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RESEARCH PAPER

The Impact of *Cannabis sativa* and *Helianthus annuus* Plants on Honeybee Colonies (*Apis mellifera* L.): *Varroa destructor* Infestation and Performances

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Abstract

The performance and *Varroa destructor* infestation percentage of the honey bee colonies placed in areas with *Cannabis sativa* and *Helianthus annuus* plants were investigated. The study was conducted in the Havza district of Samsun and Samsun Ondokuz Mayıs University campus. A total of 15 bee colonies, with five in three different areas, were used. No chemical treatment against Varroa was administered in the colonies. In various areas, significant differences were observed in the worker bee population, the brood production, and the *Varroa destructor* infestation on adult bees and pupal cells. It is determined that the amount of Varroa on adult bees of the colonies in the sunflower and campus areas three and two times higher when compared to the colonies in cannabis area. Sunflower plants had a significantly positive impact on colony development. Cannabis has significantly increased brood production at the end of summer and autumn, which is a very critical period for honey bees. The campus area had significant disadvantages due to a summer drought and lack of flora at the beginning of autumn. It has been concluded that cannabis and sunflower plants play an important role in supporting bees before winter. It is essential to examine the efficacy of pure extracts derived from cannabis in combating Varroa through clinical research.

Introduction

Varroa destructor is an arthropod that has a profound impact on honey bee populations, causing significant losses and serving as a vector for various diseases within bee colonies (Rehm & Ritter, 1989; Anderson & Truman, 2000; Morse & Fluton, 1997; Lamas et al., 2023). The mite shows genomic variation at the subspecies level, and each of them has different levels of detrimental impacts on the honey bees (Anderson & Trueman, 2000; Hua et al., 2023; Zheguang et al., 2023). Today, various methods such as heat applications, biological, genetic breeding and biotechnological approaches are employed in the management of Varroa infestations (Kumova, 2004; Girişgin et al., 2007; Çetin, 2010; Koşat, 2016; Guler, 2017; Çakmak, 2017; Seven et al., 2017; Aydın, 2018; Demirezen, 2019). Most of the biological components used for this purpose are of vegetable origins (Akyol et al., 2006; Brodschneider & Crailsheim, 2010; Damiani et al. 2011). Numerous studies showed that essential oils from herbs such as thyme, clove, mint, cinnamon, grapefruit, rosemary, marigold, laurel, eucalyptus, pine cone and tea tree have lethal effects against Varroa as well as bacteria and fungi (Sönmez, 2010; Sönmez, 2017; Varol, 2018; Bava et al., 2023; Kanelis et al., 2023; Alpay et al., 2023). It was reported that some of these herbal products are particularly beneficial in mite control (Damiani et al. 2010; Jbilou et al., 2006) and have demonstrated a wide range of biological activities of plant-derived products. These activities include toxicity, repellant or attraction, reproductive inhibition, behavioral disorder and growth regulatory effects. However, it has been reported that some organic or synthetic drugs and industrial carbohydrates negatively affect the colonies, causing stress and worker bee deaths (Guler et al., 2018; Nisbet et al., 2018a; Bava et al., 2023; Zheguang et al., 2023). For example, high doses of thymol can induce genomic cell poisoning (Glavini´c et al., 2023). This is because each extract contains a complex mixture of different phytochemicals (plant secondary metabolites). The biochemical structures of these components also show significant differences between plant species, and the composition of the extract may change depending on factors such as the harvest season, drying process, storage conditions and other factors (Damiani et al. 2010). Another issue that should not be forgotten is the presence of a uniquely balanced microbiota in the digestive system of the bee. Any negative changes in the ventricular microbiota may cause the bee not to benefit from sufficient nutrients (Brodschneider & Crailsheim, 2010; Ramsey et al., 2019; Li et al., 2022; Chenyi et al., 2022).

It is known that colonies with larvae, pupae and adults bee fed with adequate and high quality food are more healthy and productive (Seeley, 1995; Weiss, 2009; Brodschneider & Crailsheim, 2010; Sammatora & Avitabile, 2011; Jennette, 2017; Guler, 2017; Oskay et al., 2020; Li et al., 2022). Genes (AmILP-1, BRP, Vg) and gene expression structures that affect growth, development, behavior and lifespan may vary according to age and food diets (Koru, 2018; Bozkurt et al., 2022). For this reason, the quality and richness of the flora resources in the areas where the colonies are placed contribute not only to their efficiency but also to their health (Winston, 1991; Brodschneider & Crailsheim, 2010; Guler, 2017). As a matter of fact, thanks to the important fatty acids that are components of pollen, the honeycomb cells are made hygienic before the queen bee lays eggs (Winston, 1991; Öder, 1993). One of these plants is cannabis. One of the most important advantages of cannabis is that it does not need chemical control during the cultivation process (Aytaç et al., 2018). In addition, there are reports that the cannabis plant which has the tetrahydrocannabinol substance prevents the Varroa mite. Indeed, Choopracit et al. (2020) defined honey bees and the cannabis plant as sacred creatures. In addition, Dalio (2012) emphasized that the cannabis plant is an important pollen source for the honey bee during the flowering period.

Cannabis plant cultivated areas have increased day by day in our country. For this reason, there was a need to question the effect of the Varroa population and behavior on bee colonies during the flowering period of the cannabis plant and to obtain detailed data from the field.

In this study, it was aimed to determine the presence of Varroa mites, performance and some behavioral activities of bee colonies placed in a normal field, sunflower and cannabis planting areas.

Material and Methods

Material

The colonies are placed in the Cannabis sativa, sunflower cultivation in the Havza district and the campus areas of Ondokuz Mayıs University of Samsun province. The distances of the experimental fields are between 12 and 100 km. A total of 15 colonies, 5 of which were randomly selected, were placed in each area. The Black Sea genotype, which is widelykept in the region, was used. Colonies were equalized in terms of queen age, frame with bees, frame with brood, food source, chemical application and all similar features (Guler & Kaftanoğlu, 1999; Guler et al., 2018). Each of the colonies was arranged in 8 frames that were covered with bee. No chemical was applied to thecolonies in the spring agains varroa.

Method

Necessary measurements were made in the colonies before flowering, 10 days after flowering, 10 and 25 days after the end of flowering in the cannabis plant. Similarly, measurements were taken in other groups, taking into account pollen and nectar flow. Honey harvesting from the colonies was carried out considering the end of the nectar secretion of the plants and the maturation of the honey and the general practices of the beekeepers. Therefore, honey harvest was performed in August in the colonies placed in the sunflower field and in September in the cannabis planted land.

Brood Production (cm^2/colony)

The open and closed brood area on the honeycomb in the colonies was measured with the help of a ruler every 21 days over the long and wide axis. Then, the area in cm^2 was calculated by applying the length and width S=3.14xA/2xa/2 ellipse formula on the honey comb and the total brood area was determined for each colony (Guler & Kaftanoğlu, 1999; Delaplane et al., 2013; Guler et al., 2018).

Colony Population (number of frames/colony)

Frame covered with bees were counted and recorded at 21-day intervals throughout the experimental period (Sammataro & Avitabile, 2011**;** Sammataro & Weiss, 2013).

Hive Weight (kg/colony)

The hives that colonies kept in were weighed and recorded before and after the nectar flow period, and after the honey harvest.

Amount of Varroa Mite on Adult Bees (%varroa/colony)

A frame containing worker bees, without offspring or pupae, was placed in a plastic bag and the worker bees were shaken. Hot water was added to the bag and shaken for a while. When the rinsing process was finished and the worker bees, the amount of varroa on the bee and in the bag were counted and recorded. The rate of contamination (%) was determined by using the formula given below (Cobey & Lawrence, 1988; Genç, 1992; Morse & Flottum, 1997; Dietemann et al., 2013).

Infection Rate of Varroa: (Total Varroa Number/Total Worker Number)*100

Amount of Varroa Mite in Pupae Cells (%varroa/colony)

A frame with closed brood was taken from each colony and worker bees was shaken into the hive. The frame was tilted in a horizontal position and 100 pupae were removed with forceps. Varroa on the pupa and in the pupal cells were counted with the help of a light source apparatus (Aydın, 2018; Emsen, 2008; Dietemann et al., 2013).

Honey Yield (kg/colony)

Firstly, frames with honey in each colony were recorded. After leaving the required honey for the colony, the remaining was recorded as honey yield. Before the centrifugation process, the honey frames of each colony were placed in their own honeywells and weighed. After the centrifuge, the same frames were placed in their own honeywells and weighted again and their tare was found. Then, the honey amount produced by each colony (kg/colony) was found by excluding tare from the first measurement (Guler & Kaftanoğlu, 1999; Guler et al., 2018). Honey was harvested in the 3rd week of August.

Forage Bee Weight (mg/worker bees)

Ten worker bees for each colony returning from the field from the hive entrances were caught and placed in a small transparent bag. These bees were weighed on a sensitive scale and recorded as a worker bee weight.

Statistical Analysis

Statistical analyses were carried out according to the randomized block with repeated observations design. Duncan's test was used for multiple comparisons. Versus of normality was determined by Shapiro-Wilk test and the homogeneity of variances was determined by Levene test. NPMANOVA software was used to analyze the data (Anderson, 2000). It was determined that the data for all features were normally distributed (P > 0.05) and the variances were homogeneous (P > 0.05). SAS (1988 SPSSx, Customer ID: 361835) was used as a statistical program.

Result

Amount of Brood Area

There were significant differences (P <0.001) between the area and period in terms of the amount of brood area (Table 1). On average, the highest (3916.55 ± 328.28 cm²/colony) and the lowest number of brood (2122.99 ± 187.15 cm²/colony) were found in sunflowers and in the campus colonies. The overall mean was 3045.77 ± 263.34 cm²/colony. The amount of brood area varied between 468.17 to 6005.91 cm²/colony according to the periods. The highest brood area was found in the sunflower in the second period with 6005.91 cm²/colony. The lowest brood area was found on the campus in the third and fourth periods with 468.17 and 548.84 cm²/colony (Table 1).

Table 1. Mean and standard error values of brood production (cm^2/colony), colony population (number of frame/colony) and hive weight (kg/colony)

Field	Period	Brood Area	$\bar{x}\pm s$.e	Worker Bee Population	$\bar{x}\pm s$.e	Hive Weight	$\bar{x}\pm s$.e
Campus	1	4240.27±429.82^{b*}	2122.99±.187.5^{c}	7,40±0,40^{e}	6.15±0.27^{c}	13.76±0.71^{f}	14.39±1.00^{b}
	2	3235.15±123.58^{cd}		8,40±0,24^{de}		19.06±0.69^{e}	
	3	468.17±97.55^{f}		4,60±0,24^{gh}		13.62±0.63^{f}	
	4	548.84±97.65^{f}		4,20±0,20^{h}		11.12±0.24^{f}	
Cannabis	1	2876.84±345.83^{d}	3097.79±274.59^{b}	6,80±0,37^{ef}	8.10±0.43^{b}	19.46±1.16^{e}	22.73±1.16^{b}
	2	4195.64±245.61^{b}		9,20±0,49^{cd}		24.26±1.56^{d}	
	3	3862.98±282.04^{bc}		10,6±0,68^{c}		28.92±1.55^{c}	
	4	1455.70±197.84^{e}		5,80±0,20^{fg}		18.26±1.19^{e}	
Sunflower	1	4158.45±224.52^{b}	3916.55±328.28^{a}	7,80±0,20^{de}	13.20±0.54^{a}	23.6±0.93^{d}	32.99±2.96^{a}
	2	6005.91±378.22^{a}		17,2±0,86^{b}		34.9±1.01^{b}	
	3	2966.36±185.47^{d}		19,8±1,11^{a}		52.58±2.92^{a}	
	4	2535.50±106.11^{d}		8,00±0,00^{de}		20.88±0.56^{de}	
Overall average			3045.77±263.34		9.15±0.41		24.04±1.70
Sig.		<0.001		<0.001		<0.001	

$^{a,\ b,\ \ldots}$: Means with different letters are significantly different (P < 0.05).

Colony Population

The numbers of worker bee frames differed significantly (P < 0.001) across area and period (Table 1). The highest number of frames covered with worker bees were found in the sunflower (13.20 ± 0.54 frame/colony), and the lowest in the campus area (6.15 ± 0.27 frame/colony). The overall mean was found as 9.15 ± 0.41 frame/colony. The highest number of frames of worker bees (19.8 frame/colony) were found in sunflowers in the third and the lowest (4.20 frame/colony) in the campus area in the fourth period (Table 1).

Hive Weight

Hive weight showed a significant difference (P < 0.001) according to area and period. On average, the highest hive weight was found in sunflower (32.99 ± 2.96 kg/colony) and the lowest (14.39 ± 1.00 kg/colony) in the campus. Weight varied between 11.12 to 52.58 kg/colony. The overall mean was found as 24.04 ± 1.70 kg/colony. The minimum weight in the campus area was determined in the first, (13.62) and fourth periods (11.12), and the highest (52.58) was found in the sunflower area on the third period (Table 1).

Amount of Varroa Mite on Adult Bees

The mean and standard error values for the number of Varroa mites determined on the adult bee are given in Table 2. The effect of areas and periods on the rate of Varroa mite was significant (P < 0.001). The highest rate of Varroa was found in the campus area (9.46 ± 1.08%), and the lowest in the hemp area (3.08 ± 0.34%). The Varroa ratio on adult bees varied between 1.42 and 16.58 percent per colony. The highest number of Varroa was determined in the campus area in the third period (16.58%), and the lowest in the sunflower in the first period (1.42%) (Table 2).

Amount of Varroa in Pupae Cells

The effect of the areas and periods on the number of Varroa determined on the pupa was significant (P < 0.001). The highest Varroa mean (7.70 ± 0.88%) was determined in sunflower and the lowest (4.44±0.57%) in the cannabis area. The mean amount of Varroa was counted as 6.28 ± 0.77%. The number of Varroa varied between 2.5 to 12.00%. The highest number of Warroa was in the sunflower in the fourth period (12.00%). The lowest number of Warroa was in the second (3.60%) and first period (2.50%) of the cannabis area, and in the first period (3.20%) of the sunflower area (Table 2).

Table 2. Average and standard error values of Varroa amount (%) on adult bee and in pupae cell (%) in the different areas

Field	Period	*Varroa on Adult Bees*	$\bar{x} \pm s$.e	*Varroa in Pupae Cells*	$\bar{x} \pm s$.e
Campus	1	2.38±0.38^{fg*}	9.46±1.08^{a}	4.80±1.07^{cde}	6.70±0.87^{ab}
	2	8.25±1.08^{cd}		8.60±0.68^{b}	
	3	16.58±1.82^{a}		-	
	4	10.65±1.07^{bc}		-	
Cannabis	1	2.49±0.47^{fg}	3.08±0.34^{b}	4.40±1.03^{de}	4.44±0.57^{b}
	2	2.41±0.30^{fg}		3.60±0.24^{e}	
	3	2.90±0.62^{fg}		2.50±0.29^{e}	
	4	4.53±0.90^{ef}		7.50±1.26^{bc}	
Sunflower	1	1.42±0.31^{g}	6.18±0.91^{ab}	3.20±0.37^{e}	7.70±0.88^{a}
	2	5.48±0.63^{e}		7.00±0.71^{bcd}	
	3	5.85±0.66^{de}		8.60±1.57^{b}	
	4	11.96±0.75^{b}		12.00±1.22^{a}	
Overall average			6.24±0.77		6.28±0.77
Sig.			<0.001	<0.001	

$^{a, b, ..:}$ Means with different letters are significantly different (P < 0.05).

Honey Yield

The effect of the fields on honey yield was found significant (P < 0.001). There was no honey harvest in the campus area (Table 3). Honey yield averages in cannabis and sunflower cultivation areas were 5.30±0.66 and 31.40±2.45 kg/colony, respectively. The highest honey was taken from the sunflower field with an average of 31.40 kg/colony.

Forage Worker Bee Weight

The weight of the forage worker bee differed significantly (P < 0.001) according to the area. The lowest average was determined in the campus area with 90.9 ± 0.007 mg/worker bees, and the highest in sunflower and hemp fields with an average of 97.5 ± 0.004 and 97.2 ± 0.007 mg/worker bee, respectively (Table 3).

Table 3. Average and standard error values of honey yield (kg/koloni) and forage worker bee weight (mg/number)

Field	Honey Yield	Weight of Forage Worker Bee
Cannabis	5.30±0.66^{b*}	97.2±0.007^{a}
Sunflower	31.40±2.45^{a}	97.5±0.004^{a}
Campus	-	90.9±0.007^{b*}
Sig.	<0.001	<0.001

$^{a, b, ..:}$ Means with different letters are significantly different (P < 0.05).

Discussion

Colonies placed in the cannabis and sunflower and normal flora areas towards the end of summer (August, September, and October) were affected differently in terms of performance, behavior, and Varroa mite infestation. Therefore, colonies in the areas showed significant differences in terms of many phenotypes. As a matter of fact, the number of frames of worker bees, the honey yield and the amount of brood area of the colonies in the sunflower field were higher than those in the cannabis and campus areas.

Considering the performance of the colonies in these areas such as honey yield, colony worker bee population, and brood production showed similarities and differences, with many previous studies (Genç 1992; Gencer, 1996; Akyol, 1998; Guler & Kaftanoğlu, 1999; Guler et al., 2018; Nisbet et al., 2018b). These differences might have been caused by many factors such as the region, rainfall amount, flora diversity, nectar secretion level and duration, and different bee genetic resources (Korkmaz, 1997; Guler, 2017; Nisbet et al., 2018b). As a matter of fact, both nectar and pollen flow were higher in the sunflower plant. In addition, irrigation in cultivated plants also provides an important advantage. Honey was not harvested in the campus area due to a drought period. On the other hand, the reason for the low honey yield in the cannabis plant area is the low nectar production potential of the cannabis. Dalioi (2012) reported that a small amount of honey was harvested due to the fact that nectar production of the cannabis plant is generally low. However, it is known that the cannabis plant is very rich in terms of pollen sources. Thus, it has been determined that the cannabis plant, which is an annual plant, is a very good source of support, especially in late summer and autumn, when pollen sources are generally scarce. This finding has been emphasized by many researchers (Turan, 2000; Dalio, 2012; İbiş, 2020). Thus, by encouraging the queen bee to lay eggs after the honey harvest, it will increase the development of brood production and enable the colonies to enter the winter season with a stronger young worker bee population (Dalio, 2012; Guler, 2017; Chooprasit et al., 2020). It is thought that this positive effect will be further increased by supporting the colonies in the cannabis field with a small amount of syrup. As a matter of fact, as seen in Table 1, more pollen and nectar secretion in sunflower encouraged more brood production in colonies. The average production of 3916.55 ± 328.28 cm²/colony of brood in the sunflower field in August is a very significant amount. This amount is higher than those of many previous studies (Genç, 1992; Gencer, 1996; Akyol, 1998; Guler & Kaftanoğlu, 1999). While cannabis supported colony development, on the other hand, the bee supported adequate pollination of this plant. This also means more production and more income for sunflower and cannabis growers. The weight of forage worker bee was lower in the campus apiary. This is due to the high amount of Varroa infestation (Table 2), the feeding of the mite with worker bee hemolymph and body tissue and low pollen and nectar flows. In other words, the quality and richness of the pollen and nectar source in the areas where the colonies are placed are effective in their efficiency, as well as making an important contribution to the regeneration of the worker bee body fat tissues and their health (Brodschneider & Crailsheim, 2010; Nisbet et al., 2018a; Julean, 2022).

The rate of varroa in the pupae cells and on the adult bees of the colonies in the cannabis field was lower than those of in the sunflower and campus areas. It is determined that the amount of Varroa on adult bees of the colonies in the sunflower and campus areas three and two times higher when compared to the colonies in cannabis area (Table 2). It is known that the effect of licensed chemicals used in the control of varroais generally between 80% and 99% (Kaftanoğlu et al., 1992; Morse & Flottum, 1997; Tutkun & Boşgelmez, 2003; Kumova, 2004). The result of our study suggests that cannabis might have a significant impact on the Varroa. Turhan (2020), using leaves, fruit and essential oil of myrtle plant against Varroa destructor, determined the infestation level on average 16.16% in adult bees and 13.80% in larvae. Emsen (2008) investigated the effects of thymol, oxalic acid and thymol-oxalic acid mixture on the control of Varroa in colonies. The best result was determined in powder thymol (89.98%) and thymol absorbent foam group (77.15 %), while the lowest effect was determined in the oxalic acid group. Also Girişgin (2008) found Varroa rates of 81.58%, 76.28, 55.97, 18.82 and 76.57 in the oxalic acid, perizin, formic acid and lactic acid groups applied to the colonies in the autumn, respectively. In our study, the amount of Varroa detected in the cannabis area is lower than those of all application groups. Therefore, the cannabis plant caused a significant decrease in the amount of Varroa in bee colonies, and as can be seen above, this decrease was more than the therapeutic effect of many plants used in previous experiments (Chenyi et al., 2022). It was concluded that this might be resulted from the effects of chemicals substant such as tetrahydrocannabinol found in the pollen and nectar of the cannabis plant. It is believed that the effectiveness of cannabis against Varroa may be increased with the usage of pure extracts to be produced from cannabis. Additionally, the findings showed that clinical studies are needed to determine the effectiveness of cannabis plant extract against varroa mites.

Ethical Statement

The Türkiye Central and Ondokuzmayis University Ethic Committees have confirmed that no ethical approval is required for honey bee studies.

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Conflict of Interest

The authors declare that they have no potential conflict of interest in relation to the study in this paper.

Author Contributions

Ahmet Guler conceived this research, designed the experiment and paper wrote, and participated in its revisions of it. Ömer YILMAZ control, keeping of colonies, and data collection.

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RESEARCH PAPER

PCA and LDA Assessment of the Heavy Metal Contamination in Honey Bees, Bee Pollen and Honey Produced in Urban Areas of Türkiye

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Abstract

Heavy metals are of great importance in terms of environmental pollution. Environmental pollution affects not only humans but also plants and animals. Living organisms with varying sensitivity to various pollutants can be used to assess environmental pollution. Honey bees have been used as bioindicators for this purpose because they operate in very large areas in their region. The aim of this study is to investigate heavy metal pollution in honey bees and bee products in 4 different urban regions of Konya using principal component analysis (PCA) and linear discriminant analysis. In this study, when the heavy metal contents of honey bees, bee pollen, and honey samples are compared, it is seen that bees are exposed to more heavy metal contamination. Honey bees don't reflect these amounts in the honey, and the heavy metal content in honey remains in much smaller amounts. When principal component analysis (PCA) is applied to these samples, the heavy metal variation of bees and bee pollen can be explained by three components, while the variation of the heavy metal contents of honey can be explained by four components. Linear discriminant analysis was performed to distinguish the geographic locations of the samples. According to the results of the LDA analysis for heavy metal content, the bees can be assigned to 80% and the pollen and honey to 100% of their correct geographical origin.

Introduction

Environmental issues are getting worse every day, along with population increases, urbanization, industrialization, and changes in consumer habits. One of the biggest problems of our time is environmental pollution, which affects daily life negatively by disrupting the ecological balance. Heavy metals are of great importance in environmental pollution.

In considering environmental problems, the term "heavy metal" has become widely used in recent years to refer to metals with a relatively high density that are toxic even at low concentrations. Although more than sixty elements can be given as examples of heavy metals, the most common and well-known ones are mercury, manganese, iron, cobalt, nickel, copper, zinc, cadmium, arsenic, chromium, lead, silver, and selenium. Some heavy metals, like nickel, copper, iron, zinc, and chromium, are essential to organisms (not having enough or having too much of them can cause disease), while others, like cadmium and lead, are toxic in trace amounts (Goretti et al., 2020; Keil et al., 2011).

Living organisms (bioindicators or biomonitors) with varied sensitivity to different contaminants can be used to assess the environmental contamination of an area. Harmful substances accumulate in the bodies of these creatures, and high mortality rates can be seen (Jaishankar et al., 2014). Honey bees that operate over a large area around the hive are a good biological indicator because they are directly exposed to the toxic conditions in that area and are sensitive to changes in the air, plants, water, and soil in their flight areas. Since the 1970s, they have been increasingly utilized to measure the environmental contamination caused by heavy metals (Celli & Maccagnani, 2003; Satta et al., 2012). Honey bees and bee products are important indicators of environmental contamination because of residues, according to a number of researchers (Porrini et al., 2003; Taha et al., 2017; Zhelyazkova, 2012).

Multivariate statistical analyses are extensively used in the data obtained in research on animal science. In multivariate statistical analysis, p variables or features related to n experimental units are examined. If the number of these variables (p) is high and most of them are related to each other, in other words, the most important technique that can be applied is PCA. Briefly, PCA is a method for expressing the structure explained by p variables with correlation with variables that have no correlation and are linear components of the original variables in numbers less than the number of original variables. The first purpose of PCA is to eliminate the inter-variable dependency structure, and the second is to reduce its size. Therefore, after the eigenvalues are found, it is necessary to decide on the number of significant eigenvalues. Many methods have been developed for this purpose, and the simplest and most widely used method is to decide on the number of components by adding λ values until they exceed 2/3 (67%) of the total variation. Another method is to decide the number of principal components according to the slope of the scree plot graph (Ozdamar, 2004).

Linear discriminant analysis is one of the multivariate statistical methods that aims to categorize individuals into groups they belong to with the least error. Discriminant functions obtained from discriminant analysis are derived from the linear components of the forecast variables under consideration. The most “effective” discriminant that emerged as a result of the analysis is that, with the help of the function, it is possible to predict which group a newly obtained observation will be included in. LDA is widely used in biology as well as in many fields of the social sciences. LDA is used to determine the geographic or botanical origin of plant or animal products (Bassbasi et al., 2014; Choi & Lee, 2012; Jöbstl et al., 2010).

In our previous study, Bayir and Aygun (2022), we attempted to determine whether there were any differences in bees and bee products among different regions. In this study, we aimed to investigate whether bees and bee products can be distinguished based on their source using PCA and discriminant analysis. Discriminant analysis can also be used to identify which region a subsequently obtained sample belongs to. Therefore, based on our study on bees or bee products and the data obtained from subsequent discriminant analysis studies, it is possible to estimate whether unknown bees and bee products originate from areas near roadways or from regions where factories are located.

Material and Methods

Sampling Sites

In 2018, the study was conducted in the Konya region of Türkiye. Konya is located between the north parallels of 36° 22′ and 39° 08′ and the east meridians of 31° 14′ and 34° 05′. In terms of surface area, Konya is the biggest city in Türkiye, with over 41 km^2. The climate in Konya is cold and semi-arid. Konya is the province in Türkiye with the least rainfall. Five honey bee colonies (20 in total) were placed in four different locations (L1 to L4), which were around urban areas (Figure 1).

L1: 38° 02' 05'' N, 32° 30' 10'' E, 1.180 m. 1.210 meters west of the highway, on the city's north side, and in the direction of its predominant wind. There are no industrial facilities, but a small area is where fruits and vegetables are farmed.

L2: 37° 55' 12'' N, 32° 26' 10'' E, 1.140 m. 4.300 meters distant from and north of the highway, on the city's northwest side, and in the direction of the city's predominant wind. There is no industrial facility nearby, but there are agricultural activities like fruit and vegetable farming in a limited region.

L3: 37° 51' 07'' N, 32° 33' 33'' E, 1.010 m. The main wind direction is from the industrial zones, which are southwest of the city and southeast of them (Plastic packaging industry, machinery industry, marble industry, furniture industry). This location is 1.800 meters south and 1.300 meters east of one highway, respectively. Agriculture, including the cultivation of grains, vegetables, and fruits, is practiced in the neighborhood.

L4: 37° 49' 12'' N, 32° 28' 45'' E, 1.027 m. The city side is where the wind is coming from on the city's southern side. It is located 3.400 meters west of one highway and 1.000 meters south of another. Agriculture, including the growing of fruits and vegetables, is widely practiced in the neighborhood.

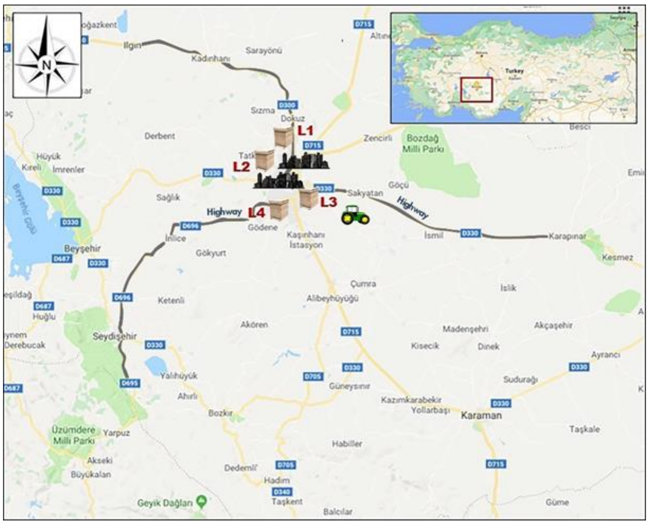

Figure 1: Locations of the honey bee colonies (sampling sites).

Colony Characteristics

Langstroth beehives with plastic bottoms and bee pollen traps were used in the study. Colonies were arranged with newly raised honeycomb and eight frame bees, and no additional feeding was given to the colonies. Honey bee, honey, and bee pollen samples were obtained from these 20 colonies, and the Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn contents of these samples were determined.

Collection and Conservation of Samples

Honey Bee: After August 15, each colony's entry hole was sealed off before midday (about 9:00–10:00), and 30 worker bees coming back from the field were apprehended at the hive entrance using plastic gloves. In glass jars, samples were kept at -18°C until analysis.

Bee pollen: Bee pollen was collected three times every 15 days in May and June, and the collected bee pollen was dried in a dark environment. 25 g (totaling 75 g) of bee pollen collected and dried from each colony at once was taken, mixed, put into glass jars, and stored at -18°C until analysis.

Honey: The honey of each colony in different locations was harvested separately between July 15 and July 20 without using a smoker. Approximately 500 g of honey was collected from each colony and stored in glass jars at room temperature and in the dark until analysis.

Preparation of Samples and Heavy Metal Analysis

To create a homogenous sample, dried bee pollen that had been kept at room temperature was ground (Kacar & İnal, 2008). A total of 2 g of ground bee pollen samples and preserved bee samples were collected and dried in a 70°C oven until they attained a consistent weight before being utilized in the study.

For the heavy metal analysis, 0.2 g of bee pollen, honey, and bee (whole bee) samples were weighed into heat-resistant teflon containers from all sites. 5 mL of concentrated HNO_3 (Nitric Acid) and 2 mL of H_2O_2 (Hydrogen Peroxide) (30% w/v) were added to the weighed samples and the samples were thawed in a microwave device (Cem MARSXpress) at high temperature (210 °C) and pressure (200 PSI). To ensure the reliability of the analysis, 1 control (blank) and 1 certified reference material (Peach Leaves, NIST, SRM 1547) were added to the 40-cell microwave set. The volumes of the thawed samples were made up to 20 ml with deionized water and filtered with blue banded filter paper. The heavy metal contents of the samples (total Pb, Cd, Cr, Zn, Cu, Ni, Mn, and Fe) were determined by an ICP-AES (Inductively Coupled Plasma Atomic Emission Spectrometry, Varian-Vista Model, Axial) device (USDA, 2004).

Statistical Analysis

ANOVA was applied to determine whether there is a statistically significant difference between heavy metals measured in honey bees, bee pollen, and honey samples. The heavy metal contents were subjected to Duncan Test, one of multiple comparison tests. Data measured from bees, honey, and bee pollen was evaluated with PCA and LDA. The number of variables was reduced by creating groups with the PCA method. After PCA analysis, geographical origins of bee honey and bee pollen were tried to be determined by LDA. Statistical analyzes were made using SPSS 19 statistical program.

Results

Table 1 shows the ANOVA results to check whether there is a statistically significant difference between bees, honey, and bee pollen in terms of heavy metal content. As a result of the analysis, it was observed that the bees contain more heavy metals than the bee products (P < 0.01). While there is no significant difference in Cr between bee and bee pollen, honey contains the least Cr (0.053 mg/kg) (P < 0.01). In all of the other heavy metals used in the study, the highest heavy metal content was found in bees and the lowest in honey, and these differences are statistically significant in all of them (P < 0.001). In this study, bees had the highest Cd (0.0189 mg/kg) and Pb (0.335 mg/kg) content compared to honey and bee pollen.

Table 1. Descriptive statistics and ANOVA results for heavy metals ($\bar{X} \pm S_x$)

Heavy metals (mg/kg)	Honey bee	Bee pollen	Honey
Cd	0.0189 ± 0.0005^a	0.0105 ± 0.0005^b	0.0065 ± 0.0003^c
Cr	0.084 ± 0.0039^a	0.075 ± 0.0029^b	0.053 ± 0.0031^b
Cu	15.06 ± 0.56^a	6.23 ± 0.22^b	0.77 ± 0.034^c
Fe	98.38 ± 1.548^a	86.61 ± 3.637^b	9.53 ± 0.847^c
Mn	30.66 ± 1.297^a	15.31 ± 0.881^b	0.98 ± 0.065^c
Ni	0.396 ± 0.0112^a	0.353 ± 0.0144^b	0.203 ± 0.0074^c
Pb	0.335 ± 0.0096^a	0.146 ± 0.0079^b	0.113 ± 0.0046^c
Zn	41.42 ± 1.347^a	18.41 ± 0.525^b	1.48 ± 0.054^c

Means with different superscript letters are significantly different (P < 0.01).

Assessment of Honey Bee Data by PCA and LDA

In this study, the principal component was determined as much as the number of eigenvalues greater than one, which is the generally accepted method. Accordingly, three principal components are suitable for the bee, and 32% of the total variance in heavy metals in bees is explained by the first PC and 68% by the first three PCs (Table 2). PC1 consists of Cr, Cu, and Fe metals and it can be said that the majority of heavy metal variance in bees can be explained by these three metals. PC2 is composed of Mn and Zn metals.

Table 2. PCA analysis results for heavy metals measured in honey bees

Heavy metals	**PC1**	**PC2**	**PC3**
Cd	0.355	0.346	**-0.383**
Cr	**0.485**	0.067	0.325
Cu	**0.324**	-0.307	-0.072
Fe	**0.448**	-0.277	-0.221
Mn	-0.052	**0.607**	-0.270
Ni	0.455	0.025	**0.470**
Pb	0.246	-0.195	**-0.623**
Zn	0.250	**0.545**	0.107
Eigenvalue	2.5424	1.8834	1.0102
Proportion	0.318	0.235	0.126
Cumulative	0.318	0.553	0.680

As a result of the discriminant analysis performed to determine the geographical origin of bees in terms of heavy metal content, three canonical functions were formed and their eigenvalues were 12.576, 2.442 and 0.350, respectively. The large eigenvalue indicates that most of the variance in the dependent variable is explained by that function. Here, the first function with the 12.576 eigenvalue explains most of the variance in the dependent variable. Wilks' lambda values are 0.016 ($p = 0.00$), 0.215 ($p = 0.131$), and 0.741 ($p = 0.690$) respectively. Wilks' lambda indicates the significance of the discriminant function. The smaller the Wilks' lambda value, the higher the discrimination power of the model (Caravaca et al., 2009; Stella, 2019). Since the significance of the first Wilks' lambda value is less than 0.05, the first separation function can separate the groups in a meaningful way. Function 1 explains 81.8% of the total variance, function 2 explains 15.9% and function 3 explains 2.3%. It can be said that the first function is more effective since it explains the majority of the variance. As a result of the classification, the bees in the L1, L2, L3 regions were classified 100% correctly, while the bees in the L4 region were classified correctly at 80%.

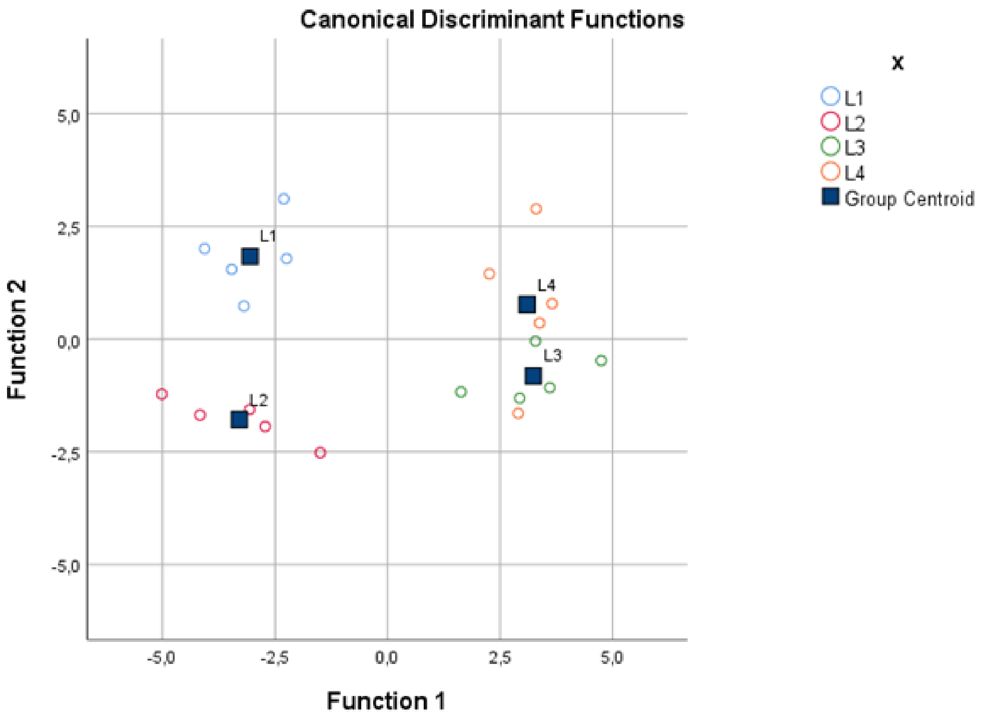

Figure 2: Canonical discriminant score plot according to the geographical origin of honey bees

Figure 2 visually shows that bees are classified according to their geographic origin. The metal with the highest coefficient in the first function is Cr (1.320), in the second function Mn (1.015) and in the third function Zn (0.662) and other coefficients are given in Table 3.

Table 3. Standardized canonical discriminant function coefficients for honey bees

Heavy Metals	Function 1	Function 2	Function 3
Cd	0.658	-0.470	-0.696
Cr	1.320	-0.003	-0.130
Cu	0.810	-0.097	0.506
Fe	-0.458	-0.176	-0.277
Mn	0.134	1.015	-0.155
Ni	-0.019	0.087	0.090
Pb	0.304	-0.062	0.489
Zn	0.359	0.194	0.662

Assessment of Bee Pollen Data by PCA and LDA

If we look at the number of eigenvalues greater than one, bee pollen is explained by three principal components. The first PC explains 36.7%, the second PC explain 55.3% and the three PC explain 68.1% of the total variance (Table 4). Cu, Fe, Pb, and Zn constitute the first principal component, while Cd, Cr, and Ni constitute the second PC and other metals constitute the third PC. The Principal Component Analysis (PCA) results obtained from bee pollen samples are clearly different from those obtained from bee samples. When the Table 4 is reviewed, Mn, unlike other elements, is found in the third component.

Table 4. PCA analysis results for heavy metals measured in bee pollen

Heavy metals	PC1	PC2	PC3
Cd	0.197	**0.448**	0.005
Cr	0.388	**0.441**	0.214
Cu	**0.334**	-0.302	-0.233
Fe	**0.450**	0.046	-0.100
Mn	-0.108	0.291	-**0.886**
Ni	0.366	**0.392**	0.129
Pb	**0.463**	-0.275	-0.288
Zn	**0.370**	-0.444	0.079
Eigenvalue	2.9359	1.4885	1.0246
Proportion	0.367	0.186	0.128
Cumulative	0.367	0.553	0.681

As a result of the discriminant analysis performed to determine the geographical origin of bee pollens in terms of heavy metal content, three canonical functions were formed and their eigenvalues were 15.185, 8.082, and 0.972, respectively. Here, the first two function with the 15.185 and 8.082 eigenvalues explains most of the variance in the dependent variable. Wilks' lambda values are 0.003 ($p = 0.00$), 0.056 ($p = 0.01$), and 0.507 ($p = 0.18$), respectively. Since the significances of the first two Wilks' lambda value is less than 0.05, the first two separation functions can separate the groups in a meaningful way. Function 1 explains 62.6% of the total variance, function 2 explains 33.3% and function 3 explains 4.0%. It can be said that the first two functions are more effective since it explains most of the variance. As a result of classification, bee pollen in all regions is classified 100% correctly.

Figure 3 visually shows that bee pollens are classified according to their geographic origin. The metal with the highest coefficient in the first function is Mn (1.255), in the second function Pb (0.741) and in the third function Zn (0.943) and other coefficients are given in the Table 5.

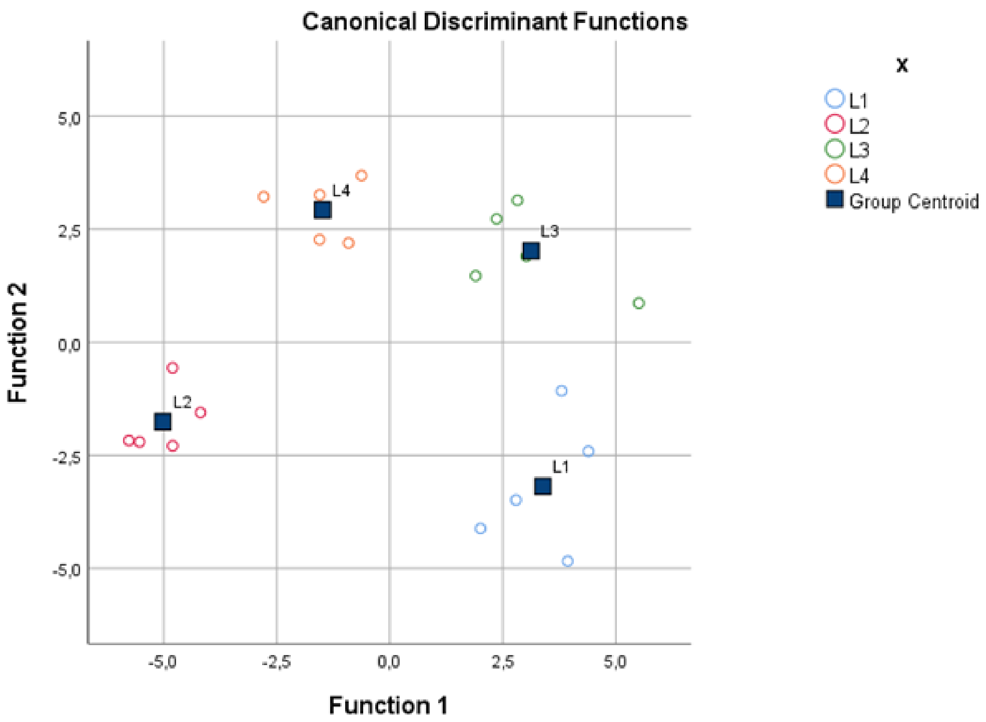

Figure 3. Canonical discriminant score plot according to the geographical origin of bee pollens

Table 5. Standardized canonical discriminant function coefficients for bee pollens

Heavy Metals	Function 1	Function 2	Function 3
Cd	0.087	0.186	-0.226
Cr	-0.268	-0.757	0.767
Cu	0.065	0.504	-0.455
Fe	-0.065	0.698	-0.050
Mn	1.255	0.035	0.188
Ni	0.871	0.461	-0.263
Pb	-0.105	0.741	-0.473
Zn	0.090	0.427	0.943

Assessment of Honey Data by PCA and LDA

The first principal component and the first four principal components together account for 27% and 74% of the total variance in honey, respectively (Table 6). Cr, Fe, and Zn are in the first basic component; Ni, Cu, Mn, Cd, and Pb are included in other components. Compared with the correlation and PCA results in bees and bee pollen, quite different results were obtained in honey. These results show that honey does not reflect the heavy metal content of the region very well.

Table 6. PCA analysis results for heavy metals measured in honey

Heavy metals	PC1	PC2	PC3	PC4
Cd	0.167	0.486	-0.286	**0.575**
Cr	**0.546**	-0.096	-0.124	-0.150
Cu	-0.303	-0.342	-0.329	-0.357
Fe	**0.518**	-0.180	-0.022	0.232
Mn	0.062	0.308	**0.597**	-0.196
Ni	-0.142	**-0.586**	-0.044	0.531
Pb	0.260	0.147	**-0.592**	-0.358
Zn	**0.471**	-0.382	0.293	-0.131
Eigenvalue	2.1324	1.372	1.3487	1.0566
Proportion	0.267	0.172	0.169	0.132
Cumulative	0.267	0.438	0.607	0.739

As a result of the discriminant analysis performed to determine the geographical origin of honey in terms of heavy metal content, three canonical functions were formed and their eigenvalues were 11.293, 4.917, and 2.164, respectively. Wilks' lambda values are 0.004 (p = 0.00), 0.053 (p = 0.01), and 0.316 (p = 0.02) respectively. Since the significance of three Wilks' lambda values is less than 0.05, three separation functions can separate the groups in a meaningful way. Function 1 explains 61.5% of the total variance, Function 2 explains 26.8%, and Function 3 explains 11.8%. As a result of the classification, the honey in all regions is classified 100% correctly.

Figure 4 visually shows that the bee pollen is located according to its geographic origin. The metal with the highest coefficient in the first function is Cr (1.134), in the second function, Mn (1.165) and in the third function Cu, (1.042) and other coefficients are given in Table 7.

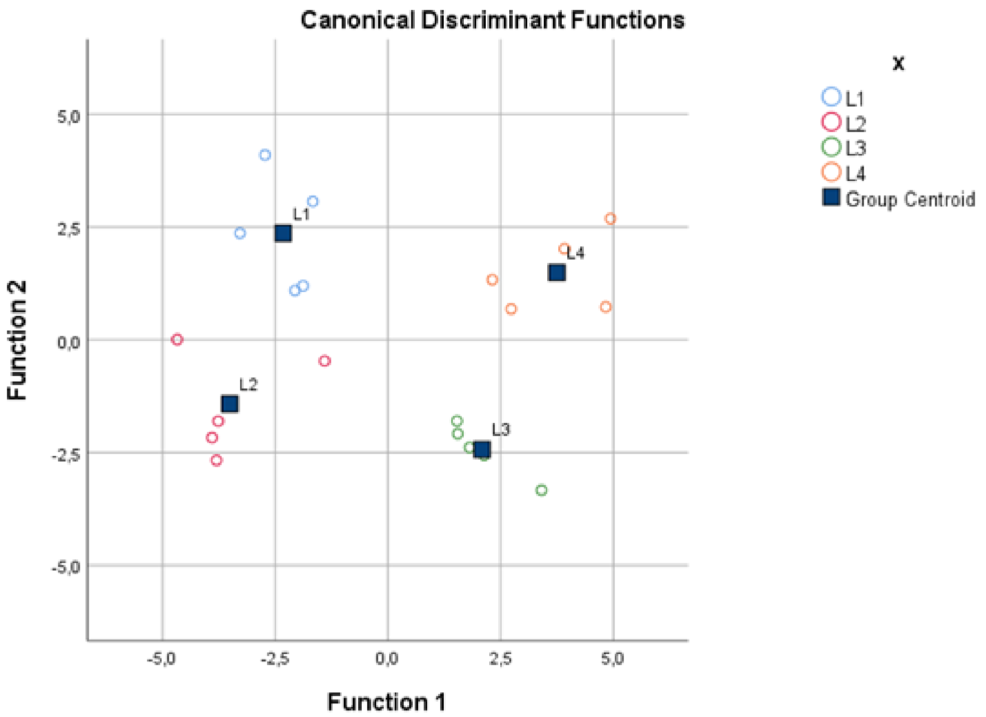

Figure 4. Canonical discriminant score plot according to the geographical origin of honey

Table 7. Standardized canonical discriminant function coefficients for honeys

Heavy Metals	Function 1	Function 2	Function 3
Cd	0.566	0.057	0.057
Cr	1.134	-0.299	-0.412
Cu	-0.102	0.214	1.042
Fe	0.882	0.610	0.475
Mn	0.500	1.165	-0.170
Ni	1.234	-0.404	-0.243
Pb	0.158	-0.395	0.129
Zn	0.359	-0.781	0.333

Discussion

Heavy metal accumulation in bees is higher than in bee products, according to studies (Al Naggar et al., 2018; Ćirić et al., 2021; Formicki et al., 2013; Sari et al., 2020). Leblebici and Aksoy (2008) reported that honey bees do not fully reflect the metal pollution they receive from plants and that it acts as a kind of natural filter. In other words, honey bees claim that it is a better bioindicator of heavy metal pollution than honey. The results obtained in this study were in agreement with those in the literature (Conti et al., 2022; Conti & Botrè, 2001; Silici et al., 2016). Cadmium and lead are highly toxic heavy metals. They are more common in industrial areas. In particular, quite a lot of Pb is emitted from automobile exhaust. For this reason, it is defined as the most common air pollutant. The mixing of air and water in these regions endangers the lives of both animals and plants. Long-term exposure, even in very low amounts, adversely affects many systems in the body. In some studies, it was determined that the Cd and Pb contents were higher in the samples taken from the city (Conti & Botrè, 2001).

In this study, bees had the highest Cd (0.0189 mg/kg) and Pb (0.335 mg/kg) content compared to honey and bee pollen. The lowest value is in honey in our study. It was determined that the Cd and Pb values of bee pollen and honey samples were within the international food standard values (Alimentarius, 2015). While Cd and Pb were found in small amounts in honey in many studies (Ahmida et al., 2012; Bayir & Aygun, 2022; Fredes & Montenegro, 2006; Manu Kumar et al., 2013; Pisani et al., 2008; Roman, 2010), some studies found no Cd in honey (Boussaid et al., 2018; El-Haskoury et al., 2018; Silici et al., 2016; Taha et al., 2017). The difference in Cd content in the studies is thought to be related to the proximity of the region where the samples were taken to the highway. The reason why Cd and Pb were found in honey in this study, albeit in small amounts, is that the samples were taken from the city center.

According to a study by Zarić et al. (2017), PCA can identify the sources of heavy metals in honey bees. Heavy metal studies in bees have also shown that PCA can successfully reduce the size of heavy metals (Di Fiore et al., 2022). According to the PCA results obtained in this study, the elements in the first two components are generally elements of natural origin. Pb and Cd, which have a higher load in the 3rd component, are found in the atmosphere, especially in areas with intense traffic and mining activities (Mohammed et al., 2011). Since the area where the samples were taken is around the city center, it is thought that these traffic-related elements are found in bee samples.

Chromium, nickel, zinc, copper, iron and manganese are abundant in nature and are essential elements for the human body. Their release to the environment is due to fossil fuels (Ni), the steel industry (Mn, Cr), smelting (Cu, Zn), and other industry activities. In cases of excessive intake, it has negative effects on many organs. In this study, the Cr content in bees and bee pollen was not statistically significant. The Cr content in bees (0.0835 mg/kg) and bee pollen (0.75 mg/kg) is higher than honey (0.053) ($P < 0.01$). Other heavy metals were found more in bees. This study's detection of the mentioned heavy metals was comparable to those of earlier studies (Boussaid et al., 2018; Conti & Botrè, 2001; Manu Kumar et al., 2013; Satta et al., 2012).

The geographic origins of bees, mostly in terms of morphological characters, were determined by discriminant analysis. (Guler & Bek, 2002; Meixner et al., 2011; Ozbakir & Firatli, 2013; Sıralı et al., 2003; Strange et al., 2008). However, a discriminant analysis study with heavy metal content in bees could not be found. As can be seen from the results of this study, geographical origins can be separated from LDA with 80% accuracy in terms of metal content.

Many discriminant analysis studies have been conducted to determine the geographic origin of bee pollen. As a result of the discriminant analysis for some toxic fatty acids (Ares et al., 2020), physicochemical properties (Pascoal et al., 2022), phenolic profiles (Kaškonienė et al., 2015), or organic matter content (Sattler et al., 2015) in the bee pollen content, geographical data were obtained. It has been determined that the origins can be separated successfully (Ares et al., 2022a; Ares et al., 2022b). According to Lilek et al. (2022), as a result of PCA and Da analysis for the 11 metal contents of bee pollen samples taken from 4 different regions of Slovenia, bee pollen samples could be successfully separated by LDA according to their geographical origins.

Considering the result of PCA, honey and bee pollen gave closer results in terms of the relationships between heavy metals, while completely different results were obtained in honey. Heavy metal pollution in the environment affects honey differently than bees in this regard. It is common practice to utilize PCA to identify potential sources of heavy metals in environmental samples (Bazeyad et al., 2019). Many studies have been conducted on the determination of the source of heavy metal contamination in soil or water with PCA (Li et al., 2006; Yang et al., 2013; Zhiyuan et al., 2011). There are very few PCA application studies on the heavy metal content of bees and their products, which are very sensitive to environmental pollutants and have bioindicator properties. Ciric et al. (2021), demonstrated that heavy metals can be explained by two components and that PCA analysis can be successfully applied to bees and bee products because of a PCA study on bees and bee products.

The physicochemical properties (Adgaba et al., 2017; Fechner et al., 2016; Karabagias et al., 2014; Serrano et al., 2004), carbohydrate profile (Nozal et al., 2005), and voltage compounds (Stanimirova et al., 2010) of honey were analyzed using LDA. It was observed that they can be successfully separated according to their geographical origins. Yayinie and Atlabachew (2022), determined 14 different metal contents of the honey they collected from seven different regions, and they saw that 93.33% correct discrimination was made as a result of PCA and LDA analysis. Fernández-Torres et al. (2005), analyzed the mineral content of honeys obtained from 4 different regions of Spain. As a result of the discriminant analysis, they concluded that the honey can be separated according to their geographical origins.

Oroian and Ropciuc (2017), determined the presence of 10 different metal contents in bee pollen samples collected from three different regions of Romania. The researchers performed a PCA-LDA analysis to assess the separation of samples based on their botanical and geographical origins. They found that the samples were successfully classified based on their botanical origin, with an accuracy of 80.8%. However, the samples could not be accurately classified based on their geographical origin, with only 21.2% accuracy.

Conclusion

In this study, it is seen that heavy metals are more common in bees than bee pollen and honey, and the heavy metal contents of bee pollen and honey samples from the same hives vary considerably. Based on these findings, it is evident that bees play a crucial role in indicating the presence of heavy metal contamination in the environment, making them the most effective bioindicators. It is seen that bees, bee pollen, and honey form different components in terms of heavy metal content because of PCA regarding the heavy metal content of the samples. In addition, when investigating the source of heavy metal contamination, it is observed that bees give better results in the evaluation of bees and bee products with PCA. The use of PCA-LDA to analyze the heavy metal levels of bee, bee pollen, and honey samples revealed that the samples could be effectively differentiated based on their respective collection sites.

Ethical Statement

Not applicable.

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Conflict of Interest

The authors state that there are no conflicts of interest.

Author Contributions

Conceptualization, H.B. and A.A.; methodology, H.B. and A.A.; validation, H.B. and A.A; formal analysis, H.B., A.A. and F.İ.; investigation, H.B. and A.A.; data curation, H.B., A.A. and F.İ.; writing-original draft preparation, H.B. and A.A.; writing-review and editing, H.B., A.A. and F.İ.; supervision, H.B., A.A. and F.İ.; project administration, A.A.; funding acquisition, A.A. All authors have read and agreed to the published version of the manuscript.

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RESEARCH PAPER

Defensive Behaviors of the Central Highland Honeybees, *Apis mellifera bandasii* against *Varroa destructor* in Ethiopia

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Abstract

The parasitic mite *V. destructor* has caused long-lasting losses to the survival of European honeybee colonies. In contrast, African honeybees are likely capable of surviving the effects of this parasitic mite with varying defense mechanisms. This study provides insights into two defense behavioral traits, including hygienic and grooming behaviors of local honeybee, *Apis mellifera bandasii* colonies against *V. destructor* mite in Ethiopia. Hygienic behavior (HB) was evaluated using the standard pin-killed brood method by calculating the dead brood removal rates (%) at 24 and 48 hrs. While grooming behavior (GB) was assessed by measuring the number of daily fallen mites and the percentage of damaged mites. The results of hygienic behavior showed greater brood removal rates of 83.1±14.3% and 97.6±3.4% at 24 hrs and 48 hrs, respectively. There were strong negative correlations between the HB and Varroa infestation rates, indicating that HB has the potential to reduce the mite population in colonies. Grooming behavior also showed higher mean daily fallen mites per colony (16.3±10.2), of which about 80% of the total fallen mites (n=488) were damaged. Ten body damage categories were identified, with most damages inflicted on mites' legs, dorsal shield, and gnathosoma because of the GB. Our study suggests that combined hygienic and grooming behaviors could be used as effective defenses against *V. destructor* infestations in *A. m. bandasii* colonies. Therefore, future selective breeding programs should integrate these specific host defenses in order to produce sustainable colonies resistant to this parasitic mite.

Introduction

The ecto-parasitic mite, *Varroa destructor*, and the honeybee are the two interacting host-parasite arthropods that have become a subject of growing interest for many scientists around the world. Honeybees are the only sources of food for the varroa mite, and the mite's life cycle coincides with the development of honeybee pupae inside the brood cells (Yang et al., 2021; Rosenkranz et al., 2010). This parasitic mite primarily feeds on the fat bodies of the bees (Ramsey et al., 2019) while also spreading other bee pathogens like bee viruses, which ultimately lead to the highest rate of honeybee mortality (Le Conte et al., 2010; Hristov et al., 2020; Traynor et al., 2020). The feeding activity of the mite and its associated secondary infections can cause huge losses to the honeybee, *Apis mellifera,* and become a threat to colony survival and beekeeping productivity around the world (Flores et al., 2021; Noël et al., 2020; O'Shea-Wheller et al., 2022). In addition to reducing the potential beekeeping production, Varroa also has negative effects on the pollination capacity of a colony, which in turn significantly affects crop production for food security (Malfroy, 2015; Abrol and Sharma, 2013). The approach so far taken to control the mite, particularly using chemical treatments over the past 30 years did not completely solve the problem due to the spread of acaricide resistant mites and the risk factors associated with acaricide residues (Kablau et al., 2020; Plettner et al., 2017). As a result, *V. destructor* remains a complex invasive parasite, crippling the Western honeybee, *A. mellifera*, in the world for many generations (Nazzi and Le Conte, 2016; Traynor et al., 2020). In contrast, the mite has been considered a harmless pest to its native host, the native Eastern honeybee *(Apis cerana)* due to their naturally evolved defensive traits developed over a long evolutionary period (Peng et al., 1987; Rath, 1999; Boecking and Spivak, 1999). Thus, a balanced host-parasite relationship has been established between *A. cerana* and the *V. destructor* mite, revealing the potential resistance of *A. cerana* honeybees to the mite infestation (Grindrod and Martin, 2023; Grindrod and Martin, 2021).

Likewise, some *A. mellifera* colonies in Africa and African-derived populations have shown varying degrees of tolerance or resistance towards the infestation of Varroa mite without the interventions of beekeepers (Locke, 2016; Castilhos et al., 2023; Büchler et al., 2010). Particularly, African honeybee populations are likely to be less threatened by the impact of *V. destructor* compared to European honeybees (Muli et al., 2014; Tibatá et al., 2021; Nganso et al., 2017). Although the mite has continued its rapid spread across many African countries over the past 15 years, there has been no report on the visible colony losses linked to Varroa mite infestation in African continent (Allsopp et al., 1997; Begna, 2014; Chemurot et al., 2016, Dietemann et al., 2009; Fazier et al., 2010; Muli et al., 2014). The long co-existence of honeybee subspecies with the mite in the absence of chemical treatment is suggestive of the potential resistance or tolerance of the honeybee populations evolving natural adaptations against the Varroa mite. Behavioral defenses such as hygienic and grooming behaviors are important natural traits that enable resistant honeybee populations to survive and co-exist with the Varroa mites, particularly in Africa (Fazier et al., 2010; Muli et al., 2014).

Hygienic behavior involves targeting, opening and removal of diseased, injured, parasitized, or dead broods by the worker honeybees (Peng et al., 1987; Boecking and Spivak, 1999; Spivak and Reuter, 2001). In particular, when hygienic behavior targets the detection and removal of varroa infested brood cells, it is referred as "Varroa Sensitive Hygienic Behavior (VSH)" (Mondet et al., 2015). On the other hand, grooming behavior involves the potential dislodging and damage of ecto-parasites from the bodies of adult bees either by themselves or by their nest mates, where they reduce the population of parasites below the danger threshold level (Mondet et al., 2020; Aumeier, 2001, Russo et al., 2020). In fact, African honeybees in general have displayed strong hygienic and grooming behaviors than their European counterparts (Muli et al., 2014; Nganso et al., 2017). Nevertheless, the diverse bee species in Africa are likely to exhibit different responses tailored to combat the negative impact of the Varroa mite (Mondet et al., 2020; Fazier et al., 2010). Moreover, colonies within the same subfamily may respond differently to parasites and pathogens, due to different factors.

In Ethiopia, the occurrence of the Varroa mite was reported about a decade ago, and currently the mite has been widely distributed across all the geographic regions of the country with varying prevalence and infestation levels (Gela et al., 2023; Shegaw et al., 2022). However, there is no report indicating that the mite has induced colony losses or a pronounced impact on the apiculture industry in the country (Gratzer et al., 2021). Due to this fact, local beekeepers do not consider the mite as a serious pest, and do not use any treatment measures against the parasite. This phenomenon suggests that the local honeybee populations have evolved some sort of resistance or tolerance traits to maintain a stable host-parasite relationship against the aggressive infestation behaviors of this mite species. However, there is limited information that explains specific natural defensive mechanisms applied by honeybee subspecies of Ethiopia against *V. destructor* mite. In fact, Pirk et al. (2016) reviewed how the treatment-free beekeeping approach in Africa has allowed honeybees to develop some natural resistance traits or behavioral adaptations to combat the negative effects of different pests and pathogens. Gebremedhn et al. (2019) also reported the failure of female varroa mites to produce adult male progeny that suppresses the population growth of Varroa mite in *A. m. simensis* colonies for the first time in Ethiopia. However, this study was limited to a specific honeybee eco-type in the northern part of the country, and it may not represent the wider geographic population of honeybees in the country. A*. m. bandasii* is the most popular geographical race of honeybees spread in the central highlands of the Ethiopia, covering more than 90% of the highland areas (Mohammed, 2002). Begna et al. (2016) investigated the non-impact of Varroa mite on population dynamics, brood rearing, as well as foraging activities of *A. m. bandasii* colonies, suggesting their survival against the destructive nature of the parasitic mite. However, the specific tolerance or resistance mechanisms employed by these honeybee populations against the mite remain unclear. Therefore, this study was designed to determine whether the hygienic and grooming behaviors of *A. m. bandasii* could contribute for the defensive mechanisms against the *V. destructor* mite infestation. Understanding such natural defense behaviors will provide salient insights into future selective breeding program and enhance resistance traits in the local honeybee stocks.

Material and Methods

Study Location

The study was conducted from September 2021 to June 2022 in the laboratory and at apiary site of Holeta Bee Research Center, Oromia, Ethiopia located at 09º03'.24" N and 038º30'.72" E about 33 kilometers in the West direction of the capital city, Addis Ababa (altitude 2400 m a.s.l.). The climate of the study area is characterized by temperate to humid weather conditions with an average temperature of 14.15°C (ranging from 6.2°C - 22.1°C), annual rainfall of 1091.51 mm that varies between 800 and 1500 mm/year and a mean relative humidity of 60.6% (Mekonnon et al., 2015). The main vegetation types in the study area include *Guzotia* spp*., Acacia* spp*., Eucalyptus globulus, Vernonia amygdalina, Trifolium* spp*., Plantago* *lanceolata, Brassica carinata* and *Isoglossa laxa* (Fichtl and Adi, 1994).

Experimental Set Up and Honeybees

A total of 15 queen- right colonies (headed by naturally mated queens) of *A. m. bandasii,* the local honeybee race, were established in standard Langstroth hives in 2021, a year prior to the commencement of the experiment. All the experimental colonies originated from locally caught swarms and were standardized to have uniform conditions, including population strength, and they were checked for the presence of Varroa mites. At the beginning of the experiment, the infestation rate of the mite was determined using the standard method of detergent wash (Dietemann et al., 2013). Thereafter, the percentage of mite infestation rate (%) in each colony was expressed as the number of mites counted per 100 adult worker bees.

Evaluation of Hygienic Behavior (HB)

Hygienic behavior was assessed in all of the established colonies (N = 15) using the standard pin-killed brood assay method as described in Büchler et al. (2013). After selecting the section of bee comb containing caped young pupae cells (white-to purple-eyed stage), this section was punctured with a circular (5 cm, ID) polyvinyl chloride (PVC) pipe to demark the entire row of cells that surrounds approximately 164 cells (Fig. 1). The number of empty cells within each circular comb section was counted and recorded. Then, every capped pupa within the marked section of comb was pin-killed with a fine insect pin (entomological pin No-2) and the combs were placed back into the test colonies.

After 24 and 48 hrs, the frame with the comb sections was taken out again from the respective colonies to record the number of removed cells and the remaining dead broods at both consecutive periods. Moreover, the marked section of the comb was photographed for later count and confirmation, as indicated in the figure below (Fig. 1). Then, the number of fully removed pin-killed pupae cells from each test frame was recorded after 24 and 48 hours and expressed as the percentage of brood removal rate (%). Lastly, the total percentage of dead brood removal rate (%) was calculated according to (Kebede, 2006) as follow (Eq-1):

$$R = \frac{K-E-C}{T-E} x\ 100 \qquad (1)$$

Where; R = Percentage of dead brood removal rate (%) in time interval

K = Number of removed dead broods in time interval

C = No. of empty cells in the section before the test

E = No. of non-removed brood cells after the test

T = Total number of cells in the demarked brood section

Then, the colonies were classified into three groups: high, medium, and low hygienic colonies based on brood removal rate after 24 hrs. Consecutively, colonies with uncapped and removed dead broods of more than 90%, 60–90%, and less than 60% were classified as having high, medium, and low hygienic behaviors, respectively (Medina-Flores et al., 2014).

Evaluation of Grooming Behavior (GB)

Grooming behavior (fallen and damaged mites) was evaluated in ten selected colonies (N=10) with uniform colony population and strength using the standard method of estimating colony strengths (Delaplane et al., 2013). Five weak colonies were excluded from the experiment in order to minimize the biased effects of varying colony populations on grooming activity. Then, the original bottom boards of selected experimental colonies were removed and replaced with modified screened bottom boards following the procedure of Pettis and Shimanuki (1999). The screens were designed to allow only the passage of mites through them on the collecting trays, but not the bees. To intercept the falling mites, mite-collecting trays on the top side were covered with white cardboard and smeared with sticky, non-toxic petroleum jelly (Vaseline®). The trays were maintained in the hives, and the fallen mites were collected every 48 hrs from the cardboard papers for three consecutive days. On each data collection day, the slide board was removed, and fallen mites were collected, cleaned, and reintroduced into the bottom boards of the hive.

Subsequently, the collected fallen mites were counted and examined for body damage under a Zeiss Primo Star light microscope, Germany (Mg. Power 40X). Each examined mite was assigned as "damaged" or "undamaged" categories for the analysis. The proportion of damaged mites (%) in each colony was expressed by dividing the number of damaged mites by the total number of fallen mites at the end of the collection time (after 48 hrs). The damaged mites were also further grouped into different damage categories following previously established classifications of damaged mites (Corrêa-Marques et al., 2000).

Statistical Analyses

All statistical analyses were carried out using R-Software version 4.1.3 (R Core Team 2021). Data were checked for normality using the Shapiro-Wilk test, and they were normally distributed. A pairwise sample t-test was used to compare brood removal rates between 24 hrs and 48 hrs, as well the percentage of damaged and undamaged fallen mites. Linear model was used to estimate the Varroa infestation rate in relation to brood removal rates at 24 hours and 48 hours separately and compare the infestation rate in relation to the percentage of damaged and undamaged fallen mites separately. To determine the relationship between hygienic and grooming behavior of *A. m. bandasii* against Varroa infestation levels, the Pearson correlation test and a linear regression model were used. The average daily fallen mite count per colony was determined by dividing the total number of naturally fallen mites to the number of mite collection days following Strauss et al. (2015).

Results

Evaluation of Hygienic Behavior

The uncapping and removal percentage of pin-killed brood from the cells was used as an indicator of the level of hygienic behavior in experimental honeybee colonies. There was a variation in removing the dead broods from the cells among the experimental colonies. The mean removal rates of pin-killed broods were 83.1± 14.3% ranged from 57.6% to 98.1% after 24 hrs, and 97.6± 3.4% ranged from 89.4 to100% after 48 hrs (Fig. 2A, Table 1).

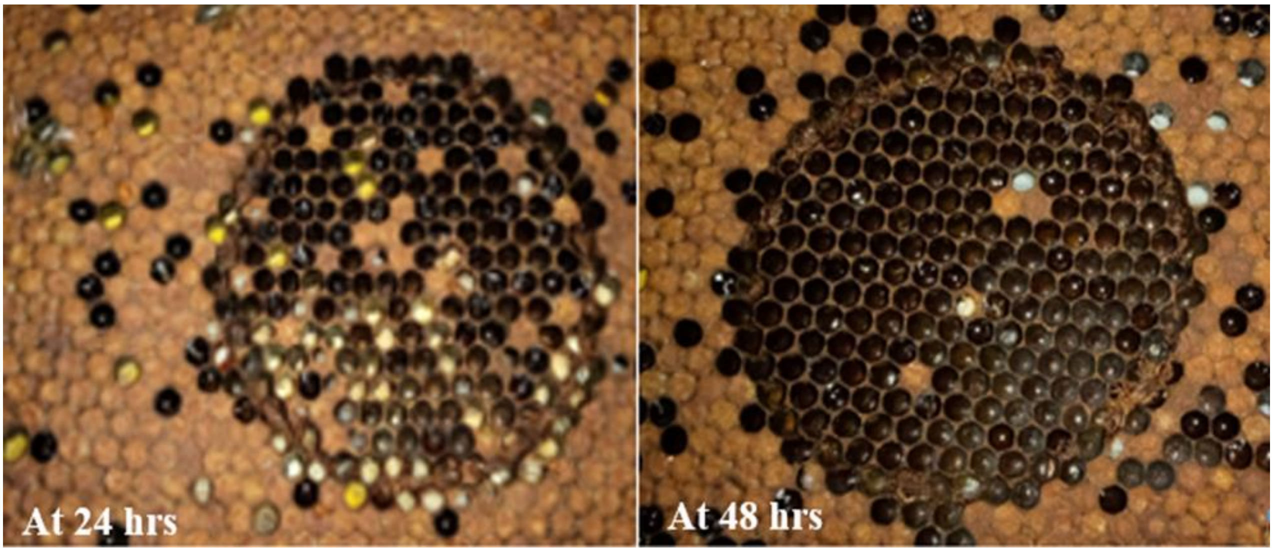

Figure 1. Sections of bee comb indicating the removal of pin-killed broods after 24 and 48 hours from *A. m. bandasii* colonies

Table 1. Description of the rates of pin-killed brood removal after 24 and 48 hours and the Varroa infestation rates, in *A. m. bandasii* colonies for the assessment of hygienic behavior

Description	N	Min	Max	Mean	Std. D
After 24 h	15	57.62	98.10	83.98	14.26
After 48 h	15	89.40	100.00	97.60	3.4
IR	15	0.16	13.43	4.06	3.78

Accordingly, 53.5% (8 of 15) of the colonies removed more than 90% pin-killed broods after 24 hrs, showing higher hygienic performances. While the other 40.0% (6 of 15) colonies removed about 60-90% of the dead broods, and were classified as medium HB. Yet, 6.5% of the colonies were able to remove less than 60% of the pin-killed broods, and classified as low HB (Fig. 2B).

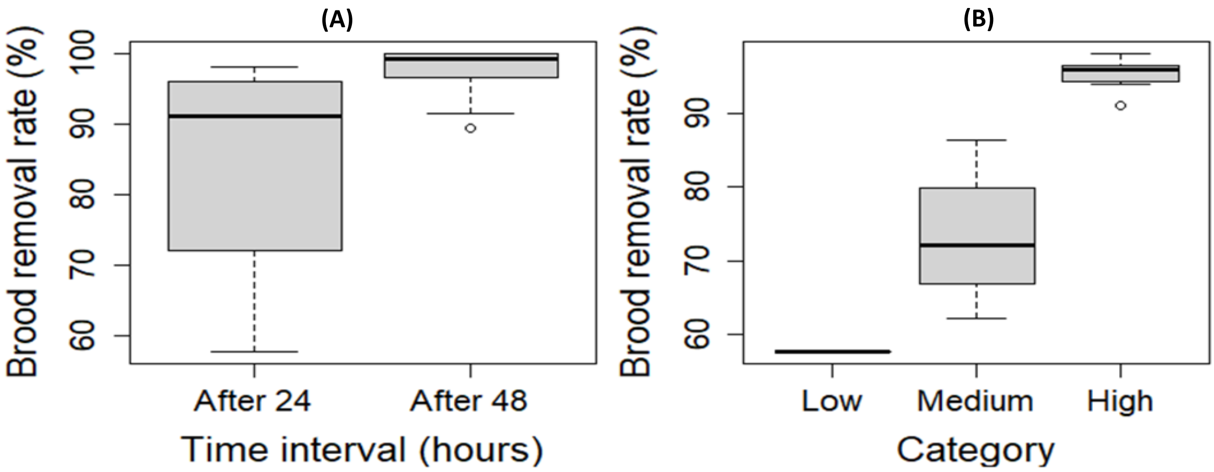

Figure 2. The mean percentage of pin-killed brood removal rates in time intervals (A) and the category of hygienic behavior for central highland honeybee, *A. m. bandasii* colonies (B)

There was negative correlation between the Varroa mite infestation rate (IR) and the proportion of pin-killed brood removal rate in honeybee colonies, both after 24 and 48 hrs of observations (Pearson correlation: r = -0.81, P = 0.001 and r = -0.483, P = 0.039, respectively) (Fig. 3A).

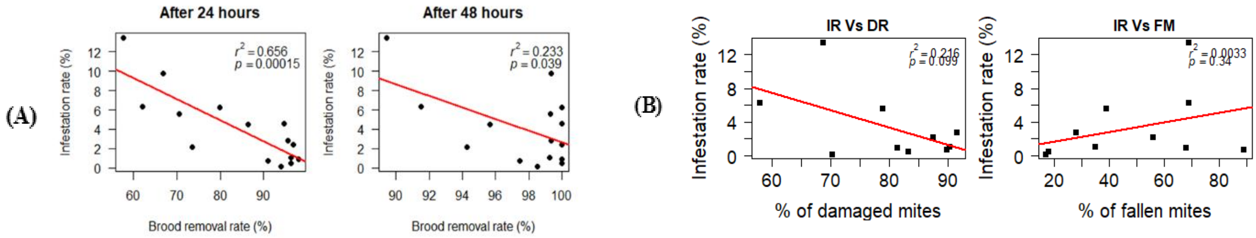

Figure 3. Correlations between Varroa mite infestation rate and brood removal rates across time intervals (A), as well as the correlations between Varroa mite infestation rate and the percentages of damaged mites and fallen mites (B) in the experimental colonies

Evaluation of Grooming Behavior

The results on the grooming behavior exhibited by the experimental colonies (N=10) in terms of fallen and damaged mites are depicted in Table 2 and Fig. 4B. Of the total 488 fallen mites collected from the bottom boards, 80.0±16.3% (Mean ± SD) were damaged, while 20±13.0% were undamaged, and these means were significantly different (t (df)= value of t; P < 0.001) (Fig. 4B). The average daily count fallen mites in experimental colonies was 16.3±10.2, ranging from 5.7±3.5 to 29.7±14.2. The higher number of daily fallen mites was recorded for colonies 6, 4 and 2, while colonies 3, 7 and 9 showed a lower number of daily fallen mite counts (Table 2).

Table 2. The mean daily count of fallen mites and the corresponding percentage of damaged mites within experimental honeybee colonies, A. *m. bandasii*

Colony code (N=10)	Daily fallen mites (Mean ± SD)	Damaged mites (Mean ± SD)	Undamaged mites (Mean ± SD)
C-1	13.0±11.4	78.9±9.2	21.1±1.8
C-2	23.0±8.2	57.9±22.0	42.1±2.1
C-3	5.7±3.5	70.4±17.9	29.6±0.4
C-4	23.0±7.8	68.8±18.6	31.2±4.1
C-5	22.7±8.7	81.4±10.2	18.6±11.3
C-6	29.7±14.2	89.9±3.8	10.1±6.2
C-7	6.0±3.6	83.3±20.8	16.7±1.7
C-8	11.7±3.2	90.3±10.9	9.7±6.4
C-9	9.3±4.2	91.7±14.4	8.3±0.7
C-10	18.7±18.7	87.5±8.5	12.5±9.1
Average	**16.3±10.23**	**80.0±16.3**	**20±13.0**
P-Value	**P < 0.001**	**P < 0.001**	

The findings indicate that there was no significant correlation between the percentage of damaged mites and infestation rate of mites ($R = r^2=0.216$, P= 0.099). Similarly, the association between the percentage of fallen mites and the mite infestation rate was not statistically significant ($r^2 = 0.0033$, P = 0.34; Fig 3B).

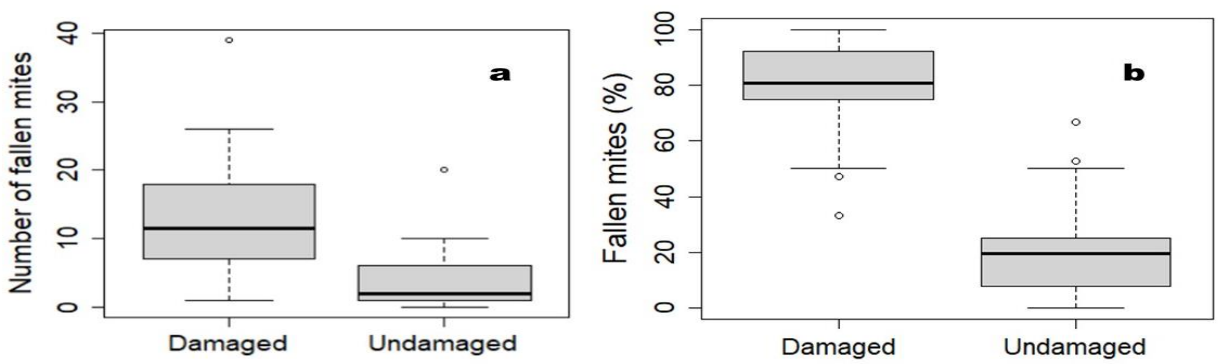

Figure 4. The average number of daily fallen mites (a) and the percentage distribution between damaged and undamaged mites (b) in local honeybees, *A. m. bandasii*

In this study, about 10 categories of damages were observed on the bodies of the mites (Fig. 5), and all injuries could be inflicted from the grooming activities of worker bees. Notably, three distinct new damage categories were observed in this study which include: carcass-empty dorsal shield + empty ventral shield; damaged legs + damaged gnathosoma + damaged shield; and hollow in the dorsal shield + damaged legs (Fig. 5D, 5H and 5J). The damaged legs (the complete or partial loss of one or more legs) (Fig. 5C) and damaged dorsal shields (Fig. 5J) were the most frequently observed (presented more than five times when examined under the microscope) body damages, with the proportion of mite injuries at 38.2% and 29.2%, respectively. Other damages represented in this assessment included combined injuries in the mite bodies and legs.

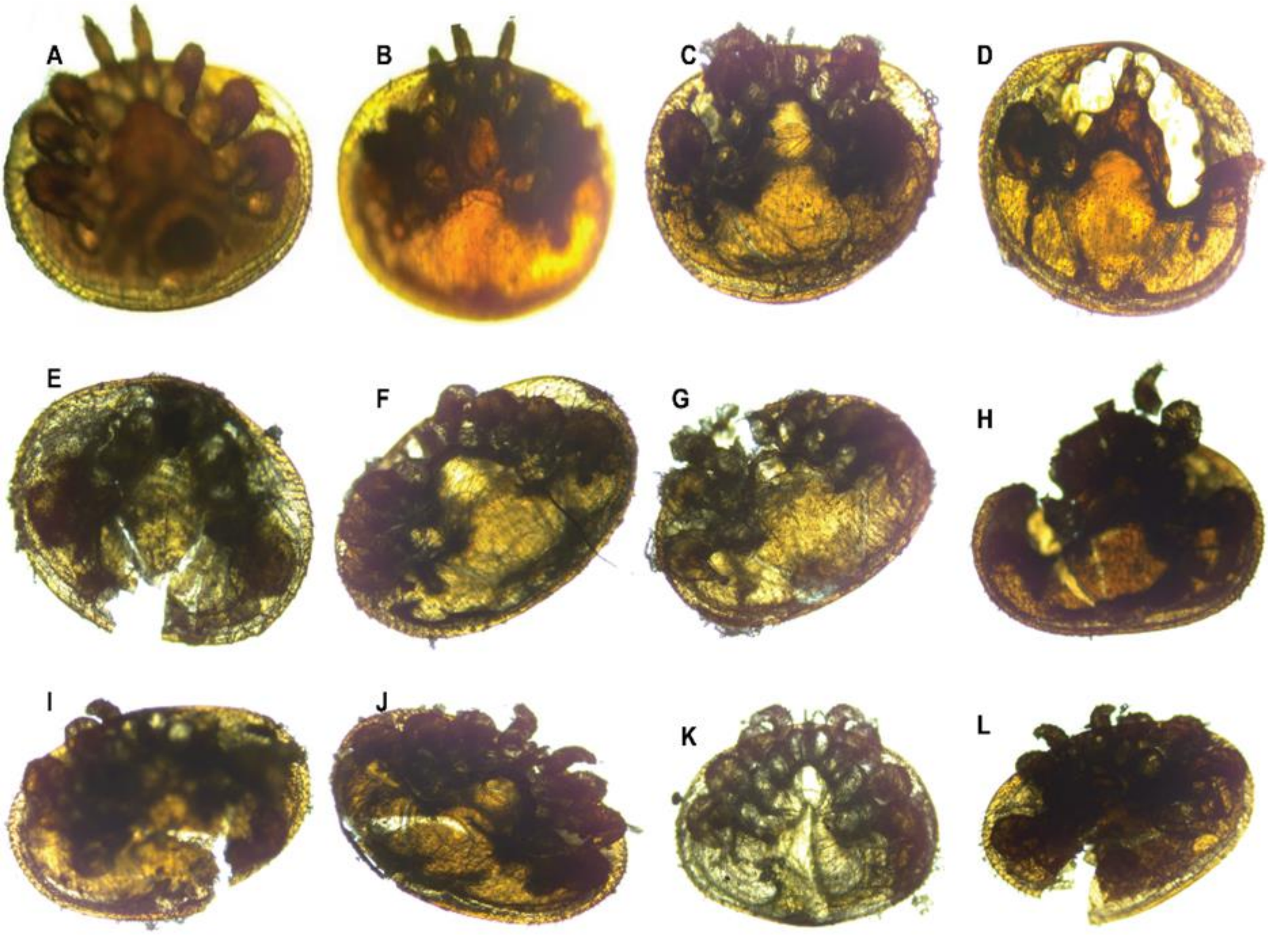

Figure 5. Morphological views of *V. destructor* mite under examination using Primo Star (Zeiss) microscope (40x Mg) during the evaluation of *A.m. bandasii* grooming behavior. The figure illustrates ventral and dorsal view of undamaged mite (A-B) and damage categories inflicted to legs (C), carcass-empty dorsal + empty ventral shield (D), damaged empty dorsal shield + missing legs (E), damaged legs + empty dorsal shield (F), damaged legs + damaged gnathosoma (G), damaged legs + damaged gnathosoma + damaged shield (H), hollow in the dorsal shield + damaged legs (I), damaged dorsal shields (J), hollow in the ventral shield + damaged legs (K) and hollow in the dorsal shield (L).

Discussions

Honeybees exhibit a wide range of natural defensive behaviors against the infestation of pathogens, pests and parasites in order to survive. Particularly, resistant honeybees display various physiological and behavioral defenses to limit the spread of pathogen and parasite infections within a colony (Mondet et al., 2020). However, such natural traits considerably vary based on bees' genetic factors and environmental conditions, as well as prevailing pathogens and parasites (Meixner et al., 2015). Several studies suggested that hygienic and grooming behaviors play key roles as defense mechanisms and enable honeybee populations to survive the effects of brood diseases and parasitic mite (Morfin et al., 2020; Boecking and Spivak, 1999; Khan and Ghramh, 2021; Nganso et al., 2017). In the present study, we assessed the potential contribution of hygienic and grooming behaviors in the central highland honeybee, *A. m. bandasii* in Ethiopia as defense strategies against the infestation of *V. destructor* mite.

Our results reveal a higher level of hygienic behavior (96.7%) expressed in the tested colonies of the *A. m. bandasii* honeybee race. This demonstrates the central highland honeybees have greater ability to detect and remove the infested broods from the comb cells. As explained by Medina-Flores et al. (2014), colonies with a HB of more than 95% within 48 hrs are genetically resistant to different infectious pathogens, and are categorized as strong hygienic colonies. However, the hygienic behaviors expressed in our study varied among experimental colonies. These variations among colonies of the same subfamilies might be influenced by factors such as the age of the worker bees performing the hygienic tasks (Panasiuk et al., 2010), or by their heritable genetic traits in the bee subfamilies (Arathi and Spivak, 2001). In fact, individual worker honeybees within the same subfamilies may also differently respond to different stressors including pest and pathogens (Roberts and Hughes, 2014; Dalmon et al., 2019). In agreement with our finding, a recent investigation by Hunde and Hora (2022) has also shown the performance of *A. m. bandasii* colonies in terms of high level of hygienic behavior (96.42%), good brood rearing, nectar production, and high aggressive behavior. Furthermore, higher percentages of HBs have been reported in different subspecies of Ethiopian local honeybees, including *A. m. scutellata* colonies (95.7%) (Shitaneh et al., 2022) and *A. m. weyi gambella* subspecies (92.16% in 24 hrs) (Aleme et al., 2017).

Compared to reports from other countries, the average hygienic behaviors displayed in Ethiopian honeybee races were higher than those reported in Kenya (81.0%) (Nganso et al., 2017), Egypt (72.5%) (Kamel et al., 2003), Ecuador (80 %) (Masaquiza et al., 2021), and Chile (20-80%) (Araneda et al., 2008). Such differences could be attributed to several factors, including the bees' genetic factors, geographic locations, climatic conditions, and seasonal variations. Although the level of hygienic behavior varies among different bee species, several studies suggested that African honeybees in general, display strong hygienic behavior that suppresses the Varroa mite reproduction cycle and population growth (Muli et al., 2014; Mondet et al., 2015; Mondet et al., 2020; Gebremedhn et al., 2019).

Interestingly, our results demonstrate a negative correlation between the HBs and Varroa mite infestation rate among the tested colonies both at 24 and 48 hrs. This supports the evidence that honeybees with higher HBs can limit the reproduction cycle of the mite, thereby reducing its infestation level in the hive colonies (Kim et al., 2018). Similarly, Muli et al. (2014) reported a strong negative relationship between hygienic behavior and the Varroa mite infestation rate in *A. m. scutellata* colonies in Kenya. This could be linked to specific tasks of worker honeybees in which they exhibit to detect and remove Varroa-infested broods from comb cells, and this specific mechanism of hygienic behavior is termed "Varroa Sensitive hygienic behavior" (VSH) (Spivak and Danka, 2021; Mondet et al., 2020; Harris et al., 2010). Several studies suggest that VSH results in reducing the reproductive potential and population growth of mites, which limits the infestation and spread of mite in colonies (Peng et al., 1987; Spivak and Danka, 2021; Kim et al., 2018). During the activities of VSH, the worker bees' antennal physiology can play a key role in detecting odor coming from Varroa-infested larvae, which then triggers the cleaning of infested broods from the comb cells (Parker et al., 2012; Mondet et al., 2015). Therefore, the local honeybees (*A. m. bandasii*) could have displayed active VSH behaviors to survive against the destructive effects of the Varroa mite by reducing the mite infestation level below the threshold damage level. However, it is worth noting that the timing of brood removal can influence the apparent resistance of honeybees, determining the overall rates of Varroa parasitism in colonies (Spivak and Danka, 2021).

Apart from hygienic behavior, the tested colonies (*A. m. bandasii)* displayed a higher level of grooming behavior in terms of mean daily fallen mites and percentage of damaged mites. The higher percentage of fallen mites and the higher proportion of damaged mites observed in this study reflect the intensive of grooming ability of *A. m. bandasii* to fight against the destructive nature of *V. destructor* mite. The percentage of grooming behavior (80.0±16.3%) recorded in this study was considerably higher when compared to the previous study for *A. m. simensis* colonies in the northern region of Ethiopia, with the GB value of 34.78% and 41.89% during active and dry seasons, respectively (Gebremedhn et al., 2019). In addition, the percentage GB of the resistant, *A. m. scutellata* colonies (21.3%) recorded in Kenya (Nganso et al., 2017) was lower than the present study. This explains a strong grooming behavior of *A. m. bandasii* stocks that could likely inflict damage to the mites' bodies and this might be contributed for colony survival. In agreement with this, Pritchard (2016) explained that colonies inflicting about 60% body damage to the total fallen mites are capable of limiting the mite infestation level and can survive the Varroa infestation without chemical treatment. Consequently, the higher intensity of grooming behavior and injuries that the bees inflict on the body of the mite can significantly influence the mites reproduction cycle and population growth in Varroa-surviving colonies (Dadoun et al., 2020; Russo et al., 2020).

In the present study, about ten body damage categories were examined from the total fallen mites. It appears that all the damages have been inflicted by the grooming activities of worker bees, which is consistent with the previous investigation by Corrêa-Marques et al. (2000). Importantly, a significant overlap of damage categories was observed between our result and the findings of recent studies in Argentina (Russo et al., 2020) and Kenya (Russo et al., 2020; Nganso et al., 2017). Although there were multiple damages examined in our study, the damaged legs (total or partial loss of one or more legs), and damaged dorsal shield were the most frequently recorded damages. This observation suggests that the primary target of worker honeybees is to destruct the legs and external bodies of the fallen adult mites, which then inhibits the mite movement and re-infestation in the hive. Such targeted damages would eventually lead to the death of mites and have potential to reduce Varroa population growth and infestation levels in the hives (Corrêa-Marques et al., 2000). In our study, the successful survival of local honeybee stocks against the Varroa mite infestation might be associated with the ability of worker bees to damage and remove mites from their nest colony. Yet, there were variations in the level of damaged mites across experimental colonies, and this would preclude us from concluding that grooming behavior is an effective mechanism of defense against mites. Because, several driving factors can considerably influence the degree of grooming behaviors among honeybee stocks, even for colonies existing in the same geographic region (Boecking et al., 2000; Masaquiza et al., 2021; Hamiduzzaman et al., 2017). Therefore, considering driving factors would be important during selection breeding programs to enhance resistant bee stocks.

Conclusion

The honeybees exhibit a wide range of resistance mechanisms in order to combat the devastating effects of the *V. destructor* mite. Our investigation into the defensive behaviors of the central highland honeybee, *A. m. bandasii* colonies in Ethiopia reveals the potential expression of hygienic and grooming behavioral traits against the *Varroa destructor* mite. While different local honeybee races may employ diverse defense mechanisms, our study highlights the pivotal roles of hygienic and grooming behaviors in withstanding the effects of the Varroa mite infestations. The strong negative correlation observed between hygienic behavior and the level of Varroa mite infestation indicates the significance of worker bees' hygienic activities in mitigating the reproduction and population growth of the mite within the colonies. Moreover, our finding revealed that *A. m. bandasii* colonies exhibit extensive grooming behaviors, leading to significant damages to the bodies of phoretic mites. This illustrates the critical part in which the combined expression of hygienic and grooming behaviors likely contributes to the survival and reduced vulnerability of local honeybee colonies to the devastating effects of the Varroa mite. These findings provide valuable insights into various natural defense mechanisms that help local honeybee populations in combating the impacts of *V. destructor* mite without the need for beekeepers' intervention. Future research should explore and identify the various defense mechanisms employed by different native honeybee subspecies in Ethiopia to combat against the parasitic Varroa mite. Understanding such natural adaptations and driving factors in local honeybee populations is crucial to design and develop effective selective breeding strategies within the country.

Ethical Statement

There are no ethical issues with the publication of this article.

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Conflict of Interest

The authors declare that there is no conflict of interest.

Author Contributions

A. Gela : Conceptualization, Investigation, Methodology, Writing original draft

A. G: Investigation, Writing, Review & Editing, Supervision

Y. W: Review & Editing, Supervision

A. A: Review & editing, Formal Analysis

Z. A: Investigation, Methodology

A. B: Supervision, Funding acquisition

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REVIEW PAPER

A Literature Review and Statistical Analysis of Turkish Agricultural Data: Assessing Crop Dependence on Insect Pollinators in Türkiye

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Abstract

The study delves into the critical role of pollination in shaping the quality and quantity of agricultural products. Insect pollination, with a primary focus on *Apis mellifera* L., is identified as an indispensable element in the development of a significant proportion of global agricultural crops. Moreover, the study underscores the multifaceted benefits of insect pollination, not only enhancing crop production but also elevating the overall quality of produce. Fruits and oilseeds are cited as beneficiaries of bee pollination, resulting in larger, higher-quality crops with extended shelf lives. Commercial crop pollination relies mainly on managed honeybees. The study elucidates the versatility, cost-effectiveness, and adaptability of honeybee colonies for pollination purposes. However, it is noted that honey bee populations are dwindling due to factors such as parasites, pesticide usage, limited floral resources, and *Nosema* spp. infection, which poses a potential risk to agricultural crop pollination. The research focuses on insect pollination due to its crucial role in the process. The goal of this study is to find out what percentage of Turkish crops depend on insects to pollinate them. Data on plants requiring insect pollination were obtained through a comprehensive literature review, while data on Turkish agricultural products were sourced from the Turkish Statistical Institute. This comprehensive study explores the vital role of insect pollinators in Turkish agriculture. This statement highlights the crucial role of plant and animal production in meeting the nutritional needs of societies, while emphasizing the importance of increasing the size of agricultural cultivation areas to enhance overall agricultural productivity.

Introduction

It is impossible to overestimate the significance of plant and animal production, as well as their quality and efficiency improvements, in providing appropriate nutrition for societies, according to the Food and Agriculture Organization of the United Nations (UNICEF, 2021). Extending the area under cultivation is a key step in the right direction for boosting agricultural output (Bhandari, 2020). Increasing yield from a given area is one of the most important variables in increasing agricultural output (Gomiero et al., 2011).

Developed nations are using intensive production tactics to increase their overall agricultural production levels (UNICEF, 2021). Good Agricultural Practices (GAP), which aims to address food-borne illnesses and environmental issues, have been popular in agriculture in recent years (Burrell, 2011).

Pollination contributes to the quality and quantity of the products. Pollination by insects is a crucial factor in the development of the vast majority of agricultural crops worldwide. Insects play a crucial role in the pollination of many fruits, vegetables, and crops; of the approximately 300 commercial crops around 84% have been pollinated by insects, primarily *Apis mellifera* L. (Klein et al., 2006). Pollination services provided by honey bees and wild bees have declined in recent decades (Kevan & Viana, 2003). Its loss is due to agricultural expansion, monoculture, disease and parasites, intensive pesticide usage, urbanization, and fragmentation (Stanton et al., 2018; Wan et al., 2021). If insect pollinators diminish or become extinct, humans will be unable to consume a variety of foods (Klein et al., 2006). Insect pollination has additional advantages for crop quality.

Pollination of plants by bees is not only essential for crop production but also improves the overall quality of the produce. Fruits that have been pollinated by bees are larger, have fewer deformities, and score higher on commercial quality scales. Fruits that have been pollinated by bees have longer shelf lives because their sugar-to-acid ratios are more optimal, and they are also more robust (Klatt et al., 2014). Pollination of oil crops by bees not only improves the quality of the fruit they produce, but it also improves the quality of the oil produced by such crops. For example, bee pollination led to an 18% rise in the weight of oil seeds and a 20% increase in their market value. Oil seeds that are pollinated by bees contain higher levels of oil and lower levels of chlorophyll (Bommarco et al., 2012).

Pollination of intensively farmed commercial crops is almost entirely dependent on managed pollinators, with only a small percentage coming from wild insects (Richards, 1993). Honey bees are the most important commercial pollinators, and they are responsible for at least 90 percent of all commercial pollination (Free, 1970; Richards, 1993). Although some species of bees, such as alkali bees, mason bees, leafcutter bees, and bumble bees, are used for commercial pollination, honey bees are by far the most important commercial pollinators (McGregor, 1976).

Honey bee colonies are the sole viable option for ensuring the successful pollination of crops in the event that wild bees do not visit the agricultural area. In comparison to other types of manageable pollinators, honey bee colonies are more adaptable, less expensive, and handier for pollination purposes (Klein et al., 2006). The number of honey bee colonies is decreasing and the most obvious reasons are due to parasites, the use of pesticides, the lack of flowers, and *Nosema* spp. infections. (Genersch, 2010; Goulson et al., 2015). This condition poses a potential risk to the pollination of agricultural crops.

This research is focused on insect pollination because they are the most significant species that play a role in the pollination process (Breeze et al., 2011). This study's objective is to determine the percentage of Türkiye's agricultural crops that rely on the activity of insects for pollination. A literature analysis of plants that need insect pollination was carried out, and the data regarding Turkish agricultural goods were gathered from the Turkish Statistical Institute.

Material and Methods

We made a rough estimate of the proportion of crop output that is attributable to insect pollination. The Turkish Statistical Institute, which is the government institution in Türkiye that is tasked with the responsibility of producing official statistics on Türkiye, was the source from which the data regarding Turkish agricultural products were gathered. Only data from 2020 was analyzed, and spices did not make the cut for the list of crops that were analyzed. The data were broken down into Türkiye's seven distinct geographical regions for analysis. There are 128 different plant species that were discovered, and these plants have been separated into nine distinct categories: leaves, stems, fruits, pods, flowers, roots, bulbs, tubers, and fungus. We individually categorized each of the 128 crops into one of two groups: those whose output did not rise with pollination and those whose production is dependent on animal pollination to at least some extent. By conducting a literature review, we were able to determine the pollination requirements of various

Table 1. Insect Pollination Requirements of Plants

Crop Name	Insect Pollination Requirement	References
Phaseolus vulgaris, Vicia faba, Glycine max, Arachis hypogaea, Gossypium hirsutum, Brassica napus, Sesamum indicum, Helianthus annuus, Papaver somniferum, Carthamus tinctorius, Solanum tuberosum, Ipomoea batatas, Pisum sativum, Vigna unguiculata, Brassica oleracea, Lactuca sativa, Cynara scolymus, Apium graveolens, Beta vulgaris, Portulaca oleracea, Petroselinum crispum, Citrullus lanatus, Cucumis melo, Capsicum annuum, Cucumis sativus, Cucumis melo, Solanum melongena, Solanum lycopersicum, Abelmoschus esculentus, Cucurbita pepo, Cucurbita moschata, Solanum muricatum, Brassica napobrassica, Allium sativum, Allium cepa, Allium ampeloprasum, Raphanus sativus, Persea americana, Musa Sapientum, Ficus carica, Citrus sinensis, Citrus reticulata, Citrus aurantium, Malus domestica, Pirus communis, Cydonia oblonga, Prunus armeniaca, Prunus avium, Prunus cerasus, Prunus persica, Prunus domestica, Eriobotrya japonica, Elaeagnus angustifolia, Ziziphus jujuba, Actinidia deliciosa, Rubus idaeus, Fragaria vesca, Vaccinium myrtillus, Rubus caesius, Prunus dulcis, Castanea sativa, Punica granatumun,	Depends upon insect pollination at least to some extent	Vaz et al., 1998; Free, 1970; Roubik, D. W.; 1995 Rhodes, 2002; Schittenhelm et al., 2006; Crane, 1991; Bichee & Sharma, 1988; Moreti et al., 1996; Dajue & Mündel, 1996; Plaisted, 1980; Jones, 1980; Smith, 1980; Somerville, 1999; Free, 1993; Abel & Wilson, 1998; Pesson & Louveaux, 1984; El-Bakatoushi et al., 2013; Stanghellini et al., 2002; Valantin-Morison et al., 2006; Jarlan et al., 1997a;b; Meisels & Chiasson, 1997; McLaren et al., 1995; Benedek et al., 2006; Slaa et al., 2006; Hamon & Koechlin, 1991; Fuchs & Müller, 2004; Kowalczyk, 2008; Schittenhelm et al., 1997; Kamenetsky & Rabinowitch, 2001; Witter & Blochtein, 2003; Gray & Steckel, 1986; Partap & Verma, 1994; Can-Alonzo et al., 2005; Ish-Am & Eisikowitch, 1993; Willson & Schemske, 1980; Gottsberger, 1999; Westerkamp & Gottsberger, 2000; Chacoff & Aizen, 2006; Sharma et al., 2003; Delaplane,

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Table 1. Insect Pollination Requirements of Plants (continue)

Crop Name	Insect Pollination Requirement	References
Camellia sinensis, Citrus paradisi, Citrus limonum, Mespilus germanica	Depends upon insect pollination at least to some extent	2000; Khan et al., 1986; Pan et al., 2011; Yadav, P K, 2021; Costa et al., 1993; Manino et al., 1991; Wickramaratne & Vitarana, 1985; Miller et al., 2005
Triticum aestivum, Zea mays, Hordeum vulgare, Secale cereale, Avena sativa, Triticosecale Wittm, Cicer arietinum, Lens culinaris, Pisum sativum, Vigna unguiculata, Oryza sativa, Beta vulgaris, Spinacia oleracea, Daucus carota, Agaricus bisporus, Vitis vinifera, Morus nigra, Corylus colurna, Pistacia vera, Jovis Glans, Ceratonia siliqua, Diospyros kaki, Olea europaea	Does not require insect pollination	Allan, 1980; Russell & Hallauer, 1980; Starling, 1980; Geiger & Miedaner, 2009; Brown, 1980; Larter & Gustafson, 1980; Gritton, 1980; Ladizinsky et al., 1984; Free, 1970; Major et al., 1993; Smith, 1980; Free, 1993; Simon, 2010; Sampson et al., 2001; Chacoff & Aizen, 2006; Mulberry, 2023; Olsen et al., 2000; Crane, 1991; Polito et al., 2004; Dafni et al., 2012; Phipps et al., 2003; Miura, 1982

crops. The plants that required insect pollination were given in Table 1.

Result and Discussion

Pollination is an essential biological mechanism for preserving the diversity and productivity of several plant species. Honey bees are among the most important pollinators for agricultural products, including fruits, vegetables, and nuts. Honey bees are extraordinarily effective in gathering nectar and pollen, and they can transport and transmit pollen grains from flower to flower. This procedure increases the likelihood of effective fertilization, seed generation, and fruit growth. Without the pollination of honey bees, many crops would experience large production losses, resulting to economic losses and food shortages (Breeze et al., 2011).

Farmers have long recognized the value of honey bees for crop pollination, and as a result, they are commonly used as pollinators in agriculture. Concerns have been expressed concerning the sustainability of food production as a result of the global fall of honey bee numbers due to factors including habitat loss, pesticide use, and diseases. Researchers have been studying alternative pollinators, such as wild bees, flies, and beetles, as well as devising measures to increase honey bee health and variety to reduce the detrimental effects of honey bee decline on agriculture (Garibaldi et al., 2014).

Table 2 gives us a more in-depth look at the proportion of crops in each region that require insect pollination to some extent, as well as the proportion of crops that do not require insect pollination to any significant degree. The percentage of all crops that require insect pollination is 72.7%, which is in line with the trend that has been observed globally, which is that insect pollinators play an important part in agriculture.

Table 2. Crop Production in Various Regions of Türkiye Depending on Insect Pollination

Geographic Locations of Türkiye	Crops, to some extent depend on insect pollination	Crops do not require insect pollination	Total number of crops produced
Mediterranean Region	72.4%	27.6%	127
Eastern Anatolia Region	70.5%	29.5%	95
Aegean Region	72.4%	27.6%	123
South-eastern Anatolia Region	71.1%	28.9%	97
Central Anatolia Region	70.2%	29.8%	104
Black Sea Region	72.7%	27.3%	110
Marmara Region	72.4%	27.6%	116
Overall	72.7%	27.3%	128

The Mediterranean region contains the greatest number of crops (127), with 72.4% of them requiring insect pollination in some capacity and 27.6% not requiring insect pollination. Similarly, in the Aegean region, 72.4% of the crops require insect pollination, compared to 27.6% of the crops that do not. Similar crop distribution exists in the Marmara region, with 72.4% of crops requiring insect pollination and 27.6% not requiring it.

Eastern Anatolia, on the other hand, has the lowest proportion of crops (70.5%), with 29.5% not requiring insect pollination. Similarly, the region of south-eastern Anatolia has a greater proportion of crops (28.9%) that do not require insect pollination. The region of Central Anatolia has a higher proportion (29.8%) of crops that do not require insect pollination, whereas 70.2% of crops are pollinated by insects.

The Black Sea region stands out in terms of the number of crops that do not require insect pollination, accounting for 27.3% of crops in the region. This could be attributable to the environmental factors and the vegetation of the region, which may favor self-pollinating crops.

In Türkiye, around 72.7% of crops require insect pollination, whereas 27.3% do not. Around 84% of the 264 crops used for food production in Europe depend on insect pollination, illustrating the importance of pollinators in European agriculture (Underwood et al., 2017). The proliferation of fruit orchards and high-value cash crops, such as oilseed rape has increased the need for insect pollination services in China (Zou et al., 2017). In some regions, the reduction of natural pollinator populations and the excessive use of pesticides pose a threat to crop output and food security (Vanbergen & Initiative, 2013).

Conclusion

One of the greatest obstacles honey bees and other pollinators face is habitat loss caused by alterations in land use patterns. As agricultural practices intensify and expand, natural habitats are transformed into monoculture fields devoid of the variety of flowering plants required to sustain pollinator populations. This may result in food shortages, economic losses, and detrimental effects on biodiversity (Kremen et al., 2007). In addition, the use of pesticides can harm pollinators, diminishing their numbers and threatening their health (Sánchez-Bayo & Wyckhuys, 2019). Additionally, honey bees and other pollinators are vulnerable to diseases and parasites, such as *Varroa destructor* (vanEngelsdorp et al., 2010).

To resolve these troubles, researchers have been studying alternative pollinators and developing strategies to improve the health and diversity of honey bees. For instance, wild bees, flies, and beetles have been identified as potential alternative pollinators, and research indicates that they can pollinate certain crops effectively (Garibaldi et al., 2014). Efforts to restore and improve natural habitats, such as wildflower meadows and hedgerows, can also provide pollinators with valuable resources and improve their health (Kremen et al., 2007).

Insect pollination is required for 72.7% of crops in Türkiye, with the highest number of crops found in the Mediterranean, Aegean, and Marmara regions. Eastern and southeastern Anatolia have the lowest proportion of crops necessitating insect pollination. The Black Sea region has the highest proportion of crops that do not require insect pollination, possibly as a result of the region's climate and vegetation. The study also emphasized the significance of pollinators in agriculture, as approximately 84% of food-producing crops in Europe depend on insect pollination. We argue that protecting honey bees and alternative pollinators is essential to maintaining crop productivity and food security.

Ethical Statement

There are no ethical issues with the publication of this article.

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Conflict of Interest

The authors declare that there is no conflict of interest.

Author Contributions

Author 1: Investigation, Writing – review & editing,

Author 2: Investigation, Writing – review & editing; Supervision, Formal Analysis

Author 3: Investigation, Writing – review & editing

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