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The Effectiveness of Smoking Against the *Varroa destructor* Mite Using Certain Medicinal Plants in Honey Bee Hives (*Apis mellifera*)

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Abstract

One of the most deadly pests of honey bees (*Apis mellifera*) is the *Varroa destructor* mite. The study aims to test the effect of four medicinal plants: Sodom Apple *Calotropis procera*, Rosemary *Salvia rosmarinus*, Camphor *Eucalyptus globulus,* and Wormwood *Artemisia vulgaris*, as eco-friendly substances through smoking to combat Varroa mites. Plant leaf powders were used at 20 g/ hive by using a smoker at 8 puffs/ hive, because exposure to more can harm the beehive. Fallen and dead Varroa mites were collected after (2, 4 and 6) days of smoking for treatments, by replacing old mite counting trays with new ones. The highest mite loss rate was 47.11 mites/hive, which represents all mites dropped for exposure periods of (2, 4, and 6) days when fumigated with *E. globulus*, while it was 15 mites/hive in the control treatment. Decrease in the shedding rate was observed over time, reaching 70.24%, 58.82%, and 31.65% mite/treatment after 2, 4, and 6 days respectively. The infection rate of honeybee brood decreased to 8% after treatment compared to 59.66% before treatment in the case of *E. globulus*, and brood growth increased by between 4.94% and 23.33% after 21 days of exposure, in the control group and in the case of *E. globulus*, respectively. We can infer from the results the effectiveness of the method of smoking plants used in this study in reducing or limiting mite density in honey bee colonies.

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Introduction

Honey bee, *Apis mellifera* L., belongs to the Apidae family and is one of the most important organisms for pollinating flowers (Maggi *et al*., 2013). Honeybees are among the most important pollinators of flowers, providing extensive environmental services, as well as producing honey (Hanley *et al*., 2015; Hung *et al*., 2018), Studies indicated to a continuous decline in the number of insect pollinators in general, and honeybees in particular, which has put the world on alert. The phenomenon of Colony Collapse Disorder (CCD) led to a sudden decrease in honeybee density (Oldroyd, 2007). (CCD) has causes many reasons like: competition between species, changes in agricultural land use,

and climate change (Mwebaze *et al*., 2018), In addition, misuse of pesticides, genetic factors, infection with pathogens including Varroa mites, beekeeping practices, and others (Zaluski *et al*., 2015).

Most of the damage is caused by a number of different pests that affect honeybees (Sajid *et al*., 2020). *V. destructor* is a dangerous mite that infests honey bees (Yang *et al*., 2021) The Varroa mite causes the death of the colony (Rosenkanz *et al*., 2010). Varroa mites are also vectors for viruses, which can lead to hive destruction in future (De Grandi *et al*., 2012).

One common method of controlling Varroa mites is using chemical pesticides, such as organophosphates and pyrethroids. However, this has led to development of resistance to these pesticides due to repeated and prolonged use, as well as contamination of honeybee products with pesticide residues (Thompson *et al*., 2002). As a result of all this, negative effects have emerged from the use of chemical pesticides such as organophosphorus compounds through the Varroa mite's resistance to their effects (Brasesco *et al*., 2017). Tosi *et al.* (2018), a three-year study In Italy, demonstrated that honeybee products were contaminated with chemical pesticides through the transfer of pollen carried by bees to the hive, indicating the indiscriminate use of chemical pesticides, which causes contamination of honeybee products as well as hive deterioration (Tette *et al.,* 2016). Therefore, there is an urgent need to replace polluting chemical pesticides with more safer materials that do not leave toxic residues (Sabahi *et al.,* 2017; Aljedani, 2021; Alsaadi *et al.,* 2024). For these reasons, need arose to use alternative methods that are safer from an environmental perspective by introducing natural plants as a control element (Li *et al.*, 2017; Sakla *et al.*, 2025) This has increased the trend towards using plant-based ingredients in many ways, including smoking in recent years due to their safety for humans (Al-Sharabi, *et al.,* 2023; Khan *et al*., 2024). The aim of this research is to evaluate the effectiveness of smoking with these plants *A. vulgaris, C. procera*, *S. rosmarinus* and *E. globulus*, which are abundant in the environment and used to combat Varroa mites in honey bee colonies.

Materials and Methods

During the bee's active period, the experiments were conducted from 10/3/2024 to 25/4/2024, in an apiary in Baqubah City- Iraq. Standard Langstroth hives, *Apis mellifera* bee colonies, were used. Each hive was equipped with seven wax-covered frames containing sealed and open brood, with adult bees covering both sides, as well as honey and pollen, according to the method by Al-Sharabi *et al*. (20 23)With a total of five treatments, including the control treatment, 15 hives were randomly divided into three hives (replicates) for each treatment.

Varroa infestation and methods of detection

1. Sugar method

In a glass container equipped with a 3mm diameter wire mesh cover, and using a soft brush, from all sections of the experiment, 200 adult bees were randomly collected. The Varroa mites mixed with the sugar were counted by turning the jar upside down until it completely emptied of sugar and Varroa mites onto a white plate, leaving only the bees in the jar. To ensure that all Varroa mites had fallen out, the process was repeated; Varroa mites were counted and recorded. The bees are placed on a piece of cloth in front of the hives to facilitate their return (Macedo *et al*., 2002).

2. Natural Varroa mite removal

A mesh screen was placed under the frames and covered with a layer of Vaseline to help Varroa, which fall from the bees during the smoking process, adhere to it., after the beehives were equipped with special bases to count the fallen Varroa mites. To prevent bees from touching the Vaseline-treated surface, the bases were covered with a mesh with 2 mm diameter holes. Data is taken for Varroa mite shedding before and after application, at 2, 4 and 6 days. Without using smoking, those bases are cleaned and then coated again with Vaseline before being returned to the beehives, in order to account for the natural shed of Varroa before use.

3. Study plants

The four medicinal plants used were: Sodom Apple *Calotropis procera*, Wormwood *Artemisia vulgaris*, Rosemary *Salvia rosmarinus,* and Camphor *Eucalyptus globulus,* each plant was collected in the early morning of March during the flowering stage to obtain the active compounds, from Baqubah city in Iraq. The leafy parts of the experimental plants were dried in a well-ventilated room for six hours at a temperature of 25°C and a humidity of 55%, then ground into small pieces and stored in containers until use (Al-Hayali *et al.,* 2025a). As shown in Table 1.

Table 1. Medicinal plants and their quantity by weight used to combat *V. destructor*

Treatment	Smoking materials	Family	Part used	Replications	Quantity Weight (gm) 20 grams/hive
T1	*Eucalyptus globulus*	Myrtaceae	leaves	3	60
T2	*Salvia rosmarinus*	Lamiaceae	leaves	3	60
T3	*Artemisia vulgaris*	Asteraceae	leaves	3	60
T4	*Calotropis procera*	Apocynaceae	leaves	3	60
T5	Control	-	-	3	0

As well as Table 2, that shows plants chemical components.

Table 2. Comprehensive composition of study plants

Medicinal plants	Component	References
E. globulus	Quinones, Volatiles, Saponins, Hansch carbohydrates, Tannins, Phenols, Flavanoids, Proteins, Fat.	(Kaur *et al*., 2019)
S. rosmarinus	Volatile oil (1.0%-2.5%), Diterpenes (up to 4.6%), Triterpenes, Phenolic acids (2%-3%), Flavonoids, Alkaloids, Tannins, Saponins, Glycolic acid and glyceric acid, Vitamin C; vitamin P, Choline	(Anadón *et al*., 2008)
A. vulgaris	Carbohydrates (40%), Volatiles, Amino acids, Phenolic compounds (9.8%), Protein (2.9%), Triterpenoids, Steroids, Glycosides, Saponins, Flavonoids.	(Melguizo-Melguizo *et al.,* 2014)
C. procera	Cardenolides, Steroids, Terpenes, Proteins, Enzymes, Flavonoids, Lignans, Esters, Volatiles.	(Kaur *et al.,* 2021)

The smoker was used, in which a piece of burlap made from jute was placed. The first treatment consisted of dried eucalyptus powder added to the jute in the smoker at a rate of 60 g (20 g/hive) for three replicates, each hive representing one replicate. The same method was used for the remaining treatments of the experiment, all openings in the hive are closed before the treatment is carried out using the blower with a rate of 8 bursts of smoke through the hive door, so that the hive door is closed to prevent smoke from leaking for eight minutes to ensure that the smoke spreads evenly throughout the beehive. Thus, the process is repeated for all the hives for the rest of the treatments, taking into account the implementation time, afternoon, which is the time when most of the worker bees that wandered back into the hive (Eischen and Wilson, 1997). After the smoking procedure, data were recorded regarding the percentage of death after two days. Thus, the smoking process was repeated in the same way, so that the data was recorded for the second time after 4 days, in addition to smoking again in the same way for the third time, and then the data was collected after 6 days. According to Goswami *et al*. (2014) deaths were using the following equation:

$$Mortality\ (\%)\ = \frac{Mortality\ in\ treatment}{(Mortality\ in\ treatment + Mortality\ in\ control)} \times 100$$

Estimation of brood infection

The area of the sealed brood was randomly calculated by selecting 3 frames from the center of the hive, at a rate of 20 eyes pertaining to the brood per hive, with three replicates. At a rate of 60 hexagonal eyes/treatment, for a total of 300 eyes. The area was calculated one day before the treatment and 22 days after it. This is the time required for an egg to transform into an adult worker bee, using a plastic divider dish, to a square inch converted to (Cm^2) by multiplying by 2.54 according to Ismail *et al*. (2006):

$$Mites\ in\ brood\ (\%) = \frac{brood\ cells\ infection}{total\ brood\ cells\ observed} \times 100 \qquad \text{(Delaplane } et\ al.\text{, 2013).}$$

The smoking by plant components, effect on brood development *A. mellifera* colonies. Brood development was identified by measuring it with the help of scale, 5 days before and after treatment both sides of the frame in the hive had brood in it measured with the help of scale.

$$\text{Brood development (\%)} = \left(\text{Final brood} - \frac{\text{Initial}}{\text{Initial}}\right) \times 100 \qquad \text{(Goswami } et\ al.\text{, 2014).}$$

Statistical analysis

Using the CRD design, the SAS statistical software was used. At a probability level of P≤0.05 the differences between the main values of the treatments were compared using Duncan's test. The data were analyzed before and after treatment using the Two Sample Paired t-test (T), (Daher-Hjaij and Alburaki, 2006).

Results and Discussion

Effect of smoking on the shedding of mites /hive

Table 3 indicates that there are statistically significant differences among treatments regarding mite shedding before and after the treatment at the level of P≤0.05. The results indicate that the highest mite

shedding after treatment reached 92.67 mite/hive during the first and two days in *E. globulus* treatment, with a significant superiority over all treatments at P≤0.05 At a rate of 47.11 mite/hive. Noting a decrease in the number of fallen Varroa mites over time showed because the first two days of application caused the largest number of fallen mites to fall. For this reason, the number of fallen Varroa mites decreased after 4 and 6 days, as the number of fallen Varroa mites was higher after two days than after four days, which in turn was higher than after six days. These results are consistent with the results reached by (Al-Sharabi *et al*., 2023).

Table 3. The effect of smoking on the shedding mite in *A. mellifera*

Treatment	Average number of dead and shedding (mite / hive)				Mean
	Before treatment	After treatment			
		2 days later	4 days later	6 days later	
E. globulus	51.33 g	92.67 a	40.33 i	8.33 o	47.11 A
S. rosmarinus	49.33 h	84.67 b	33.67 j	9.0 on	42.44 B
A. vulgaris	53.33 f	80.00 d	29.33 k	10.0 n	39.77 C
C. procera	55.00 e	81.67 c	28.33 k	9.67 n	39.89 C
Control	51.67 g	16.00 l	14.00 m	15.0 ml	15.0 D
Mean	51.93 B	71.0 A	29.13 C	10.4 D	

Similar letters on the numbers mean that there are no significant differences at p≤0.05.

Efficiency depends on the concentration used, the method of application, and the plant used in terms of the quality and quantity of aromatic compounds that result from the combustion of these substances that have mite-killing properties. The fumes disturb the mites, causing them to lose their ability to cling to the bee's body and cause them to fall to the bee bed (Koumad and Berkani, 2019). Results are consistent with those of this researcher, as the mortality rate reached 80% for rosemary leaves when smoked. It is believed that the fumes may affect the sensory organs or means that mites use to attach to bees, reducing their strength and cling and contributing to their shedding.

The effect of smoking on the death of *V. destructor* /treatment

Table 4, Data indicate that *E. globulus* was superior to all treatments with a killing rate of 65.07%, with the highest killing rate reaching 85.28% after 2 days of smoking, while the data from this table indicate a decrease in the killing rate over time, as it reached 70.24, 58.82, and 31.65% after 2,4 and 6 days, respectively.

This is evidenced by the fact that the number of dead mites decreased more after 6 days than after 4 days, and latter decreased more than after 2 days. This is due to the continued application of the treatments over three separate time periods. Studies indicate that smoke resulting from burning some medicinal and aromatic plants leads to directly toxic and irritating effect, as the smoke contains volatile molecules of essential oils, such as cineole compounds found in *E. globulus*, and other phenolic compounds affects the nervous system, act as a neuro-inhibitor and knockdown (Sendi and Ebadollahi, 2014). These compounds penetrate into the hive and affect the respiratory system of the Varroa mite, acting as contact or inhalation toxins, leading to the weakening and death of the mite (Koumad and Berkani, 2019).

Table 4. The effectiveness of smoking with medicinal plants on the mortality of the *V. destructor* that infects bee colonies *A. mellifera*

Treatment	Mite mortality (%)			Mean
	2 days later	4 days later	6 days later	
E. globulus	85.28 a	74.23 e	35.70 l	65.07 A
S. rosmarinus	84.11 b	70.63 g	37.50 k	64.08 B
A. vulgaris	83.33 d	67.69 h	40.0 f	63.67 C
C. procera	83.62 c	66.93 i	39.20 j	63.25 D
Control	0.0 m	0.0 m	0.0 m	0.0 E
Mean	70.24 A	58.82 B	31.65 C	

The number of Varroa mites inside the closed brood

From Table 5, it is clear that the highest decrease in bee brood infection was in the *E. globulus* treatment, where it reached 8 after 22 days of treatment with smoking, while it was 59.66 before smoking. Note that there were no significant differences between all treatments one day before smoking, in which the infection rate was high. On the contrary, significant differences appeared between the treatments at P≤0.05 after 22 days of smoking.

Table 5. Percentage of the spread of mite within colonies in closed broods before and after treatment

Treatment	*E. globulus*	*S. rosmarinus*	*A. vulgaris*	*C. procera*	Control	L.S.D 0.05
1 day before treatment	59.66	56.33	49.66	48.0	56.33	N.S
22 day after treatment	8.0 a	9.66 b	13.0 c	11.33 c	58.0	2.092

The results of this study agreed with findings of Al-Hayali *et al*. (2025b), who used the smoking method of *E. globulus* essential oil, which resulted in a 57.3% mortality of mites in a honey bee colony.

Brood development in *A. mellifera*

Table 6 indicated significant differences among the treatments due to the effect of smoking on the percentage of brood development. The highest increase in brood area was in the *E. globulus* treatment, which significantly outperformed all treatments including the control at p≤0.05, reaching 23.33, compared to 17.30, 15.11, and 13.61 for *S. rosmarinus*, *A. vulgaris*, *C. procera* and untreated 4.94 respectively. During 21 days, no queen and worker bee loss were observed. Results are consistent with Goswami *et al*., (2014) when fumigating with the following essential oils: Cumin oil, basil oil, turmeric oil, cinnamon oil, clove oil, and formic acid. The mortality rate for moths reached 66.54% and 77.54%, with an observed increase in brood growth ranging from 3.12% to 21.74% after 21 days of exposure.

Table 6. Effect of smoking with medicinal plants used against Varroa infestation on brood development in the colonies

Average brood growth (area in square centimeters)				
Treatments	**Before treatment**	**21 days after treatment**	**Percentage In brood development**	**Mortality of honey bees**
E. globulus	870 g	1073 c	23.33 k	Nil
S. rosmarinus	942 f	1105 a	17.30 lk	Nil
A. vulgaris	787 j	1021 d	15.11 l	Nil
C. procera	955 e	1085 b	13.61 l	Nil
Control	810 i	850 h	4.94 m	Nil

The results indicated the effectiveness of the method of smoking medicinal plants used in this study in combating Varroa.

Conclusions

We can infer from the results the effectiveness of the method of smoking plants, that used in this study, in reducing or limiting mite density in honey bee colonies, as the highest drop rate in the case of smoking with *E. globulus* powder. It also contributed to reducing the infection rate of worker brood after 22 days compared to the control treatment. The brood development Percentage also increased after 21 days of smoking. No damage was recorded to the honey bee colonies during and after application, especially the queens. Therefore, beekeepers can be recommended to use this method to combat Varroa mites in honey bee colonies, as it is safer from an environmental standpoint and does not leave toxic residues on honey bee products, thus protecting human health as one of the alternatives to using chemical pesticides. We also recommend that researchers conduct research by choosing other plants that nature abounds with to determine their effectiveness against Varroa mites.

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Conflict of interests

The authors have declared that there are no conflicts of interest regarding the publication of this manuscript.

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Author Contribution

The first and second authors conducted the experiments and wrote the original draft, and the first author also performed the statistical analysis of the results. The third author edited and finalized the manuscript. All authors read the manuscript and approved its submission to the journal.

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