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Evaluation of the effectiveness of using three volatile oils as eco-friendly materials in honey bee hives against *Varroa destructor*

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Abstract

The honey bee contributes to the pollination of a large number of plants as well as their food products, especially honey. Therefore, it is considered an important economic insect. The insect attacks a number of diseases and pests, including the Varroa mite *Varroa destructor*, the most dangerous of which is due to its resistance to chemical treatments that have a harmful effect on bees and pollute their products. For this reason, the goal of the study is to combat Varroa mites in honey bee colonies with environmentally friendly materials by evaluating the effectiveness of three types of essential oils from *Eucalyptus globulus, Calotropis procera, Artemisia vulgaris*. The research was carried out in April 2024 in Diyala Governorate, Iraq. With two exposure periods of 24, 48 hours, control was carried out in two ways, one of which was smoking using a piece of burlap saturated with oil by the smoker, and the second method was using paper saturated with oil placed under the hive to find out the most effective method, whether oil or method. The results after 24 hours of smoking with oil-saturated burlap indicated that eucalyptus was the most effective, with the average effectiveness reaching 75.5% for *Calotropis procera* oil, followed by *Eucalyptus globulus* oil at 66.5%, followed by a mixture of oils at 64.9%, then *Artemisia vulgaris* oil at 58.1%, compared to the control treatment at 24.9%. As for the oil-impregnated paper method, the highest effectiveness was *Calotropis procera* oil with an average effectiveness of 54.7%, Followed by mixed oils 53.6%, followed by *Artemisia vulgaris* oil with 41.4%, then *Eucalyptus globulus* oil with 36.2% compared to the control with 43.8%. The smoking method using burlap saturated with oil is more effective than the method of smoking paper saturated with oil. The highest average of Varroa mites was 86.5 for *Calotropis procera* oil, while the lowest average was 38 for *Artemisia vulgaris* oil. We conclude that essential oils are effective in combating Varroa mites, especially *Calotropis procera* oil, in addition to the fact that they are natural and safe for bees and their products.

Keywords: *Apis mellifera*; Honey Bees; Essential Oils; Varro

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Introduction

Honeybees are considered one of the most important insects that have benefited humanity for medical purposes and food for thousands of years. Honey bees are of great economic importance for agriculture, not only to produce honey, but also to pollinate crops [1]. And honeybees play too it plays an important role in biodiversity, and is known to increase crop yields pollinated by insects to the extent of 10 to 20 times more than its production value when not pollinated by insects, bee pollination improves the size, shape, color and taste the fruits [2].

Bees, like other living organisms, are exposed to many pests and diseases during its life, and it is one of the most common and dangerous pests that severely effects on the population and performance of bee colonies is the mite *Varroa destructor*, and it belongs this mite belongs to the family Varroidae and genus Varroa, and feeds on hemolymph the fat bodies of larvae, pupae and adult bees at all life stages infection with Varroa mites causes bees to lose weight, become deformed, or lose weight its limbs, and sometimes young bees die, and the Varroa mite is considered one of the most important destructive pests of beehives that cause damage to the beekeeping industry cannot be avoided fix it [3-5].

The mite infects bees by entering to brood cells before covering and parasitizing honeybee larvae and pupae. It becomes difficult to control; Because the majority of mites remain in the closed brood to reproduce, it is well protected from chemical treatments [6,7]. Besides the economic loss for bees and honey production, the infected hive may die or migrate [8]. This parasite destroys the protective mechanical barriers of membranes and weakens the immune system for bees [9]. Various pathogens such as viruses are likely to be transmitted acute bee paralysis, deformed wing virus and fungi to bees by Varroa [10]. Recently, six viruses have been discovered in infected bees Varroa; Therefore, it is expected that pathogenic cases in honey bees will worsen [11].

Many chemicals have been used successfully to control mites, a wide range of chemicals were highly effective; Where I killed the most of the 99% of mites found in infected colonies[12]. In recent years the problem of acaricide resistance has developed rapidly and increased tolerance has been observed Varroa is the most widely used synthetic acaricide. In addition, contamination of hive products with pesticide residues has also been reported, especially in Beeswax [13,14].This situation has led to increased concern about contamination of products bees with synthetics used against Varroa [15]. It should also be minimized use of acaricides in beekeeping; due to the decomposition of its waste and pollution honey and wax products [16].The problems associated with the use of acaricides have proven a major incentive to develop new treatment strategies and screen potential acaricides that reduces these problems, natural products that contain ingredients with different modes of action provide an effective solution to the Varroa mite problem [17-20]. Varroa is a source of concern for migratory beekeepers who depend on movement.

Essential oils are considered safer and have high toxicity to Varroa and are safe for bees. They do not have any residues on honey bee products, and do not generate resistance to their action by bees [5,21,22]. The study aimed to identify the effectiveness of three types of plant essential oils (*Eucalyptus globulus, Calotropis procera, Artemisia vulgaris*) and a mixture of these. The three oils to combat the Varroa parasite that infects honeybees, *Apis mellifera*, in two ways. The first is by using the fumigation method of a piece of burlap saturated with oil, and the second method is to place paper saturated with oil at the bottom of the cells to identify the most effective oils used and methods for combating.

Materials and Methods

Honey Bee Hives

The experiment was carried out in the beehives of the Agriculture Directorate in Diyala Governorate in February 2023. The medium-strength Langstroth hives were selected and were close in terms of bee density. Samples of bees were collected from each hive before the start of the experiment to determine the degree of Varroa infestation by selecting hives with a rate of infection of one Varroa female per 100 workers. This rate of infection is sufficient to approve the hives in the experiment [23]. And the honey bee strain is of the type *Apis mellifera*, which is considered dominant. In Iraq, in addition to its adaptation to the environmental conditions in the region [24]. Experiments were conducted in the morning from eight to ten in the morning, and it is considered the most appropriate period for conducting tests because most of the workers are wandering during this period, and it is noted that the Varroa mite (Acari: Varroidae *Varroa destructor* prefers Honey bee workers incubate inside the hives, so the focus is on this type of workers more than those that leave [25,26].

The duration of the experiments is three days, after 24 hours, 48 hours, and 72 hours. In order to count the dead Varroa individuals as a result of the control, I used A4 sheets of paper that were coated with Vaseline and placed on the bases of the honey bee hives to make it easier for the fallen and dead Varroa individuals to stick to them, so that the Varroa numbers were calculated by summing the fallen ones.

Extracting Essential Oils

Leaves of three types of plants were used. *Eucalyptus globulus*, wormwood (*Artemisia vulgaris*), and acacia

Calotropis procera. After collecting and cleaning it, it was placed at room temperature with constant ventilation and stirring to prevent it from rotting for a period of one week until it dried. Then it was ground using an electric grinder to be prepared to extract oils from it by steam distillation through a Clavenger [27]. Device to obtain samples of the oils that will be included in the experiments.

Control Methods Used

The first method is Fumigation: Use the smoker to burn a piece of burlap after supplying it with the mentioned oils at a concentration of 10 ml for each honey bee hive. By inserting the smoker's hole through the door of the hive, smoking is done towards the top, where the bees are at the top of the hive. This is done by pressing the device five regular times for each hive and repeating the process three times. For the first cell, then the second, and so on, in one day and for three days, which represents the duration of the experiment. As for the control treatment, it was fumigated with a piece of burlap that was not treated with any type of oil.

The Second Method: Oil-Saturated Paper: For this purpose, white A4 paper was used, saturated with an amount of oil. After covering the entire paper with an amount of oil, it was then placed at the base of the hive. As for the control treatment, paper coated with Vaseline was used without treating it with any type of oil. The sheets numbered according to their hive were replaced at the beginning of each treatment procedure, then each one was folded separately for a period of three days, so that all the dead Varroa individuals collected on each sheet were counted. In this way, the effectiveness of the treatment was estimated, whether for each day separately or for the three days combined.

Groups

The hive was divided into five groups

1. Control group 2- Oil treatment group (*Eucalyptus globulus*) 3- Oil treatment group (*Calotropis procera*) 4- Oil treatment group (*Artemisia vulgaris*) 5- A group treated with a mixture of the three oils used in the experiment.

Six hives were chosen for each of these groups, divided into three replicates per treatment (30 hives total), as follows: three hives for the first group, which were treated with the fumigation method, and three hives for the second group, which were treated with the oil-saturated paper method, and the same for the control treatment but without treatment with any type of oil.

Statistical Analysis

By using the equations used to calculate the effectiveness of the control material, the results were analyzed statistically [28].

1- Relative effectiveness of the control material % = (number of Varroa shedding after 24 hours of control - average natural shedding before control) x100

2- The ratio between the shedding after and before the application (double): is the number of times the Varroa shedding occurred 24 hours after the control procedure compared to the average natural shedding before the control and is calculated as follows:

The ratio between natural shedding after and before application = number of Varroa shedding after 24 hours of control/average natural shedding before control.

Percentage of Varroa shedding after an hour compared to the total shedding after 24 hours %=(Number of Varroa falling after one hour of control / Total number of Varroa falling after 24 hours of control) x 100

Using a program Statistical Package for the Social Sciences SPSS Statistical analysis was conducted (ANOVA) at the probability level $P \leq 0.05$.

Results and Discussion

In the current study, it is clear from table (1) that the average number of fallen Varroa mites before treatment ranged between 2-9 per beehive, while it reached 7-11 Varroa mites after 24 in the two groups. In the burlap smoking group before treatment, the average number of fallen Varroa mites was 24.9%, while it reached 43.8 for the group. Leaves treated with Vaseline. After 48 hours, the number of fallen mites ranged from 8 to 13 mites.

Table (2), it includes the treatment of hives with eucalyptus oil in the smoking method at an amount of 10 ml/hive, where the average shedding reached 56.5%, while the average effectiveness reached 66.5 after 24 hours. As for the group of paper saturated with eucalyptus oil, the average shedding reached 79.4% after 24 hours, which represents twice the number of Varroa shedding in the control. Which amounted to 43.8%. After 48 hours, it ranged between 24-32 per hive, while it ranged between 14-20 per hive for the first and second groups, respectively.

Table (3) shows the effective effect of using *Calotropis procera* oil using the smoking method. After 24 hours, the relative effectiveness reached 84.6%, which is higher than the control and eucalyptus treatment, and the average effectiveness reached 75.5%. As for the oil-saturated paper method, the average effectiveness reached 54.7%, and the average percentage reached 2.3 and 4.4 which is higher than eucalyptus oil and the comparison. Which amounted to 1.6 and 1.4, respectively. After 48 hours, the numbers of fallen Varroa ranged between 30-41 for the smoking treatment compared to 16-26 for the oil-saturated paper treatment.

Variables	(Group1)			(Group2)		
	Fumigation 10 ml/ hive			Oil impregnated paper/ hive		
	Number of dead Varroa mite/hive					
	hive	hive	hive	hive	hive	hive
	1	2	3	1	2	3
Natural shedding before the start of the experiment varroa/day	10	5	5	6	11	8
Number of Varroa shed one hour after application	12	9	14	7	13	11
Number of Varroa shed 24 hours after application	17	28	21	10	17	12
Comparison of Varroa shedding after 1 and 24 hours	71	32	67	70	77	92
Average%	56.5			79.4		
Relative effectiveness %	41	82	76	40	35	33
Average effectiveness %	66,5			36.2		
Varroa shedding rate after and before treatment	1.7	5.6	4.2	1.7	1.6	1.5
Average ratio	3.8			1.6		
Number of Varroa shed after 48 hours	24	32	28	14	20	18

Table 1: The number of fallen Varroa mites in the Control group.

Variables	(Group1)			(Group2)		
	Fumigation 10 ml/ hive			Oil impregnated paper/ hive		
	Number of dead Varroa mite/hive					
	hive	hive	hive	hive	hive	hive
	1	2	3	1	2	3
Natural shedding before the start of the experiment varroa/day	10	5	5	6	11	8
Number of Varroa shed one hour after application	12	9	14	7	13	11
Number of Varroa shed 24 hours after application	17	28	21	10	17	12
Comparison of Varroa shedding after 1 and 24 hours	71	32	67	70	77	91.7
Average%	56.5			79.4		
Relative effectiveness %	41	82	76	40	35	33.3
Average effectiveness %	66,5			36.2		
Varroa shedding rate after and before treatment	1.7	5.6	4.2	1.7	1.6	1.5
Average ratio	3.8			1.6		
Number of Varroa shed after 48 hours	24	32	28	14	20	18

Table 2: The effect of (*Eucalyptus globulus*) oil on Varroa mites.

Variables	(Group1)			(Group2)		
	Fumigation 10 ml/ hive			Oil impregnated paper/ hive		
	Number of dead Varroa mite/hive					
	hive	hive	hive	hive	hive	hive
	1	2	3	1	2	3
Natural shedding before the start of the experiment varroa/day	7	9	4	10	5	7
Number of Varroa shed one hour after application	10	12	17	13	6	12
Number of Varroa shed 24 hours after application	24	31	26	18	11	20
Comparison of Varroa shedding after 1 and 24 hours	42	39	65	72	82	60
Average%	48.6			71.3		
Relative effectiveness %	71	71	85	44	55	65
Average effectiveness %	75.5			54.7		
Varroa shedding rate after and before treatment	3.4	3.4	6.5	1.8	2.2	2.9
Average ratio	4.4			2.3		
Number of Varroa shed after 48 hours	30	34	41	22	16	26

Table 3: The effect of (*Calotropis procera*) oil on Varroa mites.

Table (4) shows a decrease in the relative effectiveness of *Artemisia vulgaris* oil over that of Eucalyptus and *Calotropis procera* oil after 24 hours, as it reached 58.1%. Its effectiveness percentage also decreased compared to the rest of the treatments, as it reached 2.8. This is with regard to the smoking treatment, while the effectiveness percentage with the oil-saturated paper method reached 41.4%. The numbers of fallen Varroa after 48 hours reached 14-21 and 12-16, respectively, a clear decrease from the previous treatments.

The results are shown in Table (5). When using the mixture of the three oils and fumigating the beehives by burning burlap saturated with the mixture of oils, the average effectiveness was found to be 64.9%, which is higher than the control and wormwood treatment and lower than that of *eucalyptus* and *Calotropis procera*, while the average effectiveness using the paper method saturated with the three oils was 53.6%. It is higher than other oils with the exception of *Calotropis procera* oil. While the number of Varroa shedding after 48 hours reached 22-42 and 16-27 in the smoking and oil-saturated paper methods, respectively.

Variables	(Group1)			(Group2)		
	Fumigation 10 ml/ hive			Oil impregnated paper/ hive		
	Number of dead Varroa mite/hive					
	hive	hive	hive	hive	hive	hive
	1	2	3	1	2	3
Natural shedding before the start of the experiment varroa/day	3	8	6	12	2	7
Number of Varroa shed one hour after application	6	10	8	12	8	9
Number of Varroa shed 24 hours after application	11	12	19	14	10	10
Comparison of Varroa shedding after 1 and 24 hours	55	83	42	86	80	90
Average%	60			85.2		
Relative effectiveness %	73	33	68	14	80	30

Average effectiveness %	58.1			41.4		
Varroa shedding rate after and before treatment	3.7	1.5	3.2	1.2	5	1.4
Average ratio	2.8			2.5		
Number of Varroa shed after 48 hours	17	14	21	16	12	13

Table 4: The effect of (*Artemisia vulgaris*) oil on Varroa mites.

Variables	(Group1)			(Group2)		
	Fumigation 10 ml/ hive			Oil impregnated paper/ hive		
	Number of dead Varroa mite/hive					
	hive 1	hive 2	hive 3	hive 1	hive 2	hive 3
Natural shedding before the start of the experiment varroa/day	7	11	6	8	5	10
Number of Varroa shed one hour after application	8	14	17	10	12	16
Number of Varroa shed 24 hours after application	16	31	23	13	18	20
Comparison of Varroa shedding after 1 and 24 hours	50	46	74	77	67	80
Average%	56.5			74.5		
Relative effectiveness %	56	65	74	39	72	50
Average effectiveness %	64.9			53.6		
Varroa shedding rate after and before treatment	2.3	2.2	3.8	1.6	3.6	2
Average ratio	2.8			2.4		
Number of Varroa shed after 48 hours	22	42	27	16	23	27

Table 5: The effect of using a mixture of the three oils used in the experiment in combating Varroa mites.

By comparing the average numbers of Varroa shedding using the essential oils used in this study, it was found that the highest average shedding was 86.5 using *Calotropis procera* oil, while the lowest average shedding was 38 for wormwood oil, as in Table (6). As a result of using essential oils for control, the numbers of fallen varroa gave a high moral significance equal to 0.002, and its significance limits at 95% ranged between 15,900 - 25,400, as in Table (7).

oils	Mean	Lower Bound	Upper Bound
Calotropis procera	86.5	47.35	125.65
Eucalyptus globulus	70	30.85	109.15
Artemisia vulgaris	38	0,650	75.35
mixture oils	80	40.85	119.15
control	38.5	0.35	77.65

Table 6: Shows comparison of averages of plant essential oils for Varroa mite control

	95% Confidence Interval				
	Upper	Lower	Sig. (2-tailed)	Std. Error	Mean Difference
Varroa	25.4	15.9	0.002	2.408	20

Table 7: Statistical tests to test the effectiveness of using plant essential oils to combat Varroa.

The results of the fumigation method showed that the highest effectiveness in preparing fallen varroa was for *Calotropis procera* oil, followed by the oil mixture treatment, followed by eucalyptus and then wormwood, which gave the least effectiveness in combating varroa. *Calotropis procera* oil also gave the highest effectiveness using the oil-saturated paper method. This is consistent with previous studies that when evaluating the effect of essential oils by the method of effect, whether by fumigation or by contact, it has been proven that fumigation gives better results than contact [26]. The results also showed that fumigation with a piece of burlap saturated with oil is better than paper saturated with oil, so the fumigation method is more common in controlling Varroa [29]. The use of essential oils is constantly evolving to treat Varroa [30]. Much research has indicated that essential oils have an antifungal, antimicrobial, and insecticidal effect and many pathogens and pests in the laboratory or in the field. Therefore, they are considered a good alternative to chemical pesticides because of their plant source and their broad biological anti-nutritional or toxic effect against Varroa mites [31]. There is a trend. To stay away from chemicals due to their danger to honeybees and replace them with natural materials [32]. Deosi, et al. [33] tested a group of vegetable oils and their effectiveness in combating the Varroa destructor mite. Mustard oil gave good results in maintaining the strength of the colony because it contains an antioxidant, and this is what was confirmed [34].

A number of researchers have conducted studies on the effect of other vegetable oils [35]. Observed an increase in bee coverage and brood in colonies treated with anise oil compared to those not treated. The results varied according to the concentration and method in the application used. In the fumigation method using a coil connected to a dry battery, it gave an effectiveness between 65.2% and 89.2% when fumigating at a concentration of 1 and 1.5 ml, respectively, due to its repellent effect. In a study, thyme oil was proven effective in combating Varroa [36]. Essential oils have broad activity against pathogens [37]. It also has an activity in controlling disease-carrying insects [38]. In a study, [39] fourteen types of essential oils were used by fumigation and topical treatment against Varroa and the extent of their effect on bees at the same time. It was found that the longer the exposure period led to an increase in toxicity. Against Varroa, similar results were recorded using a group of oils. After 24 hours, Thymus oil was effective in killing 50% of Varroa at the lowest dose without harmful effects on the bees [40]. This is consistent with the results of the current study, as no direct harm was observed on the bees because the oils Aromatic products are natural products with a sharp odor that are low in toxicity to bees and mammals and have no harmful effect on the environment. This is what [41] showed, as their effectiveness in combating Varroa ranged between 50 and 95% in laboratory and field tests. Found that natural oils are safer when used in the long term by combating Varroa destructor by feeding on the life of honey bees and their feeding behavior.

Conclusion

It is concluded from this study that it is possible to rely on volatile oils, especially *Calotropis procera* oil, by fumigation using a piece of burlap saturated with oil, because of its effectiveness in combating the Varroa mite, which is one of the most dangerous pests for honey bee colonies. *Calotropis procera* oil is considered safe for honey bees, as no cases of honey bee deaths have been recorded when using it Therefore, we recommend using *Calotropis procera* oil by fumigating it with a piece of burlap soaked in oil to combat it. We recommend further studies to explore the effectiveness of volatile plant oils and avoid chemicals that are harmful to the environment, humans, and bees.

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