# Study of the effect of hexane oil extract of Moringa oleifera seeds on different stages of the greater wax moth Galleria mellonella L. (Lepidoptera: Pyrallidae.(

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**Abstract**

Honey bees Apis mellifera L. are of great economic importance through their role in the pollination of agricultural crops, where they contribute approximately 75%, either completely or partially, to the pollination process, thus contributing to increasing agricultural yields. Honey bees are exposed to a variety of pests, the most important of which is the greater wax moth G. mellonella L., which causes economic losses for beekeepers. This study, conducted at the College of Technology - Al-Musayyab / Department of Biocontrol Techniques for the period 2024/2025, aimed to test effect of the hexane oil extract of Morinka M. oleifera seeds on the larvae of the greater wax moth G. mellonella L.

The results of the extract showed effectiveness in the mortality rates of the third larval stage of the greater wax moth, with significant differences between the concentrations of the extract in the mortality rates depending on the concentration and duration of exposure, as the highest mortality rate was reached at the concentration (10 mg / ml, where it reached (73.3)% within a week of treatment, while the lowest mortality rate was at a concentration of (2.5) mg/ml, reaching 40.0% during the first day of treatment, while the highest mortality rates for the fifth larval stage reached (63)% within a week at a concentration of (10) mg/ml, and the lowest mortality rate was 33% at a concentration of (2.5) mg/ml, while control mortality rate was 0.00%). This indicates the possibility of introducing the oil extract of Moringa seeds into integrated pest management programs to control the greater wax moth G. mellonella L. that infects the wax frames in honeybee hives stored in warehouses and reduce its damage. And conduct more studies on the active compounds and their effects on honeybees found in Moringa seed oil M. oleifera

**Keywords : G. mellonella L., M. oleifera Apis mellifera L, mortality rate**

## Introduction

The honeybee Apis mellifera is one of the species belonging to the order Hymenoptera, and is distinguished by its great economic importance to humans due to its production of a group of valuable products such as honey, wax, bee venom, propolis, and royal jelly, and these products have great nutritional and medical benefits [10]. Bee hives face many pests and diseases that cause economic losses, leading to a significant decrease in the number of healthy colonies for beekeepers, in addition to the loss of bee products[4,17] . The greater wax moth Galleria mellonella L. is an important pest that causes damage to wax frames, especially inside weak cells as well as stored frames. The larvae (Lrvae) feed on it because it contains honey, pollen, and the remains of the shed skins of bee larvae and pupae, which causes severe damage to the wax frames and digs tunnels. It also spins silk threads, which leads to their destruction and their use is never beneficial [15]. The use of chemical pesticides leads to the emergence of resistance in insects and also negatively affects non-target organisms. Therefore, it is necessary to find appropriate solutions to

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combat these pests, which calls for reducing the use of chemical pesticides and moving towards the use of alternative control methods with modern and developed techniques that are safe for bees and the environment and are included in the integrated management programs used to combat the greater wax moth [9] In addition to the direct and residual effects of other chemical pesticides that contribute to environmental pollution, disrupting the natural balance of other ecosystems, and their ability to cause cancer and genetic changes, as well as the problem of high economic costs in their manufacture [7.[

Researchers are seeking to find alternative, effective and environmentally friendly means to combat insect pests, by adopting new methods and techniques [6]. Among these methods, biological control has emerged, which relies on the use of bacterial pathogens of insects, in addition to predators, parasites and fungi. Modern methods also include the use of pheromone traps, physical control, and the use of plant extracts that show effectiveness against insect pests. These extracts are characterized by their low toxicity to non-target organisms, and they also limit the emergence of insect resistance compared to synthetic chemical pesticides[1,11,14] . In addition to being less harmful and less expensive than chemical pesticides by acting as anti-feeding, repellent or growth inhibitors [5] The study aims to test effectiveness of the hexane oil extract of Moringa oleifera seeds on the third and fifth stages of the greater wax moth G. mellonella L.

-2Materials and methods

-2-1Place of the experiment

The experiment was conducted in the Entomology Laboratory for Postgraduate Studies for the year (2024-2025) / Department of Biological Control / Al-Musaib Technical College / Al-Furat Al-Awsat Technical University.

2-2 -Insect rearing. Galleria mellonella L

The wax infected with the greater wax moth G. mellonella was obtained from different apiaries in the holy Karbala province . The infected wax was cut into small pieces containing the stages of the greater wax moth G. mellonella and placed in The plastic bottles were 10 cm in diameter and 30 cm in height, and were fed with some drops of honey throughout the study period and were monitored until the adults emerged. The mouth of the plastic bottles was closed with a piece of cloth and fixed with rubber bands to prevent the adults from emerging and to ensure ventilation. After the mating process, a new generation was produced, starting from the egg (Eggs), passing through the larval stages (Larvae), pupae, and then adults. After that, they were monitored every day until the larvae reached the third and fifth stages, then the third and fifth stage larvae were separated, and the rearing vessels were placed in the incubator at a temperature of 35 °C (Singh, 2014). The data were analyzed according to the factorial experiment model and Facepperiments with completely randomized Design (C.R.D) and using the least significant difference (L.S.D) test at the level of Probability 0.05) To compare the significance of significant differences between treatments, the percentage of mortality was corrected according to the Abbott equation (Abbott, 1925.

Corrected mortality percentage = □((control treatment in mortality % - treatment in mortality %)/(control treatment in mortality % - 100)) × 100

The corrected values were converted to angle values for inclusion in the statistical analysis[2.[

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The statistical program (2011) (GenStat) General Statistics was used in the statistical analysis.

-2-3Preparation of hexane oil extract of Moringa oleifera seeds

The seeds of Moringa oleifera were collected from a farm located in the Musayyib project area and 10 gm of seeds were prepared with 200 ml of hexane alcohol and placed in the Soxhlet extractor located in the laboratory of the Department of Biocontrol Technologies / Al-Musayyib Technical College. The extraction process was repeated until the desired amount was reached and used in the study. After that, the seeds were ground using an electric mill to complete the washing of secondary compounds within a period of 24 hours. After that, the sample was placed in filter paper after wrapping the filter paper in the form of (Kulla) and closing the sides of the filter paper with cotton and wrapping the filter paper with a white cotton thread. The sample was placed inside the device. After that, the extract was taken and placed in an electric oven at a temperature of 40-45 C to get rid of the alcohol. After that, it was kept in the refrigerator at a temperature of 4 C until use (Harborn, 1984). To determine the biological activity of the crude hexane oil extract of Moringa oleifera seeds, 10 ml of the oil extract was taken and dissolved with 10 ml of hexane alcohol and 6 ml of the spreading substance (Tween 20) and the volume was completed to 100 ml of distilled water [8]. The concentration of the basic solution became 10% or equivalent to 100 mg/ml and concentrations (2.5, 5, 10) mg/ml were prepared from it, respectively, and the control treatment was 10 ml of hexane alcohol and then the volume was completed to 100 ml of distilled water.

Results and discussion

-3-1Effect of the hexane oil extract of Moringa oleifera seeds on the third larval stage of the greater wax moth Galleria mellonellaL.

The results of the statistical analysis showed that the hexane oil extract of Moringa seeds M. oleifera showed effectiveness in killing the third larval stage of the greater wax moth G. mellonella L. in the laboratory, as significant differences appeared between the concentrations of the extracts and the control treatment, and the percentage of death increased with increasing the concentration of the extract and the length of exposure, and there was no significant difference between the third and fifth days. The results showed that the lowest level of death was recorded at the (2.5) mg/ml treatment, as the percentage of death of the third larval stage treated with the M. oleifera extract reached (40.0)% on the first day of the treatment, while the highest level of death reached (73.3)% at a concentration of (10) mg/ml during a week, while the control treatment did not give any percentage of death, as it reached ((0.00%), as shown in Table (1.(

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**Table (1) Effect of the hexane oil extract of Morinka M. oleifera seeds on the mortality rate of the third larval stage of the greater wax moth G. mellonella L.**

| Concentrations mg/ml | Percentage of third instar larval mortality after / day | | | | Concentration rate |
|---|---|---|---|---|---|
| | 1 | 3 | 5 | 7 | |
| 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 2.5 | 40.0 | 53.3 | 56.7 | 56.7 | 51.7 |
| 5 | 53.3 | 60.0 | 70.0 | 70.0 | 63.3 |
| 10 | 63.3 | 70.0 | 70.0 | 73.3 | 69.2 |
| Average time period | 39.2 | 45.8 | 49.2 | 50.0 | |
| L.S.D (P≤0.05) | Concentration = 8.32 period =8.32 interaction =16.63 | | | | |

2--3Hexane oil extract of Morinka M. oleifera seeds on the fifth larval stage of the greater wax moth G. mellonella L.

The results of the statistical analysis showed that the hexane oil extract of Morinka M. oleifera seeds showed effectiveness in the mortality of the fifth larval stage of the greater wax moth G. mellonella L. in the laboratory, as significant differences appeared between the concentrations of the extracts and the comparison treatment, and the mortality rate increased with increasing the concentration of the extract and the length of exposure, and there were no significant differences between the third and fifth days. The results showed that the lowest mortality level was recorded at the treatment (2.5) mg/ml, where the mortality rate of the third larval stage treated with M. oleifera extract reached (33.3)% on the first day of treatment, while the highest mortality rate was recorded (63.3)% at a concentration of (10) mg/ml during a week, while the control treatment did not give any mortality rate, reaching (0.00)%. As shown in Table (2(

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**Table (2) Effect of the hexane oil extract of Morinka M. oleifera seeds on the mortality rate of the fifth larval stage of the greater wax moth G. mellonella L.**

| Concentrations mg/ml | Percentage of fifth instar mortality after / day | | | | Concentration rate |
|---|---|---|---|---|---|
| | 1 | 3 | 5 | 7 | |
| control | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 2.5 | 33.3 | 46.7 | 46.7 | 46.7 | 43.3 |
| 5 | 43.3 | 46.7 | 53.3 | 56.7 | 50.0 |
| 10 | 43.3 | 60.0 | 60.0 | 63.3 | 56.7 |
| Average time period | 30.0 | 38.3 | 40.0 | 41.7 | |
| L.S.D (P≤0.05) | Concentration = 6.68 period= 6.68 interaction =13.37 | | | | |

Moringa seed oil contains active compounds, which are oleic acid (84%), ascorbic acid (L-(+)-ascorbic acid-2) (9.80%), octadecenoic acid (9-octadecenoic acid) (1.88%), and methyl ester-hexadecanoic acid (1.31%) (Aja et al., 2014). The cause of the death of the larvae of the waxworm G. mellonella L. may be attributed to the active compounds contained in this oil. This study agrees with what [12] did that Morinka extract has the ability to reduce or decrease the number of mosquitoes Culex gelidu and thus we conclude that it has the properties of insecticide action. This study agrees with what was mentioned by ((1990, Hoppe that the hexane extract of Morinka seeds contains active phenolic compounds, especially the compound (Eugenol) which kills by contact and fumigation. It also agrees with what was mentioned by the study that Morinka seed extract is considered an insect repellent or anti-feeding substance [12]. It agrees with what was mentioned by [13]. The ability of Morinka seed extract is due to the Morinka seeds containing chemicals that are repellent or lethal to insects, especially thymol compounds. This study is consistent with a study conducted on effect of the alcoholic extract of Moringa leaves M.oleifera on whiteflies and aphids in a laboratory experiment, where the results showed that a concentration of 3 g/L was the most effective in controlling the targeted insects, achieving mortality rates of 95% in the nymph stage and 91% in the adult stage. While a concentration of 0.5 g/L was the least effective in controlling these insects [16]. Thus, plant extracts contain active compounds, including medicinal plants, which affect the enzyme Protase in the midgut and also affect the percentage of sugar and protein in the blood of insects [3[

**-4Conclusion**

-A We conclude that effect of the hexane oil extract of the seeds of the Morinka plant M. oleifera on the third larval stage was more due to the sensitivity of the first stages, as the highest mortality rate reached 73.3% at a concentration of 10 mg/ml, while the mortality rate in the fifth stage reached ((63.3% at the same concentration. To determine the benefits of the extract in the apiary, more studies are

required according to these results. It is also necessary to know effective and biologically active compounds, which is essential to combat the greater wax moth G. mellonella L.

-B Also, increasing the concentration leads to increased mortality rates.

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