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**Dalal M Aljedani**
 Department of Biological  
Sciences, College of Science,  
University of Jeddah, Jeddah,  
Saudi Arabia

## Controlling *Varroa* mites infesting honeybees (*Apis mellifera* L.) Using some essential oils and Amitraz under colony conditions

**Dalal M Aljedani**DOI: <https://doi.org/10.22271/j.ento.2021.v9.i6a.8870>**Abstract**

The biggest difficulty for beekeepers in the globe is the *Varroa* mite. So, the honey production and the mortality of adult honeybees are damaged by this pest. Therefore, this study tested some essential oils and chemicals (Amitraz), for the control integrated on *Varroa destructor* and affected of (*Apis mellifera* L.). Five essential oils (garlic oil, Peppermint oil, Cinnamon oil, thyme oil, Lavender oil) of plant natural products and chemical pesticide (Amitraz), the *Varroa* mites infected in the honeybee colonies were examined and the falling mites were monitored by a sticky card on the base of the hive. Data was recorded after 1,3, 7, 15, 22 days of treatment, under colony conditions. The natural approaches employed in the control and spread of bee illnesses have been demonstrated to be effective. Garlic oil and thyme oil were found to be particularly efficient against *Varroa* Mites and honeybees (*Apis mellifera* L.), Garlic oil has surpassed all other treatments to reduce the amount of *Varroa* with a substantial change following treatment of (Mean± Std. Error) (9.330± 2.392) throughout the study, with increased *Varroa* mortality in all treatments compared to during the pretreatment phase. Also, the daily dead bees were counted during the study period, there were insignificant differences between honeybee colonies with different treatments in the daily dead worker bees treated with certain oils and chemicals. In addition, all the treatments were safe for worker bees at the applied dose. The natural approaches employed in the control and spread of bee illnesses have been demonstrated to be effective, and that essential oils can improve the health of bee families.

**Keywords:** Amitraz, honeybee, *Varroa* mites, essential oils, fallen mites**Introduction**

Pollinators are an important part of world biodiversity because they provide essential ecosystem services to crops and wild plants recent losses in both wild and domesticated pollinators and decreases in crops that rely on them are convincing proof, Pollinator losses can lead to pollinating services losses that have severe environmental and economic repercussions that might endanger the variety of wild plants, stability of the ecosystem, agricultural production, food safety, and human well-being [1]. The importance of (*Apis mellifera* L.) is greatly appreciated not only for their various products with nutritional and medicinal uses but also because of their important role as pollinators [2]. The health of honey bees is linked to sustainable agriculture and a variety of non-agricultural habitats, at a similar time colonies of *Apis mellifera* L., are attacked by insects, mites, and several other diseases [3]. The western honeybees are one of the most important insect species, but are, unfortunately, faced with several biological stresses, which are endangering their existence. Different diseases are common in Honey Bees, the most severe ectoparasitic mite known today being *Varroa destructor* (Anderson and Trueman) [4].

*Varroa destructor* Anderson and Trueman is an ectoparasitic mite found in honey bees (*Apis mellifera* L.), which has been responsible for colony losses [5]. In addition, *Varroa's* influence on colonies differs according to the habitat, climate, managerial practices, colony parasites and disease loads, and bee strain [6]. In the United States, most of the losses of the honeybee colony are caused by the *Varroa destructor* and the viruses it transfers; as a result, beekeepers use miticides to decrease *Varroa* populations. The miticides fluvalinate and coumaphos have developed widespread resistance. Despite a lengthy and widespread use history, *Varroa* has mostly retained susceptibility to amitraz [7]. To control this ectoparasite, synthetic miticides have been developed and registered [5]. However, there is also a strong fear that continued use of Apivar1 as a key *Varroa* control option will eventually result in resistance, leaving the beekeeping industry without viable synthetic *Varroa* control choices [7, 8].

**Corresponding Author:****Dalal M Aljedani**
 Department of Biological  
Sciences, College of Science,  
University of Jeddah, Jeddah,  
Saudi Arabia

In recent years, the ectoparasitic mite *Varroa destructor* is wreaking havoc on honeybee hives all over the world. It is believed to have a large influence on the enormous seasonal loss of colonies every year in the management of parasites, and apiculturists frequency fully takes on a restricted number of acaricides such as tau-fluvalinate or flumethrin pyrethroid, coumaphos organophosphate, and formamidine amitraz. However, the evolution of resistance in mite populations has created an unsustainable situation in which there are few options for mite control [9]. Anecdotal reports of decreasing amitraz effectiveness have been a hot topic among commercial beekeepers recently. However, a wide range of amitraz resistance was seen in apiaries across operations, ranging from little resistance to high resistance, resulting in *Varroa* control failure. Resistance ratios from *in vitro* amitraz bioassays were linked to lower Apivar® efficacy, revealing genuine *Varroa* control failures caused by amitraz resistance. As a result, protocols for monitoring amitraz resistance must be created. To ensure the long-term viability of miticide use for *Varroa* management, a resistance monitoring network should be built [7]. To decrease the danger of toxic waste in hive products and avoid the occurrence of resistance, the use of natural products like essential oils (EOs) is becoming increasingly popular with bee pathogens as a viable alternative to standard synthetic techniques such as antibiotics and fungicides [10]. Recent advancements in Essential (Eos) oils have led to a growing interest in essential (Eos) oil generated from antimicrobial plants, herbs, and spices with a wide variety of Gram-positive and Gram-negative bacteria, as well as some fungal diseases. The presence of phenolic and terpenoid chemicals, both with well-known antibacterial characteristics, largely causes this antimicrobial action. Essential oils and plant extracts have been used for the treatment of infections since ancient times [11].

Moreover, the essential oils produced in herbs are a rich metabolite source that can be poisonous or behavioral to arthropods, some of the essential oils have proven to be effective in mite control, have insecticide and repellent properties for other arthropods pests, and can actively combat the destructor from *Varroa destructor* [12]. The study by Iglesias *et al.*, [13] revealed essential oils from all four kinds (Cascade, Victoria, Spalt, and Mapuche) were harmful to the *Varroa destructor* mite in bioassays. The most harmful essential oil to *Varroa destructor* was Victoria. The oils were neither repellent nor attractants for the mites, and bee (*Apis mellifera* L.) mortality was moderate to low after complete exposure to the essential oil application.

Several researchers have reported [14] the essential oils (EOs) produced from the aromatic plant's *Artemisia annua*, *Artemisia verlotiorum*, *Cinnamomum Verum*, and *Citrus reticulata* were tested as repellents against the parasite mite *Varroa destructor*, which is found in honey bees. Except for *C. reticulata*, all EOs had substantial repellent action against the mite after 24 hours in the lab. *C. verum* was the most efficient EO against *Varroa destructor* (EC<sub>50</sub> = 1.30 L L1) and the least harmful to honeybees (EC<sub>50</sub> = 13.29 L L1). *C. verum* EO was subsequently evaluated in field trials to prevent varroosis in colonies due to its high selectivity ratio (*Apis mellifera* L.) LD<sub>50</sub>/*Varroa destructor* EC<sub>50</sub> = 10.22), the results of open field experiments, supported by the laboratory results, on the other hand. Following one week of treatment, *Varroa* infection in colonies treated with EO (65 percent at 25.0 L1) was reduced significantly and no adverse effects were reported (*Apis mellifera* L.).

Recently investigators have examined the effects of eleven essential oils extracted from Chinese crude remedies for their fumigant properties, a proper dose that did not influence honeybees was utilized for a toxicity test for *Varroa destructor* mites. Six essential oils containing odoriferous rosewood oil and fennel oil, which cause more than 650 percent fatal destructive *Varroa*, showed considerable acaricidal action against the mites. The fumigant toxicity on adult honeybee workers in all the essential oils was in the meanwhile similar to that of controls [15]. Previous studies have been reported by Aglagane *et al.*, [16] their findings show that the testing of EO combinations might be a potential option to lower effective dosages of essential oils (EO's) produced under lab conditions from *Mentha suaveolens* L. subsp. timija (Briq.) Harley *Chenopodium ambrosioides* L. and *Laurus nobilis* L. Recent data show, too, that novel essential oil products with acaricidal capacity have been identified that could assist ameliorate the poor condition of *Apis mellifera* L. [15] Some researchers have reported [17] comparing the effectiveness of six essential oils with formic acid and Apistan streaming against *Varroa* mite infestation in honeybee colonies, and they found that garlic oil was superior to all treatments in reducing the number of *Varroa* mites in honeybee colonies. The mite's resistance to and accumulation of residue from typical acaricidal treatments necessitates the use of more natural and safer options to eliminate this parasite in colonies; essential oils derived from plant leaf are one such option. The objective of this study is to evaluate the efficacy of some natural products against *Varroa* mites and Honeybees, the essential oils include; (garlic oil, Peppermint, Cinnamon oil, Thyme oil, and Lavender oil), which can maximize *Varroa* control while minimizing the side effect on colonies and the environment.

## Materials and Methods

### Study area and Honeybee Colonies

The field experiment was conducted was during March the season 2021, at the apiaries in the western region in the kingdom of Saudi Arabia. Were selected among the colonies of the honeybee *Apis mellifera jemenatica* Ruttner (Hymenoptera: Apidae), Some essential oils and chemical pesticide (amitraz) was used of evaluating their effectiveness on *Varroa* mites to test the efficiency of the control method and learn which has the most effect.

### Experimental honeybee colonies

The experimental colonies (21 colonies) were separated into seven groups, each of which had three subgroups, each of which contained three colonies as replicates. Were randomly chosen, and selection was based on vigor, some bees, and mite infestation to check for *Varroa destructor* infestation.

### Essential Oils and Chemicals

The oils were: garlic oil, Peppermint oil, Cinnamon oil, thyme oil, Lavender oil. Also, a chemical pesticide (amitraz) was used. Cotton balls were prepared and saturated by 1 mL of oil (1 mL oil/ one ball/each colony/3 replicates) and hanged between the brood combs in each colony. A Cardboard (7x15x0.3 cm) was saturated with 10 mL of amitraz and put onto the brood combs.

### Mites fall and dead bees

The numbers of fallen mites were recorded on sticky, white paper with a little layer of vaseline placed on the bottom of

the hive after 1, 3, 7, 15, and 22 days following the first treatment. The effect of treatments on worker bees was calculated daily.

### Determination *Varroa* Mites on Adult Bees

Approximately 50 bees were taken from open brood combs, if possible, and immersed in water containing detergent (washing-up liquid). The bees were caught in a wire net and shaken for 3-5 minutes before being removed. Mites would have fallen off and been discovered at the container's bottom<sup>18</sup> *Varroa* mites and bees were counted.

### Evaluation of the tested materials

Five essential oils and amitraz have been treated in each colony, untreated control colonies have been left and the test has been duplicated three times. A "sticky card," which was applied in all hives, was put before each treatment under the wire/wood frame to monitor the *Varroa* mite population before and after treatment<sup>19</sup>. Five essential oils of natural plant products were assessed against the honeybee colonies infested with *Varroa* mites. The effectiveness % was calculated using the following formula for each application of these substances<sup>20</sup>.

$$\text{Efficacy} = \frac{\text{No. of mites fallen for each treatment}}{\text{Total number of mites}} \times 100$$

### Statistical analysis

The results from the studies were evaluated statistically using SPSS software, which included an analysis of variance (ANOVA) and a Multiple Range Test to compare means, and treatment means were compared at 5% probability levels<sup>21</sup>.

### Results

Five different essential oils against mites and honeybees have been evaluated in the results of the current effectiveness research, the oils were: garlic oil, Peppermint oil, Cinnamon oil, thyme oil, Lavender oil. Also, chemical pesticide (amitraz), To evaluate its effect on the fall and death of *Varroa* mites as well as the life of honeybees.

### Fallen *Varroa* Mite

The effect of volatile oils and chemicals on *Varroa* mites number that fell during the experimental periods was depicted in Table (1) and Figure (1) Garlic oil outperformed all other treatments in terms of reducing the number of *Varroa* with a significant change following therapy of (Mean± Std. Error) (9.330± 2.392) throughout the study.

The most effective in terms of falling mites/colonies of *Varroa* after garlic oil, cinnamon oil, amitraz, and thyme oil with little differences between them, arrived at (4.262± 1.572), (4.128± 1.840), and (2.728 ± 0.723) respectively.

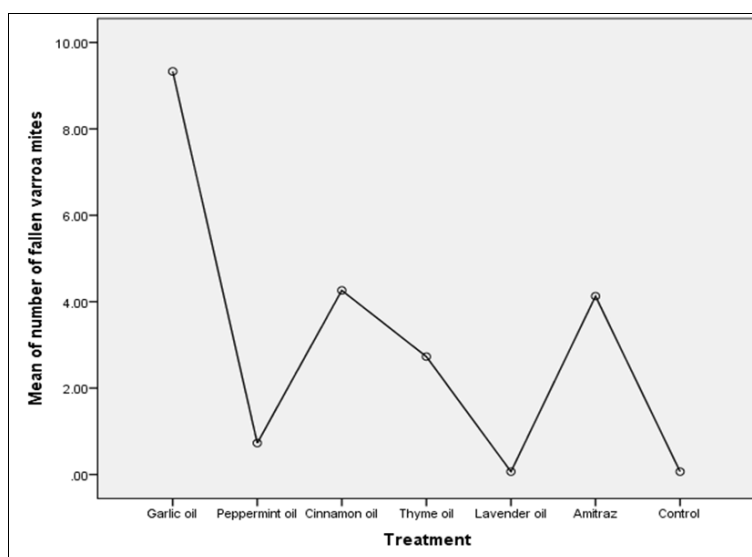
Peppermint oil and Lavender oil, on the other hand, had the lowest mean number of falling *Varroa* mites as compared to control colonies (0.728± 0.163) and (0.066 ± 0.066) fell mites/colony), in the control grope (0.066 ± 0.066).

Garlic oil has a stable and continuous effect on mites fallen during the first three days of treatment representing 14.66±1.76, 12.33±1.45, and 12.0±1.52 fallen mites/colony, respectively. The same trend was observed for both Amitraz and Cinnamon oil but with a minor effect.

It clears that, the effect of Thyme oil on fallen mites starting strongly and significantly during the first day of treatment giving (19.66±2.90) then sharply declined till the last day of treatments (22 days) represented 1.66±0.66 Figure (2).

**Table 1:** Mean some fallen *Varroa* mites after daily sequence from treatments by some essential oils.

Treatments	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
				Lower Bound	Upper Bound		
Garlic oil	9.330	5.349	2.392	2.688	15.971	1.66	14.66
Peppermint oil	0.728	0.365	0.163	0.274	1.182	0.33	1.33
Cinnamon oil	4.262	3.515	1.572	-0.103	8.627	0.33	8.66
Thyme oil	2.728	0.723	0.323	1.829	3.626	1.66	3.66
Lavender oil	0.066	0.147	0.066	-0.117	0.249	0.00	0.33
Amitraz	4.128	4.116	1.840	-0.983	9.239	0.66	11.00
Control	0.066	0.147	0.066	-0.117	0.249	0.00	0.33
Total	3.044	4.063	0.686	1.648	4.439	0.00	14.66



**Fig 1:** Percent efficacy of naturally some essential oils against *Varroa* mites

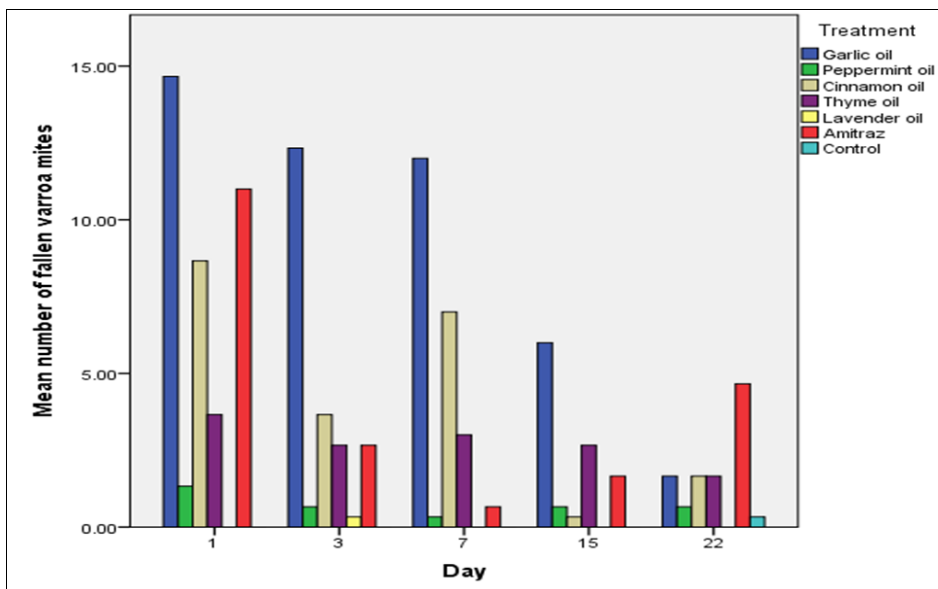


Fig 2: The percentage of the effect of some different essential oils against *Varroa* mites during the days of the study

**Bee Mortality**

Table (2) illustrated the mean number of dead worker bees after treating the honeybee colonies with different treatments. The daily dead bees were counted during the study period. There were insignificant differences between honey bee colonies with different treatments in the daily dead worker bees treated with certain oils and chemicals. In addition, all the treatments were safe for worker bees at the applied dose. Data reveals that colonies treated with Amitraz gave the highest number of dead bees (1.928 ±0.818). Followed by colonies treated with Thyme oil, Cinnamon oil, and

Peppermint oil represented 1.130±0.402, 1.000±0.633, and 0.728±0.163, respectively. Additionally, the highest number of dead bees was observed mainly during the first day of application for all treatments. The number of dead bees in all treatments is not in big concern. So, it could be concluded that all the treatments can be used safely for bees, Figure (3). It clears that, the effect of Amitraz on the death of honeybees started strongly and significantly on the third day of treatment giving (19.66±2.90) then sharply declined on the seventh day of treatments, after that, its effect on honeybees returned to days 15 and 22 days represented, Figure (4).

Table 2: Mean the number of dead honeybees after daily sequence from treatments by some essential oils.

Treatments	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
				Lower Bound	Upper Bound		
Garlic oil	0.000	0.000	0.000	0.000	0.000	0.00	0.00
Peppermint oil	0.7280	0.36561	0.16351	0.274	1.182	0.33	1.33
Cinnamon oil	1.0000	1.41716	0.63377	-0.759	2.759	0.00	3.34
Thyme oil	1.1300	0.89939	0.40222	0.013	2.246	0.00	2.33
Lavender oil	0.000	0.000	0.000	0.000	0.000	0.00	0.00
Amitraz	1.9280	1.83006	0.81843	-0.344	4.200	0.00	4.66
Control	0.000	0.000	0.000	0.000	0.000	0.00	0.00
<b>Total</b>	<b>0.6837</b>	<b>1.10426</b>	<b>0.186</b>	<b>0.304</b>	<b>1.063</b>	<b>0.00</b>	<b>4.66</b>

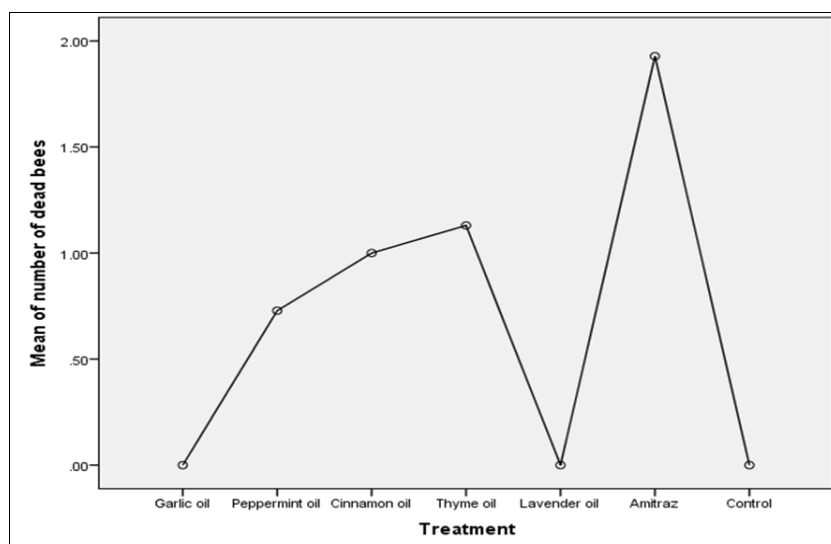
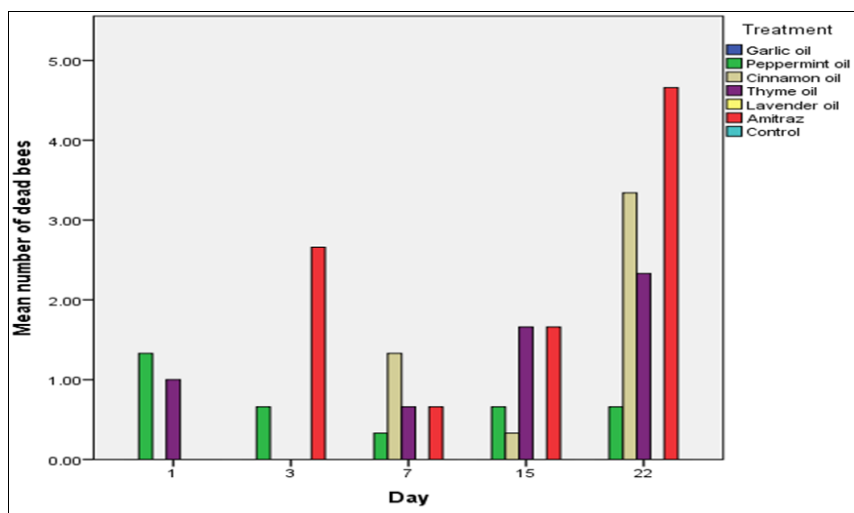


Fig 3: Percent mortality of honeybee after treatments by some essential oils.



**Fig 4:** The percentage of the effect of some different essential oils on honeybees during the days of the study.

## Discussion

Today, aromatic herbs and oils play an important role in the fight against disease caused in farmed animals, and many synthetic medicines against bee illnesses and pests are used in beekeeping. Several sorts of studies have revealed a mortal impact on mites, germs, and fungus on plants and essential oils. Aromatic oils, when applied at suitable dosages, do not have harmful effects on the bee colony and do not adversely affect colony development, in addition, the use of medicinal aromatic herbs and oils in beekeeping supports organic production, as well as preventing residue problems in bee products (honey, beeswax, and others) [22]. *Varroa* withes infesting in colonies of honeybees make bees more susceptible to other diseases such as viruses and bacteria. This necessitates the implementation of effective *Varroa* mite control techniques. Chemical application by beekeepers is the most extensively used *Varroa* control strategy. A chemical application does not give comprehensive control against *Varroa* mites due to the development of acaricide resistance in *Varroa* mites and chemical residues in honeybee products. To avoid this, natural solutions like essential oils and organic acids, as well as biotechnical procedures like mite traps, are among the alternatives. Furthermore, many studies have used combinations of different control strategies in an integrated pest management program that are successful against *Varroa* mites. As a result, proper treatment can reduce economic losses by preventing excessive chemical use and residual concerns in bee products [23].

From the results of the current study, Garlic oil outperformed all other treatments in terms of reducing the number of *Varroa* mites after treatment, with a significant difference of (Mean  $\pm$  Std. Error) (9.330  $\pm$  2.392) for the study. This is consistent with what was found when the effects of fresh garlic, onion, a combination of them, garlic oil, and onion oil against *Varroa* mite in honey bee colonies were evaluated, the treatment with fresh garlic resulted in the highest percentage of fallen *Varroa* mite with 94.29 percent of the total *Varroa* present in the bee colony [24]. Also, in the work by El-Nagar *et al.*, [25] when they were studied to evaluate the effect of some botanicals-based food mixtures added to sucrose syrup and offered to honeybee colonies on the development of *Varroa* infestation, brood rearing activity, and clover honey, summarized results are as follow: The food mixture composed of lemon juice + garlic extract reduced significantly the rate of *Varroa* infestation as compared to the control in all seasons

of the year by 37.02-55.40%, also, the food mixture composed of lemon juice + garlic extract induced the highest brood rearing activity in all seasons of the year.

This study confirms Peppermint oil and Lavender oil, on the other hand, had the lowest mean number of falling *Varroa* mites as compared to control colonies (0.728  $\pm$  0.163) and (0.066  $\pm$  0.066) fell mites/colony), these results match those observed in earlier studies by El-Sayed *et al.*, [26] through which it was proven a diet that has been mixed with mint oil proved to be better to be offered to bees during winter, feeding on gave the highest means of sealed brood area (1348.0  $\pm$  13.93 inch<sup>2</sup>), number of eggs/queen/day (1195.64  $\pm$  12.35 eggs), and the number of frames covered with bee (9.0  $\pm$  0.00 frames/hive), and the fallen mites increased recording high mean numbers of 176.0 and 201.0 mites (effectiveness= 80.11 and 80.33%) (2019 season) and 201.0 and 161.0 mites (effectiveness= 87.06 and 83.85%) (2020 season), respectively. In at same study, Thyme oil-impregnated papers were proved to be more effective during February of both seasons recording effectiveness of 77.84 and 83.95%, followed by mint oil (75.16 and 81.37%, respectively), while mustard oil-impregnated papers were proved to be the least effective. Residues of the evaluated essential oils in honey were absent and that because these compounds quickly decreased due to their volatility, thus there cannot be any food safety risk for the consumer.

Was clear from the results of the current study, Cinnamon oil has a stable and continuous effect on mites fallen during the days of treatment but with a minor effect, this is consistent with what was found in studies that have demonstrated that natural techniques for controlling and spreading bee diseases are beneficial over time, which has sparked an ongoing interest in finding new options. In bee colonies, essential oils have been investigated for their ability to prevent or treat diseases. However, Cinnamon's main mode of action with insects as a pesticide appears to be as a repellent, it possesses biocidal properties at higher levels. Cinnamon's phytotoxic properties make it a potential herbicide, but they also limit its foliar application to crops. Cinnamon and its essential oils have antibacterial properties and can prevent germs and fungi from growing [27].

Thyme is a valuable economic herb grown in the Mediterranean and temperate areas. Because the plant's leaves, flowers, and oils are commonly used as food items in a variety of cuisines, they are deemed safe. Thyme, thyme oil,



and its principal active ingredient thymol have antimicrobial, antifungal, and insecticidal capabilities for pest control [28]. Reference to previous research by da Silva *et al.*, [29] *Apis mellifera* Linnaeus (Hymenoptera: Apidae) were exposed to ginger, mint, oregano, and thyme essential oils across three pathways to determine their LC<sub>50</sub>, LD<sub>50</sub>, and LC<sub>90</sub>, LD<sub>90</sub>, oregano, and thyme were more harmful to (*Apis mellifera* L.) through contact and topically, whereas mint and ginger were less hazardous. In the present study, the effect of Thyme oil on fallen mites started strongly and significantly during the first day of treatment giving (19.66±2.90) then sharply declined till the last day of treatments (22 days) represented 1.66±0.66. *In vitro* studies with adult bees Calculations of each compound LC<sub>50</sub> on (*Apis mellifera* L.) and *Varroa destructor* revealed that each chemical has a different harmful effect on both species. Thymol and Phellandrene killed mites in lower quantities than they killed bees. The toxicity of the binary mixture of these two compounds differed from that of each pure chemical, as it was highly selective for mites in bioassays after 24 hours of total exposure [30].

According to recent research, these oils could be used for food preservation and insect control, among other things, although phenolic components have been ascribed to different activities, the medicinal and pharmacological properties of lavender are mostly related to the essential oils, especially linalool and linalyl acetate, the principal essential ingredient of oils, the major and minor substances of essential oil operate together, to give a range of health advantages. Much current research is on assessing the biological activity of essential lavender oils for possible applications in conventional and supplementary medicinal goods, food systems, formulations of cosmetics and fragrances, and pest control products [31]. These results reflect the results seen in other research, which used biomarkers to assess changes in the physiology of honeybees in high and low-weight colonies before and throughout the lavender hybrid season [32].

Data in the present study reveals that colonies treated with Amitraz gave the highest number of dead bees (1.928 ±0.818), this is consistent with what was found in another study by Chaimanee *et al.*, [33] the amitraz residue and its metabolites in honey products are still a matter of worry when they have proved that amitraz can kill mites in honey bee colonies. Although the study utilized high dosage to create worst-case scenarios, the amitraz metabolite residue detected in honeybee products was not over the maximum residue levels (MRLs). In the studies by Guo *et al.*, [34], the amitraz and its main metabolite are potentially active for all four mite octopamine receptors and to increase amitraz sensitivities of Oct2R, showing that its relative insensitivity is the major mechanism for the resistance of amitraz by honeybees, replacing three bee-specific residues.

Amitraz was frequently used in various areas, which resulted in *Varroa destructor* resistance to it. Some efficient field experiments in France revealed that amitraz reduced treatment effectiveness. Some mites obtained from various apiaries show resistance to amitraz and tau-fluvalinate (71 percent of mite samples indicate resistance to amitraz and 57 percent to tau-fluvalinate), and if mite resistance to acaricides continues to rise, mite resistance to acaricides will become a problem [35]. Honeybees and their products are good bioindicators because they are inextricably linked to the natural environment they inhabit; the beekeepers should be interested in identifying bee-sensitive and beekeeping sites to obtain good products and free from any environmental contaminants. So, further data collection is required to determine exactly. Because of the recommendation that the quality and safety of honeybees and their products are related to the health and

safety of human beings, so it is necessary to be paid attention to it [36].

## Conclusions

In conclusion, Various methods, and materials for *Varroa* control have been proposed. Because insecticides do contaminate honeybee products, they are not recommended to be used directly in honey bee colonies. The efficacies of several essential oils and a chemical pesticide (amitraz) were measured in honeybee colonies. The levels of *Varroa* infestation were measured before and after the treatments. The overall *Varroa* mortality was dramatically raised by all the essential oils examined. Garlic oil and thyme oil, which displayed longer and more constant activity, had the best efficiencies after 22 days post-treatment. This study discovered that all the treatments resulted in higher *Varroa* mortality than the mortality of natural. The toxicity of essential oils against *Varroa* mites in adult honeybees in colonies was consistent, while amitraz was relatively more harmful to worker bees. essential oil compounds have emerged as a viable alternative to standard acaricides; however, the toxicity of these mixtures is still unknown. As a result, essential oils that can be utilized to control insect pests in pollinators like honeybees need to be researched further.

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