

Phytochemical Determination and Insecticidal Activity of some plants Extracts against Varroa pest *Varroa destructo*

Kamila Ward Shaher

Ibtisam Hussein Awwad

University of Baghdad / College of Agriculture

Abstract

This study was conducted in the College of Science for Girls / University of Baghdad for the period from 4/1/2022 to 31/5/2022 to demonstrate the effect and effectiveness of plant extracts (Peganum, cinnamon, propolis) by testing concentrations (1%, 2%, 3%, 3.25%) g /L as well as the use of Irwin , In addition to the control treatment (spraying with water) on the vitality of the Beehive and on the Varroa parasite, Where the laboratory experiment was conducted in the garden of the vegetable herb laboratories in the Department of Seed testing and Certification and the selection of the effective concentration on Varroa for all alcoholic and hexane extracts to see its effect on Varroa and bees. The results of the laboratory experiment of alcoholic and hexane extracts of plants (Peganum, cinnamon, propolis) showed that concentrations (1), (2) g /L do not affect the fall of Varroa pest, and that the concentration 3 g / L leads to the fall of Varroa without affecting the movement and shedding of workers. As for the concentration of 3.25 g /L , and through testing it was found that it leads to nervous movement of the workers and the death of some of them, so it was preferred to use the concentration of 3 g /L for all treatments.As for the results of the field experiment of alcoholic and hexane extracts of plants (Peganum, cinnamon, propolis), the best extracts effective in killing the Varroa pest was the alcoholic extract of Peganum, Where the average percentage of pest killing was 53.9%, which was significantly superior to all extracts.It was followed by the hexane cinnamon extract with a killing percentage rate of 47.3%, which was also significantly excelled on some other extracts but not others. The lowest percentage killing Varroa was 20.3% for the compared pesticide Irwin.There was no negative or harmful effect of all the plant extracts used in the experiment on the individuals and brood of bees and their products.

Introduction

Apis Mellifera L. A type of social insect that dates back to, APIS, Apidae family, Hymenoptera, and the known types of it are more than 20 thousand species, distributed around different countries and regions of the world, it lives in huge, cooperative and consistent groups in between them, this type of species has been prepared for human attention since previous ages (Al -Ali, 2011). Honey bees are infected with many fungal, bacterial and viral diseases as well as insect and non -insect pests, including the rhyme of the parasite of the Varua that affects the larvae, virgins and the whole insects of honey bees, Bees are infected by transferring brood from one cell to another or met during the soul, or as a result of the placement of the cells close to each other, or by chasing and sometimes due to migration (Al -Hasnawi,

2019), The Male brood is preferred because it contains the compounds of Benhu (5-6) twice the bosom of the workers, as well as the largest area and the longest period of reproduction of male costumes compared to the custody of the workers who move to the modern works at the age of (3-12) day (Dillier, 2006, Rosenkranz, 2010 ; Al-Jouraniet al, 2004,). The infection of honey beehs in Varua caused great economic losses, as thousands of sects in China and in Europe have deteriorated almost the late 1970s, while in Russia it was the largest rate of death in the winter, the losses of bee cells reached 100 thousand sects for the year 1971 (SAND, 2015), Varroa parasite ranks first in the Arab world in terms of the danger between the pests that affect honey bees, according to the questionnaire of the Arab Organization for Agricultural

Development (Hajij, 2014; Al-Hujaimi, et al, 2012,). Several chemical compounds were used in control Varroa , including amitraz is one of the locally popular pesticides. This pesticide is found in a solid or solution in a solution, and Apistan is used in the form of tapes (Muhammad, 2011.(The repeated and wrong use of it led to the emergence of the status of resistance, as well as pollution of bee products such as honey, wax, royal food, and propolis (Rosenkranz, 2010), Given the emergence of control against some of these pesticides, the use of natural alternatives, including plant extracts (GAREDEW et al, 2004.(Many plant extracts were used to combat Varroa, including: alcoholic extract for black cumin seeds, which gave Mortality rate high to Varroa , and the extract of thyme, mint and scarf, as the sheikh extract gave the highest Mortality rate to the Varroa , and the oil extract of thyme and the calamity gave thyme oil the highest rate of killing the paragraph of the Varroa , as well as the alcoholic extract of the Rosemary and other extracts of garlic, thyme and mint, in which the alcoholic extract of garlic excelled it by giving it the highest killing of the Varroa , as well as a large number of researchers who used types of plant extracts to combat the parasitic parasite (Hadi and others, 2012, Salibi, 2015, Al -Harami, 2017, Radi, 2021(And based on the data of previous research in the field of using plant extracts in treating the Varroa , plant extracts have suggested to study their effect on Varroa, Including the Cinnamon plant, peganum and propolis, a field and a laboratory against this pest .

Materials and methods:

1- Preparation of plant samples :

Peganum harmala L. seeds and Cinnamon Cassia peels were purchased .From the local markets, as for Propolis, it was purchased from some apiaries. The samples were prepared for extraction, where they were ground separately with an electric mill, the opening of which was 30-40 Inch..

2- Total crude extraction:

A - Crude alcoholic extraction:

Take 100 gm of powdered seeds and Cinnamon Cassia peels and propolis separately and add 250 ml of ethyl alcohol 85% in a 1 liter glass beaker at laboratory temperature. Leave the sample for 72 hours on an electric shaker. The extract was filtered using a Buchner funnel and What manNO.2 filter paper using vacuum. The filtrate was concentrated using a rotary evaporator with vacuum pump and at a temperature of 40-45 m to obtain the final extract, according to the method (Harpne, 1973).This process was repeated for several samples to obtain the sufficient quantity for the research, and the percentage of the extracted materials was calculated, and it was (6.2%, 4.3%, 3.2%) for the above models, respectively. The samples were kept in glass vials, labeled and kept in the freezer until the biocalcification process.

B- Crude hexane extraction:

The above method was followed except for the use of a 96% hexane solvent instead of ethyl alcohol. The models were concentrated at a temperature of 40-35 °C to obtain the crude hexane extract (Harbourne, 1973). This process was repeated for several samples to obtain the sufficient quantity for the research, and the percentage of the extracted materials was calculated, so it was (6.3%, 5.9%, 4.8%) for the above models, respectively.

3- Effect of concentrations of plant extracts and selection of effective concentration on Varroa:

For the purpose of determining the effective concentration on the Varroa pest, several concentrations were tested for the purpose of determining the most effective concentration on Varroa, and accordingly, the concentrations were tested (1%, 2%, 3%, 3.25%) g / L and for all alcoholic and hexane extracts of the selected plants(Peganum, cinnamon, propolis) preliminary concentration test was conducted to coundected the effective concentration by preparing 4 small nuclei of alcoholic and

hexane extracts to see their effect on Varroa and bees. It was found that the concentrations (1) and (2) g/L do not affect the fall of Varroa pest, and that the concentration 3 g/L leads to the fall of Varroa without affecting the movement and shedding of workers, As for the concentration of 3.25 g /L , and through testing it was found that it leads to nervous movement of the workers and the death of some of them, so it was preferred to use the concentration of 3 g /L for all treatments.

4- Laboratory study:

(12) wooden boxes were prepared with dimensions (length 23 cm, width 15 cm, height 14 cm). It contains an opening for ventilation from the front. The top cover of the box is a flap, inside it are wooden frames with dimensions (11 x 12) cm. A piece of wax was

fixed on it. The box contains a hole along the base with a height of 2 mm to allow placing white paper greased with Vaseline inside the box for the purpose of calculating fallen Varroa. Almost 30 infected and uninfected bees were placed in the box in each repetition and for each treatment. The small boxes were provided with pollen grains and a sugar solution to feed them, with 3 boxes for each treatment, representing three replicates, with a concentration of 3 g / liter. infected and healthy bees were sprayed at a distance of 15 cm with a hand sprayer, each separately, with an amount of (25) ml of the extract solution. The control was conducted in the garden of the vegetable herb laboratories of the Seed Examination and Certification Department on 1/4/2022, and the results were recorded by calculating the number of fallen Varroa.



(A) An internal view of the box with the waxy frame. (B) An external view of the breeding box

Figure (1) The wooden box with the wax frame for the laboratory experiment (23 x 14 x 15)

5- Field experiments:

Hive Initialization and Varroa Infection:

(24) Hive of hybrid Iraqi bees *L. Apis mellifera* were prepared from Iraqi apiaries, each parcel containing (5) frames of their queens fertilized in the spring of 2020. The parcels were placed in the University of Baghdad / College of Science for Girls to

conduct experiments on them. It was infected with the Varroa parasite by bringing two brood tires infected with Varroa from one of the infected apiaries in Abu Ghraib and Rashidiya, after making sure that they were infected with the Varroa parasite. The two tires were placed in two parcels prepared for the study. The appearance and spread of Varroa were monitored in them on a weekly basis, and after the appearance of the infection

and the observation of Varroa, the infection occurred in the rest of the packages (in the same way as before). The development of the infection was followed up by testing the cells weekly by taking the readings by placing a white paper greased with Vaseline at the bottom of each parcel for the purpose of calculating the number of fallen Varroa.

Use of various plant extracts in the fight against Varroa:

The frames of the parcels and between them and for all treatments were sprayed with (50 ml) of each extract solution separately, using a special hand sprayer for each solution (in addition to the three comparison parcels sprayed with water only and the same amount), After placing a white paper smeared with Vaseline under the frames of the parcels in order to stick the fallen Varroa on it, the numbers of Varroa that fell after spraying with the treatment solution were calculated in periods (72 hours, one week, two weeks), The results were analyzed statistically according to the design (R.C.B.D), the relative efficiency of the treatments was extracted according to the equation of Henderson (& Tilton, (1955):

Relative

Efficiency

$$\% = \left(1 - \frac{\text{The number of individuals after the treatment} \times \text{the number of individuals in the comparison before the treatment}}{\text{The number of individuals before the treatment} \times \text{the number of individuals in the control after the treatment}}\right) \times 100$$

The number of individuals after the treatment × *the number of individuals in the comparison before the treatment*

The number of individuals before the treatment × *the number of individuals in the control after the treatment*

100

7- Statistical design and analysis:

The data were analyzed statistically for all the studied traits of the field experiment according

to the The Randomized Complete Block Design (RCBD) and the results were compared using the significant difference test (L.S.D) at the level (0.05) to test the significance in comparing the results (Steel, 1980).

Results and discussion :

Effect of effective concentration 3 g/L water of different plant extracts on precipitation of Varroa in laboratory:

Table (1) that the best extracts effective in precipitation of Varroa are alcoholic Propolis extract, hexane cinnamon extract, alcoholic cinnamon and hexane propolis, where the precipitation rates of Varroa reached (3.6 and 4) Varroa, respectively. Statistical analysis indicated that there were no statistical differences. significant among them, but it excelled on all other extracts. As for the lowest rate of varroa precipitation, it was 2.6 varroa and for the alcoholic propolis extract, As for the rest of the rates of the other extracts, they fall in the middle between them, with the presence of significant statistical differences between some and not the others, where it is clear from the table that the average of the two solvents for the extracts for both alcohol and hexane, the total precipitation of Varroa reached 3.3 Varroa for both solvents, and the statistical analysis indicated that there were no differences significant statistic between them.

Table (4) Effect of effective concentration 3g/l water of different plant extracts on precipitation of Varroa in laboratory

average	The individuals' number of fallen Varroa	extracts	extraction method
3.3	4	Peganum	Alcohol
	3.3	cinnamon	
	2.6	propolis	
3.3	3.3	Peganum	hexane
	3.6	cinnamon	
	3.1	propolis	
1			control

- The least significant difference between the extracts at the level of 5% = 0.32

- The least significant difference between the solvents at the level of 5% = 0.15

The effect of using plant extracts (cinnamon, Propolis, propolis) and Irwin pesticide on field precipitation of Varroa in Jadriya:

It is clear from Table (2) that the best extract effective in killing Varroa pest is the alcoholic Peganum extract, as the average percentage of pest killing reached 53.9%, which was significantly excelled on all extracts. It was followed by the hexane cinnamon extract with a killing percentage rate of 47.3%, which was also significantly excelled on some other extracts without others, and this is due to the compounds contained in these extracts that are effective in killing Varroa pest, where the Propolis plant contains compounds (harmalin, harmine, (harmalol (Majrashi, 2006), as the cinnamon plant contains compounds, and these compounds may affect the behavior of Varroa pest or its neurological effect, and thus lead to its paralysis and fall, because it does not remain in its balance, and among these

compounds (Cinnamic aldehyde, Ugenol, Ugenol acetate, Polyphenols, Turbines, terpene alcohols) (Hariri, 2016). As for the lowest percentage rates of killing Varroa, it reached 20.3%, and the comparative pesticide Irwin, while the effect of other extracts is in the middle between them. This is clear in the table, as the increase occurs with the progression of the exposure periods (Shaaban, 1993, Al-Adel, 1973). This means that the pest inhaled a larger amount of chemicals, and with an increase in exposure (the length of the period) leads to an increase in the fall of Varroa with the expiration of the exposure period or in addition to the period of direct exposure (direct spraying), it also leads to disturbing the Varroa pest and causing it to fall to the bottom of the hive, or to both methods together, and this is more likely. It is also clear from the table that the periods had a clear effect on the percentage rate of killing Varroa, where the highest percentage rate of killing for the periods was at the two-week period, at an average of 71.1%. Followed by the period of one week, which was significantly superior to all other periods, with no significant statistical differences between one another and not the other.

Table (2) the relative efficiency of the effect of extracts and Irwin pesticide on the precipitation of Varroa numbers in Baghdad / Jadiriyah

average	Two weeks	week	72 hour	48 hour	24 hour	1 hour	0	The average of pest numbers before spraying	Treatments
53.9	83.1	87.3	73.7	77.2	56	0	0	4.3	Hexane cinnamon
40.4	72	68.6	49.5	66.8	26	0	0	5.3	Hexane Peganum
27.4	84.2	87.6	20.1	0	0	0	0	3.3	Hexane Propolis
40.5	79.7	81.1	47.7	48	27.3	0	0		averages
47.3	78.3	72	66.6	69.8	45	0	0	6	Alcoholic cinnamon
38.2	71.7	65.1	66.4	27.6	30.8	0	0	4.6	Alcoholic Peganum
24.2	59.8	61.3	34.5	14.1	0	0	0	7.6	Alcoholic propolis
36.5	69.9	66.1	55.8	3.8	25.2	0	0		averages
20.3	48.7	36.7	37	20.2	0	0	0	6.6	Irwin exterminator
	5.3	4.6	4	3.3	5.6	0	0	6.3	control

The least significant difference between the treatments at the 5% level = 4.6

- The least significant difference between the periods at the level of 5% = 8.1

- The least significant difference for the interaction between treatments and periods at the level of 5% = 14.5

- The least significant difference between the two solvents at the level of 5% = 3.3

- The least significant difference between the two solvents and Irwin pesticide at the level of 5% = 9.8

It is also clear from the table that the interaction between the extracts and the periods had a clear effect on killing the Varroa pest. The highest rate of the percentage of killing Varroa was 83.1% and for the alcoholic Peganum extract at the two-week period, which was significantly superior to all the interactions. The lowest rate of the percentage

of killing Varroa was zero for all extracts and at the two periods (zero, hour), and zero for the hexane propolis extracts, alcoholic propolis, and Irwin pesticide at the 24-hour period, and zero for the hexane propolis extract at the 72-hour period. It is also clear from the table that the total rate of relative efficiency for both the hexane and alcoholic solvents in dropping Varroa was 40.5% Varroa and 36.5% Varroa for both solvents, respectively. Irwin, which averaged 20.3% Varroa. As for the rest of the extracts with little effect, it is likely that they will not have a clear effect, and this may be due to the lack of the active substance in them or their effect is weak compared to the other extracts mentioned previously, or to the lack of purity of the solvent in extracting the compounds in a better way or in terms of proportionality between the amount of solvent and its concentration and the amount of the extracted substance (Nasr Abu Zayed, 2000) As for the comparative pesticide Irwin, its weak effect in the fall or killing of the Varroa pest may be

attributed to the fading or expiration of the effectiveness of other compounds in it against this pest, despite the presence of a mark indicating the expiration date for use, and this may be due to the poor storage conditions of the pesticide.

References

Al-Jourani, Reda Sakkab, Kamila Ward Shaher, Alaa Sharif Abbas, 2004, Evaluating the Efficiency of Some Means for Controlling American Foul Brood Disease (AFB) on Honeybees, Iraqi Journal of Agricultural Sciences, 35 (4): 100-95

Al-Hujaimi, Kamila Ward Shaher, Taher, Intisar Jabbar, 2012, Pollution of honey bees with lead and cadmium, Iraqi Journal of Agricultural Sciences. 43(15): 68-63

Al-Hijami, Hassanein Taher Karim, 2017, Testing the effectiveness of thyme and eucalyptus oils coated with starch and glycerol on the vital performance of honey bees *Apis mellifera* L. and the protection of the colony from the tit of varroa drusctor and the greater wax moth. *Galleria melamonella*, PhD thesis, College of Agriculture, University of Baghdad.

- Al-Hasnawi Montaser Sabah Mahdi, 2019, Spatial analysis of the spread of Varroa parasite and its effect on honey bees in Iraq. Master thesis / College of Arts / University of Kufa. p. 209

- Al-Ali, Abdul-Baqi Muhammad, 2011, Book of Beekeeping, Plant Protection Department / College of Agriculture - University of Baghdad, pg. 434.

Hajjij, Zaher, Nouredine Youssef, 2014. The use of ant acid in the control of Varroa on honey bees. Arab Journal of Plant Protection: pp. 63-57.

- Radi, Hala Kazem Muhammad, 2021, Controlling Varroa varroa destructor by using some plant extracts in Babylon Governorate, Master Thesis, Technical College - Al-Furat

Al-Awsat University - Department of Bioresistance Techniques. p3

- Muhammad, Khawla, Alia Tamim, 2011, Study of the methods of colonies of indigenous honey bees *Apis mellifera* L. and their treatment with various pesticides and their residual effect on some bee products (honey and wax), Tishreen University for Research and Scientific Studies - Biological Sciences Series, Volume 4, Issue 4.

- Majrashi, Ali Abdullah Ali, 2006, Study of the genetic effects of the extract of the Peganum plant on the meristem cells of the apex of the roots of the faba bean plant, Ain Shams University, Egypt, p. 53

Hadi, Marza Hamza, Sakr, Seylan Hassan, Abbas Ghanem Hamza, 2012, Effect of aqueous and alcoholic extract of black seed in controlling Varroa Varroa destructor mite on honey bee *Apis mellifera* (L.)

- **Harborne, J . B;1973.** Phytochemical methods, Halsted press. John Wiley and Sons. New York. 278 pp

- **Hariri, M. and Ghiasvand, R., 2016.** Cinnamon and chronic diseases. *Drug discovery from mother nature*, pp.1-24.

- **Henderson, C.F. and TILTON, E.W., 1955.** Tests with acaricides against the brown wheat mite. *Journal of economic entomology*, 48(2), pp.157-161.

- **Barbara.C; Rossella.B. and Francesca .C; 2020 .** Essential oils against varroa destructor a soft way to fight the parasitic mite of *Apis mellifera* .

- **Dillier, F.X., Fluri, P. and Imdorf, A., 2006.** Review of the orientation behaviour in the bee parasitic mite Varroa destructor: sensory equipment and cell invasion behaviour. *Revue suisse de zoologie*, 113(4), pp.857-878.

- **Reza, A.P., Sohbat, B., Mehdi, A.P. and Asgar, E., 2012.** Toxic effect of a selection of medicinal plant products against the parasitic

bee mite Varroa destructor. *Journal of Medicinal Plants Research*, 6(14), pp.2807-2811.

- **Rosenkranz, P., Aumeier, P. and Ziegelmann, B., 2010.** Biology and control of Varroa destructor. *Journal of invertebrate pathology*, 103, pp.S96-S119.

- **Sand , H ; 2015 .** The Animal and Plant Health Agency National Agri- Food Innovation Campus pp44.

- **Steel, R.G.D. and Torrie, J.H., 1980.** *Principles and procedures of statistics, a biometrical approach* (No. Ed. 2). McGraw-Hill Kogakush-Garedew, **A., Schmolz, E. and Lamprecht, I., 2004.** The energy and nutritional demand of the parasitic life of the mite Varroa destructor. *Apidologie*, 35(4), pp.419-430.

a, Ltd..