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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 (cm²/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	

$^{a, b, ...:}$ 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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