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# Studying the Effect of Some Plant Extracts Using Hexane on *Varroa Destructor* and *Apis Mellifera* in Laboratory

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***Abstract.*** *This study was conducted in the laboratories of the Technical College of Al-Mussaib, AL-Furat Al-Awsat Technical University for the period of time (1/9/2019 -1/4/2020), The experiment was in a laboratory to test the efficiency of hexane extract of garlic, thyme, and* mentha, *in controlling Varroa destructor on honey bees, Apis mellifera. The results showed that the thyme hexane extract had a high effect on Varroa mites that infected honey bees, where the mortality rates were (0.78, 2.67, 2.78, 3.00,and 3.33) mites / hour after (1, 24, 48, 72, and168) hour respectively. In a row with little effect on bees, It achieved the lowest mortality rate (2.44, 3.00) bees / hour after (24, 48) hours respectively . While the hexane extract of garlic showed less effect on varroa mites where it reached (0.11, 1.44, 2.00, 2.11,and 1.89) varroa / hour after (1, 24, 48, 72,and 168) hours of treatment, which was not significantly different from the hexane extract of* mentha *. While the effect was high on bees, reaching (4.78, 5.22) bees / hour after 48,168 hours of treatment. respectively* .use (while) too much in all paper

***Keyword:*** *bees; Varroa parasite; garlic*; *honey.*

## 1. INTRODUCTION

The Western honey bee (*Apis mellifera*) is one of the most common types of honey bees in the world, and it is adopted mainly in Iraqi apiaries for its suitability to the natural environment and for its economic importance and ease of dealing with it, especially in modern methods of breeding. Despite this importance, the profession of beekeeping in Iraq is still facing many problems that prevent its development, which made it unable to meet the population's need for bee products, especially honey [1]. Honey production is an essential resource for many people working in this profession [2] that honey bees are important in many fields and in various parts of the world, as it has become a science and a profession at the same time, and bees, like other organisms, are exposed to many pests and diseases, including the varroa mite (Varroa destructorIt). It is considered one of the main pests on honey bees, where the bee community deteriorates significantly in colonies infected with Varroa [3]. Varroa parasite attacked honey bees at the beginning of the twentieth century in areas of East Asia [4]. Varroa plays a major role in the loss of bee colonies due to the damage caused by the Varroa mite within colonies, which leads to a reduction in the percentage of reproduction, mortality of Larval, distortion of embryos, as well as the transmission of many fungal, bacterial and viral diseases [5],[6]. Many chemical pesticides have been used to combat it, but the widespread misuse is, It led to contamination of its products and its impact on the consumer as well as the appearance of resistance trait in Varroa mite to these pesticides [7]. Therefore, the

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search for safe alternatives to the consumer and the environment began, so natural materials were used, and their first use was with bees in 1965.Where menthol was used to control Varroa, the use of plant extracts continued and it took an advanced role in controlling the Varroa parasite, especially the use of essential oils such as cumin oil,Thyme and other oils [8] Numerous studies have shown that essential oils have an anti-microbial effect and are toxic to fungi and insects for many pathogens and pests under laboratory and field conditions [9]. Thus, oils have become a desirable alternative to chemical pesticides due to their plant source and their wide biological toxicological, harmful and inhibitory effect on parasite feeding and its development [10]. Therefore, this research aimed to study the effect of these hexania plant extracts and to study their effect on Varroa and bees in a laboratory.

## 2. MATERIALS AND METHODS

### *2.1. Laboratory study*

To demonstrate the effect of various treatments on the Varroa mite, wooden boxes have been prepared with special dimensions (length 23 cm, width 15 cm, height 14 cm) and inside them are wax frames and the box contains an opening in the front and the top cover of a flip box. Where (20 healthy bees and 10 infected bees) were placed in each box (one infected bee contains one mite parasite) and provided with pollen and a sugar solution to feed it, and (9) boxes were made with (3) boxes per treatment, (three concentrations for each type of extracts). Vegetable for garlic, thyme and mentha. Where the infected and healthy bees are sprayed at the same time at a distance of 15 cm by spraying the treatments separately and then the readings were taken after (1, 24, 48, 72, and 168) hours of treatment, Based on previous studies (Majid Mahmoud) to find out residues of the extracts.

### *2.2. Collecting and preparing samples of the plants under study*

Plant samples (garlic, mentha, thyme) were collected from local markets and washed separately to remove impurities from them. Then they were dried at room temperature with stirring constantly to prevent rotting. Then they were milled with an electric grinder at a speed of 2500 rpm. The plant powders were formulated separately. Based on previous studies that showed their effect in controlling Varroa, because they contain repellants or killers.

### *2.3. Extraction of hexane plants*

The hexane extract of plants (garlic, mentha, and thyme) were prepared. 300 gm of vegetable powder were taken separately and put in a 1000 ml glass beaker and then hexane was added to it both separately.The plant material was mixed with an electric mixer (Electric Shaker) for 15 minutes and then leave the solution for 24 hours (for a better extraction), filter the solution with a damp cloth or cotton used a Buechner funnel and take the filtrate. Then the foreign materials were deposited using a Centrifuge at 3000 rpm for 10 minutes. Then it was placed in a vacuum evaporator (Vacunm Rotary evaporatore) at a temperature of (40-45) C for the purpose of obtaining the raw material for the plant extract. Then it was stored in small opaque glass bottles and were sealed and placed in the refrigerator until use. According to Al-Mansour [3] with some modifications, the method of spraying with plant extract was applied.

### *2.4. Statistical analysis*

The experiment results were conducted according to Completely Randomized Design (C.R.D). Then, the data were statistically analyzed for all the studied traits and for each experiment separately in the Gnestat program, and the results were compared by using the lowest significant difference test L.S.D at the level of (0.05) to test the significance in comparing results [11].

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## 3. RESULTS AND DISCUSSION

*3.1. The effect of using hexane extract of plants (garlic, thyme,* mentha*) and Varroa strips on the numbers of Varroa in the laboratory*

The results in Table (1) showed that there were significant differences between the hexane extract (for garlic, thyme, and mentha) in the mean numbers of Varroa killed in the laboratory.The thyme hexane extract achieved the highest average (0.78, 2.67, 2.78, 3.00,and 3.33) varroa / hour. Compared to the garlic hexane extract , which achieved the lowest average of (0.11, 1.44, 2.00, 2.11,and 1.89) varroa / hour, which did not significantly differ from the hexane extract of mint after (1, 24, 48, 72, and168) hours of consecutive treatment. Since mentha leaves are often used to repel mosquitoes, and mentha oil is used as an insecticide for its ability to kill common insects, they used garlic to prevent insects from approaching such as mosquitoes [12]. It appears from the results in Table (1) that there are no significant differences between the concentrations of plant extracts (1000, 2000,and 3000 ppm) in the mean numbers of Varroa killed laboratory (1, 24, 48, 72,and 168) hours of treatment, respectively.The effect of plant extracts on the number of killed Varroa was independent of the effect of the concentration used. As for the interaction between plant extracts and their concentration, the effect was not significant on the number of mites killed in the laboratory.

*3.2. The effect of using hexane extract of plants (garlic, thyme,* mentha*) and Varroa strips in preparing laboratory killed honey bees*

Table (2) showed that there was a significant effect of plant extracts on the numbers of laboratory killed bees. The hexane extract of thyme achieved the least effect in preparing the killer bees, where it reached (2.44, 3.00) bees / hour after (24, 48) hours of treatment, respectively. While the hexane extract of mint gave the highest average of (3.22, 4.33) bees after (24, 72) hours of treatment respectively . The garlic hexane extract gave the highest average (4.78, 5.22) varroa / hour (48, 168) hours of treatment. The plant extracts will not differ significantly in the effect on this trait after an hour of treatment, and the reason may be due to the oils contained in the materials used have a high correlation and thus slow activity, which leads to the release of the active substance in the form of successive layers that express the release of the substances with biological activity associated with a time Longer than the natural time for the work of these materials and this time is days and even years [13],The concentration (1000, 2000, and 3000) ppm differed significantly by the effect on preparing the killed bees experimentally, where the concentration (3000 ppm) achieved the highest average, reaching (3.56, 4.33, 4.67, and 5.22) bees per hour. In compared to the concentration (1000 ppm), which achieved the lowest average of (2.44, 3.33, 3.11,and 3.56) bees / hour after (24, 48, 72, and168) hours of treatment, respectively. The significance of concentration did not differ in this traits after one hour of treatment. From the results in tables (1 and 2) it appears that thyme hexane extract (2000 ppm) and concentration (1000ppm) were the best two treatments affecting the effect of the killed varroa preparation, as well as they were the least effective in preparing the killed bees in the laboratory, which may be due to thymol and Carvacrol. They cause high mortality in Varroa without having a significant negative effect on bees [14]. It appears from the results in Table (2) that there is no significant effect of the interaction between the two study factors on the average number of bees killed laboratory.

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**Table 1. The effect of using hexane extract of the plant (garlic, thyme, mentha) and Varroa strips on the number of Varroa killed in laboratory**

| After week | | | | After 72 hour | | | | After 48 hour | | | | After 24 hour | | | | After hour | | | | Extract type |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Average | Concentration ($mg.L^{-1}$) | | | Average | Concentration ($mg.L^{-1}$) | | | Average | Concentration ($mg.L^{-1}$) | | | Average | Concentration ($mg.L^{-1}$) | | | Average | Concentration ($mg.L^{-1}$) | | | |
| | 3000 | 2000 | 1000 | | 3000 | 2000 | 1000 | | 3000 | 2000 | 1000 | | 3000 | 2000 | 1000 | | 3000 | 2000 | 1000 | |
| 1.89 | 2.00 | 2.00 | 1.67 | 2.11 | 2.33 | 2.00 | 2.00 | 2.00 | 2.33 | 2.00 | 1.67 | 1.44 | 1.67 | 1.33 | 1.33 | 0.11 | 0.33 | 0.00 | 0.00 | **Garlic** |
| 3.33 | 3.67 | 3.00 | 3.00 | 3.00 | 3.33 | 3.00 | 2.67 | 2.78 | 3.00 | 2.67 | 2.67 | 2.67 | 3.00 | 2.67 | 2.33 | 0.78 | 1.00 | 0.67 | 0.67 | **Thyme** |
| 2.44 | 2.67 | 2.33 | 2.33 | 2.33 | 2.67 | 2.33 | 2.00 | 2.11 | 2.33 | 2.00 | 2.00 | 2.00 | 2.33 | 2.00 | 1.67 | 0.33 | 0.33 | 0.33 | 0.33 | **mentha** |
| 0.81 | n.s | | | 0.63 | n.s | | | 0.43 | n.s | | | 0.60 | n.s | | | 0.47 | n.s | | | **L.S.D 0.05** |
| | 2.78 | 2.56 | 2.33 | | 2.78 | 2.44 | 2.22 | | 2.56 | 2.22 | 2.11 | | 2.33 | 2.00 | 1.78 | | 0.56 | 0.33 | 0.33 | **Average** |
| | n.s | | | | n.s | | | | n.s | | | | n.s | | | | n.s | | | **L.S.D 0.05** |

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**Table 2. The effect of using hexane extract of the plant (garlic, thyme, mentha) and Varroa strips on the numbers of killed bees in laboratory**

| After week | | | | After 72 hour | | | | After 48 hour | | | | After 24 hour | | | | After hour | | | | Extract type |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Average | Concentration ($mg.L^{-1}$) | | | Average | Concentration ($mg.L^{-1}$) | | | | Concentration ($mg.L^{-1}$) | | | Average | Concentration ($mg.L^{-1}$) | | | Average | Concentration ($mg.L^{-1}$) | | | |
| | 3000 | 2000 | 1000 | | 3000 | 2000 | 1000 | | 3000 | 2000 | 1000 | | 3000 | 2000 | 1000 | | 3000 | 2000 | 1000 | |
| 5.22 | 6.33 | 5.33 | 4.00 | 4.11 | 5.33 | 4.00 | 3.00 | 4.78 | 5.33 | 5.00 | 4.00 | 3.11 | 3.67 | 3.00 | 2.67 | 0.11 | 0.33 | 0.00 | 0.00 | **Garlic** |
| 3.44 | 4.00 | 3.33 | 3.00 | 3.22 | 3.67 | 3.33 | 2.67 | 3.00 | 3.33 | 3.00 | 2.67 | 2.44 | 3.00 | 2.33 | 2.00 | 0.00 | 0.00 | 0.00 | 0.00 | **Thyme** |
| 4.56 | 5.33 | 4.67 | 3.67 | 4.33 | 5.00 | 4.33 | 3.67 | 3.89 | 4.33 | 4.00 | 3.33 | 3.22 | 4.33 | 3.00 | 2.67 | 0.22 | 0.33 | 0.33 | 0.00 | **Mentha** |
| 0.74 | n.s | | | 0.66 | n.s | | | 0.79 | n.s | | | 0.38 | n.s | | | n.s | n.s | | | **L.S.D 0.05** |
| | 5.22 | 4.44 | 3.56 | | 4.67 | 3.89 | 3.11 | | 4.33 | 4.00 | 3.33 | | 3.56 | 2.78 | 2.44 | | 0.22 | 0.11 | 0.00 | **Average** |
| | 0.74 | | | | 0.66 | | | | 0.79 | | | | 0.38 | | | | n.s | | | **L.S.D 0.05** |

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## 4. CONCLUSIONS

The hexane extract of thyme was distinguished by its percentage of killing Varroa, but It did not continue to shed the Varroa linearly, but change it the effect faded after 72 hours.While it had less effect on the numbers of killed bees, while change it the hexane extract of garlic and mentha gave less effect on Varroa and higher for bee numbers.

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