurea urvillei			5.08 (IP)	
Cirsium			0.25 (MP)	
Cyanus	1.27 (MP)	9.04 (IP)	0.76 (MP)	
Carthamus	0.25 (MP)			
Inula	2.03 (MP)		0.25 (MP)	
Silybum		0.20 (MP)		
Taraxacum		0.20 (MP)	1.02 (MP)	
Brassicaceae	7.61 (IP)	1.41 (MP)		
Brassica		21.29 (SP)		
Isatis	0.76 (MP)	2.41 (MP)		
Boraginaceae				
Echium	0.25 (MP)	0.80 (MP)		
Caryophyllaceae				
Silene				0.74 (MP)
Cistaceae				
Cistus	12.69 (IP)	12.05 (IP)		
Helianthemum	5.08 (IP)			

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Table 1. Pollen taxa identified from pollen loads and their RA *(continued)*

	Muş	Tunceli	Bitlis 1	Bitlis 2
Convolvulaceae				
Convolvulus			0.51 (MP)	1.12 (MP)
Crassulaceae				
Sedum		2.61 (MP)		
Ericaceae	0.25 (MP)			
Fabaceae				
Astragalus	0.51 (MP)	3.01 (IP)		
Lathyrus	0.25 (MP)	0.20 (MP)		0.37 (MP)
Melilotus		1.00 (MP)	0.76 (MP)	7.43 (IP)
Onobrychis		16.87 (SP)		
Trifolium pratense				1.12 (MP)
Trifolium	2.79 (MP)	0.20 (MP)		
Fagaceae				
Quercus	15.74 (SP)	0.60 (MP)	3.81 (IP)	5.95 (IP)
Fumariaceae				
Fumaria	1.02 (MP)			
Hypericaceae				
Hypericum			9.14 (IP)	15.61
Juglandaceae				
Juglans		0.20 (MP)	2.03 (MP)	1.86 (IP)
Lamiaceae	0.25 (MP)	0.20 (MP)		
Lamium	4.31 (IP)			0.37 (MP)
Moraceae				
Morus	0.51 (MP)		1.02 (MP)	0.74 (MP)
Papaveraceae				
Papaver	22.59 (SP)	2.21 (MP)		0.37 (MP)
Plantaginaceae				
Plantago	0.76 (MP)	0.20 (MP)	0.51 (MP)	
Polygonaceae				
Rumex	0.51 (MP)	0.20 (MP)		
Poaceae		0.80 (MP)	0.25 (MP)	
Poa	0.76 (MP)			
Ranunculaceae				
Nigella				0.37 (MP)
Ranunculus	2.28 (MP)			0.37 (MP)
Rhamnaceae				
Paliurus spina christi	0.76 (MP)			
Rosaceae	0.76 (MP)	4.02 (IP)		0.74 (MP)
Crataegus	1.52 (MP)			
Potentilla	0.51 (MP)			
Pyrus			5.08 (IP)	3.72 (IP)
Rosa		0.20 (MP)		
Sanguisorba	0.51 (MP)	0.20 (MP)	0.51 (MP)	
Rutaceae				
Citrus	1.27 (MP)			
Salicaceae				
Salix	3.30 (IP)	12.05 (IP)	60.41 (PP)	47.96 (PP)
Scrophulariaceae				
Verbascum	2.28 (MP)			
Xanthorrhoeaceae				
Eremurus		0.20 (MP)	2.03 (MP)	

In this study, the most common pollen grains from the plant families of Salicaceae (30.93%), Fabaceae (8.63%), Brassicaceae (8.37%) and Cistaceae (7.45%) were found in pollen samples, respectively. The results found are compatible with the literature (Bay et al., 2021). Taxa of *Salix* sp., *Papaver* sp., *Cistus* sp. appear with high incidence in bee pollen samples. The pollen diagram shows the percentage of pollen grains found for each taxa. According to the diagram, pollen loads was divided into two groups according to the similarity between the plant taxa determined as a result of the melissopalynological analysis. The first group consists of Bitlis 1 (1), Bitlis 2 (2) and the greatest similarity was found in this group. The second group Muş (3) and Tunceli (4) pollen loads, contained 15 common plant taxa.. *Salix* sp., *Hypericum* sp. and *Quercus* sp. plant taxa pollen grains were most common in Bitlis 1 and Bitlis 2. Pollen grains of *Cyanus* sp., *Brassica* sp., *Cistus* sp., *Onobrychis* sp., *Quercus* sp., *Papaver* sp., *Salix* sp. plant taxa were found in Muş and Tunceli. Pollen grains belonging to the *Cistus* taxon were found in the pollen loads taken from Muş and Tunceli (Figure 1). This suggests that the beekeepers who produce these pollens can do wandering beekeeping. Because *Cistus* taxon does not belong to the Eastern Anatolian bee flora.

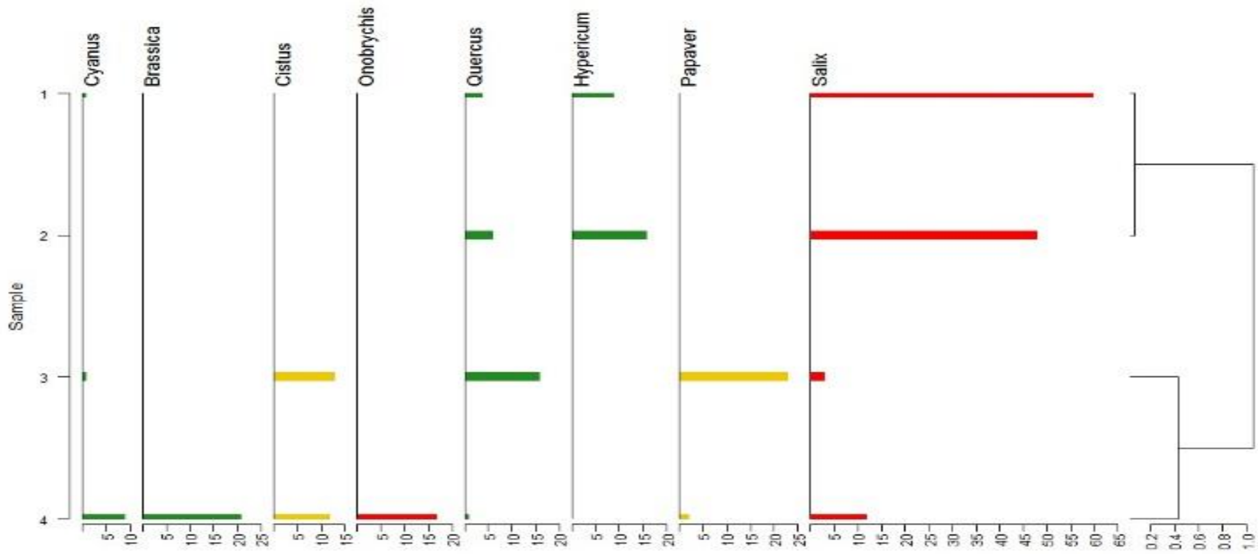

Figure 1. Pollen diagrams of pollen loads

According to PCA of melissopalynological analysis, there are two significant axes. The first principal component (PC1) explains 57.1% of the total variance, while the second component (PC2) explains 40.7%. Fig.2 shows that *Onobrychis, Brassica, Cyanus, Cistus, Papaver* taxa constitute the positive part of PC1. All plant taxa except, *Quercus, Papaver, Cistus* constitute the positive part of PC2.

The pH, electrical conductivity and color values of bee pollen samples are summarized in Table 2.

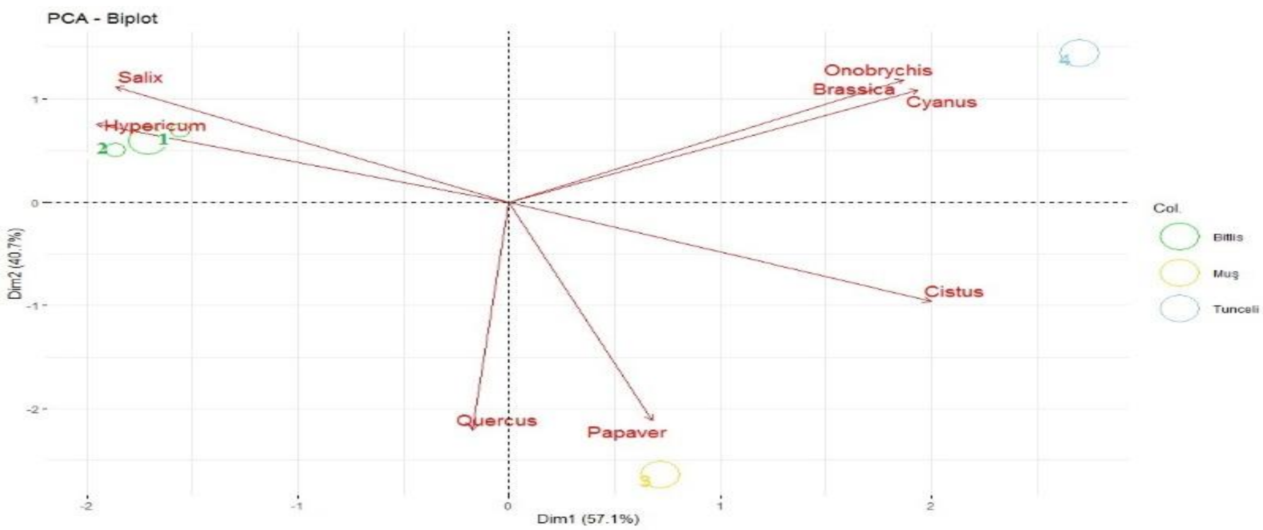

Figure 2. PCA biplot for melissopalynological analysis

Table 2. Physicochemical parameters of bee pollen sample

Bee Pollen Samples	pH	Electrical Conductivity mS/cm	L*	a*	b*
Bitlis 1	4.86 ± 0.005	2830.7 ± 48.7	58.3 ± 0.02	11.5 ± 0.01	51.6 ± 0.01
Bitlis 2	4.80 ± 0.015	2933.7 ± 55.3	57.4 ± 0.01	11.8 ± 0.02	51.3 ± 0.03
Mus	4.62 ± 0.015	2371.0 ± 22.6	56.6 ±0.02	12.2 ± 0.02	50.9 ± 0.01
Tunceli	4.70 ± 0.006	3008.0 ± 22.9	59.9 ± 0.01	12.0 ± 0.00	53.1 ± 0.03

The pH values of pollen samples varied between 4.62±0.015 - 4.86±0.005. The lowest pH was observed in the pollen sample from the province of Muş, while the highest pH was observed in the pollen sample from the province of Bitlis. However, the pH values of the pollen samples were very close to each other and the pollen showed acidic properties. The highest electrical conductivity (EC) value was found to be 3008.0 mS/cm in the Tunceli sample. The EC of the pollen samples was in the range of 2371.0-3008.0 mS/cm and the lowest EC was determined in the pollen sample of Muş province. EC value is a concept related to the presence of organic acids, proteins, sugars and minerals. The EC values of the analyzed pollen samples were found to be relatively high. This suggests that pollen samples may be a good source of organic acids and minerals. In the study investigating the physicochemical properties of bee pollen samples collected from countries such as Italy, Denmark, Poland, Spain, and Lithuania, pH values were found in the range of 4.3-5.2, similar to our study. The EC values were found to be 444-836 µS/cm and these values were found to be well below the pollen samples examined in our study (Adaškevičiūtė et al., 2019). In a study examining bee pollen samples from Portugal, pH values were close and found in the range of 4.5-5.1 (Feas et al., 2012). Color analysis of bee pollen samples using the Minolta color device. The results are given in Table 2. in terms of L*, a*, b*. The L* value was found to be between 56.6±0.02 - 59.9±0.01, the a* value was between 11.5±0.01 - 12.2±0.02, and the b* value was between 50.9±0.01 - 53.1±0.03. The L* value is related to lightness-darkness, and it was found to be higher than 50 in all of the pollen samples examined, so it can be said that all of the samples are light colored. A* is red (+)-green (-), and b* is yellow (+)-blueness (-). It can be said that the color of the pollen samples is close to red according to the a* value, and the color of the samples is close to yellow according to the b* value. As a result, in general, pollen samples can be said to be red-yellow light colored. It is seen that the color values of the pollen samples belonging to the provinces examined are close to each other (Figure 3).

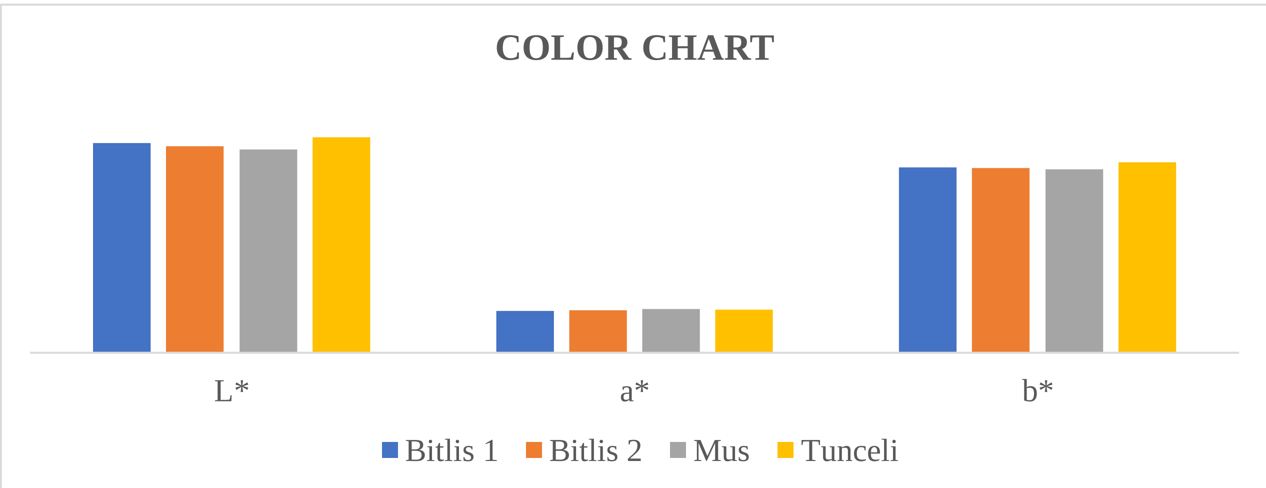

Figure 3. Color chart of bee pollen samples

This situation can be associated with the fact that the pollen samples were obtained from nearby provinces and those provinces belonged to the same region. In a study examining the color values of 9 different pollen samples taken from the Eastern Black Sea Region Bayburt province, the L* value was determined in the range of 73.1-74.9. These values were higher than the pollen samples that were examined in our study. In addition, the a* value was determined in the range of 1.05-2.44, and the b* value was determined as 56.13-58.47. According to these values, it can be said that the samples have less redness and more yellowness than the pollen samples in our study (Saroglu, 2018). Thus, it can be thought that the color values of pollen samples may change depending on the changing regional differences.

Total phenolic contents (TPC) of bee pollen samples were determined as gallic acid equivalents and DPPH free radical scavenging activity was determined as trolox equivalents and is given in Table 3.

Table 3. TPC and DPPH activity of bee pollen samples

Bee Pollen Samples	Total Phenolic Content (TPC) µg GAE/ mg bee pollen	DPPH µg TE/ mg bee pollen
Bitlis 1	15.05 ± 0.46	15.94 ± 0.15
Bitlis 2	14.41 ± 0,59	16.93 ± 0.92
Muş	9.81 ± 0.59	13.38 ± 0,64
Tunceli	11.57 ± 0.48	15.42 ± 0.93

The phenolic contents of the pollen samples were found to be between 9.81±0.59 µg GAE/mg and 15.05±0.46 µg GAE/mg. Among the pollen samples, the lowest and the highest total phenolic content belonged to Muş and Bitlis 1 respectively. In a study examining the pollen samples obtained from Bayburt province, the highest total phenolic content was found to be 7.69 mg GAE/g, and this value is lower than in our study (Saroglu, 2018). For *B. napus* subsp. *napus* L. pollen, the TPC value was found to be 1383.67 mg.kg^{-1} (Fatrcová-Šramková et al., 2013). In another study examining the bee pollen from Portugal and Spain, TPC was detected in the range of 18.55-32.15 mg GAE/g (Pascoal et al., 2014). Considering the studies, it can be thought that the total phenolic content varies depending on the plant and geographical source of the bee pollen. In addition, within the scope of our study, it is seen that the phenolic content of pollen changes very little with regional proximity. The DPPH free radical scavenging activities of bee pollen samples were between 13.38±0.64 and 16.93±0.92 µg TE/mg bee pollen. While the Muş sample showed the lowest activity, the Bitlis 2 sample showed the highest activity. In parallel to the total phenolic content of the Muş sample, DPPH activity was also found to be low. In a study examining bee pollen from Portugal and Spain, DPPH activities were found in the range of 2.98-6.69 mg/g extract. It is seen that the DPPH activities of the pollen samples we used in our study were higher than in this study (Pascoal et al., 2014). According to the PCA of physicochemical analysis, there are two significant axes. The first principal component (PC1) explains 63.9% of the total variance, while the second component (PC2) explains 30.9%. Fig.4 shows all parameters except a* constitute the negative part of PC1. TPC, pH, DPPH constitute the positive parts of PC2.

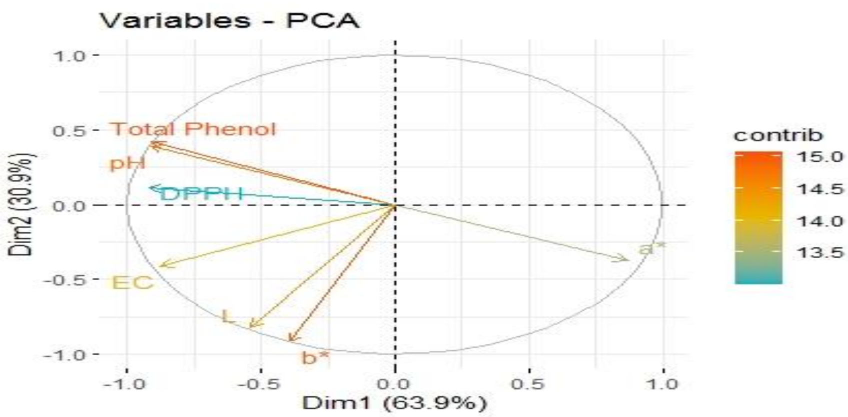

Figure 4. PCA biplot of physicochemical analyses of pollen loads

Conclusion

Bee pollen load content varies depending on geographical origin, botanical source and beekeeping practices. According to palynological analysis, the pollen content of the samples obtained from Bitlis reflects the Eastern Anatolian flora, but the samples obtained from Muş and Tunceli do not. Muş and Tunceli samples were purchased from the market and Bitlis'samples were obtained from beekeepers. In our study, the pollen grains belonging to *Cistus* sp. were determined in the pollen loads from Muş and Tunceli provinces. *Cistus* taxon is not one of the bee plants belonging to the Eastern Anatolia region. It has been proven once again with our study that it can be determined whether the bee products sold in the market belong to that region or not by palynological analyzes. Many different test methods were used to determine the antioxidant activity of pollen samples. Each of these methods is a very important indicator for determining the antioxidant activity of the bee pollen loads. According to the results obtained in this study demonstrated that bee pollen possesses good antioxidant activity suggesting that it could be useful in prevention of diseases in which free radicals are implicated.

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RESEARCH PAPER

A Seasonal Perspective of Nosemosis; the Presence of *Nosema* spp. in Honeybee Colonies During Summer Months in Ankara, Turkey

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Abstract

Nosemosis is known as a serious disease of adult honey bees, *Apis mellifera* L. (Hymenoptera: Apidae) caused by *Nosema apis* and *N. ceranae* which are obligate intracellular microsporidian parasites. The parasites infect the epithelial cells of honey bee ventriculum and lead to critical changes in midgut mucosa that cause digestive and metabolic disorders. Accordingly, the infestation causes the death of adult honey bees and leads to great economic losses for beekeeping industry worldwide. Seasonal patterns of nosemosis are consistent and mostly observed in the spring or autumn months, with the highest spore counts and viability. The aim of this work was to evaluate the seasonality of nosemosis in the light of previous literature and specifically investigate the presence of *Nosema* spp. during the summer season including June, July, and August from various locations in Ankara. Honey bee individuals were collected from 80 apiaries located in 14 different areas in Ankara. The samples were analyzed from pools of ten adult honey bees per population using digestion methods. Before analysis, the anesthesia was induced by cold (30 sec, -80 ℃) on the bees. According to obtained data, 12 out of 80 (15%) sampled apiaries were infected with *Nosema* spp. spores. Infected apiaries were mainly located in the central and north parts of Ankara. Results show nosemosis might be detrimental to honey bee colonies and its productivity in the summer months. Therefore, the treatment might be needed when infections of *Nosema* spp. reach to an infectious level even in summer.

Introduction

Microsporidia are eukaryotic, obligate intracellular parasites that invade vertebrates and invertebrates. They are spore-forming organisms and classified as fungi. Spores are the infective stage of microsporidian parasites and keep them surviving outside of the host (Adl et al., 2005; Higes et al., 2006). The western honey bee, *Apis mellifera* L. (Hymenoptera: Apidae), is mostly infected by two microsporidia species, *Nosema ceranae* (Fries et al., 1996) and *Nosema apis* (Zander, 1909) (Microsporidia: *Nosema*tidae), causing *Nosema* disease. Although *N. ceranae* and *N. apis* both infect honey bees, *N. ceranae* dominates its distribution geographically in the world (Fries et al., 2006; Higes et al., 2013b).

Nosemosis is known as one of the most significant diseases of adult honey bees. The infections possess decreasing honey production, foraging behavior, and pollination productivity (Higes et al., 2006). In severe cases, it can cause bee mortality and even colony collaps (Cox-Foster et al., 2007; Higes et al., 2008; Martín-Hernández et al., 2007).

Nosema spp. are transmitted orally with contaminated nectar, pollen, honey, and bee feces (Smith, 2012). When the spores of *N. ceranae* reach the infected level, infections lead to cytopathological changes in the midgut epithelial cells by degenerating columnar cells, disrupting microvilli, damaging goblet cells. Additionally, *N. ceranae* inhibits programmed cell death, apoptosis by inducing the genes involved in apoptosis in order to prevent cell death to limit pathogen growth (Ceylan et al., 2019; Higes et al, 2013a; Kurze et al., 2018; Martín-Hernández et al., 2017).

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Since the first description of *N. ceranae* in 1996 (Fries et al., 1996), the prevalence, distribution, and seasonality have been determined and showed variability of seasonal patterns in the world. However, seasonal patterns of Nosemosis are consistent and mostly observed in the spring or autumn months, with the highest spore counts and viability (Brenna et al., 2012; Gisder et al., 2010; Traver and Fell, 2011). On the other hand, a study from Spain reported that *N. ceranae* raised the number of winter hive losses (Martín-Hernández et al., 2007). Rarely, consistent high levels of summer infection prevelance were demonstrated (Pernal et al., 2010). Many significant gaps remain in our understanding of the variations in seasonal patterns of Nosemosis.

This study investigates the seasonal pattern of *Nosema* spp. infection in the light of literature and focuses on the presence of Nosemosis in honey bee colonies during the summer months in Ankara, Turkey.

Materials and Methods

Collection of Bee Samples

Samples were requested from beekeepers in the different locations of Ankara, which are registered in the Bee Registration System. Adult bee samples collected in the study were taken from a total of 80 hives from 23 beekeepers who agreed to participate in the study from Ankara districts (Ayaş, Bala, Beypazarı, Çankaya, Çubuk, Gölbaşı, Güdül, Kalecik, Kazan, Kızılcahamam, Nallıhan, Polatlı, Haymana, Yenimahalle). Sample was taken once from the hives in which beekeepers thought there was a disease and deaths were seen in June, July and August months. In order to detect the presence of nosemosis, 50 bee samples per hive were taken from the outermost frame of the hive.

Detection and Counting of *Nosema* spores

Counting was performed with a Neubauer slide for the detection of *Nosema*. For the preparation of samples for *Nosema* counting, 10 bees were randomly taken from the groups after inducing the anesthesia by cold (30 sec, -80 °C) (Tutun et al., 2020). The abdomens of the bees were separated from their bodies with the help of forceps and collected in a container. Abdominal pieces collected in the container were crushed to allow the intestinal contents to come out. A homogeneous mixture was obtained as a result of crushing using 1 mL of distilled water per bee. Then, the body parts were separated by filtering with 3 layers of gauze patch. Furthermore, the mixture was placed in 15 mL centrifuge tubes and centrifuged at 5000-6000 rpm for 10 min. The supernatant was removed from the tube and counting was performed by adding 1 mL of distilled water per bee (Güzerin, 2013; Terrestrial Manual of the OIE, 2018).

Safranin (1%) and Giemsa (5%) stained smears were prepared for a more detailed examination. For both methods, the smears were firstly fixed with 100% methanol. Then, they were stained with Safranin and Giemsa, 30 and 45 min., respectively. Stained smears were rinsed with water, air-dried, and examined under a light microscope with 100x objective and immersion oil.

Results

Within the scope of this research, bee samples were collected from different locations in Ankara to determine the level of nosemosis infestation in the summer. *Nosema* agent was found to be positive in 12 out of 80 hives in total, and the infestation rate was determined to be 15% in the collected samples. Nosemosis positive samples were obtained from Çubuk, Gölbaşı, Kalecik, Kazan, Kızılcahamam and Yenimahalle districts (Figure 1).

Discussion

Although Nosemosis caused by *N. cerenae* and *N. apis* is known as a serious disease of adult honey bees worldwide, some gaps still remain in our understanding

Figure 1: Map of sampling locations of Honey bee populations from beekeeping areas in Ankara. The colors, green, red indicate the positive and negative samples, respectively.

of their biology, particularly its seasonal features. Therefore, the current study aimed to provide information about the seasonal features of nosemosis, focusing on summer status in Ankara, Turkey. The occurrence of *Nosema* infection was first described in 1986 in Turkey (Tutkun & Inci, 1992), and, so far, the infestation rate has been reported in different ratios such as 26.4%, 60%, 45.8% in 1990, 2005, 2016 respectively (Kutlu & Kaftanoğlu, 1990; Aydın et al., 2001; Özkırım et al., 2019). One of the studies conducted in Ankara showed the existence of nosemosis and 93.75% prevalence of *N. ceranae* and 6.25% *N. apis* infection (Utuk et al., 2016).

The results of previous studies showed that seasons influence the abundance of nosemosis in hives. A high spore abundance was detected in spring and autumn (Brenna et al., 2012; Gisder et al., 2010; Traver & Fell, 2011). On the other hand, rare studies have existed about *Nosema* spp. infection in winter and summer stuation (Martín-Hernández et al., 2007; Pernal et al., 2010). According to the findings of a present study, *Nosema* infections have still appeared and are a problem in the summer months in line with Pernal et al. (2010) and Martín-Hernández et al. (2009).

In Turkey, Basar (1990) reported that the maximum level of infection was seen in spring and winter in the Trakya region, Muğla and İstanbul. Another study conducted in the winter season in Hatay reported a 10% *Nosema* infection between 2010 and 2011 (Muz et al., 2012). In addition, the highest infestation level was observed in June and July in the Eastern Black Sea region of Turkey (Tosun & Yaman, 2016). In our study, 15% (12 out of 80) prevalence was determined during the summer season in Ankara, Turkey. Additionally, infected apiaries were mainly located in the central and north parts of Ankara.

Conclusion

Detection of nosemosis in the hives is very important to prevent colony losses and financial damage. Even though the presence of *Nosema* infection is mostly known in spring and autumn, summer infection prevalence was demonstrated rarely. The results of the present study provide a minor evidence for summer infection. Therefore, more attention should be paid to the presence of infestation and treatment necessity in all-season including summer.

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RESEARCH PAPER

Influence of Different Classes of Insecticides on Honey Bee Survival

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Abstract

Pesticides used to prevent or control unwanted pests are often being considered as a cause of the decline of the honey bee (*Apis mellifera*) population. Exposure to insecticides, or a group of pesticides, has many negative effects on honey bees. Here we elucidated whether feeding carbon microparticles, designed to absorb pesticide residues, improved to the survival of honey bees exposed to pesticides. Honey bees were exposed to different classes of insecticides (thiamethoxam, chlorpyrifos, and carbaryl) for 10 days. The study shows that feeding carbon microparticles didn't ameliorate the survival of honey bees exposed to insecticidal compounds.

Introduction

Pesticides are an indispensable part of modern agriculture because of their role in reducing pest numbers, improving yields, and quality of the crops (Damalas & Eleftherohorinos, 2011). However, the extensive use of pesticides in agricultural areas is a major concern for honey bees that are integral for pollination service a wide variety of plants (vanEngelsdorp & Meixner, 2010). Since forager bees visit many pesticide treated crops to gather pollen and nectar, more than one hundred different residues of pesticides including miticides, insecticides, fungicides, and herbicides have been identified in hive products (Mullin et al., 2010). Such exposure to pesticides is a well-known factor that causes colony health problems and leads to population decline or loss (Doublet et al., 2015; Schneider et al., 2012; Urlacher et al., 2016; Yang et al., 2008)

Insecticides are the main risk group of pesticides to honey bees. In modern agriculture, different classes of insecticides including organophosphates, neonicotinoids, and carbamate have been using against target organisms and each class has a different mode of action on honey bees. These insecticides used to control insect pests do not only affect the target organism but can also affect numerous beneficial insects, such as the honey bee, when exposed to these insecticides in treated fields.

Mitigating the exposure to pesticides is important, but unintended sublethal exposure also poses a risk for honey bees. Therefore, we investigated the functionality of a newly developed carbon microparticle materials that was designed with the goal to adsorb ingested pesticide residues for the purpose of protecting bees. The carbon microparticles can be mixed into the sugar syrup used to feed honey bee colonies (US Fed News Service, 2018).

The goal of this study was to determine the effects of carbon microparticle on honey bee survival while exposing bees to three widely used insecticides. Our study revealed carbon microparticle food did not negatively or positively affect honey bee survival under pesticide exposure during laboratory experiment.

Materials and Methods

Chemicals

Thiamethoxam (THX), Chlorpyrifos (CHL), and Carbaryl (CRB) (all insecticides 99% purity) were purchased from Sigma–Aldrich (Missouri, United

States). Initially, the powder form of each insecticide was solved in acetone (40 mg/L) to prepare a stock solution. Then the stock solutions were diluted into 50% W/V sugar syrup to a final concentration of 40 µg/L. The percentage of stock solution in the syrup was equal to 0.1% (V/V).

Experimental design of laboratory exposures

Unknown age worker honey bees were collected from frames of a colony located in a WSU apiary site in Pullman, WA and transported to the laboratory. Worker bees were randomly distributed into plastic cages (14 x18.1 x11.1 cm) containing approximately 70 bees each cage. The cages were held in an incubator at 27 ± 2°C and 70% relative humidity during the experiment.

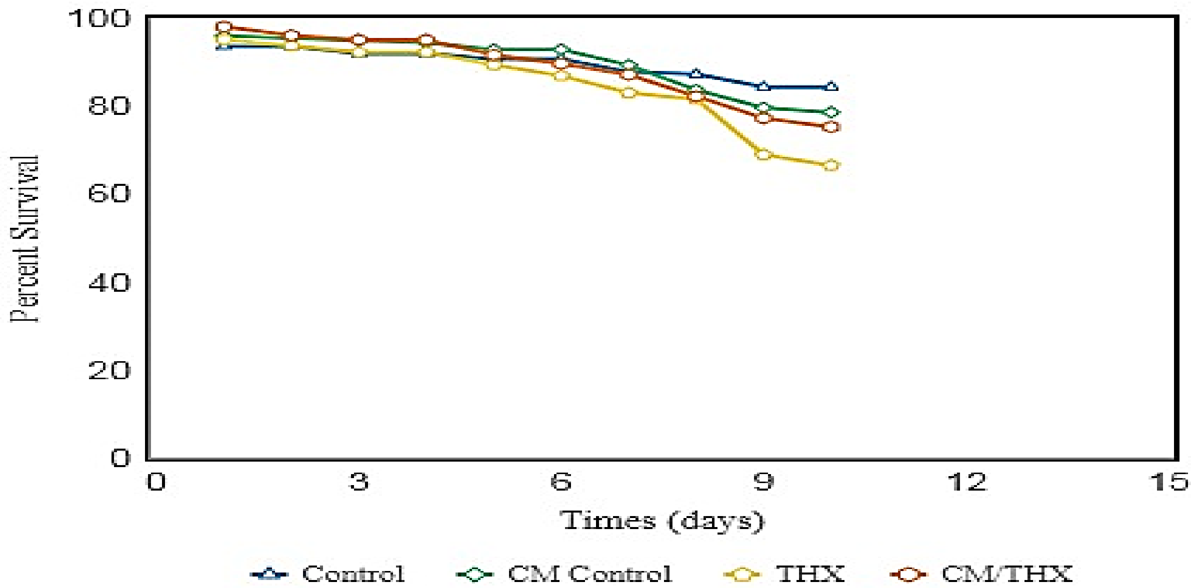
Honey bees in cages were subjected to one of eight treatments over 10 days. Each treatment group was comprised of three replicate cages. Two of the groups were treated as controls, with one group fed just sugar syrup (50% W/V) and the other was fed syrup containing 0.1% carbon microparticles. Three of the groups (TXM, CHL, and CRB) were fed the syrup (50% W/V) at first for 12 hours and then the feeders in the cages were swapped with the feeders containing 40 µg/L thiamethoxam, chlorpyrifos, and carbaryl for 12 hours. Feeder swapping was repeated over 10 days for each 12 hours period. As for CM treatments, unlike the previous three groups, these three groups (CM/TXM, CM/CHL, and CM/CRB) were fed with syrup containing 0.1% CM at first for 12 h period. Then, the groups were fed the syrup containing 40 µg/L thiamethoxam, chlorpyrifos, and carbaryl for 12 h period until the next feeder change during the experiment. Feeders in the cages were measured to estimate average consumption of the syrup for per bee for the last five days of experiment. Dead bees in the cages were counted every day for 10 days.

Statistical analysis

Analyses were conducted in the online application for survival analysis (OASIS 2) program. The 10 day honey bee survival data among groups were analyzed using Kaplan-Meier survival analysis. Differences between survival curves of treatment and control groups were determined using Log-Rank test. The gallic acid equivalent of bee pollen samples was calculated using the gallic acid curve (Saroglu, 2018).

Results

In this experiment, We recorded the survival of honey bees fed with CM food while exposing them to three different insecticides (Thiamethoxam, Chlorpyrifos, and Carbaryl). The percent survival in the control and CM/control groups were 84.30% and 78.57%, respectively over 10 days (Figure 1). There was no significant difference between the survival of control and CM/control (*P*>0.05 by log-rank test) (Table 1).



Control: Group of honey bees fed without carbon microparticles. CM Control: Group of honey bees fed with carbon microparticles. THX: Group of honey bees exposed to thiamethoxam and fed without carbon microparticles. CM/THX: Group of honey bees exposed to thiamethoxam and fed with carbon microparticles.

Figure 1. Honey bee survival ratio comparisons between the control groups and treatment groups exposed to thiamethoxam.

Table 1. Survival analysis between groups treated with and without carbon microparticles by Kaplan-Meier Procedure.

	n	Survival (%) at 10 d	Mean	S.E.	95% CI	*P* (Log -Rank)
Control	172	84.30	9.12	0.18	8.75-9.16	1
CM/Control	196	78.57	9.19	0.15	8.90-9.48	
THX	206	66.50	8.83	0.17	8.50-9.16	0.86
CM/THX	202	75.24	9.12	0.14	8.84-9.40	
CHL	232	79.74	9.01	0.16	8.69-9.63	0.27
CM/ CHL	251	75.29	9.04	0.14	8.76-9.31	
CRB	210	49.04	8.17	0.19	7.80-8.53	0.17
CM/CRB	255	53.72	8.51	0.16	8.19-8.83	

Control: Group of honey bees fed without carbon microparticles. CM/Control: Group of honey bees fed with carbon microparticles. THX: Group of honey bees exposed to thiamethoxam and fed without carbon microparticles. CM/THX: Group of honey bees exposed to thiamethoxam and fed with carbon microparticles. CHL: Group of honey bees exposed to chlorpyrifos and fed without carbon microparticles. CM/CHL: Group of honey bees exposed to chlorpyrifos and fed with carbon microparticles. CRB: Group of honey bees exposed to carbaryl and fed without carbon microparticles. CM/CRB: Group of honey bees exposed to carbaryl and fed with carbon microparticles.

The average consumption of the syrup was 0.056 and 0.052 mL/bee day for the control and CM/control group respectively. The average consumption of the syrup containing insecticides among other groups ranged from 0.045 to 0.061 mL/bee day in the experiment. Based on the consumption of syrup including insecticides, We could estimate the consumption over the last 5 days per bee. Average consumption of insecticides for each treatment per day were: CRB: 0.0018 µg/bee; THX: 0.0021 µg/bee; CHL: 0.0016 µg/bee; CM/CRB: 0.0015 µg/bee; CM/THX: 0.0022 µg/bee; CM/CHL µg/bee: 0.0024 µg/bee.

When exposed to thiamethoxam (40 µg/L), the percentage of survival of groups exposed to THX syrup and pre fed CM prior to exposure to THX was 66.50% and 75.24%, respectively (Figure 1). The survival of THX and CM/THX did not differ significantly from each other ($P>0.05$ by log-rank test) (Table 1). However, the survival analysis indicated that exposure to thiamethoxam induced a significant decrease in honey bee survival compared to the control group of honey bees ($P<0.05$ by log-rank test) (Table 2).

The groups (CRB and CM/CRB) exposed to carbaryl (40 µg/L) showed the lowest survival at the rate of 49.04% and 53.72%, respectively (Figure 3). The survival of bees exposed to carbaryl demonstrated a noticeable reduction in survival compared to the control group ($P<0.05$ by log-rank test) (Table 2). However, the survival between groups CRB and CM/CRB did not significantly differ considerably from each other ($P>0.05$ by log-rank test) (Table 1 and Table 3).

The survival of the bees exposed to chlorpyrifos (40 µg/L) was 79.74% and 75.29% for group CHL and CM/CHL respectively after 10 days (Figure 2). Similarly, there was no significant difference in the survival of between-group CHL and CM/CHL ($P<0.05$ by log-rank test) (Table 1 and Table 3). Contrary to thiamethoxam and carbaryl, the exposure to chlorpyrifos did not significantly change the survival of both groups compared to control group ($P<0.05$ by log-rank test) (Table 1).

Discussion

The survival of honey bees when feeding small doses of insecticides varied, likely a reflection of the fact that LD50 values also differ for each of the tested insecticides. The oral LD50 value of carbaryl, thiamethoxam, and chlorpyrifos is 0.15, 0.005, and 0.24 µg/bee, respectively, and all of them are highly toxic to bees (Sanchez-Bayo & Goka, 2014). Our concentrations of the insecticides was lower than these LD50 concentrations. However, the exposure to low level or sublethal doses of pesticides may cause stress that makes the bee colony weak and susceptible to pathogenic infection and can also reduce the lifespan of foragers (Pettis et al., 2012; Vidau et al., 2011; Wu et al., 2011).

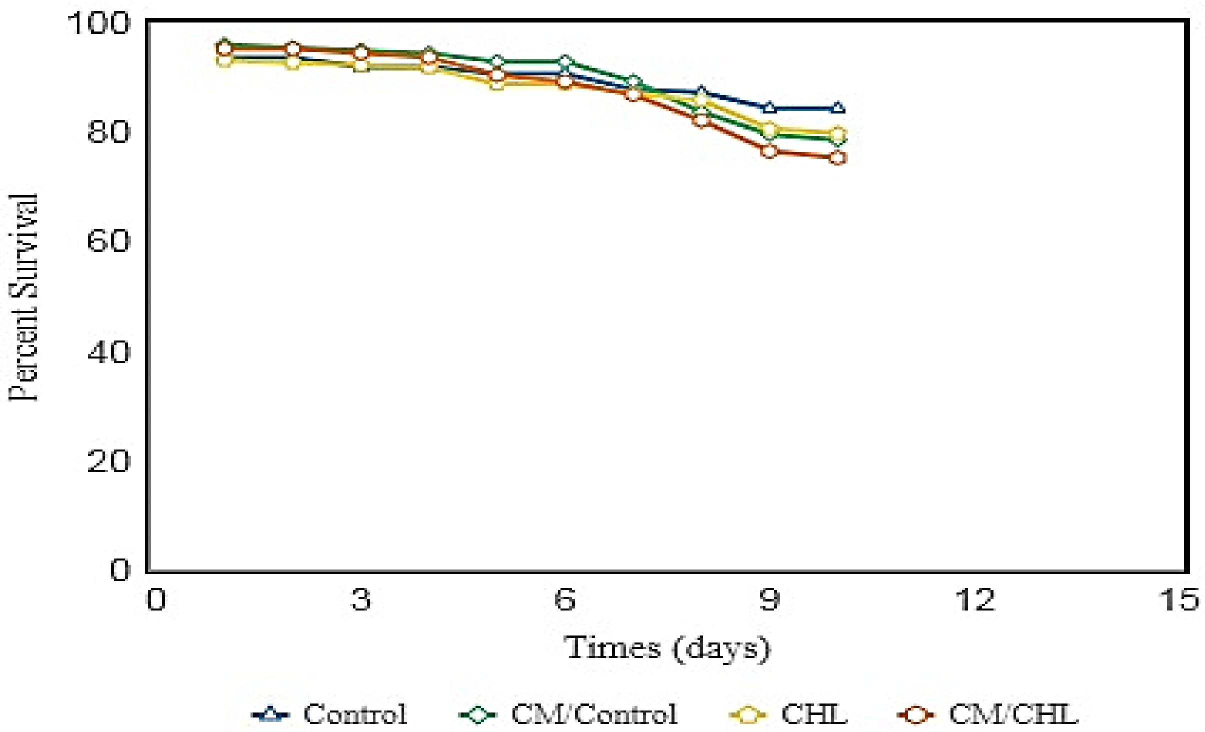
The survival of CM+ and CM- groups exposed to thiamethoxam, carbaryl, and chlorpyrifos showed a similar decline for 10 days. Therefore, feeding honey bees with carbon microparticles did not mitigate bee mortality during exposure to the insecticides. In addition, feeding honey bees with sugar syrup containing carbon microparticles did not negatively impact survival during the experiment.

The survival of honey bees was significantly reduced by carbaryl at a concentration of 40 µg/L for 10 days (Figures 3). In the experiment, the cumulative oral dose of carbaryl for 10 days was estimated at 0.018 µg/bee; eight times lower than the reported LD50 values (Sanchez-Bayo & Goka, 2014). Although our concentration was less toxic to honey bees, approximately half of the group was dead after 10 days. Such a high number of dead bees might have been due to physical contact. A previous study has been demonstrated that body contact with the carbaryl killed more bees than oral contact (Tarek et al., 2018).

Table 2. Survival analysis between control and treatment groups exposed to different pesticides by Kaplan-Meier Procedure.

	n	Survival (%) at 10 d	Mean	S.E.	95% CI	*P* (Log -Rank)
Control	172	84.30	9.12	0.18	8.75-9.16	0.0002
THX	196	66.50	8.83	0.17	8.50-9.16	
Control	172	84.30	9.12	0.18	8.75-9.16	0.26
CHL	232	79.74	9.01	0.16	8.69-9.33	
Control	172	84.30	9.12	0.18	8.75-9.16	0
CRB	210	49.04	8.17	0.19	7.80-8.53	

Control: Group of honey bees fed without carbon microparticles. THX: Group of honey bees exposed to thiamethoxam and fed without carbon microparticles. CHL: Group of honey bees exposed to chlorpyrifos and fed without carbon microparticles. CRB: Group of honey bees exposed to carbaryl and fed without carbon microparticles.



CHL: Group of honey bees exposed to chlorpyrifos and fed without carbon microparticles. CM/CHL: Group of honey bees exposed to chlorpyrifos and fed with carbon microparticles.

Figure 2. Honey bee survival ratio comparisons between the control groups and treatment groups exposed to chlorpyrifos.

Therefore, bees inside the cage might have external contact with the carbaryl and, consequently, a higher percentage of dead bees for the 10 day exposure.

It has been reported that a concentration of 100 µg/L thiamethoxam causes acute effects for individual bees (Overmyer et al., 2018). However, in the present study, exposure to the lower concentration of thiamethoxam (40 µg/L) caused a significant decline in the honey bee survival (66%) over 10 days (Figure 1). A constant exposure to the low dose of thiamethoxam over time may have led to increased bee mortality.

The acute of oral toxicity LD50 values at 24 hours for thiamethoxam was 4.4 - 4.7 ng/bee (Laurino et al., 2011; 2013). The estimated exposure to thiamethoxam for 10 days in my experiment was 21 ng/bee, which is about four-fold higher than the documented 24-hour LD50 concentration and led to 34% of the honey bees dying during the experiment. The estimated "daily" exposure rate would be 2.1 ng/bee in the current study, about half of the 24-hour LD50 value. Laurino et al. (2011; 2013) used a commercial formulation of thiamethoxam (Actara®: 25.0% pure a.s., hydro dispersible granules) in their experiment but it was used thiamethoxam (Sigma-Aldrich) with a purity of 99% in this experiment. Commercial pesticides may include chemical adjuvants that are harmful to bees and increase the toxicity of pesticides to honey bees (Chen et al., 2019). Therefore, the same concentration of thiamethoxam used in this study might be less toxic to honey bees than previous studies utilizing commercial formulations.

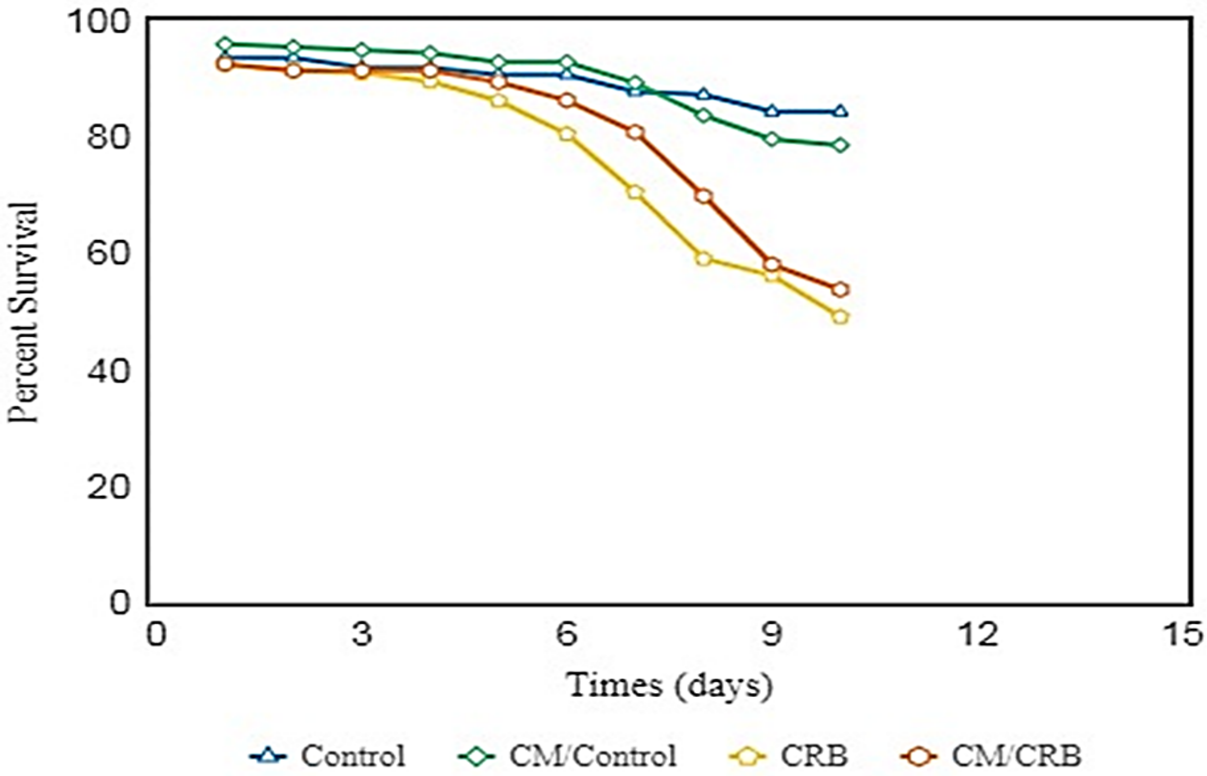
In contrast to carbaryl and thiamethoxam, chlorpyrifos insecticide showed no reduction in honey bee survival in this experiment. Chlorpyrifos has the least toxic LD50 value (0.24 µg/bee) of the three insecticides used in this study, although it is still considered highly toxic to bees (Sanchez-Bayo & Goka, 2014). In our experiment, the estimated cumulative dose of chlorpyrifos for 10 days was around 0.020 µg/bee. The amount of active ingredient delivered daily to the bees was, therefore, approximately 12 times less than the published LD50 value. Such a low concentration of chlorpyrifos might not lead to a measurable reduction in honey bee survival. Although our concentration of chlorpyrifos did not reduce honey bee survival, exposure to chlorpyrifos to amounts 10-times lower than the LD50 values has been shown to have a negative impact on the immune system of honey bees (Christen & Fent, 2017).

In summary, pesticide toxins in plants and hive products are a major risk for honey bee colonies. This study focused on the survival of honey bees exposed to three insecticides fed with and without carbon microparticles. Carbon microparticles did not affect the survival of honey bees after exposure to putatively sub-lethal amounts of the three insecticides tested. Further studies may be warranted to better understand how feeding carbon microparticles impact honey bees exposed to insecticides.

Table 3. Survival analysis between control group fed with carbon microparticles and treatment groups exposed to different pesticides by Kaplan-Meier Procedure.

	n	Survival (%) at 10 d	Mean	S.E.	95% CI	*P* (Log -Rank)
CM/Control	196	78.57	9.19	0.15	8.90-9.48	0.44
CM/THX	202	75.24	9.12	0.14	8.84-9.40	
CM/Control	196	78.57	9.19	0.15	8.90-9.48	0.41
CM/CHL	251	75.29	9.04	0.14	8.76-9.31	
CM/Control	196	78.57	9.19	0.15	8.90-9.48	1.6e-7
CM/CRB	255	53.72	8.51	0.16	8.19-8.83	

CM/Control: Group of honey bees fed with carbon microparticles. CM/THX: Group of honey bees exposed to thiamethoxam and fed with carbon microparticles. CM/CHL: Group of honey bees exposed to chlorpyrifos and fed with carbon microparticles. CM/CRB: Group of honey bees exposed to carbaryl and fed with carbon microparticles.



CRB: Group of honey bees exposed to carbaryl and fed without carbon microparticles. CM/CRB: Group of honey bees exposed to carbaryl and fed with carbon microparticles.

Figure 3. Honey bee survival ratio comparisons between the control groups and treatment groups exposed to carbaryl for 10 days.

Conclusion

Exposure to pesticides is a major concern for honey bee health. However, the use of pesticides is considered widely necessary because they enhance productivity, protect against crop losses, provide vector disease control and assure quality food for humans (Aktar et al., 2009). Concern about the hazar of pesticide use on honey bees has led to investigating new method for researchers to minimize pesticide impact. Feeding honey bee colonies with carbon microparticles might protect honey bees against exposure to pesticides.

The study has shown that feeding honey bees with carbon microparticles didn't affect their survival when they exposed to thiamethoxam (THX), chlorpyrifos (CHL), and carbaryl (CRB) for ten days. The survival of honey bees between CM+ and CM- groups demosrated similarities for 10 days. Additional studies are required for a better understanding whether carbon microparticles could protect honey bees exposed to pesticides.

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RESEARCH PAPER

Classification of the Provinces of the Black Sea Region in Terms of Beekeeping

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Abstract

This study aims to group the Black Sea provinces, which hold 27.48% of the honey production in Turkey, by cluster analysis. For this purpose, the number of beekeeping enterprises in the Black sea region, the total number of colonies (traditional and modern), honey production, and beeswax production data gathered from the Turkish Statistical Institute (TURKSTAT) between 2016 and 2020 were used. As a result of the analysis, the Black sea region provinces were clustered into three groups based on their colony count and production. The first group consisted of; Amasya – Gümüşhane, Bayburt – Tokat, Düzce, Bolu – Karabük provinces, and Zonguldak – Çorum, Sinop provinces. The second group consisted of; Artvin, Kastamonu – Samsun, Bartın, Giresun – Trabzon, Rize provinces. The only province in the third group was Ordu. Assessing their development levels in regards to beekeeping practices may provide an opportunity for grouping provinces that are similar in terms of their product type, especially for apitherapy, health, cosmetics, and edible products to help them specialize in certain products. Determining these groups might also make it possible to carry out training and extension programs in a more organized way. Thus, it is thought that the contribution to the country's economy will increase by providing sustainable and economic production.

Introduction

Turkey, due to its geographical structure, has suitable climate conditions and a wide variety of plants available for honey bees (*Apis mellifera* L.), and therefore is a suitable ecosystem for beekeeping. In addition to the production of different bee products, diversity can be seen in those products (Akkaya, 2007; Özkırım, 2018; Sorkun, 2007). These advantages of our country have supported beekeeping and contributed to the country's economy with the produced goods. (Fıratlı et al., 2000). In addition to the advantages of the diverse vegetation, Turkey has an important position in the world with its genetic resources (Bodenheimer, 1942; Kandemir et al., 2000; Smith et al., 1997). Moreover, Turkey is one of the leading countries in the world in the apiculture sector with more than 8 million colonies and advanced beekeeping practices (FAO, 2021; TURKSTAT, 2021).

The presence of genetic resources has seen as a Caucasian race (*A. m. caucasica*) in the Northeastern Anatolia Region and the Eastern Black Sea Region (Akyol et al., 2006; Bodenheimer, 1942; Dodoloğlu & Genç, 2002; Genç et al., 1997; Palmer et al., 2000; Ruttner, 1988; Smith et al., 1997). Studies imply that the Western Black Sea Region is one of the important gene regions. The fact that the region is not on the migratory beekeeping migration routes provides an advantage in terms of breeding and preservation of breeding material.

Beekeeping activities can be carried out in every region of Turkey with its vegetation, suitable climate, social and economic structure. Based on these facts some differences occur both in regions and in provinces depending on the advantages and disadvantages of the regions. Examples of these differences are the number of colonies, amount, and variety of products produced. In the Black Sea Region, specific vegetation has been formed depending on geography and related climatic condition, and as a result of these region-specific monofloral kinds of honey have been produced (Sıralı & Cınbirtoğlu, 2018; Sorkun, 2007). According to 2021 Turkish Statistical Institute data, the Black Sea region accounted for 27.34 percent of the entire Turkish beekeeping enterprises. 20.74% in terms of the number of hives, 27.48% of honey production, and 17.32% of beeswax production are made by businesses in the Black Sea region provinces. 20.74% of the number of hives, 27.48% of honey production, and 17.32% of beeswax production are produced by enterprises in the Black Sea region provinces (TURKSTAT, 2021).

According to 2021 Turkish statistical institute data, Ordu province has 11.77% of the number of enterprises in the Black Sea region. The highest number of hives with a rate of 33.79% and 60,18 percent of honey produced from enterprises in Ordu. It corresponds to approximately 16.54% of the total honey production in Turkey. Even though the second number of hives are in Trabzon province around 9.89 percent, the second-highest production is in Rize province. The lowest number of beehives (0.89%) and honey production (0.38%) in the region is in Karabük (TURKSTAT, 2021). Therefore, the number of colonies is an effective factor in the amount of product produced.

Financial supports for the beekeeping sector started in 2003 (Çevrimli & Sakarya, 2018) and the Beekeeping Registration System (AKS) was valid after 2009 (Anonymous, 2020). In the apiculture sectors such as queen bee breeding, bumblebee breeding and use, honey production and beehive, and export have started to receive financial support (Çevrimli & Sakarya, 2018). In addition, in some cases, some municipalities and beekeepers' associations are providing financial support to beekeepers, and also the Agriculture and Rural Development Support Institution (TKDK) supports beekeeping projects. However, there is no financial support to beekeepers whose bees died off due to illnesses and this creates a challenging situation for beekeepers (Anonymous, 2020). However, in recent years, production losses in beekeeping have been increasing due to the increase in input costs, forest fires, and global warming. It is thought that the same amount of support given to all provinces will not prevent these losses. For this reason, providing region-based financial support for regions similar in production might enable beekeepers to develop competitively in their own regions. Determining these groups might also make it possible to carry out training and extension programs in a more planned way. For this reason, in this study, we set out to group provinces in the Black Sea region by assessing the number of the beekeeper and the production amount together. For this purpose, we grouped the provinces in the Black Sea region based on the number of beekeeping enterprises, number of colonies, amount of honey, and beeswax production by performing cluster analysis.

Materials and Methods

Cluster analysis is one of the multivariate statistical methods that divide unknown variables into similar subgroups (Özdamar, 2004). It is aimed to classify the provinces in the Black Sea region where honey and honey products are produced. The number of beekeeping enterprises, the total number of hives (traditional and modern), the amount of honey and beeswax produced in the provinces between the years 2016-2020 obtained from TURKSTAT were used (TURKSTAT, 2021). The normality assumption of the data was analyzed with the Anderson Darling method, it was determined that while the number of enterprises showed normal distribution ($P>0.05$), other variables did not show normal distribution ($P<0.05$). In addition, since the units of the variables used were not the same and there were outliers in the data (due to Ordu province data), 0 - ≤1 standardization was applied to the data. Afterward, Euclidean Distance values were calculated. The "Similarity Between Groups" dendrogram, which shows the similarities and differences of the provinces with each other, was obtained by performing cluster analysis according to the Average Linkage method in order to have the maximum distance between the groups and the high similarity within the group. Minitab 21 package program (trial version) was used in the analysis of the data (Minitab, LLC., 2021).

Results and Discussion

The clusters formed as a result of the clustering analysis, the similarity rates, and the findings of the number of observations in the new cluster are given in Table 1.

The dendrogram of the clusters formed as a result of the clustering analysis is given in Figure 1.

Table 1. Amalgamation Steps

Step	Number of clusters	Similarity level	Distance level	Clusters joined		New cluster	Number of obs. in new cluster
1	17	98.4987	0.02979	5	9	5	2
2	16	97.7692	0.04427	4	15	4	2
3	15	96.5228	0.06901	4	6	4	3
4	14	96.4725	0.07001	17	18	17	2
5	13	96.1447	0.07651	1	8	1	2
6	12	93.6908	0.12522	1	4	1	5
7	11	93.6129	0.12676	10	13	10	2
8	10	91.4496	0.16970	14	17	14	3
9	9	90.9000	0.18060	1	5	1	7
10	8	90.2746	0.19302	2	10	2	3
11	7	89.2590	0.21317	7	16	7	2
12	6	86.8799	0.26039	2	3	2	4
13	5	86.5511	0.26692	1	14	1	10
14	4	81.2578	0.37197	7	12	7	3
15	3	77.6000	0.44456	2	7	2	7
16	2	70.0817	0.59378	1	2	1	17
17	1	13.8742	1.70931	1	11	1	18

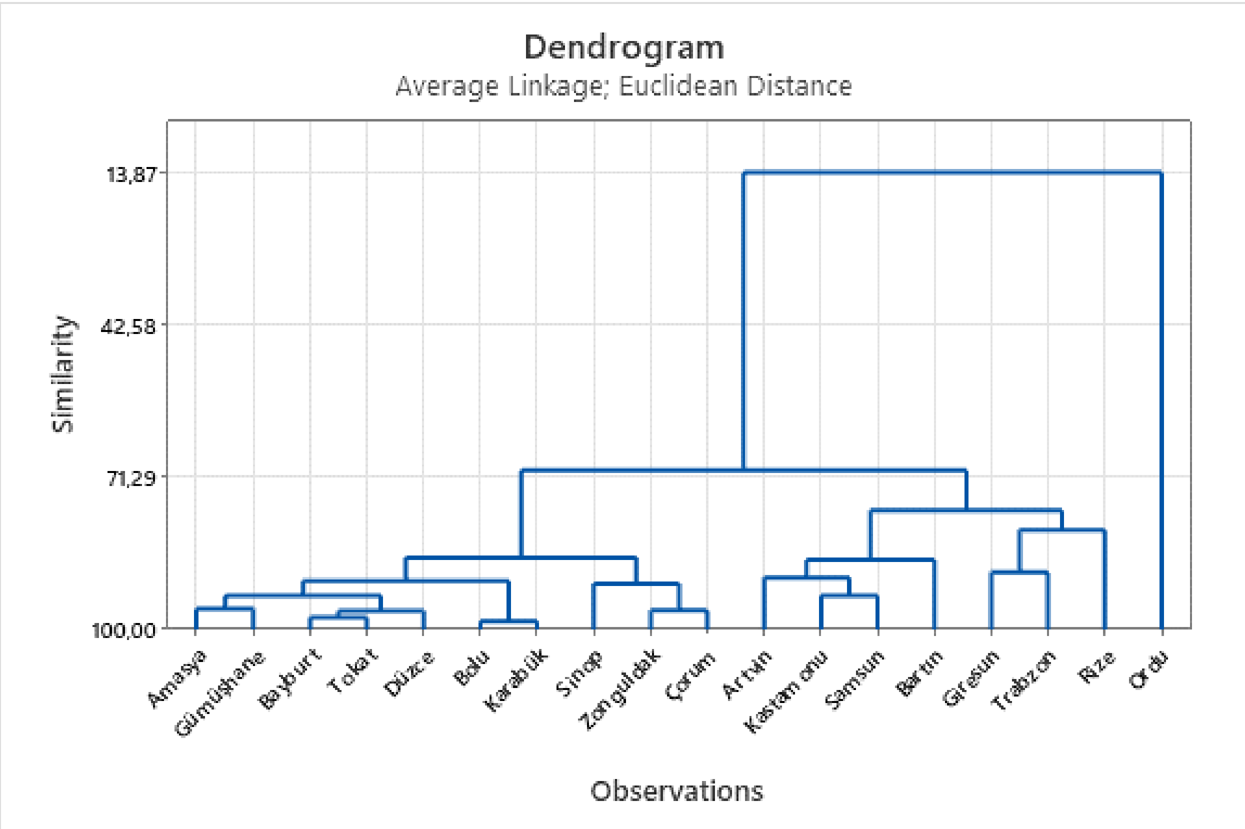

Figure 1. Dendrogram of Cluster Analysis

According to the results of the analysis, the similarity between the provinces in the Black Sea region can be grouped into different three clusters. Amasya – Gümüşhane provinces formed a subset with 96.15% similarity. The subset of Bayburt and Tokat provinces is similar to each other with a rate of 97.77%. Düzce was included in these provinces with a similarity of 96.53%. It was determined that the similarity between Amasya – Gümüşhane provinces and Bayburt, Tokat Düzce provinces was 93.69% and a new subset was formed. Bolu and Karabük provinces formed a subset with 98.51% similarity, and Bolu - Karabük provinces were found to be included in Amasya - Gümüşhane, Bayburt - Tokat, Düzce provinces with 90.91% similarity. Zonguldak – Çorum provinces formed a subset with 96.48% similarity, and Sinop province was included in this subset with 91.45% similarity. Amasya - Gümüşhane, Bayburt - Tokat, Düzce, Bolu - Karabük provinces, Zonguldak - Çorum, Sinop provinces were included with 86.55% similarity, and the first cluster consisting of 10 provinces was formed.

A subset was formed with a similarity of 93.62% to Kastamonu-Samsun province, Artvin and Bartın were included in this subset with a similarity of 90.28%, 86.88% respectively. Giresun and Trabzon formed a subset with 89.26% similarity and Rize entered this subset with 81.26% similarity. The similarity between the provinces of Artvin, Kastamonu - Samsun, Bartın, and Giresun - Trabzon, Rize was determined to be 77.61% and the second cluster consisting of 7 provinces was formed.

It was determined that beekeeping and bee products production in Ordu province differed from other provinces and Ordu province alone formed the third cluster.

The beekeeping sector is developing day by day in Turkey and the world, and its economic return and product diversity are increasing. The number of hives and honey production in Turkey increases every year, but there is a decline in productivity (Çevrimli & Sakarya, 2018). For this reason, Abacı et al. (2020) reported that the number of hives will increase in Turkey in the next 5 years compared to 2018, and they were successful with an average deviation of 0.51% in their predictions for the first 2 years of this prediction. In addition, a 7-year prediction was made by Burucu and Gülse Bal (2017) for honey production and it was stated that honey production would increase, and it was determined that honey production increased from 2017 to 2020 according to the TURKSTAT 2021 data. Compared to 2018, there was a 1.82% decrease in 2019 and a 7.15% decrease in 2020 (Abacı et al., 2020; TURKSTAT, 2021).

Keleş et al. (2019) in the study conducted by the Agriculture and Rural Development Support Institution (TKDK) on the investigation of the effectiveness of beekeeping grants within the scope of the European Union Pre-Investment Assistance Instrument Rural Development Program (IPARD) in Trabzon, IPARD's supports could not fully achieve their goal. The reasoning is that the errors originate from the enterprise and the institution.

Günbey and Cengiz (2021) investigated the performance of some honey bee genotypes under regional conditions. Okuyan et al. (2020) determined the antioxidative effects of propolis collected from Samsun province. Kuvancı et al. (2017) investigated the status of beekeeping activities (migratory beekeeping, local beekeeping, production, loss) in the Eastern Black Sea region. They suggested that beekeepers should follow the developments and innovations in beekeeping to increase their knowledge level and get technical support when necessary. Güler (2021), in his study to determine the efficiency of beekeeping according to the provinces in Turkey, found that the provinces with enterprises with more than the average number of hives in Turkey are productive. However, it has been reported that the efficiency value is high in the provinces above the honey yield average of Turkey (13 kg). These results show that efficiency values differ according to the scale of the enterprise and honey yield and that large-scale beekeeping enterprises are advantageous in terms of efficiency. For this reason, Turkey and the regional beekeeping sector have many technical and economic problems, especially low yield.

Conclusion

Supports given to beekeeping are made per hive. Despite the increase in the number of hives, the decrease in the yield per colony increases the production costs. In addition, small businesses do not fully take advantage of the supports. Therefore, the supports cannot reach their purpose completely.

The provinces of the Black Sea region, which accounted for 27.48% of the total honey production in Turkey, are divided into 3 clusters in terms of beekeeping and production. The first cluster consists of Amasya - Gümüşhane, Bayburt - Tokat, Düzce, Bolu - Karabük provinces and Zonguldak - Çorum, Sinop provinces. The second cluster consists of the provinces of Artvin, Kastamonu - Samsun, Bartın, Giresun - Trabzon, Rize. The third cluster consisted only of Ordu province.

Starting from the lowest groups of the clusters determined in this study, plans should be made to ensure competitiveness with support policies, production plans, and producer organizations of beekeeping products in similar provinces. By determining the development status of these clusters in terms of beekeeping, provinces similar to each other can be specialized, especially in terms of products produced in the field of apitherapy, health, cosmetics, and food. With the determination of these regions, training and extension studies can be carried out in a more planned way to adopt production and innovations in those regions. Moreover, cooperation can be established between breeders in sub-cluster provinces. With the specialization of the producers in the provinces, a higher quality product can be provided for the consumers. Thus, producers can produce products with high economic returns. It can be thought that sustainable and economic production will much contribute to the country's economy.

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RESEARCH PAPER

Some Physical and Chemical Properties of Raw Flower-Honey from Erzurum Highland

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Abstract

In this study, the physical and chemical properties of the highland flower raw honey produced in different locations of Erzurum province were investigated. In 2019, 12 highland flower raw honeys sold during the honey harvest season were purchased from local vendors. Moisture, color, HMF, proline, acidity, pH, conductivity, diastase number, invertase activity, C13, C13 protein-honey, C4 analysis and sugar components were analyzed in honey. It has been evaluated whether the research findings are in compliance with the Turkish Food Codex Honey Communiqué and whether they are similar to the findings of previous studies The average values of the honey samples examined in this study respectively are; moisture is 15.8%, color is 41 mm pfund, HMF content is 4.1 mg/kg, proline amount is 661.2 mg/kg, acidity value is 20.4 meq/kg, pH value is 3.5, conductivity is 0.23 mS/cm, diastase number was determined as 17.8 DN and invertase activity as 189.9 U/kg. The sugar ratios in honey samples were respectively average fructose 34.6%, glucose 27.7%, sucrose 0.5%, turanose 2.6%, maltose 2.8%, isomaltose 2.9%, erlose 0.3%, fructose+glucose 62.3%, fructose/glucose 1.24, glucose/water 1.6 and trehalose, meritose, maltotriose were not detected. The difference between the protein and crude honey delta C13 values was found to be -0.29% and C13 value -29% and the C4 sugar ratio was 1.8%. As a result, it has been seen that the multifloral highland flower raw honey produced and sold in Erzurum region has high biological activity values and is in accordance with the Honey Communiqué.

Introduction

Honey is a valuable animal originated food known for its nutritional value and medicinal properties for centuries. The components in its content determine the nutritional value of honey. The composition of honey varies depending on the geographical and botanical origin, the material the bees feed on, the climatic conditions, the nectar density, the manipulations of the beekeepers, the packaging procedure, and the storage conditions (Thrasyvoulou et al., 2018). Countries determine their own regulations for the production and sale of honey in accordance with consumer health, and producers are obliged to comply with this regulation. Honey production in Turkey increased by 8.2 percent compared to the previous year in 2020 rose to 104077 tonnes (Burucu, 2021). With this amount it produces, it ranks second in honey production in the world. The aim of the Turkish Food Codex Honey Communiqué is to determine the characteristics of honey in the stages of producing, preparing, processing, storing, transporting and placing on the market hygienically and in accordance with its technique. In the codex, flower honey is defined as honey obtained from plant nectar (Turkish Food Codex, 2020). Honey must be free of all inorganic and organic additives that are not found in its natural composition. Although honey-specific regulations vary from country to country, basically the principle is that honey cannot have a unique foreign taste and odour, cannot be artificially acidified or heated in a way that will break down or significantly inactivate the natural enzymes it contains, and honey-specific components such as pollen cannot be removed from honey. Although the taste and aroma of honey varies depending on the source of the honey and the type of plant from which it is produced, honey should have a distinctive smell and taste. Moisture, which is a quality parameter related to the shelf life of honey, may be at different levels in honeys from different botanical origins (Machado De-Melo et al., 2018). Honey with high humidity is prone to fermentation because the osmotic pressure of the sugar is not strong enough to prevent the growth of osmophilic yeast (Bogdanov & Martin, 2002). Hydroxymethylfurfural (HMF) is a furanic complex caused by the breakdown of sugar and is a factor of honey freshness (Gökmen, 2007). Honey is a supersaturated liquor where carbohydrates make up 95% of the dry matter, and its basic nutritional and physicochemical properties such as energy value, sweetness, granulation and viscosity depend on the compounds of these sugars (Bogdanov et al., 2008; Sabatini, 2007). Glucose and fructose are the main sugars in honey. A small quantity of other monosaccharides such as galactose have also been detected in honey varieties (Ruiz-Matute et al., 2010). The major disaccharides found in honey are α-glucosyl derivatives of monosaccharides (Machado De-Melo et al., 2018). More than 45 disaccharides, trisaccharides, oligosaccharides and polysaccharides have been detected in trace amounts in honey (Lazaridou et al., 2004). On the other hand, sugars such as lactose, galactose, and raffinose are toxic to honeybees because they cannot digest these sugars (Herbert, 1992). Proteins such as globulins, albumins, proteases and nucleoproteins in honey originate from both bee salivary glands and plant pollen. Diastase is the most heat stable honey enzyme, so it is widely included in the honey legislation of countries as an indicator of honey bloom (Doner, 2003). The aim of this study was to investigate the physical and chemical properties of raw honey of highland flower produced in different locations of Erzurum province.

Materials and Methods

Collection of honey samples

After the honey harvest in 2019, 12 honey samples were purchased from beekeepers engaged in honey production and sales in 6 different districts of Erzurum (Oltu, Şenkaya, Olur, Uzundere, Tortum, Narman). In the analyzed samples, the statement 'raw honey from highland flowers' on the package was taken as basis. Purchased honey samples were stored in glass jars at room temperature in a darkened environment until analysis.

Analyzes

The physical and chemical properties of raw flower-honey samples were determined according to the standarts. The moisture content of honey is determined by Bogdanov et al. (2002). Sugar analysis of the honey samples was done by using HPLC - Refractive Index detector, accordance to the method proposed by Bogdanov et al. (2002). pH and acidity levels of the honey samples were identified accordance to the method proposed by Bogdanov (2009). Electrical conductivity levels were analysed by handling a conductivity meter (Meterlab-CDM230, Turkey) by the method of by Bogdanov et al. (2002). Analysis of HMF the honey samples was done by handling HPLC-UV detector (Shimadzu UV-1800, Japan) according to Turkish Standards Institute method (TSE 3036, 2002). HPLC-UV was calibrated with the solution of Hydroxymethylfurfural (HMF). Analyses were done by handling the C18-reversed-phase column below the isocratic mobile phase terms with the inclusion of 90% distilled water-10% methanol by a flow rate of 1 mL/min and the injection volume was 10 µL (Korkmaz & Küplülü, 2017). Diastase analyses of honey samples were identified by handling UV-Spectrophotometer (Shimadzu UV-1800, Japan), according to the method recommended by TSE method (TSE 3036, 2002). The absorbance levels of samples were measured at the 600 nm range of UV-Spectrophotometer for detection of the diastase activity (Bogdanov et al., 2002). The invertase activity of honey samples was determined according to German Institute for Standardization method based on the spectrophotometric measurement of p-nitrophenol (DIN 10754, 2002). Color analyzes of honey samples were made according to the method based on photometrically reading the color in terms of Pfund Scale (AOAC, 2005; Kolaylı et al., 2013). Proline contents of honey samples were analysed in accordance with the IHC method (Bogdanov, 2009). C13, C13 protein-honey, C4 analyses of honey samples were determined in accordance with the Official Methods of Analysis 998.12 (Amor, 1978). All data were analyzed statistically using SPSS 22.0 (SPSS Inc., Chicago, IL, USA) software. All results exist as the minimum, the maximum levels and mean ± standard deviation.

Results and Discussion

The quality of honey is determined by its sensory, physical, chemical and microbiological properties. Determining the properties of honey is very important to know the quality and naturalness of honey. Some of these features are; moisture, color, acidity, pH, conductivity, hydroxymethyl furfural content, diastase activity, reducing and non-reducing sugar content, invertase activity and proline content.

In this study, the moisture content of raw flower honey was between 15.0% and 15.9%, and the average value was determined to be 15.8±0.7% (Table 1). Moisture content of honey may vary according to place of production, climatic conditions, degree of maturity and season (Amor, 1978). In a study conducted in 2013

Table 1. Analysis results of honey samples

Parameter	Unit	Minimum	Maximum	Mean±SD
Moisture	%	15.0	15.9	15.8±0.7
Color	mm pfund	39	42	41±0.5
HMF	mg/kg	4.0	4.3	4.1±0.4
Proline	mg/kg	660.2	662.4	661.2±0.6
pH	-	3.4	3.6	3.5±0.4
Acidity	meq/kg	20.0	20.8	20.4±0.6
Invertase activity	U/kg	187.7	188.9	189.9±0.2
Diastase number	DN	17.0	18.8	17.8±0.4
Conductivity	mS/cm	0.22	0.24	0.23±0.2
C13 Honey	%	-30.91	-25.82	-29±0.03
C13 Protein/Honey	%	-0.29	-0.26	-0.29±0.7
C4	%	1.7	1.9	1.8±0.6
Fructose	%	34.6	36.7	34.6±0.9
Glucose	%	27.7	29.2	27.7±0.6
Sucrose	%	0.5	0.9	0.5±0.4
Turanose	%	2.6	3.1	2.6±0.3
Maltose	%	2.8	3.7	2.8±0.5
Isomaltose	%	2.6	3.8	2.9±0.4
Trehalose	%	ND	ND	ND
Meritose	%	ND	ND	ND
Maltotriose	%	ND	ND	ND
Erlose	%	ND	0.4	0.3±0.2
Fructose+glucose	%	59.4	64.8	62.3±0.8
Fructose/glucose	-	1.12	1.58	1.24±0.6
Glucose/water	-	1.2	1.8	1.6±0.2

HMF: Hydroxymethylfurfural SD: Standard deviation ND: Not detected

examining the moisture content of honey, the average moisture content of honey produced in Erzurum was reported as 15.35%, and this data of the researchers is similar to the findings of our study. The moisture values measured in the honey samples in this study were below the 20% limit determined by the Turkish Food Codex Honey Communiqué and were determined to be in compliance with the standard (Turkish Food Codex, 2020).

Invertase is one of the enzymes released from the cephalic and thoracic glands of honey bees and has the highest activity in the maturation of honey (Al-Sherif et al., 2017). When the invertase values obtained within the scope of this study are compared with the ratio of IU≥73.45 (Bogdanov & Martin, 2002) recommended by the International Honey Commission (IHC) in terms of proof and freshness of honey not being heat-treated; It was observed that all samples labeled raw honey fit very well with this measurement scale with an average of 188.5±0.2 U/kg (Table 1). In another scientific study investigating the invertase activity of six fresh raw flower honey; invertase values were found to be 178.187 U/kg on average (Şahin et al., 2020).

Diastase is an enzyme found naturally in honey and its amount varies depending on the origin of the flora, and is also an indicator of the applied heat (Çiftci, 2014; Çiftçi & Parlat, 2018). According to the Turkish Food Codex Honey Communiqué, the number of diastases in flower honey should be 8 or more (Turkish Food Codex, 2020). In the thesis study conducted by Korkmaz and Küplülü (2017) they reported that the diastase number in five different flower honeys was between 10.8 and 14.1 on day zero. The number of diastase in honey analyzed in this study was measured between 17.0 and 18.8. When the research findings of Özgüven et al. (2020) were examined, the number of diastase in twelve honey samples varied between 9.0-25.4. The difference in the number of diastase seems to be quite variable according to the region of production, storage temperature and time.

Electrical conductivity is a parameter used to distinguish between flower and secretory honeys. It was seen that the electrical conductivity of the flower honeys examined in Özgüven et al. (2020) study varies between 0.18 and 1.05 mS/cm. In the raw flower honeys examined in this study, the electrical conductivity is vaule average 0.22±0.3 mS/cm, which complies with the Turkish Food Codex Honey Communiqué (Turkish Food Codex, 2020). The electrical conductivity (0.41 mS/cm) found by Albu et al. (2021) in multifloral raw honey samples in Romania is considerably higher than the findings of this study.

In this study, it was observed that the average HMF values of raw honey samples varied between approximately 4.0-4.3 mg/kg, and the average was determined as 4.2±0.4 mg/kg (Table 1). Storing honey at an unsuitable temperature or applying heat treatment creates HMF compound depending on the bond between sugars and amino acids contained in honey (Gökmen, 2007). In the Turkish Food Codex Honey Communiqué, it is allowed to contain HMF in honey up to a maximum of 40 mg/kg. The HMF values of none of the raw flower honeys analyzed in this study were found to be above 40 mg/kg. In our study, the highest HMF value was measured as 4.3 mg/kg. Batu et al. (2013) reported in their study that the HMF value of the honey sample from Rize region was 4.04 mg/kg, the HMF value of the honey sample from Kars region was 0.15 mg/kg, the HMF value of the honey sample from Malatya region was 0.96 mg/kg and the HMF value of honey sample from Erzurum region is 1.91 mg/kg.

The amount of proline, one of the amino acids found in honey, should be higher than 300 mg/kg (Turkish Food Codex, 2020). It was determined that the average amount of proline in the honey samples analyzed in this study was 660.2±0.6 mg/kg and all samples were in compliance with the Turkish Food Codex Honey Communiqué. Erez et al. (2015) reported the proline amount of flower honey obtained from Pervari region as 192-234 mg/kg in 2015. It was seen that the amount of proline in the honey analyzed by the researchers is considerably lower than the amount of proline in the honeys analyzed in this study.

The acidity of honey may vary depending on the plant source and the production region. According to the Turkish Food Codex Regulation, the total acidity of honey should not exceed 50 meq/kg (Turkish Food Codex, 2020). In our study, it was determined that the acidity average values of raw flower honeys (20.01±0.2 meq/kg) were in accordance with the Turkish Food Codex Honey Communique. In the study conducted by Sorkun et al. (2002) they reported the acidity of flower honey as 15 meq/kg. In another study by Erdoğan et al. (2004) the acidity of honey produced in İspir district of Erzurum province was between 25.50-29.0 meq/kg. The researchers' findings are consistent with the findings of this study.

The sugar ratios in honey samples were respectively average fructose 34.6%, glucose 27.7%, sucrose 0.5%, turanose 2.6%, maltose 2.8%, isomaltose 2.9%, erlose 0.3%, fructose+glucose 62.3%, fructose/glucose 1.24, glucose/water 1.6 and trehalose, meritose, maltotriose were not detected (Table 1). Average sugar rates in honey are in compliance with the Turkish Food Codex (Turkish Food Codex, 2020). The fructose/glucose ratio in honey is a parameter that shows both the crystallization tendency of honey and its origin. According to the Turkish Food Codex Honey Communiqué, the fructose/glucose ratio of raw flower honey should be between 0.9-1.4 (Turkish Food Codex, 2020). The average fructose/glucose ratio of honey samples analyzed in this study was 1.24±0.06. Çiftçi and Parlat (2018) reported fructose/glucose ratio in flower honey as 1.05-1.19 in their research. The results of the researchers are similar to the results of this study.

The difference between the protein and crude honey delta C13 values was found to be -0.29±0.03% and C13 value -29±0.7% and the C4 sugar ratio was 1.8±0.6% (Table 1). The results of the raw flower honey samples used in the study, the protein in honey and raw honey. The difference between delta C13 values and the average C4 calculated from raw honey delta C13 sugar ratios are in accordance with the Turkish Food Codex Honey Communiqué found (Turkish Food Codex, 2020). In addition, our results are in harmony with the results reported by Çiftçi and Parlat (2018) on flower honeys in Konya province.

Conclusion

The physical and chemical parameters of honey, which has started to take its place in the market in recent years, defined as raw honey and not exposed to heat treatment and advanced mass production processes, were evaluated with this study. This study showed that the physical and chemical properties of Erzurum highland flower raw honey are higher than the other honeys produced in the region. This study supplies helpful data for the characterization of highland flower raw honey of Erzurum province. In this region, researchs should be carried out with a larger number of samples in a wider area.

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RESEARCH PAPER

An Online Survey to Determine Breeding Activities and Main Issues in Turkey's Beekeeping Enterprises

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Abstract

This study was conducted online survey between November 2019 and January 2020, with the objective of determining the production characteristics and sectoral issues of beekeeping enterprises in Turkey. According to the survey, the Black Sea region accounts for 28% of the total; the Central Anatolia region accounts for 18%; the Marmara region accounts for 17%; the Eastern Anatolia region accounts for 13%; and the Aegean region accounts for 9%. It was conducted with a total of 200 participants, 8.5% of which were beekeepers from the Mediterranean region and 6.5% from the Southeast Anatolia region. 82% of the participants are for income; 18% of them are involved in production activities for backyard purposes. The enterprises have an average of 140 colonies and produce 17.29 kg of honey per colony; 31.5% of the enterprises consider beekeeping to be their first job, and 49% use the migratory beekeeping model. 67% of them attended beekeeping classes. The enterprises collectively produce 90.5% extracted honey, 56.5% comb honey, and 23 % natural honey comb. 37% of them work with Caucasian or crossbred bees, and 32.5 % with Anatolian bees. It has been determined that 63.0% of enterprises meet their queen bee demands solely via their own operations, and 60.6% of enterprises are exposed to the varroa infection. According to our findings, one of the most important concerns for enterprises in the industry is marketing, which accounts for 24%. Following issues, accommodation (17.7%), diseases and pests (15.7%), safety (15.7%), transportation (10.4%), and pesticide applications (7.1%).

Introduction

Beekeeping is an important agricultural activity that produces products such as honey, pollen, royal jelly, beebread, and propolis, which have been used by people over the years for their food and pharmacological properties. Honey bees pollinate plants and ensure that agricultural products are greater in quantity and quality, in addition to the bee products they produce (Free, 1993). It is known that approximately 20000 of the more than 250000 flowering plant species spread in the world are visited by bees (Kaufman, 1989). 90 percent of the foodstuffs are acquired from 82 plant species and 63 (77%) of these plant species require pollination by bees. Furthermore, 1/3 of human food is made up of plants that require bee pollination, either directly or indirectly (Güler, 2006). It is estimated that Turkey has 10000 natural plant species, 900 of which are indigenous, and 500 of these plants provide nectar and pollen. The plant diversity and vegetation in Turkey are highly diverse, and beekeepers may produce monofloral and polyfloral honeys nearly all year. In Turkey, roughly 81000 beekeeping enterprises produce 109330 tons of honey with 8128360 colonies (Anonim, 2021a). Turkey, which has the third-highest number of colonies in the world after India and China, also in second rank in terms of honey harvest (Anonim, 2021b). In 2020, 6011 tons of honeys were sold to numerous nations throughout the world, including Germany, the United States, and Saudi Arabia. Turkey covers 0.84% of global honey exports with this quantity (Anonim, 2021a). Although beekeeping in Turkey is in an increasing trend in terms of honey production and colony amount over the years, the low yield per colony, the problems experienced in the fight against diseases and pests, the insufficient variety of production, the instability of prices and the inability to reach the desired levels in the export of bee products can be shown among the biggest problems of the sector. Many local and national scholars have conducted scientific studies to pay attention to these issues (Kekeçoğlu & Rasgele, 2013; Çelik & Turhan, 2014; Kutlu, 2014; Emir, 2015; Akdeniz et al., 2015; Karahan & Karaca, 2016; Kuvancı et al., 2017; Borum, 2017; Öztürk, 2017; Sert, 2017; Çevrimli & Sakarya, 2018; Seğmenoğlu, 2018; Aktürk & Aydın, 2019; Güneşdoğdu & Akyol, 2019; Turhan, 2019; Tabur & Gül, 2019; Kutlu & Kılıç, 2020). The purpose of our study, which is one of the first online surveys conducted with beekeeping enterprises in Turkey, is to determine the general structure of backyard and income-oriented beekeeping enterprises at the national level, to define differences in colony management, and to reveal the present condition of beekeeping in Turkey from various perspectives.

Materials and Methods

An online survey was conducted with the Google online form between 8 November 2019 and 30 January 2020 in order to determine the production characteristics and sectoral problems of beekeeping enterprises in Turkey. Beekeepers were reached via online surveys and social media platforms such as Facebook, Twitter, and Instagram. Although 212 beekeepers took part in the study, only the surveys of 200 beekeepers were considered due to inadequate completing of the questions or inconsistencies in the replies. Following the completing the survey, the data collected were categorized and sorted, and qualitative data were applied to the remaining data using the Excel applications.

Results and Discussion

Regional distribution of beekeepers taking part in the survey; 28.0% Black Sea area; 18.0% Central Anatolia region; 17.0% Marmara region; 13.0% Eastern Anatolia region; 9.0% Aegean region; 8.5% Mediterranean region; and 6.5% South East Anatolia region. 55 different provinces participated in our online survey study. These are the Black Sea provinces of Artvin, Giresun, Gümüşhane, Kastamonu, Ordu, Rize, Samsun, Sinop, Zonguldak, Bayburt, Bartın, Düzce, Trabzon; the Central Anatolia provinces of Ankara, Eskişehir, Kayseri, Konya, Nevşehir, Niğde, Yozgat, Karaman, Kırıkkale; the Marmara provinces of Balikesir, Bilecik, Bursa, Çanakkale, İstanbul, İzmit, Sakarya, Tekirdağ, Yalova; the Eastern Anatolia provinces of Elazığ, Erzincan, Erzurum, Muş, Van, Ardahan, and Iğdır; the Aegean provinces of Afyon, Aydın, İzmir, Kütahya, Manisa, Muğla; the Southeastern Anatolia provinces of Adıyaman, Gaziantep and Şanlıurfa; the Mediterranean provinces of Adana, Antalya, Hatay, Isparta, Mersin, Kahramanmaraş. The provinces with the largest involvement are 8% Ordu, 7.5% Konya, 4.5% Erzurum, 4% Istanbul, 4% Artvin, 4% Giresun, 4% Adıyaman, 3% Balıkesir, 3% İzmit, 3% Bursa, 3% Ankara and 3% İzmir.

The rate of interest in beekeeping among the young people is a significant factor in the long-term sustainability of beekeeping enterprises. In our study, the average age of the examined beekeeping enterprises was determined as 41.02 years (Table 1), and according to Güneşdoğdu and Akyol (2019) the resulting average age value was 47.77 in the Adana province; Onuç et al. (2019) reported 47 in Kemalpaşa district of İzmir province; Öztürk (2017) found 54 in Muğla; Söğüt et al. (2019) 47.3 in the province of Bingöl; Kadirhanoğulları et al. (2016) found 52 in Iğdır; Kekeçoğlu et al. (2014) reported 50.38 in Düzce; Kuvancı et al. (2017) 52.34 in the Eastern Black Sea Region; Aktürk and Aydın (2019) found 54.71 in Çanakkale; In the Aegean region of Çevrimli and Sakarya (2018), 50.08; It is seen that it is lower than the average age reported by Tabur and Gül (2019) as 53.19 in Uşak. Previous researches have found an age gap, which may be explained by the young population's participation in our online surveys, since they use social media significantly more frequently. In our study, income-oriented beekeeping enterprises had an average experience length of 13.07 years, whereas backyard beekeeping enterprises had an average experience length of 5.91 years (Table 1). The average experience period of the examined beekeeping enterprises was reported in previous studies; Onuç et al. (2019) 18.08 years; Öztürk (2017) 26 years; Söğüt et al. (2019) 18 years; Kadirhanoğulları et al. (2016) 20 years; Kuvancı et al. (2017) 24.28 years; Aktürk and Aydın (2019) 19.37 years; Çevrimli and Sakarya (2018) 17.52 years; Tabur and Gül (2019) were determined to be 18.47 years, less than their experience period.

Table 1. The average age and professional experience of the beekeeping enterprises surveyed

	General		Income-oriented enterprises		Backyard beekeeping enterprises	
	n	**Average, year**	**n**	**Average, year**	**n**	**Average, year**
Age	200	41.02	164	41.05	36	40.89
Beekeeping experience	200	11.79	164	13.07	36	5.91

In our study, the average colony number of income-oriented enterprises was calculated as 167. On the other hand, Backyard beekeepers were calculated as 13.64 and, it was determined that the enterprises, in general, had an average of 140 colonies (Table 2). The average colony number of beekeepers in Adana was 293.21 (Güneşdoğdu and Akyol, 2019). The average number of colonies of enterprises in Adıyaman are 102.4 (Özbakır et al., 2016). The average colony number of beekeepers in Muğla province is 258 (Öztürk, 2017). In the Eastern Black Sea region, the average colony number is 101.56, and the highest average colony number is in Ordu with 228.41 colonies, and the lowest average colony number is in Gümüşhane with 56.33 colonies has been reported by (Kuvancı et al., 2017). Within the scope of our study, the average honey yield of income-oriented enterprises and backyard beekeepers were calculated as 17.41 kg and 16.75 respectively. In general, the average honey yield of the enterprises is 17.29 kg (Table 2). The average honey yield of income-oriented enterprises (95 enterprises) engaged in migratory beekeeping is 19.46 kg. The average honey yield of backyard beekeepers (3 enterprises) is 19.00 kg. The average honey yield of income-oriented enterprises (69 enterprises) that are not engaged in migratory beekeeping activities and backyard beekeepers (33 enterprises) was 14.58 kg and 16.55 kg respectively. In our study, it is seen that the average honey yield per colony of income and backyard enterprises and migratory and non-migratory beekeeping enterprises is higher than the Turkey average of 13.45 kg. In a study conducted by Onuç et al. (2019) in the Kemalpaşa district of İzmir province, honey yield per colony was calculated as 19.27 kg. Honey yield per colony of the enterprises in Adıyaman was calculated as 7.7 kg (Özbakır et al., 2016). Honey yield per colony in Yığılca district of Düzce province was calculated as 5.67 kg (Kekeçoğlu & Rasgele, 2013). Honey yield per colony in Ordu, Artvin, Gümüşhane, Giresun, Bayburt, Trabzon and Rize provinces was 38.54 kg, 17.01 kg, 16.82, 16.32 kg, 15.00 kg, 14.36 kg, 11.45 kg respectively (Kuvancı et al., 2017). Honey yield per colony in Çanakkale was calculated as 16.24 kg (Aktürk & Aydın, 2019). Honey yield per colony of beekeepers in Konya was calculated as 20-30 kg (Çelik & Turhan, 2014). In the Hizan district of Bitlis province, it has been reported that 58% of beekeepers have a honey yield between 4-10 kg, 30% between 11-15 kg, and 12% per colony of 3-6 kg (Kutlu et al., 2016). In Afyon province, 72.37% of the enterprises have a honey yield between 5-21 kg, 15.79% over 21 kg, and 11.84 of them have honey yield per colony below 5 kg have been reported by (Karahan et al., 2019). It is seen that honey yields per colony of migratory beekeeping enterprises are higher than non-migratory beekeeping enterprises (Uzundumlu et al., 2011; Kekeçoğlu et al., 2014).

Table 2. The average number of colonies and honey yield of the beekeeping enterprises surveyed

	General		**Income-oriented enterprises**		**Backyard beekeeping enterprises**	
	n	**Average**	**n**	**Average**	**n**	**Average**
Number of colonies	200	140	164	167	36	13.64
Honey yield (kg)	200	17.29	164	17.41	36	16.75

In our survey, 31.5 percent of the enterprises considered beekeeping to be their first job. The priority occupations of the remaining enterprises are 19% government workers, 11.5% farmers, 10.5% tradesmen and 27.5% of them are retired, self-employed, workers and private sector employees. 41.7% of backyard beekeeping enterprises are run by government workers, 41.7 % by retirees, self-employed, workers, and private enterprise people (Table 3).

Table 3. The distribution of priority professions of the beekeeping enterprises studied

	General		**Income-oriented enterprises**		**Backyard beekeeping enterprises**	
	n	**Frequency, %**	**n**	**Frequency, %**	**n**	**Frequency, %**
Beekeeper	63	31.5	63	38.4	-	-
Farmer	23	11.5	19	11.6	4	11.1
Government worker	38	19	23	14.0	15	41.7
Tradesmen	21	10.5	19	11.6	2	5.6
Other	55	27.5	40	24.4	15	41.7
Total	**200**	**100**	**164**	**100**	**36**	**100**

In Adana province, 59% of beekeepers, 18% farmers, 12% retired, 7% tradesmen, 4% civil servants; In the province of Konya; 21% are beekeepers, 46% are farmers, 17% are retired, 9% are tradesmen and 7% civil servants (Karahan & Karaca, 2016); When the activity areas of beekeepers in Muğla province are examined; 60% of them are only engaged in beekeeping, 15% of them are farming besides beekeeping, 7.5% tradesmen, 15% retired, 2.5% civil servants (Öztürk, 2017); In the province of Konya, 91.11% of the enterprises owners are farmers, 4.45% are tradesmen, 2.22% are retired and 2.22% are workers, and in Konya, the enterprises are mostly engaged in beekeeping activities (Çelik & Turhan, 2014); In the Hizan district of Bitlis province, apart from beekeeping, 6% of the enterprises are civil servants, 8% are farmers, 13% are tradesmen and 73% are workers (Kutlu et al., 2016); In a study conducted in the eastern Black Sea region, the ratio of enterprises that see beekeeping as the main source of income is 27.45%, while the ratio of enterprises that see it as a source of secondary income is 72.55%. Among the provinces in the region that consider beekeeping as the main source of income, Ordu province enterprises rank first with a rate of 50.62%; 90.90% of the businesses that see it as the highest side income source are located in Gümüşhane (Kuvancı et al., 2017); In Iğdır province, 37.60% of beekeeping operators use beekeeping as their primary source of income, 43.50% as an additional income source and 18.90% for hobby purposes (Kadirhanoğulları et al., 2016).

In our survey, we discovered that 57.9% of income-oriented enterprises and 8.3% of backyard beekeeping enterprises were involved in migratory beekeeping activities. In general, 49% of beekeeping enterprises use the migratory beekeeping concept (Table 4).

Table 4. Production model of the examined beekeeping enterprises

	General		**Income-oriented enterprises**		**Backyard beekeeping enterprises**	
	n	**Frequency, %**	**n**	**Frequency, %**	**n**	**Frequency, %**
Yes	98	49.0	95	57.9	3	8.3
No	102	51.0	69	42.1	33	91.7
Total	**200**	**100**	**164**	**100**	**36**	**100**

An average of 2.93 income-oriented enterprises engaged in migratory beekeeping operations; however, backyard enterprises remain at an average of 2.00 points. 94.8% of beekeepers in Adana (Güneşdoğdu & Akyol, 2019); 89% of the enterprises in Konya (Karahan & Karaca, 2016); 53.5% of the beekeepers in Adıyaman province (Özbakır et al., 2016); 96.05% of enterprises in Afyon province (Karahan et al., 2019); 95% of the beekeepers in the Eastern Black Sea region (Kuvancı et al., 2017); 87.36% of beekeepers in Çanakkale (Aktürk & Aydın, 2019); 31% of beekeepers in Hizan district of Bitlis province (Kutlu et al., 2016); 39.2% of the enterprises in Uşak (Tabur & Gül, 2019) are engaged in migratory beekeeping activities. In Düzce, 46.8% of beekeepers are interregional, 9.00% intra-provincial, 13.10% intra-district (Kekeçoğlu et al., 2014); In the Yığılca district of Düzce province, 19.1% of them were migratory beekeepers (Kekeçoğlu & Rasgele, 2013). The fact that the flowering periods of nectar and pollen source plants for honey bees in Turkey change due to altitude differences necessitate migratory beekeeping activities. In our study, the rate of enterprises engaged in migratory beekeeping, Güneşdoğdu and Akyol (2019), Karahan and Karaca (2016), Çelik and Turhan (2014), Karahan et al. (2019), Kuvancı et al. (2017), Aktürk and Aydın (2019), lower than the rates reported by; Kutlu et al. (2016), Tabur and Gül (2019), Kekeçoğlu and Rasgele (2013). It is thought that these differences are due to the fact that our study was carried out at the national level, and the literature studies were carried out at the specific regional or provincial level.

It was shown that 67% of the surveyed enterprises enrolled in technical education. The rate of involvement in technical education by income-oriented enterprises was found to be greater than that of backyard beekeeping-purpose enterprises (Table 5).

Table 5. The technical education status of the investigated beekeeping enterprises

	General		**Income-oriented enterprises**		**Backyard beekeeping enterprises**	
	n	**Frequency, %**	**n**	**Frequency, %**	**n**	**Frequency, %**
Yes	134	67.0	114	69.5	20	55.6
No	66	33.0	50	30.5	16	44.4
Total	**200**	**100**	**164**	**100**	**36**	**100**

While it was reported that 16.3% of the beekeepers in Adıyaman took a 20-hour course, 40.7% took an 80-hour course, and 36% did not attend any course or training (Özbakır et al., 2016), 45.01% of the enterprises in Uşak were beekeeping. they gained professional knowledge from their courses and seminars (Tabur & Gül, 2019); In Konya province, 57.11% of beekeepers attend beekeeping courses (Çelik & Turhan, 2014); Among the sources of information about beekeeping in the province of Elazig, the rate of choosing beekeeping courses in the first degree was 49.2%, while the rate of preference was found in the second place by 39.7% (Seven & Yeninar, 2010). According to the information obtained from 27% of the beekeepers in Gaziantep, beekeeping courses are very effective on starting and learning beekeeping (Kutlu, 2014).

42% of the enterprises surveyed produce just extracted honey, 8% produce only honeycomb, and 27% produce both extracted honey and honeycomb. Cumulatively, 90.5% extracted honey, 56.5% honeycomb and 23% natural honeycomb are produced in the enterprises. Cumulatively, the enterprises produce 90.5% extracted honey, 56.5% honeycomb, and 23% natural honeycomb. Backyard beekeeping enterprises extracted honey and honeycomb production rates were found to be higher than income-oriented enterprise rates. Cumulatively, 93.2% of income-oriented enterprises produce extracted honey, 58.5% honeycomb and 27.4% natural honeycomb. On the other hand, backyard enterprises produce 77.8% of extracted honey, 47.2% of honeycomb and 2.8% of natural honeycomb cumulatively. It is seen that income-oriented enterprises produce more natural honeycomb than backyard-purpose enterprises (Table 6). In the Kemalpaşa district of İzmir province, 94.34% of beekeepers produce honeycomb, 22.64% natural honeycomb (Onuç et al., 2019). In addition, it has been reported that all beekeepers in Gaziantep produce honeycomb and extracted honey (Kutlu, 2014).

Table 6. Production types of the examined beekeeping enterprises

	General		**Income-oriented enterprises**		**Backyard beekeeping enterprises**	
	n	**Frequency,%**	**n**	**Frequency,%**	**n**	**Frequency,%**
Extracted honey (EH)	84	42.0	65	39.6	19	52.8
Honeycomb	16	8.0	8	4.9	8	22.2
EH + Honeycomb	54	27.0	46	28.0	8	22.2
EH + Naturel Honeycomb	3	1.5	3	1.8	-	-
Honeycomb + Naturel Honeycomb	3	1.5	3	1.8	-	-
Honeycomb + EH + Naturel Honeycomb	40	20.0	39	23.8	1	2.8
Total	**200**	**100**	**164**	**100**	**36**	**100**

In this study, 37.4% of the enterprises had mixed blossom honey, 15.3% thyme honey, 12.2% milkvetch honey, 12.2% chestnut honey, 10.6% pine honey. It was determined that 4.1% of them produced citrus honey and 8.3% of them produced other monofloral and polyfloral honeys, especially lavender, sunflower, cotton and oak. The ratio of mixed flower honey and monofloral chestnut honey produced by backyard beekeeping enterprises was found to be higher than that of income-oriented enterprises (Table 7).

Table 7. Distribution of honey produced in the examined beekeeping enterprises

	General		**Income-oriented enterprises**		**Backyard beekeeping enterprises**	
	n*	**Frequency,%**	**n***	**Frequency,%**	**n***	**Frequency,%**
Blossom honey	166	37.4	138	35.9	28	46.7
Milkvetch honey	54	12.2	51	13.3	3	5.0
Thyme honey	68	15.3	61	15.9	7	11.7
Pine honey	47	10.6	44	11.5	3	5.0
Chestnut honey	54	12.2	43	11.2	11	18.3
Citrus honey	18	4.1	16	4.2	2	3.3
Other	37	8.3	31	8.1	6	10.0
Total	**444**	**100**	**384**	**100**	**60**	**100**

*Multiple responses were considered.

In the Kemalpaşa district of İzmir province, 98.11% of beekeepers produce pine honey (Onuç et al., 2019); beekeepers of Konya province concentrate on blossom, citrus and pine honey production and they go to the Mediterranean and Aegean regions, where pine honey is produced, especially at the end of August (Çelik & Turhan, 2014); the plant diversity used by the migratory beekeepers to get nectar in the Yığılca district of Düzce; 46.7% chestnut, 26.6% chestnut and rhododendron, 13.3% rhododendron, 6.7% linden and 6.7% wildflower (Kekeçoğlu & Rasgele, 2013). Migratory beekeepers in Ordu province stated that they carry out their beekeeping activities 20% at Muş, 15% at Erzincan, 12% at Erzurum, 10% at Yozgat, 9% at Sivas, 7% at Kars, 6% at Kars, 3% at Hakkari, 1% at Çankırı, and 15% at more than one location to follow the blooming seasons, in addition 19% of the beekeepers in the Eastern Black Sea region stated that they produced honey in Erzurum province and 35% in the Eastern black sea location (Kuvancı et al., 2017).

It has been realized that 33.5% of the surveyed enterprises produce mainly pollen and honey, and 40% produce pollen, honey and other bee products. Together with honey, 19.0% of the enterprises are produced beeswax; 1.5% produced propolis, 1.0% produced royal jelly and 0.5% produced bee venom. It has been determined that the product variety of bee products in income-oriented enterprises is higher than in backyard enterprises. Together with honey, income-oriented enterprises produced 73.6% of were pollen, 56.6% beeswax, 29.8% propolis, 7.8% royal jelly and 1.2% bee venom produced (Table 8).

Table 8. Production diversity of the examined beekeeping enterprises

	General		**Income-oriented enterprises**		**Backyard beekeeping enterprises**	
	n	**Frequency,%**	**n**	**Frequency,%**	**n**	**Frequency,%**
Pollen	67	33.5	54	32.9	13	36.1
Propolis	3	1.5	2	1.2	1	2.8
Beeswax	38	19.0	30	18.3	8	22.2
Royal jelly (RJ)	2	1.0	2	1.2	-	-
Bee venom	1	0.5	1	0.6	-	-
Pollen + RJ	1	0.5	1	0.6	-	-
Pollen+ Beeswax	30	15.0	24	14.6	6	16.7
Pollen + Propolis	13	6.5	9	5.5	4	11.1
RJ + Beeswax	1	0.5	1	0.6	-	-
RJ + Propolis	1	0.5	-	-	1	2.8
Beeswax + Propolis	7	3.5	7	4.3	-	-
Pollen + RJ + Beeswax	2	1.0	2	1.2	-	-
Pollen + RJ + Propolis	2	1.0	2	1.2	-	-
Pollen + Beeswax + Propolis	27	13.5	24	14.6	3	8.3
Pollen + RJ + Propolis + Beeswax	4	2.0	4	2.4	-	-
Pollen + Beeswax + Propolis + Bee venom + RJ	1	0.5	1	0.6	-	-
Total	**200**	**100**	**164**	**100**	**36**	**100**

In the Kemalpaşa district of İzmir province, together with honey production, 94.34% of beeswax, 47.17% of pollen and 18.87% of propolis are produced by beekeepers (Onuç et al., 2019). In Adıyaman province, the income of the enterprises is obtained from honey, beeswax and pollen, respectively, and only 4.7% of the enterprises produce pollen (Özbakır et al., 2016). In Afyon province, 80.26% of beekeepers were produced beeswax, 63.16% pollen, 19.74% propolis, and 5.26% royal jelly (Karahan et al., 2019). In Çanakkale province, 35.63% beeswax, 34.48% pollen, 5.75% propolis and 4.59% royal jelly were produced by beekeepers (Aktürk & Aydın, 2019). 6% of beekeepers in Ordu produce pollen. 7% of enterprises in Giresun province produced pollen, propolis and royal jelly. 33% of Trabzon province enterprises produce pollen and propolis. 17% of Artvin province enterprises produce pollen and propolis. 61% of Gümüşhane province enterprises produce pollen. 10% of the enterprises in Bayburt province produce pollen (Kuvancı et al., 2017). Beekeepers of Bitlis Hizan province produce 15% pollen and 3% royal jelly (Kutlu et al., 2016). It has been reported that 88.88% of the enterprises in Konya produce beeswax and 15.55% produce pollen (Çelik & Turhan, 2014). It has been seen that the product diversity of the enterprises examined in our study is higher than in the previous studies. The reason for this high rate can be explained by the fact that the study was carried out at the national level and the use of online surveys.

The queen bee's quality is one of the most critical factors affecting a colony's productivity. 37% of the examined beekeeping enterprises were Caucasian or hybrid, 32.5% Anatolian bee, 18% Carniolan bee, 8.5% Belfast bee, 2.5% Muğla ecotype, 1% of beekeepers stated that they work with Italian bees and 0.5% with Yığılca ecotype. While income-oriented enterprises use 37.8% Caucasian bees, 32.9% Anatolian bees, and 15.9% Carniolan bees, backyard enterprises use 33.3% Caucasian bees, 30.6% Anatolian bees, and 27.8% Carniolan bees (Table 9). According to Karahan and Karaca (2016), in the province of Adana; 56% Hybrid bees (61% Anatolian hybrid), 12% Anatolian bee, 12% Italian bee, while 37% Caucasian bee and 35% Hybrid bee (54.3% Caucasian hybrid) were used in Konya. According to a study conducted in Adiyaman, 53% of the beekeepers work with Caucasian crossbreeds and 41% with Caucasian bee breeds (Özbakır et al., 2016). According to a research conducted by Karahan and Karaca (2019) in Afyon province, 47.37% of beekeepers use Anatolian bees, while 38.16% use hybrid bees (Aegean, Muğla, Italian, and Caucasian hybrids). Kekecoğlu et al. (2014) stated that, native breeds appropriate to the region are used by 61.50% of beekeepers in Düzce, whereas Caucasian hybrids are used by 34.70 percent. According to Aktürk and Aydın (2019) the local bee breed is preferred by 41.38 percent of producers in Çanakkale, 37.93 percent prefer Anatolian bees, and 21.84 percent prefer Carniolan bees. In a study conducted by Akdeniz et al. (2015) in the province of Antalya, 36.84% of migratory beekeeping enterprises use Caucasian hybrids, 21.05% use Muğla bees, 5.26% use Anatolian bees, and 36.84% use multiple bee breeds in their enterprises, moreover in farms with mixed race colonies, 92.85% Caucasian crossbreeds, 14.29% Anatolian Bees, 42.86% Belfast bees, 50% Muğla bees, 35.71% Italian bees, 21.43% Carniolan bees were reported. In a study conducted throughout Turkey, according to the information obtained from beekeepers, 65% hybrid honey bee, 12% Caucasian bee, 9% Muğla bee, 6% Anatolian bee, 4% Carniolan bee, 2% Thrace bee and 1% Buckfast are generally used (Emir, 2015). In our study, it is seen that the domestic and foreign queen bee races and ecotypes used in the production activities of the enterprises we examined are similar to the data obtained from the studies conducted in different geographical regions.

Table 9. The evaluated beekeeping enterprises' genotype distribution of reared queens

	General		**Income-oriented enterprises**		**Backyard beekeeping enterprises**	
	n	**Frequency,%**	**n**	**Frequency,%**	**n**	**Frequency,%**
Anatolia bee	65	32.5	54	32.9	11	30.6
Belfast bee	17	8.5	16	9.8	1	2.8
Italian bee	2	1.0	2	1.2	-	-
Caucasian bee	74	37.0	62	37.8	12	33.3
Carniolan bee	36	18.0	26	15.9	10	27.8
Anatolian bee (Muğla Ecotype)	5	2.5	3	1.8	2	5.6
Anatolian bee (Yığılca Ecotype)	1	0.5	1	0.6	-	-
Total	**200**	**100**	**164**	**100**	**36**	**100**

According to our research, 63.0% of the analyzed enterprises fulfilled their queen demands solely from their own operations, 18.5% entirely from commercial enterprises, and 18.5% achieved their needs by both producing and purchasing their own queen bees. Moreover, income-oriented enterprises produce more queens and use less commercial queens than backyard-purpose enterprises (Table 10). In the study conducted by Güneşdoğdu and Akyol (2019) in Adana province, reported that 77.05% of the enterprises require the queen bee, from natural queen capscap for swarming, 13.66% by larvae transfer, larvae grafting, and 9.29% commercially purchased. In other survey studies, conducted in the Kemalpasa region of Izmir province, 94.35% of beekeepers produce their own queen bees (Onuç et al., 2019), in Adıyaman, 48.8% of the enterprises stated that they produced their queen bee needs demands from their own colonies, while 27.9% stated that they achieved their queen bee needs by purchasing them from commercial queen bee producers (Özbakır et al., 2016). In Muğla province, 45% of bee breeders use natural swarm and 55% use artificial swarm to reproduce their colonies (Öztürk, 2017), 90.8% of beekeepers in the province of Bingöl reproduce their colonies by division method, 6.9% by natural swarm, and 2.3% by commercial queen bee

(Söğüt et al., 2019). In the Yığılca district of Düzce province, 8.2% of the beekeepers use artificially produced queen bees and 91.8% use the queen bees created naturally by the colony itself (Kekeçoğlu & Rasgele, 2013). In Konya province, 46.67% of the enterprises produce the queen themselves, 37.78% buy them from the enterprises producing queen bees, 6.67% are from the beekeepers in the region and 8.88% are providing the queen bee through cooperatives and unions. (Çelik & Turhan, 2014). In the province of Antalya, 36.84 of the migratory beekeeping enterprises breed the queen naturally and 44.74% by transferring larvae; It has been reported that 18.42% of them obtain their queen bee needs by purchasing them from enterprises that sell commercial queen bees (Akdeniz et al., 2015). In a study conducted in the provinces of the Eastern Black Sea region, 70.43% of the breeders produced their own queen bees and 24.50% purchased commercial queens (Kuvancı et al., 2017). Contrary to other provinces and our study results, it has been reported that 84.5% of beekeepers in Malatya purchased commercial queen bees (Kutlu & Kılıç, 2020).

Table 10. Queen supply of the examined beekeeping enterprises

	General		**Income-oriented enterprises**		**Backyard beekeeping enterprises**	
	n	**Frequency, %**	**n**	**Frequency, %**	**n**	**Frequency, %**
Own production	126	63.0	107	65.2	19	52.8
Purchased	37	18.5	30	18.3	7	19.4
Both of them	37	18.5	27	16.5	10	27.8
Total	**200**	**100**	**164**	**100**	**36**	**100**

In our research, we noticed that 60.6% of the investigated enterprises were affected by varroa parasites, 14.3 percent by brood diseases, 11.7 percent by nosema, and 9.8 percent by chalkbrood. Furthermore, it has been found that the frequency of varroa parasites is greater in backyard-purpose enterprises than in income-oriented enterprises (Table 11). In a study conducted in Adana, enterprises faced the highest number of *Varroa destructor* parasites (87.6%) in their colonies, followed by brood diseases (11.2%), nosema (7.1%), and chalkbrood (4.7%) (Güneşdoğdu & Akyol, 2019). In another study conducted in Adana province, 78% of the enterprises were found to have varroa, 27% had nosema, 14% had brood diseases and 13% had chalkbrood (Karahan & Karacan, 2016). On the other hand, 63% of the enterprises in Konya are exposed to varroa, 22% to chalkbrood, 22% to brood diseases rot and 18% to nosema diseases and pests (Karahan & Karaca, 2016). In Afyon province, the problem that beekeepers suffer the most is varroa with 76% (Karahan et al., 2019). In the province of Bingöl, it has been reported that 86.2% of the beekeepers were exposed to varroa, 9.2% to brood diseases, 2.3% to nosema and 2.3% to chalkbrood (Söğüt et al., 2019). It has been determined that the primary problem of all the enterprises in bee health is *Varroa destructor* parasite. Varroa parasites are spread by bees by natural swarming or predatory activities of colonies. Moreover, the ability of drones to roam between colonies has also been reported as an important factor in the rapid spread of the varroa (Aydın, 2012).

Table 11. Diseases and pests encountered by the examined beekeeping enterprises

	General		**Income-oriented enterprises**		**Backyard beekeeping enterprises**	
	n*	**Frequency, %**	**n***	**Frequency, %**	**n***	**Frequency, %**
Varroa	191	60.6	158	58.7	33	71.7
Brood diseases	45	14.3	38	14.1	7	15.2
Nosema	37	11.7	37	13.8	-	-
Chalkbrood	31	9.8	28	10.4	3	6.5
Other	11	3.5	8	3.0	3	6.5
Total	**315**	**100**	**269**	**100**	**46**	**100**

**Multiple responses were considered.*

According to our findings, one of the most important concerns for enterprises in the industry is marketing, which accounts for 24% of all concerns. After the marketing problem, the major problems faced by enterprises are accommodation (17.7%), diseases and pests (15.7%), safety (15.7%), transportation (10.4%), and pesticide applications (7.1%). While the marketing problem is foregrounded with a share of 25.3% in income-oriented enterprises, it has been determined that the biggest problem in enterprises engaged in production for backyard purposes is honeybee diseases and pests with a share of 19.3% (Table 12). It has been determined that the most important problem encountered by beekeepers in Adana is the accommodation problem, besides, in the regions where the beekeepers go, there are important problems such as forager bee deaths caused by pesticides, theft incidents, transportation problems, demanding rent for the land and fire (Güneşdoğdu & Akyol, 2019). According to a survey performed by Onuç et al. (2019) in the Kemalpasa region of Izmir, the most fundamental challenge in beekeeping is difficulties in marketing bee products, with a rate of 32.08%. This is followed by the absence of effective treatments against diseases and parasites with a rate of 26.42%, the use of intensive pesticides with a rate of 13.21%, the inability to sufficiently introduce the benefits of bee products to consumers with a rate of 13.21%, and the inability to standardize bee products with a rate of 5.66%. Öztürk (2017), questioned beekeepers in Muğla province Ula district what the most significant difficulty of beekeeping in Turkey is, 70% of the producers responded that they had marketing challenges due to low honey prices and that they had accommodation problems in migratory beekeeping. Other concerns include high input prices, limited supports, the varroa problem, a lack of fuel support for transportation, placing a colony in the same place above its capacity, and bee mortality caused by pesticides. In the Konya province, 57.78% claimed difficulty with marketing, 22.22% in production, and 20.00% with disease and pest control (Çelik & Turhan, 2014). In Bitlis province's Hizan district, 43% of beekeepers reported pesticide problems, 32% reported problems with place availability and accommodation, 13% reported problems with foreign migratory beekeepers, 7% reported theft, and 5% reported problems with the headmen in their accommodation (Kutlu et al., 2016). Although there are variations in the rankings in terms of regional differences and priorities, the findings we obtained in our study are similar to the findings given in the literature information. In general, beekeepers highlight marketing, diseases and pests, bee fatalities due to intensive pesticide application, and difficulties encountered during accommodation and transportation.

Table 12. The main problems faced by the examined beekeeping enterprises in the sector

	General		**Income-oriented enterprises**		**Backyard beekeeping enterprises**	
	n*	**Frequency, %**	**n***	**Frequency, %**	**n***	**Frequency, %**
Accommodation	87	17.7	79	18.2	8	14.0
Transportation	51	10.4	48	11.1	3	5.3
Security	77	15.7	69	15.9	8	14.0
Usage of Pesticides	35	7.1	29	6.7	6	10.5
Diseases and Pests	77	15.7	66	15.2	11	19.3
Packaging	10	2.0	9	2.1	1	1.8
Marketing	118	24.0	110	25.3	8	14.0
Other	36	7.3	24	5.5	12	21.1
Total	**491**	**100**	**434**	**100**	**57**	**100**

**Multiple responses were considered.*

Conclusion

The marketing problem is one of the most serious issues confronting beekeeping operations in Turkey. In particular, the presence of imitation and adulteration bee products in the market causes the price stability of the products to not be ensured, and therefore the products produced by the enterprises cannot be offered at their ideal value. The Ministry of Agriculture and Forestry's identification of counterfeit and adulterated products and enterprises on the market, as well as the application of regulatory sanctions on these enterprises, helped beekeepers breathe a little easier. The establishment of honey cooperatives through Beekeepers' Unions and assuring honey price stability by governmental organizations are among the primary recommendations brought to the table by beekeepers in the marketing of bee products. Another problem faced by enterprises is the inability to effectively control honey bee diseases and pests. Moreover, diseases and pests cause a significant amount of colony losses and yield losses for enterprises. Furthermore, it has been noticed that the effectiveness levels of licensed treatments available on the market do not satisfy the requirements of enterprises. In particular, the potential of beekeepers to turn to unlicensed drugs due to commercial concerns will cause residue problem in products. It is critical to identify the efficacy levels of licensed treatments with different active components on the market, as well as the rates of parasite resistance to these treatments in particular. Communication should be established between relevant government institutions and organizations, non-governmental organizations, and universities. Pesticide application in agricultural products is another threat to enterprises, since it causes a decrease in colony populations, yield losses, and residues in bee products. Pesticide treatments should be carried out during times when honey bees do not flight activity, with the cooperation of beekeepers, non-governmental organizations, and Agriculture and Forestry Departments. It is required to produce various bee products, such as apiteraphy products, other than honey in order for the companies to be financially successful. The positive effects of apitherapy products on human health make bee products consumption more paying attention by the day. It is obvious that strategically guiding breeders to alternative bee products with the appropriate support policies, would make major contributions to the country`s economy. On the other hand, solving the problem of quality breeding queens with breeding studies, more effective work of non-governmental organizations, dissemination of vocational training, increasing state supports and inspections are other issues that are expected to be solved by enterprises.

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