*Open Veterinary Journal*, (2024), Vol. 14(2): 692-698
ISSN: 2226-4485 (Print)
ISSN: 2218-6050 (Online)
*Original Research*
DOI: *10.5455/OVJ.2024.v14.i2.9*

Submitted: 18/11/2023 Accepted: 26/01/2024 Published: 29/02/2024

# Some essential oils as potential control agents for varroa mite (*Varroa destructor*) in infected honey bees (*Apis mellifera*)

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**ABSTRACT**

**Background:** Ecto-parasite, varroa mite, (*Varroa destructor*), is the primary pest affecting the apiculture sector globally in various regions.

**Aim:** This study examined the toxicity of nine essential oils to *Apis mellifera* L. and the acaricidal impact of those oils against *V. destructor*.

**Methods:** The acaricidal effects of nine essential oils, extracted from plant materials were used. In the screening experiment, 10 mg of the active ingredients of the plant material extracts were prepared in an alcohol solution with concentrations of 5%, 10%, and 15%. For each type of plant extract, five female *V. destructor* were transferred to a Petri dish with five worker bees incubated at 70% humidity and 33℃–34℃ for 2 days, for each treatment four replicates were used compared to the control. Forty-eight hours following treatment, the number of dead and live mites was counted to determine the mortality rate. In the second assay experiment, the best five essential oils of the previous experiment were selected to re-assess their effectiveness on varroa mites and honeybee workers by using a concentration of 15%. Five females of *V. destructor* were transferred to a Petri dish with 10 adult bees and treated with the solution of the selected oils. Five replicates and control treatments were taken for each sample simultaneously. Dead and live bees were counted for each replicate at 48 hours after treatment.

**Results:** There were no significant differences between the concentrations used of each oil on the rate of death of mites, and its effectiveness ranged between 70.0% and 53.3% compared to the control groups. In addition, the best oil used was bitter melon, with a death rate of 80% at a concentration of 15%, while peppermint oil showed the lowest death rate of 45% at a concentration of (5%). However, all these treatments were statistically highly significant compared with the natural death rate in control (2%). In the second test, the results of the statistical analysis indicated that there were highly significant differences ($P_{0.05}$<0.0001) in the average numbers of dead varroa mites compared to the control when using a 15% concentration of five selected oils. On the other hand, there was no statistically significant difference in the honey bee workers' mortality rate between the treatment and control groups ($P_{0.05}$<0.3390), and it was relatively low for all treatments except the basil oil, where the bee mortality rate was 16% compared to the control (10%).

**Conclusion:** It is clear from this experiment that bitter melon oil can be used to control varroa mites and it can be considered safe for honey bees as well as for the environment.

**Keywords:** Essential oils, *Apis mellifera*, Control, *Varroa destructor*.

## Introduction

In Libya and around the world, beekeeping is regarded as one of the most important agricultural and industrial endeavors (Crane, 1990). The colonies of honey bees, *Apis mellifera* L., are vulnerable to a variety of pests and diseases that can lead to significant reductions in honey yield and even the collapse of the colony (Nixon, 1982; Nagaraja and Rajagopal, 2009). The ectoparasite *Varroa destructor* (Anderson and Trueman, 2000), Acari: Varroidae, is considered one of the ultimate serious threats to honey bee colonies in managed beekeeping (Baker and Peng, 1995; Rosenkranz *et al.*, 2010; Nazzi and Le Conte, 2016; Brasesco *et al.*, 2017). Most commercial colonies often die if they are not treated, after 1–2 years of continuous varroa mite infestation (Gregorc *et al.*, 2018).

The presence of mites in colonies of honey bees raises the risk of infection by several diseases because the mites serve as carriers of honey bee pathogens (Ball, 1994). As a result, bee colonies typically die from varroa infestations very quickly in the absence of mite control (Ritter, 1981), consequently, beekeepers mostly depend on synthetic acaricides to bring the varroa population down to non-damaging levels (Caron, 1999; Melathopoulos and Farney, 2002). Although acaricides such as pyrethroid pesticides have been effective in

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controlling varroa mite populations; however, due to frequent use of these preparations, mites have become resistant to several classes of synthetic acaricides in many geographic regions (Milani, 1995; Spreafico *et al.*, 2001; Ellis, 2005). Moreover, acaricide residues have been contaminating honey bee products such as honey and bee wax (Wallner, 1999).

Several components derived from plants, including plant oils, have been shown to have biocidal activity (Masry et al., 2020; Sabahi *et al*., 2020). These compounds are thought to be more environmentally friendly acaricides and have been investigated as potential varroa mite control (Imdorf *et al.*, 1999; El-Hady *et al*., 2015; Abu Bakar *et al*., 2017; Bendifallah *et al*., 2018; Kadhim *et al*., 2022). Approximately 150 essential oils have been evaluated in laboratory screening tests (Imdorf *et al.*, 2006; Bava *et al.*, 2023) very few of them, however, have proven successful in controlling *V. destructor* when tested in field trials.

The toxicity of essential oils to mites and bees was evaluated under laboratory and field conditions (Ghasemi *et al.*, 2016, Hýbl *et al.*, 2021), as well as the repellent or attractive effect of these extracts toward mites, and their effects on mite reproduction (Colin, 1990; Imdorf *et al.*, 1995, 1999; Elzen *et al.*, 2000; Aljedani, 2021).

In Libya, the people have been practicing beekeeping traditionally for a long time (Crane, 1999); however, *V. destructor* was introduced to Libya through infested bee packages imported from Bulgaria in 1976, first reported in the east regions (Crane, 1979), and then spread rapidly throughout the country (Keshlaf and Mirwan, 2019; Keshlaf *et al.*, 2023). As it has elsewhere, chemical acaricide misuse has contributed to the development of mite resistance (Milani, 1995). Finally, several beekeepers in Libya have expressed concern about the significant decline in honey production, the collapse of honey bee colonies, and the negative impact of these developments on agriculture and food production (Keshlaf, 2017; Keshlaf and Alfallah, 2018, 2019).

Some beekeepers in Libya used different materials or extracts of the roots or leaves or even pods of local plants, they claimed success in controlling mites in their apiaries, moreover, they utilized different ways of application; however, the methods and results are unclear (Keshlaf, 2017).

In that respect, this work was to explore the toxicity of nine essential plant oils against varroa mite infested the honey bee workers under laboratory conditions and to determine the mite mortality, and bee mortality after treatment with essential oils.

## Materials and Methods

The experiments were carried out at the Honey Bees Laboratory, Department of Plant Production of the Faculty of Agriculture, University of Tripoli (UOT), Libya. To study the efficacy of some plant essential oils against varroa mite, *V. destructor* infested worker bees. Nine plant materials were used to extract their volatile oils: Bitter melon *Citrullus colocynthis* (fruits), Garlic *Allium sativum* (bulbs), Basil *Ocimum basilicum*, Thyme *Thymus vulgaris*, Mint *Mentha spp.*, Eucalyptus *Eucalyptus spp.*, and Rosemary *Rosmarinus officinalis* (leaves), which collected from local area of the city of Al Khums-Libya, and Mustard *Sinapis arvensis* (seeds), and Clove *Syzygium aromaticum* (buds) were purchased from local market of the city of Al Khums-Libya.

***Extraction and characterization of essential oils***

The extraction of the essential oils was made at Elmergib University, Libya. The essential oils were extracted from the above-mentioned plant materials using a Soxhlet Extractor (Zygler *et al.*, 2012).

***Mite collection***

Female adult varroa mites were collected from *A. mellifera* colonies from the apiary of the Department of Plant Protection, at the Research and Experimental Stations in the Faculty of Agriculture, UOT- Libya, using colonies that had not been treated with miticides for at least 1 year. The *V. destructors* were separated from the bees by shaking them in a jar with two tablespoons of powdered sugar and collecting them in a sieve (Macedo *et al.*, 2002). At the laboratory, mites showing normal movement after 15 minutes of their re-motion were used for the experiment test.

***Bees' collection***

Approximately 200 workers of mixed age from healthy colonies of *A. mellifera* were collected from the experimental apiary mentioned above. Bees were collected from frames by brushing them into a 120 × 80 mm insect breeding dish (Williams *et al.*, 2013) and transported to the laboratory for toxicity bioassay for the first assay. In the second test, a frame of sealed brood showing emerging new bees was incubated a day before the experiments, this procedure would facilitate dealing with young worker bees and guarantee the age similarity. The collected bees were maintained at 25℃ ± 2℃, 60% ± 10% RH, and provided with a 50% sucrose solution until the start of the bioassay.

***Toxicity to mites***

Plastic Petri dishes with a diameter of 9 cm were equipped with a 5 cm circular hole in the cover of the dish then closed by a net for ventilation. A filter paper with a diameter of 9 cm was placed at the bottom of the dish, and five worker bees were placed on it, then five mites were taken, at the rate of one individual for each worker bee. A filter paper scrap (2 × 2 $cm^2$) was saturated with oil (concentrations of 5%, 10%, and 15%) and placed underneath the cover of each Petri dish. The plate was covered with oil-loaded filter paper by Parafilm after placing it on the net (to prevent oil volatilization), leaving an opening for ventilation. Four replicates for each concentration were placed in an incubator at 70% RH and 33℃–34℃ for 2 days. Two grams of candy paste was added to feed the bees. The dead and alive mites were counted 48 hours after the treatments.

***Toxicity to bees***

The same experiment was repeated using the best five oils, which showed the highest mortality rate of the varroa mite, the oils of bitter melon, basil, garlic, mustard, and thyme. Ten adult bees, 1 day old, newly emerged from the frame were placed inside a Petri dish containing candy for bees feeding. Five female mites were introduced with a brush directly onto the bee's body. A piece of filter paper (2 × 2 $cm^2$) was saturated with oil at a concentration of 15%. Four replications of each tested oil and control were placed in an incubator at 70% RH and 33℃–34℃ for 2 days.

Dead and alive bees and mites in each Petri dish counted 48 hours after the treatments. Data were analyzed by ANOVA for each oil, only considering the oil dose effect and means were compared by Duncan's Test. Data were analyzed by using the Statistical Analysis System SAS, 2003 (SAS, 2003).

***Ethical approval***

This study was approved by the Graduate School of the UOT, Faculty of Agriculture, Department of Plant Protection. All animal welfare protocols were followed.

## Results

***Efficacy of nine essential oils on varroa mites mortality***

The percentage ratios of dead female mites after 48 hours of treatment with essential oils were compared to the control using three concentrations of each oil. All oils used in the first experiment showed high effectiveness in killing female mites, with an average death rate of 70.0% for bitter melon and garlic, 66.6% for basil oil, 61.7% for mustard and thyme oil, 58.3% for clove oil, and 55.5% for eucalyptus oil. The death rate for peppermint and rosemary oil was 53.3%, while the natural death rate in the control was 2% (Table 1).

The results of the statistical analysis indicated significant differences ($P_{0.05}$ <0.001) in the means of dead female mite numbers compared to the control. The Duncan test for means isolation showed that all oils were effective in killing the mites, with bitter melon oil and garlic showing the highest death rate compared to the control.

Considering the different concentrations of the same oil, the highest killing rate was recorded when using a 15% concentration of bitter melon oil, reaching approximately 80%, however, when used at lower concentrations (5% and 10%), the death ratio decreased to (60% and 70%), making its effect similar to the other tested oils. There were no significant differences between the concentrations of each oil on the mite death rate, even for peppermint oil, in which the mortality ratio decreased to 45% at a concentration of 5% compared to a mortality ratio of 60% to 65% at concentrations of 10% and 15%, respectively.

***Efficacy of five essential oils on varroa mites and bees' mortality***

The results in Table 2 showed the mean value and average percentage of dead worker bees *A. mellifera* (*n* = 10 individuals), and varroa mites *V. destructor* (*n* = 5 individuals), for 5 essential oil treatments with a concentration of 15% administered by saturated filter paper, at 48 hours, and indicated an increase in the rate of varroa mites death, when using essential oils with 15% concentration compared to the control. The statistical analysis showed highly significant differences ($P_{0.05}$ <0.001) for the average number of dead female mites compared to the control, with the efficacy of bitter melon oils (80%), then thyme oil (76%), garlic (72%), mustard (64%), then basil oil (60%), compared to the control (12%).

The results for the treatment of the five selected essential oils on worker honey bees showed that the percentage of dead bees after 48 hours of treatment was very low. The statistical analysis indicated that there were no significant differences (*p* = 0.3390) in

**Table 1.** Average percentage of dead mites *V. destructor* (5 replications, *n* = 5 individuals) for nine essential oil treatments at different doses administered by saturated filter paper, at 48 hours.

| Treatments | Mean percentage of dead mite/concentrations | | | Mean |
|---|---|---|---|---|
| | 5% | 10% | 15% | |
| Bitter melon oil | %60$^{abc}$ | %70$^{abc}$ | %80$^{a}$ | %70.0 |
| Garlic oil | %70$^{abc}$ | %65$^{abc}$ | %75$^{ab}$ | %70.0 |
| Basil oil | %65$^{abc}$ | %60$^{abc}$ | %75$^{ab}$ | %66.6 |
| Thyme oil | %55$^{abc}$ | %70$^{abc}$ | %60$^{abc}$ | %61.7 |
| Mustard oil | %60$^{abc}$ | %60$^{abc}$ | 65%$^{abc}$ | %61.7 |
| Cloves oil | %50$^{abc}$ | %70$^{abc}$ | %55$^{abc}$ | %58.3 |
| Eucalyptus oil | %50$^{abc}$ | %60$^{abc}$ | %55$^{abc}$ | %55.5 |
| Rosemary oil | %50$^{abc}$ | %50$^{abc}$ | 60%$^{abc}$ | %53.3 |
| Mint oil | %45$^{c}$ | 60%$^{abc}$ | %55$^{abc}$ | %53.3 |
| Control | 2%$^{d}$ | | | |

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

**Table 2.** The mean value and the average percentage of dead worker bees *A. mellifera* ($n$ = 10 individuals) and mites ($n$ = 5 individuals), for five essential oil treatments with a concentration of 15% administered by saturated filter paper, at 48 hours.

| Treatments | Mean no. dead bees/10 worker | Mortality (%) | Mean no. dead mites/5 mites | Mortality (%) |
|---|---|---|---|---|
| Basil oil | 1.6 [a] | 16% | 3.0 [a] | 69% |
| Mustard oil | 1.2 [a] | 12% | 3.2 [a] | 64% |
| Bitter melon oil | 1.0 [a] | 10% | 4.0 [b] | 80% |
| Garlic oil | 0.6 [a] | 6% | 3.6 [ab] | 72% |
| Thyme oil | 0.6 [a] | 6% | 3.8 [b] | 78% |
| Control | 1.0 [a] | 10% | 0.6 [c] | 12% |

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

the average number of dead workers compared to the control treatment. However, the highest number of bee mortality was observed after being treated with basil oil (16%), then mustard oil (12%), bitter melon oil (10%), thyme oil, and garlic oil (6%), compared to the control (10%) (Table 2).

## Discussion

The results of the initial screen test of nine essential oils extracted from some local plants (Bitter melon, garlic, basil, thyme, mint, eucalyptus, and rosemary) and others extracted from mustard seeds and clove buds showed that they were all effective in killing female varroa mites. The death rate of varroa mites ranged between 80% and 45% under laboratory conditions. Compared to the control, the concentrations used for these oils (5%, 10%, and 15%) showed the same efficiency in controlling varroa mites, with the exception of bitter melon oil, which had the highest effect when used at a concentration of (15%), and the least effective was mint oil at a concentration of (5%), which confirms that all these natural oils are effective in their effect on the death rate of varroa mites. The results of this study are consistent with several previous studies which concluded that essential oils were effective in controlling varroa mites (Imdorf *et al.*, 1999; Abd El-Wahab *et al.*, 2012; Rashid *et al.*, 2012; Islam *et al.*, 2016; Abu Bakar *et al.*, 2019; Hýbl *et al.*, 2021).

Although it is difficult to compare the results of the current study with other studies due to the differences in oils tested, as well as the treatment method and concentrations used, it is useful to refer to a laboratory study by Rashid *et al.* (2014) to evaluate the efficiency of clove oil, neem, olive, garlic, and tobacco extracts and applied with different concentrations. They found that the mixture of clove oil and tobacco extract was the most effective compound at a concentration of 5%, in addition to that the results of the field experiment recorded the highest rate of daily fall of varroa mites when using the same mixture at the same concentration. Moreover, when testing the effectiveness of essential oils of *Eucalyptus* sp., *Tagetesminuta*, and *Heterothecalatifolia*, plants at concentrations of 3%, 4%, and 5% for each oil, the results showed that all concentrations were effective in killing mites and there were no statistically significant differences between them for all concentrations (Ruffinengo *et al.*, 2007). Abd El-Wahab *et al.* (2012) evaluated the effectiveness of four oils with a concentration of 50% and 100% compared to formic acid at 65% against varroa mites, and the results showed that the use of thyme, anise, cinnamon, lemongrass, and formic acid at a concentration of 100% led to a decrease in the incidence of infection. at a rate of 44.8%, 41.6%, 40%, 38.1%, and 37.9%, respectively.

The results of the confirmatory test for the best five oils in the initial test indicated that they were all effective in controlling female mites under laboratory conditions compared to the control when used at a concentration of 15%. Accordingly, the three promising oils, which gave the highest death rate for female varroa mites, bitter melon oil (80%), thyme oil (76%), and garlic (72%), should be evaluated under field conditions. These results should be assessed in further tests since Taha *et al.* (2020) stated that using garlic oil decreased varroa mites numbers by 90%, this disagrees with the results of this experiment, where the effects of garlic oil reduced the number of the mites by only 72% and were not superior as they mentioned; however, the results of this assessment were agreed with what Faraj *et al.* (2021) found. A mixture of two or more essential oils extracted from plant material could lead to a reduction of varroa mites damage, where Begna *et al.* (2023) claimed that mixing some essential oils, a 1:1 mixture of thymol and carvacrol, decreased the number of *V. destructor*, rather than using single oil. The agreement of the results of the second experiment with the results obtained from the initial test confirms the possibility of benefiting from these materials in controlling varroa mites.

## Conclusion

It is worth noting that most of the previous studies related to evaluating the efficacy of natural products in controlling varroa mites were *in vitro* (Bunsen, 1991;

Sammataro *et al.*, 1999; Ruffinengo *et al.*, 2007), while others were conducted directly in the field without prior verification of their effectiveness nor specifying the mechanism for determining the concentrations used in the studies (Islam *et al.*, 2016; Aljedani, 2018; Müller *et al.*, 2022); therefore, this study included laboratory evaluation to verify the best oil that can be used and the appropriate concentration to verify the effectiveness of the oils in controlling varroa mites in the field using one essential oil with only one concentration. However, all the materials used could be applied as part of integrated pest management, and are highly recommended to improve the beekeeping industry and pollination services.

**Acknowledgment**

The authors thank Dr. Salem Drah for supervising the oils extractions and Dr. Khaled Ben Nasr for providing the statistical analysis.

***Conflict of interest***

The authors declare that there is no conflict of interest.

***Funding***

No external funding for this study.

***Authors contributions***

The authors contributed to this study. All authors read and approved the final manuscript.

***Data availability***

All data are provided in the manuscript.

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