

Study of the Effectiveness Ofessential Oils (Anise, Clove)Solution in Controlling Varroa Mites (*varroa destructor*) on Honey Bees *apis mellifera*

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Abstract. This study was conducted at the AL- Musayyib Technical College / Department of Biological-Control Techniques for the period from 1/10 /2021 to 1/4 /2022. The study aimed the effectiveness of essential oils (anise, clove)solution in controlling Varroa mites (*Varroa destructor*) on honey bees *Apis mellifera*. The results indicatd that there are significant differences between all treatments of the essential oil solution (cloves, anise) and the treatment of its Varroa mites in comparison with the control treatment and the natural shedding of Varroa mites after (24, 48) hours of treatment, except for the treatment of cloves and anise at concentration (10). mg/ml, where there were no significant differences between them in the average mortality of Varroa mites. The results also showed in table (1) that there were no significant differences between the treatments of the solution (cloves, anise) in all concentrations in the time periods after (72, 96, 144) hours of treatment.It also showed that there were no significant differences between the treatments of cloves and anise at a concentration of (10) mg/ml after (48) hours of treatment. The results showed that there are significant differences between the treatments of cloves and anise at the concentration (40) mg/ml and the control treatment in the average mortality of field honey bee workers.The treatment of the essential oil solution of cloves with a concentration of (10) mg/ml achieved the lowest average death rate for the workers, which amounted to (2.42) after (15) hours of treatment, while the treatment of the essential oil solution of cloves at a concentration of (40) mg/ml gave the highest average death. It reached (6.58) after (15) hours of treatment, but it differs significantly with all transactions in Table (2) after 15 hours of treatment. The results revealed showed that there were no significant differences between the two treatments (cloves and anise) in the concentrations (10, 20) mg/ml after (15) hours of treatment in the average mortality of worker bees. Through the results of the statistical analysis in Table (2), it was found that there were no significant differences between all treatments (cloves, aniseed) and the control treatment in the average death of workers in the time period after (40,64) hours of treatment. The results show that a solution of essential oils with concentration (40)mg/ml has a small effect on the worker bees compared to the great effect on Varroa.

Keywords. Control, *Syzygium aromaticum*, *Pimpinella anisum*, *Varroa destructor*, *Apis mellifera*.

1. Introduction

honey bee insect(Hymenoptera: Apidae) *Apis mellifera* L.considered as living social insect [1]. The importance of honey bees comes first in pollinating a third of the agricultural crops that feed the world [2]. The other importance is the production of a lot of honey bee products such as (honey, wax, royal jelly, bee venom, bee gum and pollen), which are considered as food, medical and industrial products [3]. Honey bee breeders are facing significant losses in their colonies in many countries due to the Varroa mite, *V. destructor* [4]. and associated pathogens which are the most devastating [5]. Varroa mite is considered one of the most dangerous pests and diseases that affect honey bees in the world, as it leads to their destruction or weakening of many colonies and reducing their production[6]. The use of chemical pesticides in the control of the Varroa mite in an irregular and continuous manner for several years leads to the contamination of the products of honey bee colonies, as it can be found in honey, pollen and beeswax more commonly, and the Varroa mite gains resistance to pesticides[7]. This means that honey bee products are considered unsuitable for use, as these residues have recently become a global concern as they enter the human food cycle and cause diseases [8]. Therefore, it is necessary to rely on modern methods and new methods that include relatively cheap and safe materials for humans, honeybee colonies and the environment and biodegradable to control varroa mite,Using biological control, which represents the global trend, such as control with plant essential oils (clove, anise) and *B. thuringiensis* (Bt).Which are considered one of the safest ways to controlling this pest [9].Where researchers confirmed that the effectiveness of some essential oils may reach 94-98% [10].

Aims of the study:

- Study of the effectiveness of essential oils (anise, clove)in controlling Varroa mites (*Varroa destructor*) on honey bees*Apis mellifera*.
- Study of the effectof essential oils (anise, clove) on honey bees (*Apis mellifera*).

2. Materials and Methods

This study was conducted in the laboratories of the Musayyib Technical College / Department of Bio-control Techniques on 1/10/2021.

The field experiment was applied in the apiary researcher of the non-controlling with Varroa pesticides last year from 1/10/ 2021 to 1/12/2021. All beehives prepared for the experiment were from the Iraqi hybrid bees *A. mellifera* and experiments were conducted on it with three replicates for each concentration in addition to the control treatment where the study was conducted using (27) beehives, each containing (8) wax cells all covered with bees. Newly fertilized queens (20201) and the colonies (cells) were all similar in strength and from one lineage, and the queens fertilized with the same age, cell box and frames from one type of wood, shape and measurements of the same Langstroth measurements and free from diseases and pests to avoid the negatives that affect their activity and effectiveness inside and outside the cell. The bases of the hive boxes are provided with a movable chute that contains a wire clip resembling a sieve through which the Varroa parasite can pass without the bees Where A3 white paper greased with Vaseline® was placed in moving galaxies [11]. To facilitate the process of collecting fallen varroa without opening the beehive and ensuring that it does not return to the brood and worker bees again. The cells were checked weekly to follow up and calculate the severity of infection in The colony in particular and the apiary in general, by collecting 100 worker bees from the brood area in a plastic box with a wire cover, then adding sugar powder [12]. and according to the equation of [13]. Varroa mite samples were diagnosed at the University of Baghdad \ College of Science \ Natural History Research Center and Museum \ Department of Insects and Invertebrates. It was diagnosed as *V. destructor* [14].

2.1. The Method of Extracting Essential Oils from the Studied Plants

The reproductive parts of cloves and anise plants seeds (flower buds of *Syzygium aromaticum* from the family Myrtaceae, and seeds of the anise plant *Pimpinella anisum* from the family Apiaceae). were collected from local markets and the samples were well dried. Grind 100 gm of the reproductive parts of cloves and anise plants separately in an electric mill with a speed of 2500 r/min before a short

period of time before extracting the essential oil from them. The extraction process was carried out at the College of Agriculture - University of Al-Qadisiyah - Laboratory of Horticulture and Gardening Department using a modified distillation steam clavier (fine herm) type Chinese. As 100 grams of the powder from which the essential oil is to be extracted were immersed in 700 ml of distilled water in the 1-liter glass beaker for the device, and the temperature of the device was regulated to the boiling point of water (100) ° C. Then, after 20 minutes of operating the device, the temperature was reduced and regulated to 65 °C for the extraction process to take place by evaporation and condensation. It was sealed and kept in the refrigerator at 3°C and without aqueous sodium sulfate was added to it to pull out the water droplets suspended in the oil so that the concentration of the extracted essential oil was 100%. The process of extracting the essential oil was repeated several times to obtain an adequate amount for the study [15].

2.2. Study of the Effectiveness of Essential Oils Soaked with Cotton in Controlling Varroa Parasite

The experiment was carried out by placing cotton soaked with clove and anise essential oils at a concentration of 100% each separately inside the cell on a moving Hungary plate and with three replications for each concentration in addition to the control treatment, a total of 12 cells for the anise oil treatments and 12 cells for the clove oil treatments. The concentrations used were (0, 0.5, 1, and 2) ml/cell for each of clove oil and anise. Where the experiment was applied in the field on honey bee colonies at sunset time to ensure the presence of all bees infected with the Varroa destructor inside the cell during the application of the treatment for a period of two months (October and November). One (1) transaction per week. Then the readings were taken with the number of Varroa falling on the moving galaxies and their leaves (A3) greased with Vaseline for all treatments and after (24, 48, 72, 96 and 144) hours of applying the treatment [16].

2.3. Control with the Chemical Pesticide Apistan in the Form of Strips

The pyrethroid pesticide Apistan, the active substance fluvalinate 10%, was used, which is an effective pesticide by contact and through the stomach. To compare it with the results obtained when applying vegetable oil treatments, as the pesticide strips were used and placed inside the cells with two strips of the pesticide in each cell according to the instructions and they were suspended between the frames containing the brood. Where the pesticide treatment was applied for a period one month of autumn 1/10/2021 with three replications. Then the readings were taken with the number of Varroa falling on the moving galaxies and their leaves (A3) greased with Vaseline and after (24, 48, 72, 96 and 144) hours of applying the treatment [17].

2.4. Calculation of the Natural Shedding of Varroa Parasites

The experiment was conducted according to the method [18].

2.5. Statistical Design and Analysis

After collecting and tabulating the data for the studied traits, they were statistically analyzed using the (Genstat) program, where a Complete Randomized Blocks Design (R.C.B.D) was used for field trials, and the least significant difference (L.S.D) test was used to compare the means at a probability level of (0.05) [19].

3. Results and Discussion

3.1. Effectiveness of Spraying 20% Essential Oil Solutions (Clove, Anise) in the Average Fatality of Varroa Mites in Field

The results in Table (1) indicate that there are significant differences between all treatments of the essential oil solution (cloves, anise) and the treatment of its Varroa mites in comparison with the control treatment and the natural shedding of Varroa mites after (24, 48) hours of treatment, except for the treatment of cloves and anise at concentration (10). mg/ml, where there were no significant

differences between them in the average mortality of Varroa mites. The results also showed in Table (1) that there were no significant differences between the treatments of the solution (cloves, anise) in all concentrations in the time periods after (72, 96, 144) hours of treatment. It also showed that there were no significant differences between the treatments of cloves and anise at a concentration of (10) mg/ml after (48) hours of treatment.

Table 1. Shows the effectiveness of spraying 20% essential oils solutions (cloves, anise) in the average field death of Varroa mites and comparing it with the Apistan pesticide and natural shedding of Varroa mites.

Treatment	concentration mg/ml	Reading rate after 24 hours	Reading rate after 48 hours	Reading rate after 72 hours	Reading rate after 96 hours	Reading rate after 144 hours
Control						
Distilled water and alcohol	0	10.13 g	4.25 f	3.79 cd	3.96 bc	6.00 b
clove oil solution	10	17.58 e	5.87 e	3.71 cd	3.38 c	5.75 b
anise oil solution		14.21f	5.17 ef	3.37 d	3.67 bc	5.92 b
clove oil solution	20	24.92 c	11.38 c	4.29 bcd	3.71 bc	5.58 b
anise oil solution		18.96 d	9.08 d	4.00 cd	3.25 c	5.33 b
clove oil solution	40	32.25 b	15.38 b	4.96 b	3.63 bc	5.71 b
anise oil solution		23.79 c	11.46 c	4.54 bc	3.25 c	5.50 b
Comparison natural precipitation	without treatment	4.33 h	4.54 f	3.92 c	4.04 b	5.83 b
Comparison Apistan	2 bare	34.25 a	34.00 a	31.46 a	27.04 a	42.25 a
L.s.d	0.05	1.29	1.02	0.95	0.65	1.30

The averages with different letters within the same column have significant differences in comparison with L.S.D at the level of significance 0.05.

3.2. Effect of Spraying 20% Essential Oils Solutions (Cloves, Anise) on Average Field Mortality of Honey Bee Workers

The results in Table (2) indicate that there are significant differences between the treatments of cloves and anise at the concentration (40) mg/ml and the control treatment in the average mortality of field honey bee workers.

The treatment of the essential oil solution of cloves with a concentration of (10) mg/ml achieved the lowest average death rate for the workers, which amounted to (2.42) after (15) hours of treatment, while the treatment of the essential oil solution of cloves at a concentration of (40) mg/mol gave the highest average death. It reached (6.58) after (15) hours of treatment, but it differs significantly with all transactions in Table (2) after 15 hours of treatment.

The results in Table (2) showed that there were no significant differences between the two treatments (cloves and anise) in the concentrations (10, 20) mg/ml after (15) hours of treatment in the average mortality of worker bees.

Table 2. Shows the effect of spraying 20% essential oils solutions (cloves, anise) on average field mortality of honey bee workers and its comparison with the control treatment and natural death.

Treatment	concentration mg/ml	Reading rate after 15 hours	Reading rate after 40 hours	Reading rate after 64 hours
Control distil water and alcohol	0	2.83 d	2.83 bc	2.58 a
clove oil solution	10	2.42 d	3.25 ab	2.67 a
anise oil solution		2.33 d	2.83 bc	3.25 a
clove oil solution	20	4.00 c	3.00 bc	2.92 a
anise oil solution		3.67 c	3.17 b	3.00 a
clove oil solution	40	6.58 a	2.92 bc	3.25 a
anise oil solution		5.92 b	2.58 c	3.00 a
Comparison natural precipitation	without treatment	2.58 d	3.75 a	3.67 a
L.s.d	0.05	0.52	0.58	N.S

Means of different letters within the same column there are significant differences between them according to compared with L.S.D at the level of significance 0.05.

4. Discussion

The results in Table (1) showed the superiority of the pesticide treatment and it differed significantly with all treatments, and the reason is due to the active substance Fluvalinate 10%, which is considered a dream pesticide that works by contact and the digestive system. However, the purpose of its use is to compare it with the results obtained from treatments of essential oils (cloves, anise). This is agreement with [20]. that natural essential oils can be an alternative to pesticides in controlling Varroa mites. The reason for the superiority of the clove oil solution may be attributed to the active compounds contained in the oil, especially Eugenol, which kills by contact and evaporation [21].

As for the effectiveness of anise essential oil, it may be due to its containing active compounds, especially the compound (anthol), which is characterized by its repellent and anti-nutritional properties on Varroa mites [22]. The results of this study agree with what was mentioned by [23]. that anise oil is effective in controlling Varroa mites by both fumigation and contact methods.

In table (2) the reason for giving the essential oil solution (clove, anise) at a concentration of (40) mg/ml to the highest average fatality is that these solutions contain high concentrations of active compounds (eugenol, alantole) that affected the workers compared to the great effect on the Varroa mites.

The results of this study agree with what was reached [24]. who stated in his laboratory study that the treatment of workers with clove essential oil by contact (spray) did not exceed the percentage of bee deaths (2%).

The results are consistent with [25]. No negative effects were observed on bees and brood as a result of the control with anise oil, as it was applied in the correct proportions. It also agrees with the study [26]. where he noticed a remarkable increase in bee coverage and brood in colonies treated with essential oils compared to non-treated colonies.

Conclusions

- We conclude from the study that the use of essential oils (anise, clove) solution different concentrations they had a great impact in the controlling Varroa mites At concentration (40)mg/ml and the effectiveness lasted for two days.
- clove oil outperformed aniseed oil In all concentrations after two days of treatment In controlling Varroa mites.
- The effectiveness of clove oil solution at concentration (40) mg/ml after a day of treatment was to some extent equal to the effectiveness of epestanicide in controlling Varroa mites.
- the effect of essential oils(anise, clove) solution on adult honeybees it was very little.

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