

## EFFECT OF HEXANE EXTRACT OF *MORINGA OLEIFERA* AND *ROSMARINUS OFFICINALS*, ON THE RATE OF DESTRUCTION OF THE *VARROA DESTRUCTOR* OF HONEY BEES IN THE FIELD

Mohammed Ali Abdulhasan, Marzah Hamzah Hadi and Adil Abaed Hassoni

AL-Musyib Technical College, Al-Furat Al-Awsat Technical University, Iraq.

Emails: [mhmedaaligmil.com@gmail.com](mailto:mhmedaaligmil.com@gmail.com) , [Marzarussia@gmail.com](mailto:Marzarussia@gmail.com) , [dr.adil\\_aa@yahoo.com](mailto:dr.adil_aa@yahoo.com)

### Abstract

The study was conducted in the province of Babylon in one of the apiaries in the village of Al-Mahnawiya. The aim of the study was to evaluate the efficacy of the hexane extract of Morinca and rosemary plants on the rate of destruction of the Varroa parasite and its effect on honey bees. The concentrations used for both extracts were (1, 1.5, 2) g/cell, respectively. The results showed the superiority of the hexane extract of the Morinca plant at a concentration of 2 g/cell, 1 g/cell, and 1.5 g/cell over rosemary extract at a concentration of 2 g/cell, 1 g/cell, and 1.5 g/cell, with a significant difference between all concentrations and for all time periods. The highest rate of death for Varroa at a concentration of 2 g/cell was 22.16 after 48 hours, and the lowest rate of death for Varroa after 144 hours for rosemary extract at a concentration of 1 g/cell 0.66. As for the effect of hexane extract of moringa and rosemary plants on honey bees, it was the highest effect For Morinca hexane extract, after 40 hours, at a concentration of 2 g/cell, it reached 2.41, and the least effect was for the hexane extract of rosemary plants, after 64 hours, it was 0.10 at a concentration of 1 g/cell. The results indicated that as the time increases, the effect decreases.

**Keyword:** hexane extract, *Moringa oleifera*, *Rosmarinus officinals*, *Varroa destructor*, honey bees.

### Introduction

The honey bee, *Apis mellifera*, belongs to the family Apidae and the order Hymenoptera. It is a social insect that lives in a sect in protected places. Which are misleading, as they are chosen by scout bees, or human-made cells, as they are self-explanatory typical specifications (Li *et al.*, 2016). These sects continue to work throughout year, but it is affected by the weather and environmental conditions surrounding the hive in terms of the abundance of cover Vegetation, Temperature and Humidity (Humagain, 2016).

There has been a decline in the number of European honey bees since the year 1970 of the last century The decline has continued to this day (Lozano *et al.*, 2019). Many have confirmed From scientific research and studies at the global level, which reached many factors Which is responsible for reducing the numbers of bees, and one of the most important and most important of these factors is the Varroa parasite *Varroa destructor* (Trueman and Anderson, 2000) as well as diseases that It is caused by *Varroa mites* on honey bees, which are more effective on bees (Kusza and Zakar, 2014). Many beekeepers suffer huge losses due to *Varroa destructor* As it is one of the most important and dangerous pests that affect honey bees and cause many diseases Which eventually lead to the destruction of honey bee hives and reduce their production (Mikheyev and

Eliash, 2020). Because of the large losses, the beekeepers were forced to use chemical pesticides. It is considered one of the most common methods (Haber *et al.*, 2019).

And the excessive use of these products led to contamination of bee products from honey and wax, as well as led to the acquisition of Varroa. The ability to resist the pesticide, and there are also chemical residues in the cells that were detected. These residues are Amitraz, Bromopropylate, Coumaphos, Fluvalinate and CAN. The presence of these substances in honey and its products (Jamal *et al.*, 2020). Thus, products from bees are unfit for human consumption because they cause many diseases (Lozano *et al.*, 2019). Therefore, all studies tended to reduce the use of chemical pesticides and use the means relatively safe, which does not cause harm to bees or their products, as well as the inability of the Varroa parasite to form the characteristic of resistance to these products, through natural materials that are amenable. It is degradable and does not leave residues on bee products and does not cause harm to the environment or to humans. Among these products are natural plant extracts, which are considered one of the safest methods of control (Refaei, 2011; Adang *et al.*, 2014).

#### **Aim of the present study :**

Evaluation of the efficacy of the hexane extract of *Moringa oleifera* and *Rosmarinus officinalis* on the rate of *Varroa destruction* and its effect on honey bees.

#### **Materials and methods**

##### **Collect plant samples**

The plants used in the study were obtained from nurseries located in Babylon Governorate Hilla Center and Al-Musayyib district, after the plants were diagnosed by Prof. Dr. Huda Jassim Muhammad Al-Tamimi, a teacher at the College of Girls' Sciences at the University of Babylon, and diagnosed her as both vegetarian *Moringa oleifera* and *Rosmarinus officinalis*.

##### **Plant extracts**

##### **Hexane extract of Moringa and Rosemary**

After the plants were diagnosed, the plant parts required to be dried were dried, and seeds were used. The dried Moringa leaf as well as the leaves of the Rosemary plant were then placed into the vegetable parts. It is required to prepare the extract from it in an electric mill and grind it into small parts, but it should not be powdery so that it does not run out of filter paper, then make a tumble using filter paper. And roll the paper in the form of a cylinder and there is the weight of the powder to be extracted. It was placed in the Soxhlet apparatus, and then hexane alcohol was used, and it was placed inside the beaker, and it was a degree. The boiling point of the alcohol is 60 C. The weight of the sample was 20 grams per 200 ml of alcohol. The extraction process took place after 48 continuous hours until the color of the alcohol reached the sample to white. This indicates that the extraction process has been successfully completed and the extract has been collected from the beaker at the bottom of the device and placed in petri dishes, then the dishes were placed in the oven at a temperature of 35 degrees Celsius until the liquid reached a solid or viscous consistency, and this indicates that the alcohol has been evaporated. After that, the extracted material was placed in a tightly closed glass tube and placed in a container Refrigerate at 3°C in darkness until use (Harbourene, 1999). The required concentrations in the study were 1, 1.5 and 2 g/cell.

### **Detection of infection in bee hives**

The infection was detected in beehives located in Al-Mahnawiya district in Babylon Governorate. By using powdered sugar, samples were collected from infected bees and placed in containers that have a volume of 1000 ml and the packaging contains a clip that allows Varroa permeation falling on the board. A sheet of A3 paper was put on it, greased with Vaseline, to help Varroa adhesion. The number of bees used in each detection time was 75 bees, and the total 225 bees from each hive. The bees were placed in the plastic package and the mouth of the box was closed. Then powdered sugar was sprinkled for the purpose of separating Varroa from bees by shaking the bees in the box for two minutes. Then the package was placed upside down so that it would fall Varroa from the mouth of the can containing a clip that allows Varroa to penetrate from inside the can. After that, the severity of the injury was calculated according to the equation (Allen, 2004).

### **The severity of the infection in the cell:**

$$\text{Infection intensity of the cell} = \frac{\text{Varroa group numbers}}{75 \text{ adult bees}} \times 100\%$$

### **Prepare the cells for the study**

The cells were prepared for the study in order to maintain the strength of the cells during the study period. As I used the Turkish type candy dough, which consists of pollen and sugar and vitamins and used a sugar solution consisting of water and sugar 50% and put inside the cell for a month throughout the study period (Somervillw, 2000).

### **Study the effect of hexane extract of Morinca seeds and rosemary leaves on the Varroa parasite**

The hexane extract obtained from the previous paragraph was used. The required concentrations were prepared from it in the study, and the required concentrations were 1, 1.5, 2 g/cell, then put the required concentration in a 50 ml sprayer. This sprayer is sufficient for single cell treatment, spraying was done at a distance of 15 cm after the sprayer from the cells (Hajij *et al.*, 2004). Each hive was sprayed from the inside of the wood and tires as well as wax and workers and the queen in full and readings were taken after 24, 48, 72, 96, 144 hours and for a month if the treatment was repeated for each cell 4 times during the study period if it was treated cell 4 times throughout the study period. Then the results were taken and recorded in a special record and analyzed statistically.

### **Study the effect of hexane extract of moringa seeds and rosemary leaves on Honey bee workers**

After the cells were treated with hexane extract to show its effect on Varroa. The effect of the extract on honey bee workers indoors was also studied. The cells were taken by placing the queens barrier at the entrance to the cells. The reading was recorded weekly after each treatment for a period of (15, 40, 64) hours. The results are in a special table and analyzed statistically.

### **Results and discussion**

**Table (1): Effect of the hexane extract of Moringa and Rosemary plants on the rate of destruction of the Faroe parasite.**

Treatment	Cocentration cfu/ml	Reading rate after 24 hours	Reading rate after 48 hours	Reading rate after 72 hours	Reading rate after 96 hours	Reading rate after 144 hours
MO H	1 g	4.08	3.91	3.75	2.66	1.33
	1.5	7.16	9.08	8	5.41	4.08
	2	20.66	22.16	17.91	12.66	8.25
RO H	1	2.25	2	1.5	1.16	0.66
	1.5	5.83	6.25	4.91	3.41	2.41
	2	15.66	17.33	14.25	9.66	6.16
Control		1.58	1.66	0.25	0.83	0.33
Natural shedding	Without treatment	1.25	0.83	0.91	1	1.08
Pesticides	2Bars	49.25	48.58	37.08	33.03	25.16
LSD P< 0.05		2.035	1.478	1.687	1.765	1.235

The results showed table ( 1 ) the superiority of the apistan pesticide treatment over all treatments and in all time periods, and the reason for using the pesticide for the purpose of comparing the results of any of the extracts gave a high mortality rate. The Varroa parasite affects it either through contact or through the stomach, as the highest effect of the pesticide after 24 hours was 49.25 and the lowest effect was after 144 hours 25.16. Followed by the Hexane Morinca seed extract, as it was superior to the Hexane rosemary extract in all concentrations and time periods, as the highest effect of the Morinca seed extract after 48 hours was 22.16, and the lowest concentration after 144 hours was 8.25. Moringa’s dream-killing effect is due to its containment of thymol, which is considered an insect repellent (Sarhan *et al.* 2022).

Followed by the rosemary treatment, as it outperformed the natural precipitation treatment and the comparison treatment with a significant difference As the highest death rate after 48 hours was 17.33, and the lowest death rate after 144 hours was 6.16. The reason for this is due to the fact that rosemary contains cineole and camphor, which is considered a mite killer (Hcini *et al.*, 2013).

**Effect of hexane extract of Morinca seeds and rosemary leaves on the mortality rate of honey bees**

The results of table ( 2 ) showed the effect of the hexane extract of Morinca seeds on the worker honey bees, as the treatment of Morinca seeds with a concentration of 2 g/cell was superior to all the study treatments with a significant difference, as the highest effect of Morinca hexane seeds was after 15 hours at a concentration of 2 g/cell if the mortality rate was 2.5 And the least effect of the same concentration after 64 hours 2 , thus superior to the two natural precipitation treatments

and the comparison treatment, as well as the rosemary treatment. As for the treatment of rosemary hexane extract, there was no effect on worker bees Honey as it was outperformed by the natural precipitation treatment in all time periods and for all concentrations of the hexane extract of rosemary leaves.

**Table (2): Effect of the hexane extract of rosemary and Moringa plants on worker bees destruction of the Varroa parasite.**

Treatment	Cocentration cfu/ml	Reading rate after 15 hours	Reading rate after 40 hours	Reading rate after 64 hours
MO H	1 g	0.58	0.40	0.35
	1.5	1	0.56	0.44
	2	2.5	2.41	2
RO H	1	0.39	0.22	0.10
	1.5	0.40	0.19	0.16
	2	0.5	0.33	0.25
Control		0.38	0.29	0.20
Natural shedding	Without treatment	1.25	1	0.91
LSD : P< 0.05		0.600	0.566	0.423

### Conclusion's

The results showed the superiority of the hexane extract of *Moringa oleifera* plant over The effect of hexane extract of *Moringa oleifera* and rosemary *Rosmarinus officinals* on honey bees.

The highest effect of Moringa hexane extract was after 40 hours at a concentration of 2 g/hive.rosemary extract *Rosmarinus Officinals* at a concentration of 2 g/cell, 1 g/cell, and 1.5 g/cell.

### References

- Anderson DL & Trueman JWH. *Varroa jacobsoni* (Acari: Varroidae) is more than one species. *Experimental and Applied Acarology*. 2000;24(3):165-189 .
- Li, C. C., Wu, J. N., Yang, Y. Q., Zhu, R. G., & Yan, S. Z. (2016). Drag reduction effects facilitated by microridges inside the mouthparts of honeybee workers and drones. *Journal of Theoretical Biology*, 389, 1–10.
- Humagain, S. (2017). Life cycle of honey bee and uses of honey. Retrieved September 30, 2018, from <http://onlinesciencenotes.com>
- Adang, M. J.; Crickmore, N . and Jurat-fuentes, J. L. (2014). Diversity of *Bacillus thuringiensis* crystal toxins and mechanism of action. *Adv. Insect Physiol.* 47 ( 22): 39–87.
- Haber, A. I., N. A. Steinhauer, and D. vanEngelsdorp. (2019). Use of chemical and nonchemical methods for the control of *varroa destructor* (Acari: Varroidae) and Associated Winter Colony Losses in U.S. Beekeeping Operations. *J. Econ. Entomol.* 112: 1509–1525.

- Jamal**, M., Aziz, M.A., Naeem, M., Iqbal, Z., Khalid, A., Siddique, F., Khan, K.A. and Ghramh, H.A., (2020). Detection of flumethrin acaricide residues from honey and bees wax using high performance liquid chromatography (HPLC) technique. *Journal of King Saud University-Science*, 32(3), pp.2229- 2235.
- Zakar**, E., A. Jávora, and S. Kusza. (2014). Genetic bases of tolerance to *Varroa destructor* in honeybees (*Apis mellifera* L.). *Insectes Soc.* 61: 207–215.
- **Refaei**, G.S. (2011). Evaluation of Some Natural Substances Against *Varroa Destructor* Infesting Honeybee, *Apis mellifera* in Egypt. *Journal Agricultural Research*, 89(1): 169–175.
- **Eliash**, N. and Mikheyev, A., (2020). *Varroa* mite evolution: a neglected aspect of worldwide bee collapses?. *Current opinion in insect science*, 39, pp.21-26.
- **Lozano**, A., Hernando, M. D., Uclés, S., Hakme, E., & Fernandez-Alba, A. R.(2019). Identification and measurement of veterinary drug residues in beehive products. *Food Chemistry*, 274(April 2018), 61–70.