The Use of essential oils to Control Varroa Mite, *Varroa destructor* in Honeybee Colonies

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استخدام الزيوت العطرية لمكافحة حلم الفاروا Varroa destructor في طوائف نحل العسل

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المستخلص:

هدفت هذه الدراسة إلي تقييم فعالية الزيوت الأساسية المستخلصة من ثلاثة نباتات: الخردل (Citrullus colocynthis)، الزعتر (Thymus vulgaris)، والثوم (Allium staivum) في مكافحة حلم الفاروا (Varroa destructor)، والذي يعد أحد الأسباب الرئيسية لفقدان طوائف النحل. تم تقسيم عشرين طائفة نحل إلى أربع مجموعات: مجموعة الشاهد و ثلاث مجموعات تجريبية. تم معاملة كل مجموعة ب 40 مل من الزيت النباتي بتركيز 15٪. تم تقدير مستويات الإصابة بالحلم باستخدام طريقة الغسل بالكحول، كما تم استخدام قاعدة الخلية ذات الشباك المعدني لمراقبة معدلات سقوط الحلم. أظهرت النتائج أن جميع الزيوت الأساسية المختبرة كانت فعالة في مكافحة الفاروا. بلغت فاعلية الزيت في خفض نسبة الإصابة بالفاروا على النحل البالغ 86٪ للخردل، 85٪ للزعتر، و 78٪ للثوم. أما بالنسبة لحضنة النحل، فقد كانت الفاعلية أعلى نسبياً، حيث بلغت 96٪ للخردل، 90٪ للزعتر، و 70٪ للثوم. تم رصد أعلى معدلات سقوط للحلم بعد المعاملة الأولى لجميع الزيوت المختبرة، مع ذروة فاعلية بعد 24 ساعة من المعاملة. سجل زيت الخردل أعلى معدل سقوط أسبوعي للفاروا بنسبة 52٪ ، يليه الزعتر 47٪، بينما سجل زيت الثوم أدنى معدل سقوط بنسبة 34٪. تشير هذه النتائج إلى أن الزيوت الأساسية يمكن أن تكون بديلاً فعالاً وأمناً في إدارة حلم الفاروا بالمناحل.

الكلمات المفتاحية: *الزيوت الأساسية، نحل العسل، حلم الفاروا، إدارة الأفات، ليبيا .*

Abstract:

This experiment aimed to evaluate acaricidal activity of essential oils extracted from bitter melon (*Citrullus colocynthis*), thyme (*Thymus vulgaris*) and garlic (*Allium sativum*) against varroa mites (*Varroa destructor*), a major cause of honeybee colony loss. Twenty honeybee colonies were divided into four groups, one control group and four treatment groups. Each treatment group received 40 ml of plant extracts at a 15% concentration. Mite infestation levels were assessed using the alcohol wash method, and hive bottom boards were utilized to monitor mite drop rates. All tested essential oils were effective against mites. The efficacy rates on the adult bees were 86% for bitter melon, 85% for thyme and 78% for garlic. On the brood, the rates were slightly higher, with 96% for bitter melon, 90% for thyme and 70% for garlic. The highest mite drop rates were observed after the first application of all tested oils, with peak toxicity occurring 24 hours post-treatment. Bitter melon treatment led to weekly varroa drop rates of 52%, followed closely by thyme at 47%. The lowest drop rate was recorded with garlic oil treatment at 34%. These findings indicate that essential oil could serve as practical alternative acaricides in the management of varroa mites in apiaries.

Keywords: *Essential oil / Apis mellifera / Varroa destructor / Pest management / Libya.*

Introduction:

The honeybee, *Apis mellifera* L., has been one of the most beneficial insects to humans, serving nutritional and medicinal purposes for centuries (Klein et al., 2007). Honeybees face numerous threats from invertebrate pests, including various insects and mites (Morse & Flottum, 1997). Over the past two decades, the ectoparasitic mite *Varroa destructor* has posed a significant threat to the beekeeping industry in Libya (Keshlaf, 2017). The presence of *V. destructor* was first reported in Libya in 1976 (Crane, 1979), after it was introduced through infested bee packages imported from Bulgaria to Jebel Akhdar. This introduction led to the rapid spread of the mite throughout the country (Keshlaf & Mirwan, 2018; Keshlaf et al., 2023).

Parasitic varroa mite causes severe complications in beekeeping worldwide. In cases of heavy infestation, bee pupae are unable to develop into adult bees (Shimanuki and Knox, 2000). The mite also reduces a colony's ability to pollinate plants and produce honey (De Jong et al., 1984). Additionally, varroa mites are vectors for several pathogens, including the deformed wing virus and the acute bee paralysis virus (Chen & Siede, 2007).

To manage mite populations, beekeepers have traditionally relied on synthetic insecticides. Fluvalinate and Amitraz are among the most widely used chemicals due to their effectiveness and ease of application. However, these synthetic compounds can negatively impact honeybee health (Abousharra et al., 2017) and contaminate hive products (Wallner, 1999). Furthermore, *V. destructor* has been rapidly developing resistance to these chemicals, an issue increasingly reported in recent years (Milani, 1995; Panini et al., 2019; Rinkevich, 2020; Elzen et al., 2000b; Spreafico et al., 2001). Keshlaf & Alfallah, 2019a) suggested that modified bottom boards might be effective in controlling *V. destructor* in honeybee colonies. Additionally, (Keshlaf & Alfallah, 2019b) demonstrated that Libyan honeybees exhibit higher resistance behavior against Varroa mites. Natural insecticides provide a desirable alternative to synthetic chemicals, offering lower toxicity to mammals and minimal environmental impact. Organic acids and essential oils are among the most recognized natural products with acaricidal activity (Eguaras et al., 1996; Flamini, 2003).

Different components of essential oils have been tested for their efficacy against *V. destructor* (Sammataro et al., 1998; Colin, 1990). In Libya, (Alsaadi et al., 2024) recently evaluated the toxicity of nine locally extracted essential oils against *V. destructor* under laboratory conditions. They found that oils of bitter melon, garlic, and thyme were the most toxic to mites while being safe for adult bees. Based on these findings, this study was conducted to evaluate an effective field management strategy for controlling mite populations using the most promising essential oils.

Materials and Methods:

The experiment was conducted during the summer of 2020 in a private apiary located in Al-khomas, Libya. Twenty honeybee colonies, naturally infested with mites and similar in strength, were selected for the study. Each colony was headed by a queen of the same age. The colonies were randomly assigned into four groups (n=5), with three groups receiving the essential oil treatments and one group serving as a control.

Leaves of thyme (*Thymus vulgaris*), fruits of bitter melon (*Citrullus colocynthis*), and bulbs of garlic (*Allium sativum*) were collected from their natural habitats in Libya. These plant materials were air-dried, ground using a ZM 100 ultra-high speed mill, and sieved according to respective Pharmacopoeia monographs. Essential oils were extracted at Elmergib University, Libya, using a Soxhlet extraction instrument with a solvent controller, following the steam distillation method described by (Benthin et al., 1999). The extracted oils were stored in sealed plastic tubes and kept refrigerated at 4°C.

To administer the oils, cotton balls were used as carriers for the botanical volatile oils. A sample of 40 ml of diluted volatile oil (15%) was applied to saturate the cotton balls, which were then placed in small petri dishes (9 cm) covered with a screen mesh. These dishes were positioned on top of the brood combs inside the hive. The treatment was applied weekly for four consecutive weeks, with the cotton balls being replaced every seven days (Grant & Jackson, 2004).

Mite Mortality and Bee Colony Infestation Rate:

The infestation rate of mites was assessed before and after treatment for each colony. Approximately 300 adult worker bees were sampled from each colony, placed in a vial containing about 100 ml of alcohol, and transported to the Honey Bee Laboratory (University of Tripoli). The vials were shaken for 30 minutes to dislodge the ectoparasites, which were then separated from the bees by sieving (Dietemann et al., 2012). The number of *V. destructor* mites was counted, and the infestation rate was calculated, dividing the number of mites on the number of honeybees per sample and multiplying by 100.

For sampling parasitic mites in brood cells, two frames with recently sealed brood were selected from each colony. One hundred sealed brood cells were randomly chosen, uncapped, and examined. Any female mites present were counted, and the total number of adult mites was recorded as a percentage of infestation in sealed brood.

The reduction percentages of infestation on adult bees and brood were calculated using the (Henderson & Tilton, 1955) formula:

$$\text{Reduction }\% = [1 - (\frac{Ta}{Tb} \times \frac{Cb}{Ca})] \times 100$$

Where:

Ta= Treatment after   Ca= Control after

Tb= Treatment before   Cb= Control before

Varroa Dropping Rate

Bee hives were equipped with screened bottom boards and a drawer (closed bottom) covered with paper smeared with a thin layer of Vaseline oil to capture fallen mites (Gregorc & Sampson, 2019). The number of fallen mites for each colony was recorded at three intervals (24 hours, 48 hours, and 7 days post-treatment) after each of the four treatment applications.

The obtained results were analyzed using mixed model analysis of variance (ANOVA) with SPSS® for Windows™ Version 14. Prior to analysis, each variable was visually tested for normality using a P-P plot, and Levene's test was employed to assess the equality of error variance (Levene, 1960). If significant differences between treatments were detected, their means were separated using Duncan's multiple range test. A significance level of 0.05 was used for all statistical analyses.

Results and Discussion:

The evaluation results demonstrated the effectiveness of the three oils in field conditions compared to the control over the study period. Results presented in Table 1 indicate a reduction in *V. destructor* mite infestations in both worker bees and brood, as influenced by the tested oils in honeybee colonies, compared to the control treatment. After treatment with oils of *Momordica charantia*, *Thymus vulgaris*, and *Allium sativum*, the varroa infestation rates were 1.0, 1.0, and 1.2 mites per 100 bees, respectively. These rates showed a significant reduction ($P<0.01$) compared to the pre-treatment measurements. In contrast, the number of mites in the control colonies (3.6 mites per 100 bees) did not differ significantly from the pre-treatment measurement. Regarding sealed brood, the varroa infestation rate after treatment with the oils of *M. charantia*, *T. vulgaris*, and *A. sativum* was 0.4, 0.6, and 1.0 mites per 100 brood cells, respectively, which also showed a significant reduction ($P<0.001$) compared to pre-treatment measurements. Meanwhile, the number of mites in control colonies (4.4 mites per 100 brood cells) did not show significant differences from pre-treatment values.

The highest reduction in infested worker bees, compared to the control treatment, was observed with *M. charantia* and *T. vulgaris*, showing reductions of 86.8% and 85.9%, respectively. *A. sativum* showed a lower reduction rate of 78.3%. Similarly, the highest reduction in infested sealed brood was recorded with *M. charantia* and *T. vulgaris* at 96.5% and 90.3%, respectively, while the least reduction was observed with *A. sativum* at 70.8%. Table 2 highlights the effect of weekly repeated applications of essential oils on the number of dropped *V. destructor* mites in honeybee colonies, compared to the natural mite fall rate (pre-treatment). The greatest increase in dropped mites was noted after the first and second applications of all tested oils. After the third application, the number of dropped mites from treated colonies slightly decreased, reaching the natural falling rate after the fourth application. Statistical analysis indicated that the efficacy of the essential oils decreased with repeated applications, though this reduction was only significant ($P<0.01$) after the fourth application. Logically, the number of dropped mites after the fourth application was similar to the normal mite mortality rate observed before treatment, as well as in control colonies.

The mean number of dropped mites after four successive applications was 52.1, 47.6, 34.6, and 20.5 mites per week in colonies treated with oils of *M. charantia*, *T. vulgaris*, *A. sativum*, and the control colonies, respectively (Table 2). Statistical analysis revealed a significant difference ($P<0.01$) in mite fall rates, with *M. charantia* and *T. vulgaris* showing superior efficacy, followed by *A. sativum*, with the lowest mite fall rate observed in the control colonies (natural fall).

The mean number of daily dropped mites over the four successive applications for three periods (1st day, 2nd day, 3rd-7th day) was 33.3, 11.2, and 7.6 mites for *M. charantia*; 29.4, 10.8, and 7.4 mites for *T. vulgaris*; 20.0, 9.2, and 5.4 mites for *A. sativum*; and 7.2, 6.7, and 6.6 mites for control colonies (Table 3). Statistical analysis showed a significant difference ($P<0.03$) in mite fall rates, with the highest number of mites falling on the first day of application, followed by the second day. The lowest mite fall rates were observed during the third period, which was similar to the natural fall in control colonies.

Given the documented resistance of Varroa mites to chemical acaricides such as fluvalinate, apistane, coumaphos, and amitraz (Milani, 1995; Panini et al., 2019; Rinkevich, 2020; Elzen et al., 2000b; Spreafico et al., 2001), the use of plant extracts as alternative acaricides has been explored. Some of these extracts have shown significant efficacy (Alssadi et al., 2024; Elzen et al., 2000a; Elroby & Darwish, 2018; Bakar et al., 2017; Mahmood et al., 2014; Calderon & Spivak, 1995).

In this study, treatment with 40 ml of essential oils at a 15% concentration led to a rapid catabolic effect and subsequent death of Varroa mites. (Enan, 2001) reported that the octopamine nervous system is considered the target site of action for essential oils in arthropods, though this remains to be confirmed for Varroa mites. Of the three essential oils tested, *Momordica charantia*, which had not previously been reported as effective against Varroa mites, exhibited the highest acaricidal activity. While several studies have confirmed the insecticidal activity of *M. charantia* L. leaf extract on pests such as the Mung Bean weevil (*Callosobruchus chinensis* L.) (Wahyutami & Aisyah, 2022), the maize weevil (*Sitophilus zeamais*) (Adesina, 2013), and the larvae of the armyworm (*Spodoptera litura* Fab.) (Wardhani et al., 2015), this is the first study to report its efficacy against mites.

The second most effective oil was *T. vulgaris*, which has previously been reported to be effective against Varroa (Baggio et al., 2004). A recent study identified carvacrol and thymol as the main chemical components of the essential oils of *Thymus* spp. (Rahimi et al., 2017), and several reports have highlighted the activity of these compounds against *V. destructor* without significant risk to honeybees (Calderone & Spivak, 1995; Imdorf et al., 1995a, 1995b). Additionally, the essential oil of *Acantholippia seriphioides*, which mainly consists of thymol and carvacrol (Ruffinengo et al., 2005), and *Zataria multifera*, containing thymol, and *Saturea hortensis* L., containing carvacrol, have shown high mite mortality without significant harm to adult bees.

Although garlic oil (*A. sativum*) exhibited the least acaricidal activity (78%), it was still deemed effective. These findings align with those of (Mazeed & El-Solimany, 2020), who reported that two applications of garlic oil could eliminate approximately 70% of total mites. (Elroby & Darwish, 2018) found that garlic fumigation resulted in high efficacy against Varroa mites, with an 89.8% reduction in adult infestation and 86.1% in brood infestation, while spraying garlic extract also showed high efficacy with an 88.82% reduction in adult infestation. (Taha et al., 2020) reported that garlic oil was more effective in eliminating Varroa mites in both brood and worker bees than formic acid, clove oil, and apistan strips.

Regular monitoring of Varroa populations is crucial for integrated Varroa control. Many studies have relied on the level of mite infestation on adult bees or brood as a measure of the oil's effectiveness in killing Varroa mites. Despite its importance, this measure does not provide other necessary information, such as the survival period (effective time), which is critical for determining the ideal dose and the frequency of treatment applications. This underscores the importance of testing for mite fall rates at the bottom of the hive.

Regarding the timing of application (Table 2), the highest number of mites that fell from the hive after essential oil treatment was observed on the first day (24 hours after application). The effectiveness then decreased significantly over time (second day and the rest of the week) with each repeated treatment. This pattern was consistent across all three oils, albeit with varying levels of efficacy. These findings are supported by (Abdel-Rahman & Rateb, 2008), who observed that the number of dead Varroa mites was highest after 24 hours, compared to after 48 and 72 hours, which showed lower numbers. Similarly, (Rahimi et al., 2017) found that Varroa mite mortality began five hours after the application of thyme extract, reaching its peak 24 hours post-application. However, these results differ from those of (Calderone & Spivak, 1995) and (El-Zemity et al., 2006), who reported that essential oils showed good results against Varroa mites after 48 hours of exposure. (Bakar et al., 2017) also noted an increase in the mortality rate at 24, 48, and 72 hours post-application.

Regarding the application frequency (Table 3), the number of dead mites that fell was higher after the first two applications of the tested oils. Subsequently, there was a decline in the fall rate following the third treatment. However, this decrease was not significantly different from the previous two periods. By the fourth application, the fall rate in the treated colonies reached its lowest level, becoming comparable to the natural fall rate observed in the control hives. This trend suggests that the population density of mites in the experimental colonies was progressively diminishing with each treatment, as evidenced by the marked difference in the number of mites that fell during the first week compared to the fourth week. These findings align with the study by Islam et al. (2016), which also observed a significant reduction in Varroa infestation rates after four oil treatments, with the highest mite fall occurring after the first and second applications. Similarly, (Mazeed & El-Solimany, 2020) reported that treating colonies with 50 g of fresh garlic resulted in the highest reduction rate after the first application, followed by a smaller but still significant reduction after the second treatment (6 days), with reduction percentages of 44.92% and 21.67%, respectively. Following these treatments, the number of fallen mites gradually decreased in subsequent applications.

In conclusion, volatile oils offer effective control of Varroa mites but tend to degrade rapidly in nature. They are target-specific and show less resistance development compared to synthetic insecticides. Therefore, incorporating the most promising essential oils into beekeeping practices presents a safer and more effective strategy for managing mite populations. This approach offers cost advantages and minimizes health and environmental risks for both consumers and beekeepers. Further research into the active ingredients and mechanisms of action of these oils could support their development as a promising commercial solution within integrated pest management programs.

Table 1. Infestation rate of *V. destructor* mites on adult honeybees and sealed brood in colonies treated with essential oils.

Treatments	Mean no. Mite/ 100 Worker bee			Mean no. Mite/ 100 Brood cell		
	Pre-treat	Post-treat	decreased rate (%)	Pre-treat	Post-treat	decreased rate (%)
Bitter melon oil	^{a}11.8±1.1	^{b}1.0±0.2	86.8	^{a}14.0±1.5	^{b}0.4±0.02	96.5
Thyme oil	^{a}11.0±0.9	^{b}1.0±0.2	85.9	^{a}7.6±0.7	^{b}0.6±0.02	90.3
Garlic oil	^{a}8.6±0.7	^{b}1.2±0.1	78.3	^{a}4.2±0.3	^{b}1.0±0.09	70.8
Control	^{a}5.6±0.4	^{a}3.6±0.5		^{a}5.4±0.7	^{a}4.4±0.5	

Means in each row followed by different letter(s) are significantly different.

Table 2. Mean numbers of dropped *V. destructor* mites from infested honeybee colonies after repeated applications of essential oils.

Treatments	Average number of dropped mites / colony					
	Pre- treat	Post treatment Application				
		1st	2nd	3rd	4th	Average**
Bitter melon	c23.2±2.7	a69.2±5.7	a67.8±8.7	ab44.4±6.7	c27.0±3.7	a52.1±2.7
Garlic	c21.4±2.2	a63.2±5.3	a57.6±2.7	ab44.4±2.7	c25.2±2.4	ab47.6±3.3
Thyme	c18.0±3.5	ab43.0±4.9	ab42.4±2.7	b33.0±2.7	c20.0±3.6	b34.6±1.7
Control	c20.9±2.9	c20.6±2.7	c19.8±2.1	c19.8±1.6	c21.8±1.3	c20.5±2.4

* Means in each row followed by different letter(s) are significantly different.
**Average column, values followed by different letter(s) are significantly different.

Table 3. Mean daily number of dropped *V. destructor* mites from infested honeybee colonies after repeated applications of essential oils.

Treatments	Mean no. of dropped mites/colony		
	Day 1	Day 2	Day 3-7
Bitter melon oil	a33.3±2.1	b11.2±1.6	c7.6±0.8
Thyme oil	a29.4±1.9	b10.8±0.9	c7.4±0.4
Garlic oil	a20.0±1.2	b9.2±0.7	c5.4±0.9
Control	c7.2±0.7	c6.7±0.4	c6.6±0.5

* Means in each row followed by different letter(s) are significantly different.

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