

Evaluation the effect of some different alcoholic extracts concentrations in eliminating the *Varroa* mite parasitizing in honeybee hives at Al-Diwaniyah governorate – Iraq

Reyam J. Khadim¹, Hadi M. Al-Mayali², Abbas K. Hamza²

¹Department of Anatomy, College of Medicine, University of Al-Qadisiyah, Iraq .

²Department of Biology, College of Education, University of Al-Qadisiyah, Iraq.

*Corresponding author email. : hadi.hamza@qu.edu.iq

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Abstract— This study was conducted in Twelve apiaries in Al-Diwaniyah Governorate from May 2024 - August 2025 to demonstrated the efficiency of natural extracts as safe alternatives to chemical pesticides in eliminating the *Varroa* mite parasitizing in honeybee hives . *Eucalyptus* oil was extracted using a Soxhlet device, and garlic extract was obtained by enzymatic hydrolysis and maceration. The compound 1,8-cineole was purified and analyzed using HPLC. The field study included twelve apiaries in Al-Diwaniyah Governorate, divided into four groups: three treatment groups (garlic 10%, eucalyptus 10%, cineole 2%) and one control group. The hives were sprayed three times over 10 days, and the therapeutic efficacy of the extracts in controlling *Varroa* mites was measured by counting the number of falling mites periodically. Garlic extract (10%) was the most effective treatment with high therapeutic efficiency, the percentage ranged between 97.0% - 97.2% compared to the control group . *Eucalyptus* extract at a concentration of 10% showed good efficacy, reaching 90.9%, but it remained second only to garlic. Cineole -1,8 at a concentration of 2% came in third place with a lower therapeutic efficiency of 84.7%, which may be attributed to its low concentration used in the study. Conclusions: This study showed that garlic extract (10%) was the most effective treatment and showed that these extracts do not cause any side effects to honeybees, and no abnormal mortality rates were observed in the treated hives.

Keywords — Garlic, Eucalyptus, Cineole, *Varroa* mite, Honeybee, Al-Diwaniyah.

INTRODUCTION

Honeybee (*Apis mellifera* L.) farming is a cornerstone of global ecosystems and agriculture, given its vital role in crop pollination and ensuring food security. However, this industry faces significant threats from insect, mite, and parasitic pests, most notably the *Varroa destructor* mite, which causes the

collapse of bee colonies and substantial economic losses globally, resulting in the loss of millions of colonies worldwide (1). While synthetic acaricides are widely used to control *Varroa* infestations, their overuse has led to the emergence of resistant strains and adverse effects on the host, creating an urgent need for a new acaricide that is effective, cost-effective, and safe for use on bees (2).

Varroosis in honey bees is currently the most serious problem in beekeeping. This parasitic species is called *Varroa* spp. The Varroidae family comprises several distinct species. Varroosis, the medical term for the disease caused by these parasites feeding on bee larvae in the hive, is a significant concern. These mites have a devastating impact on all stages of honeybee life (3), infecting brood and adult bees. They primarily feed on the host's fat, causing deformities and shortening the lifespan of adult bees. The mites are also vectors for viral bee diseases such as bag brood and acute paralysis (4).

Over the past decades, strategies for controlling these mites have relied mainly on synthetic acaricides such as fluvalinate and amitraz. While initially effective, the intensive use of these substances has led to the emergence of resistant mite strains, as well as the risks associated with the accumulation of toxic chemical residues in bee products like honey and wax, posing a threat to consumer health. The urgent need to develop highly effective and environmentally safe natural alternatives has become apparent (2).

Essential oils extracted from medicinal plants are among the most promising alternatives, particularly garlic, eucalyptus, and 1,8-cineole the main component of *eucalyptus* spp. oil. Studies have shown that cineole possesses acaricidal properties and is effective against many other insect pests through multiple mechanisms of action. It inhibits acetylcholinesterase in the pest's nervous system (5,6). Field research indicates that cineole-rich extracts, when applied at controlled concentrations, can achieve eradication rates exceeding 96% against *Varroa* mites while preserving the health of bees and hives (7,8).

In addition to its effectiveness Against *Varroa*, cineole has shown remarkable efficiency in controlling other agricultural pests such as red spider mites, making it an ideal candidate to be a cornerstone of Integrated Pest Management (IPM) programs as a bio-based alternative to traditional chemical pesticides. However, further research is still needed regarding the stability of this substance and the impact of climatic conditions and ambient temperatures on its effectiveness during field application. This study aimed to evaluate the efficiency of the aforementioned natural extracts as safe bio-alternatives in combating the *Varroa* mite, which infests honeybee hives, while comparing the effectiveness of these components at different concentrations and determining their ability to reduce infestation rates in field apiaries.

MATERIALS AND MTHODS

Extraction of Essential Oil from *Eucalyptus* Leaves

The essential oil was extracted from eucalyptus (*Eucalyptus Globulus*) leaves using a Soxhlet apparatus (BIOBASE, China) according to standard procedures ISO6571:2008 (9) with minor modifications. An organic solvent (Acetonitrile grade (HPLC) and Cynor Laboratories) was used at a ratio of 1:6 w/v in a round-bottom flask, and the extraction process continued for 6 hours until the active ingredient was completely depleted. The resulting extract was treated using a rotary evaporator under reduced pressure at 40°C to completely remove the solvent. The oil yield was calculated based on the dry weight of the plant material using the following equation:

$$\text{Yield (\%)} = \frac{\text{Mass of extracted oil (g)}}{\text{Mass of dry plant material (g)}} \times 100$$

The oil was then stored in opaque glass bottles at 4°C, protected from light.

Extraction of Active Compounds from Garlic

To prepare the garlic oil (*Allium sativum*) according to (10), AOAC Method 920.39 (2019). The filtrate was concentrated using freeze-drying or vacuum evaporation to obtain a highly concentrated crude extract, which was then stored under freezing conditions (-20°C) to ensure the stability of allicin and other sulfur compounds.

Purification and Analysis of 1,8-Cineole by HPLC

The purification and quantitative analysis of 1,8-Cineole were performed using high-performance liquid chromatography (HPLC) according to protocol (10). Chromatography (HPLC) Rotary evaporator Waters Corporation USA) to ensure the accuracy of the laboratory results.) The sample was prepared by dissolving the extract in an HPLC-grade solvent, followed by aching and filtration using 0.45 µm micro-injection filters. The protocol employed a mobile phase and a constant flow rate through the column under controlled thermal conditions. Cineole was identified and its purity determined by matching the retention time to standard reference values. The concentration was calculated based on the chromatographic peak area using the instrument's analytical software, allowing for highly accurate final graphs.

Spray Method

Twelve (12) randomly selected apiaries from several apiaries in Al-Diwaniyah Governorate were chosen from the Agricultural Preparatory Station, Al-Furat District, Al-Siniyah District, Nafar District (three apiaries), Al-Daghara District, Al-Budair District, Sumer District (two apiaries), and Al-Hamza Al-Sharqi. The selection period was from March 25, 2024 - August 25, 2025. The apiaries were confirmed to be infested with *Varroa* mites through direct visual inspection. Infestation and uniformity of *Varroa* mite severity were confirmed in all selected apiaries. A 1:1 sugar solution was provided to all colonies throughout the experiment, and temperature and relative humidity were measured.

The spraying process was carried out according to the method of (11) with some minor modifications, such as the type of extract, concentration, and treatment duration, as follows: The apiaries were divided into four experimental groups: three treated groups (each group containing three apiaries for each extract) and a fourth control group. Each frame of the apiary was sprayed. The bee-covered hives were sprayed with approximately 20 ml of a 10% garlic extract, prepared by distillation, after being manually lifted. The total volume of each hive was approximately 60 ml throughout the experiment. The inner frames of the hive were sprayed using a 500 ml handheld plastic sprayer, ensuring a uniform mist throughout the hive. Each hive was sprayed three times (3 replicates) over a 10-day period (days 1, 5, and 10). After each treatment, *Varroa* mites were observed falling onto petroleum jelly-coated plates placed at the bottom of the hives. These plates were replaced and checked periodically between sprays to count the number of fallen (dead) mites and calculate the average and efficiency rate of the extract. The same spraying process was repeated for the remaining extracts (eucalyptus 10% and cineole 2%) in the remaining hives at the same time and location. The control hives were sprayed with the same volume of distilled water for the same duration (11).

Statistical Analysis:

The replicates were used. Replications were used to reduce random error, and ANOVA was performed to determine the significant differences between the treated colonies and the control group. The Least Significant Difference (LSD) test was also used, in addition to calculating the arithmetic mean and efficiency ratio to measure the effectiveness of the extracts compared to the control group.

1-Arithmetic Mean Equation and Efficiency Percentage (Abbotts formula): This is used to calculate the mite fallout rate in the different replicates (treatments) for each colony type according (11)

$$\bar{x} = \frac{\sum x}{n}$$

$$\sum x = \text{total of replications}$$

$$n = \text{numbers of replications}$$

2- Efficiency Percentage) Abbotts formula) .

$$\text{Efficiency \%} = \left(\frac{mt - mc}{mt} \right) \times 100$$

mt = Mortality rate in the treatment group.

mc = Natural mortality rate in the control group

to convert the ratio to a)A constant mathematical coefficient :100
(.percentage

RESULT

Effect of the studied plant extracts on the infestation and mite drop rate in honeybee hives

Effect of 10% garlic extract

The results of the current study on the effect of 10% garlic extract, as shown in Table (1), demonstrated the superiority of the extract over all other extract treatments in reducing the number of *Varroa* mites infesting bees and falling on the filter paper placed at the bottom of the hive after treatment. This was observed in all three treatments, with a 10-day interval between treatments. The number of mites decreased significantly in all three colonies where the garlic extract was used. The results showed that the garlic extract achieved the highest *Varroa* mite fall rates and the highest therapeutic efficiency. The total number of mites falling in the four colony (A, B, D, and C) ranged between 132 and 139, with hive (B) recording the highest average fall rate of 46.3 ± 2.52 . As for the therapeutic efficiency, the percentage ranged between 97.0% and 97.2% compared to the control group, which are very similar percentages in all colonies and confirm the high effectiveness of garlic in reducing *Varroa* infection.

Table 1. Effect of 10% garlic oil extract on the rate of *Varroa* mite drop and therapeutic efficiency.

Colony No.	Mite drop No.			Total mite drop	Mean \pm LSD	T.E. %
	T. 1	T. 2	T.3			
A	47	45	44	136	45.33 ± 1.53	97.1
B	49	44	46	139	46.33 ± 2.52	97.2
C	50	47	42	139	46.3 ± 4.04	97.2
D	45	45	42	132	44.01 ± 1.73	97.0

T.1,2,3= Treatments T.E.= Therapeutic efficiency

Effect of 10% *Eucalyptus* Extract

The effect of 10% *eucalyptus* extract showed high efficiency in all treatments and all colonies, but less than that of garlic extract, with the total number of mites falling mite ranging from 27 to 43. Colony (B) recorded the highest average falling mite at approximately 13.32 ± 14.3 , while the highest therapeutic efficiency rate was 90.9% in colony (B), and the lowest was 85.6% in colony (D), compared to the control group. The average total number of mites falling at the bottom of the colony was found to increase, reaching 33, 43, 31, and 27 respectively after the third treatment in all colonies, as shown in Table (2).

Table 2. Effect of 10% *eucalyptus* extract on the rate of *Varroa* mite drop and the percentage of therapeutic efficiency

Colony	Mite drop No.	Total	Mean	T.E.
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No.	T. 1	T. 2	T. 3	mite drop	\pm LSD	%
A	22	9	2	33	10.15 ± 11.00	88.2
B	29	11	3	43	13.32 ± 14.33	90.9
C	21	8	2	31	9.71 ± 10.33	87.4
D	19	7	1	27	9.0 ± 9.17	85.6

T.1,2,3= Treatments T.E. = Therapeutic efficiency %

The effectiveness of *eucalyptus* extract in the current study came in second place with a mean therapeutic efficiency of (88.0%). This is because the presence of the compound 1,8-cineole as a main component, in addition to the presence of the various components of *eucalyptus* oils, and not just the cineole alone. This was confirmed by conducting an HPLC examination of *eucalyptus* leaves to identify the most important chemical compounds. This experiment in the current study revealed that the cineole compound reached 63.1%, as shown in Figure (1), which enabled these leaves to eliminate the *Varroa* mite. The results suggest that 1,8-cineole may contribute significantly to the observed acaricidal activity against *Varroa* mite . However, the overall effectiveness of the *eucalyptus* extract could also be attributed to the synergistic effects between 1,8-cineole and other minor volatile constituents present in the essential oil.

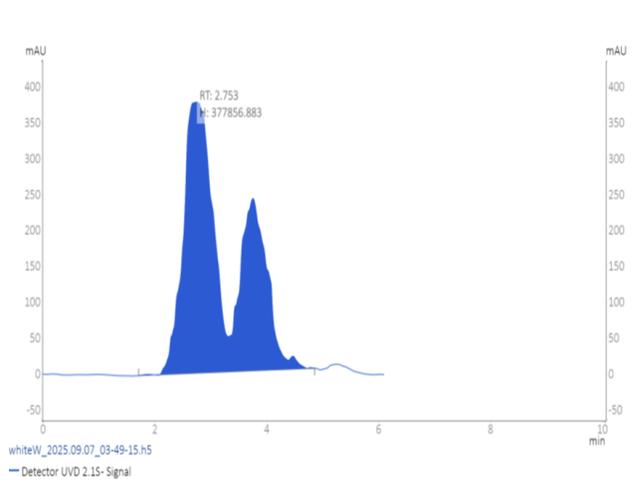


Figure 1. Chemoanalysis of eucalyptus leaves by HPLC explained the presence of cineole as a major component.

Effect of 2% 1,8- Cineole

A 2% concentration of 1,8-Cineole ranked third in terms of effect, as shown in Table (3). The total number of falling mite mites ranged between 9 and 17, and (B and D) colonies recorded the highest average falling mite at 84.7. However, the percentage of therapeutic efficiency varied significantly, with colonies (B and D) recording a rate of 84.7%, while it decreased in colony (A) to 71.1%. (Table 3).

Table 3. Effect of 2% cineole on *Varroa* mite drop rate and therapeutic efficiency.

Colony No.	Mite drop No.			Total Mite drop	Mean \pm LSD	T.E. %
	T. 1	T. 2	T. 3			
A	7	2	0	9	3.61 \pm 3.00	71.1
B	12	5	0	17	6.03 \pm 5.67	84.7
C	9	3	0	12	4.00 \pm 4.58	78.3
D	13	4	0	17	6.66 \pm 5.67	84.7

T.1,2,3= Treatments T.R.= Therapeutic efficiency %

Table 4. ANOVA results for the effect of garlic, eucalyptus and cineole extracts on mite drop and therapeutic efficiency.

Extract	Con. %	Average of Mite drop \pm SD	Mean T.R. (%)	Significant difference (LSD)
Garlic	10	45.55 \pm 1.05 a	97.1 a	A
Eucalyptus	10%	11.17 \pm 2.26 b	88.0 b	B
Cineole	2%	4.58 \pm 1.30 c d	79.7 c	cd
Control	--	1.11 \pm 0.38 d	0.00 d	d
LSD (P<0.05)	--	6.42*	--	--

T.R.= Therapeutic efficiency %

* significant differences at (P<0.05) df= 3 df error = 12

LSD(P<0.05) \approx 6.20 T.R.= Therapeutic efficiency %

Control Group

The results in the control group and the treatment with distilled water, as shown in Table (5), showed a very natural decrease in the falling of the mite, as the total number of falling mite mites did not exceed 2-4 mites only in all colonies, and the percentage of therapeutic efficiency reached 0.00% for all colonies, which confirms that the mite drop was natural and not the result of any therapeutic efficiency, unlike the rest of the treatments with plant extracts.

Table 5. The rate of Varroa Mite drop in the control group (Distilled water).

colony No.	Mite drop No.			Total Mite drop	Mean \pm LSD	T.R. %
	T. 1	T. 2	T. 3			
A	1	1	0	2	0.67 \pm 0.58	71.1
B	1	1	2	4	1.30 \pm 0.53	84.7
C	1	1	2	4	4.00 \pm 4.58	78.3
D	1	1	2	4	0.58 \pm 1.33	0.0
Total					0.67 \pm 0.58	0.0

T.1,2,3= Treatments T.R.= Therapeutic efficiency %

As for the results of the statistical comparison, according to Table (4), which shows the comprehensive comparison between the different extracts and the control group, clear and significant differences appeared at the probability level ($P \leq 0.05$) and an LSD value of 6.20. Garlic extract significantly outperformed the other treatments, recording the highest average mite fallout (45.5%) and the highest therapeutic efficiency (97.1%). *Eucalyptus* extract came in second, significantly ahead of garlic and cineole, with a therapeutic efficiency of (88.0%). Cineole recorded the lowest therapeutic efficiency among the substances used (78.97%), but it remained significantly superior to the control group. The results demonstrated clear statistically significant differences between all groups, indicating varying therapeutic efficiency of these substances in controlling *Varroa* mites.

Through statistical significance and variance between replicates, the statistical analysis and LSD value (6.20) showed that the differences between treatments were highly significant, supporting the hypothesis that each extract has a different mechanism of action. The consistency of the garlic oil results (between 97.0% and 97.2%) indicates high reliability in field application, while the noticeable variability in the cineole results (between 71.1% and 84.7%) may be due to the sensitivity of this substance to intracolonyular distribution or the varying strength of the initial infestation in those replicates. These results indicated that the garlic extract had a consistent and continuous effect on the deciduous mite during the three treatments, reaching 1.05 ± 45.50 . This indicates that the highest reduction in *Varroa* mites was achieved using the garlic extract, with an efficiency of 97.1%. The same trend was observed for both eucalyptus and cineole extracts, but with a lower effect, with an efficiency of 0.88% for eucalyptus and 7.79% for cineole, as shown in the table (4).

Discussion

Herbs and essential oils play a significant role in combating bee diseases and pests, offering an alternative to synthetic chemical pesticides. These essential oils do not cause any harmful effects on bee colonies or negatively impact their growth. Furthermore, the use of herbs and essential oils supports organic production and prevents contamination of bee products such as honey, beeswax, and others with waste. At the same time, they are highly toxic to *Varroa* mites, leave no residue on hive products, and do not induce resistance in bees. These essential oils are considered safer for bees (12).

Effect of Garlic Extract

The results were consistent with the findings of the study by (12) to evaluate the effectiveness and persistence of the effect of some essential oils such as garlic, turmeric, ajoene, cinnamon, lavender, and formic acid against *Varroa* mites in honeybee colonies in India. The results revealed that the highest mortality rate was 77.54% with the highest rate of brood development when using garlic oil. Colony's treated with the other oils also showed good persistence of the effect; *Varroa* mortality rates ranged between 66.54% and 77.54%, and an increase in brood development after three weeks of exposure to the treatment. This also aligns with the findings of the study by (13), which evaluated the effectiveness of six

volatile essential oils compared to formic acid and apistan strips against Varroa mites. The results showed that garlic oil was superior in reducing Varroa mite populations after treatment, with a significant improvement in the bees' defensive behavior. This also agrees with the study of (14) who found that using garlic extract in treatment led to a clear 90% reduction in Varroa mite populations. The high efficacy of garlic extract is primarily attributed to the mechanical crushing of bulbs, which triggers the Alliinase enzyme to convert alliin into Allicin. Allicin is a potent organosulfur compound that exerts a strong neurotoxic and respiratory effect on Varroa mites upon contact or inhalation. Furthermore, the 24-hour maceration process and storage at -20°C ensured the stability and high concentration of these active sulfur components (12).

Effect of Eucalyptus Extract

The results of the current study are consistent with the study by (5), which aimed to determine the chemical composition, toxicity, and field efficiency of the essential oil. Distilled from eucalyptus leaves, the study focused on the essential oil of the species *Eucalyptus globulus*, prevalent in Algeria, as an acaricide for Varroa mite control. Its toxicity was measured on bee colonies infested with the mites. Field tests confirmed the toxicity and efficiency of cineole oil, extracted from the leaves by steam distillation, as a Varroa mite control agent. The study concluded that combining eucalyptus oil (1,8-cineole) and thymol achieves greater efficiency than using either alone, making it a promising alternative to traditional commercial treatments due to its effectiveness and safety.

This aligns with the findings of the study by (16), which aimed to determine and guide the efficiency of thyme, eucalyptus, and oxalic acid oils in controlling Varroa mites on honeybees, evaluating the therapeutic potential of these natural substances as alternatives to traditional chemical pesticides. The results showed that all tested substances had a lethal effect on the Varroa mite, except for oxalic acid. Oxalic acid oil recorded the highest efficacy, followed by thyme oil, while eucalyptus oil was the least effective, ranking third. (17) also indicated that eucalyptus oil had the lowest Varroa mortality rate at 65%, after peppermint, and a bee mortality rate of approximately 10%, making it less attractive for specialized control compared to peppermint.

Effect of 1,8-Cineole Compound

The current study, through compound analysis using HPLC, revealed that 1,8-Cineole in eucalyptus leaves is the dominant compound, comprising 63.1%, as shown in Figure (1). This enabled the leaves to eliminate the Varroa mite, consistent with the findings of (5). The moderate efficacy of the 2% cineole treatment compared to the whole eucalyptus oil and garlic extract may be attributed to the synergistic interaction between 1,8-cineole and other minor constituents present in the crude oil, which are absent in the purified form. Additionally, the superior performance of garlic extract (10%) suggests that organosulfur compounds, such as allicin, possess higher acute toxicity against Varroa mites than terpenes at the tested concentrations.

These conclusions also align with those of (18), who stated that the efficiency of essential oils depends primarily on hive

temperature and temperature. The active ingredient evaporates, as cineole requires higher concentrations or slow-release techniques to achieve results comparable to sulfur compounds. The results of the current study are consistent with those of (1) and (8), who found that this effect is related to the chemical composition of these plants. This aligns with the findings of (5), who found that the aromatic eucalyptus extract has moderate toxicity, meaning it has a moderate effect in killing the parasite. The aim of selecting these essential oils was to demonstrate the potential of using eucalyptus as a natural means of controlling the Varroa mite without harming the environment or bees.

Alsadi *et al.* (19) demonstrated the lethal effect of nine essential oils on the Varroa mite. Using oils extracted from plant materials at varying concentrations, the results showed the oils' effectiveness against the mites while preserving bee health. This suggests the possibility of using these oils as natural control agents and reducing reliance on synthetic chemicals. Similarly, Alaux *et al.* (20) indicated that Varroa mite infestation in bee colonies makes bees more susceptible to other diseases, such as viruses and bacteria. They found that the use of chemicals by beekeepers to manage Varroa mites is the most common control method. However, this method does not lead to complete control. Garlic extract may be an effective alternative to formic acid, a chemical treatment widely used in honeybee colonies against Varroa mites. This suggests significant differences in treatment for each extract at a probability level of ($P \leq 0.05$). Overall, this study investigated the use of these extracts against mites. Varroa mites within honeybee colonies are highly effective, and the use of these essential oils for Varroa treatment is constantly evolving. This aligns with the findings of (21), who discovered that a mixture of two or more essential oils extracted from plant materials can reduce Varroa mite populations. This is also consistent with the findings of (22).

Based on these results, this study demonstrates that the use of natural extracts, particularly garlic oil at a concentration of 10%, represents a safe and effective alternative to manufactured chemical pesticides. The high drop rate in the treated groups compared to the control group, which recorded 0.00% effectiveness, confirms that these natural substances possess acaricidal properties capable of disrupting the parasite's life cycle and reducing its economic damage to honeybee colonies. In conclusion, Varroa mites should be managed continuously (monthly) within honeybee colonies using mechanical methods. However, the concentration of these extracts and the appropriate timing of treatment are two important factors that must be considered in the apiary.

Conclusions

The results of this study showed that the tested plant extracts possess effective Varroa mite control properties under field conditions. Garlic extract (10%) and eucalyptus oil achieved the highest therapeutic efficacy, with statistically significant differences ($P \leq 0.05$) compared to the cineole compound group (2%) and the control group, according to the least significant difference (LSD) test. These results indicate that the efficacy of these natural treatments is highly

dependent on the concentration used and is affected by environmental conditions, specifically the high temperatures characteristic of the study area in Diwaniyah, which may have contributed to increased volatilization of the active compounds. It is important to note that although high mite mortality rates were recorded, this study did not directly measure the cleaning or hygiene behavior of honeybees; therefore, the results obtained are primarily attributed to the direct chemototoxicity (via contact and inhalation) of the extracts. This study recommends further researches to evaluate these treatments across different seasons and at varying concentrations to improve their use within integrated pest management programs.

Authors' Contribution

Conceptualization: Hadi M. Al-Mayali, Reyam J. Khadim, Abbas K. Hamza.

Data curation: Hadi M. Al-Mayali, Reyam J. Khadim.

Formal analysis: Hadi M. Al-Mayali.

Investigation: Reyam J. Khadim, Abbas K. Hamza.

Methodology: Hadi M. Al-Mayali, Abbas K. Hamza.

Project administration: Hadi M. Al-Mayali, Abbas K. Hamza.

Resources: College of Education, University of Al-Qadisiyah.

Software: Hadi M. Al-Mayali, Reyam J. Khadim, Abbas K. Hamza.

Supervision: Hadi M. Al-Mayali, Abbas K. Hamza.

Validation: Hadi M. Al-Mayali, Reyam J. Khadim.

Visualization: Reyam J. Khadim.

Writing—original draft: Reyam J. Khadim.

Writing—review & editing: Hadi M. Al-Mayali.

Competing Interests

The authors declare that they have no competing interests that could have influenced the outcome of this study.

Consent

Consent was obtained from all beekeepers who participated in this study. Each participant was informed about the objectives and procedures of the investigation, and verbal consent was given before sample collection.

Ethical Approval

This study was approved by the Scientific Committee of the College of Education at the University of Al-Qadisiyah, Iraq. All sampling procedures were performed in accordance with ethical guidelines for research involving animals and the Declaration of Helsinki (1964) and its later amendments. Beekeepers were informed about the study's purpose and assured that all data and locations would remain confidential.

References

- 1) Calderone, N. & Spivak, M. (1995). Plant Extracts for Control of the Parasitic Mite *Varroa jacobsoni* (Acari: Varroidae) in Colonies of the Western Honey Bee (Hymenoptera: Apidae). *Journal of Economic Entomology*. 88. 1211-1215. 10.1093/jee/88.5.1211.
- 2) Begna, T., Ulziibayar, D., Bisrat, D., & Jung, C. (2023). Acaricidal Toxicity of Four Essential Oils, Their Predominant Constituents, Their Mixtures against *Varroa Mite*, and Their Selectivity to Honey

- Bees (*Apis cerana* and *A. mellifera*). *Insects*, 14(9), 735.
- 3) Abdullah Z. Alshehri, Najeeb M. Almasoudi; Ahmed A. Zaitoun and Abo-Elyousr, K.A.M. (2023). Acaricidal efficacy of four plant materials for controlling varroa mite in hives of honeybee. *Fresenius Environmental Bulletin* 32:07: 2729-2734.
- 4) Gutiérrez CLC, Tapia RMZ and Velázquez FD (2024). Comparison of the use of essential oils for the control of varroa (*Varroa destructor*) in *Apis mellifera*. In: Zafar MA, Abbas RZ, Imran M, Tahir S and Qamar W (eds), *Complementary and Alternative Medicine: Essential oils*. Unique Scientific Publishers, Faisalabad, Pakistan, pp: 123-128.
- 5) Atmani-Merabet, G., Belkhiri, A., Dems, M., Lalaouna, A. E. D., Khalifaoui, Z., Mosbah, B., & Mohamed Abdesslem, D. (2018). Chemical Composition, Toxicity, and Acaricidal Activity of *Eucalyptus Globulus* Essential Oil from Algeria. *Current Issues in Pharmacy and Medical Sciences*, 31.
- 6) Čmiková, N., Galovičová, L., Schwarzová, M., (2022). Chemical Composition and Biological Activities of *Eucalyptus globulus* Essential Oil. *Plants*, 12(5), 1076.
- 7) Choi W, Lee S, Park H, Ahn Y.(2004).Toxicity of plant essential oils to *Tetranychus urticae* (Acari: Tetranychidae) and *Phytoseiulus persimilis* (Acari: Phytoseiidae). *J Econ Entomol*. 97(2):553-8.
- 8) Clarkson, Cailean & Maharaj, Vinesh & Crouch, Neil & Grace, Olwen & Pillay, Pamisha & Matsabisa, Motlalepula & Bhagwandin, Nireesh & Smith, Peter & Folb, Peter. (2004). In vitro antiplasmodial activity of medicinal plants native to or naturalised in South Africa. *Journal of Ethnopharmacology*. 92. 177-91. 10.1016/j.jep.2004.02.011.
- 9) International Organization for Standardization. (2008). Spices, condiments and herbs — Determination of volatile oil content (hydrodistillation method) (ISO Standard No. 6571:2008).<https://www.iso.org/standard/41299.html>.
- 10) AOAC International. (2019). *Official Methods of Analysis of AOAC International* (21st ed.). Gaithersburg, MD: AOAC International.
- 11) Sakla, R. S., El-Gendy, R. M., Ali, J., & Abdel Hafez, M. M. (2025). Stinging nettle (*Urtica dioica*) as a potential control agent for *Varroa mite* (*Varroa destructor*) in honeybee colonies (*Apis mellifera*). *BMC Plant Biology*, 25, 578.
- 12) El-Nagar, A. & Mohamed, Z. & Alkherb, W. ; Alhashdi, D. & Mohsen, A. (2025). The effects of *Varroa destructor* infestation and botanical oils on physiological characteristics and mechanism of defensive behavior of Carniolan and Italian *Apis mellifera*. *Journal of Asia-Pacific Entomology*. 28. 102435. 10.1016/j.aspen.2025.102435.