Bionatura
Latin American Journal of Biotechnology and Life Sciences

Bionatura Issue 4 Vol 8 No 1 2023

Article

Evaluation of the efficiency of some vegetable oils and bio-fungi in controlling Aphis fabae Scopoli of black bean insect.

Layla A.Benyan[1], Jinan M. Kalaf [2,] and Dawood S. Hamid[3]*
[1] *Department of Plant Protection, College of Agriculture, University of Basrah Basrah, Iraq;*
[2] *Department of Plant Protection, College of Agriculture, University of Basrah Basrah, Iraq;*
[3] *Department of Plant Protection, College of Agriculture, University of Basrah Basrah, Iraq;*
***** *Correspondence: Jinanmalik66@gmail.com.*
Available from: http://dx.doi.org/10.21931/RB/CSS/2023.08.04.90

ABSTRACT

The experiment was conducted to evaluate the efficiency of some vegetable oils such as watercress oil, aloe vera oil and eucalyptus oil in the percentage of nymphs of black broad bean Aphis fabae Scopoli on the chard plant Beta vulgaris subbsp. cicla. The mortality rate was 83.88, 90.56, and 93.89 % for oils used in the laboratory, while the percentage of loss in the field was 82.91. 88.19 and 89.86%, respectively, the results showed that vegetable oils had an effect on the destruction of whole black broad bean insects, and the increase of this effect with the increase of the time and concentration factor.

Keywords: Aphis fabae; Plant oils; Biological fungi.

INTRODUCTION

The aphid Aphis fabae Scopoli is one of the most dangerous insects that infect barley and many economic plants. The danger of aphids lies in the speed of reproduction and their spread on many weeds, crops and various plant families. Aphids infect both surfaces of the leaf due to the nature of the sucking penetrating mouth of the insect, so it sucks the plant juice from the leaves, which leads to the leaves turning yellow as the infection intensifies and the affected leaves wrinkle. It shows mold that causes the leaves to stop photosynthesizing and transmits many viral diseases [1]. Aphids need a large amount of protein in the plant juice, so they absorb the juice to get enough protein substances, and then they excrete the excess water and sugars in the form of honeydew. Swiss chard (Beta vulgaris subbsp. cicla) is a type of hybrid leafy vegetable of the sage family spread all over the world. The first species belonged to the island of Sicily. Its leaves and roots are eaten at other times. It contains minerals, vitamins A, B, C, K1, K2, folic acid and iron. The chard can be harvested before its green leaves are ripe. However, it can also be harvested after maturity. It has bright green leaves and is threatened to be eaten by various birds and insects due to its good and sweet taste. It can be planted at any time of the year with water and fertilizer, so it is considered one of the profitable economic plants [2]. Bio-control is one of the promising modern strategies in integrated control to reduce the impact of pests and their spread, not to leave adverse effects on the agricultural ecosystem, not to disturb the ecological balance, and to be safer and more stable in controlling insect pests. Many fungi were used to combat aphids

biologically. Many fungi were isolated from insects, including Penicillium chrysogenum, Cladosporium oxysporum, Beauveria bassiana, and Penicillium compactum[3]. [4] nine types of fungi were used, the best of which were Beauveria bassiana and Cladosporium oxysporum, the bacterial suspension of them at a concentration of 1 × 10^3 spore/ml achieved a mortality rate of 39.02 and 35.68% for the two fungi, respectively. Another alternative method is the use of vegetable oils. The effect of the killer, attractant and repellent of cactus oil, watercress and camphor in adults of the flour beetle Tribolium castaneum varied. The essential oils of Achillea wilhelmsii L. and Freula assafoetida L. were also used against aphid nymphs as pesticides of plant origin and have toxic and repellent efficacy, where the mortality rate increased with the increase in concentration and exposure time [5].

MATERIALS AND METHODS

Preparation of The Oil Extract

Different concentrations of commercial oils were prepared by taking 1 ml of the original oil and adding to it 5 ml of ethanol alcohol, then completing the volume to 100 ml of distilled water after adding 1 ml of liquid paraffin and two drops of tween 80 to make the original solution 1% ml of the original solution and bring the volume up to 100. As for the control treatment, 5 ml of ethanol alcohol was added to it, 1 ml of liquid paraffin and two drops of Tween 80 were added, then the volume was completed to 100 ml with distilled water, as the following oils were used as shown in Table 1.

Oils	Name
Rocket salad	Eruca vesicaria
Cactus	Aloe marlothii
Camphora	Cinnamomum camphora

Table 1. The oils used in the study.

Effect of Vegetable Oils on The Percentage of Insect Nymphs and Adults of Black Bean in Vitro

The above vegetable oils were added to the chard leaves at a concentration of 2, 4 and 6% for each oil separately. They were placed in sterilized plastic dishes for 2 ml per plate, and 10 insects were added to each plate with 3 replications for each concentration. As for the control treatment, 5 ml of ethanol alcohol was added, 1 ml of liquid paraffin and two drops of Tween 80 were added. The volume was completed to 100 ml with distilled water. 2 ml of alcohol was added to each plate in the control treatment.

Effect of Vegetable Oils on The Percentage of Whole Insect Mortality of Field Black Bean

The above vegetable oils were sprayed on the leaves of the chard planted in the field at a concentration of 2, 4 and 6% for each oil separately and with 3 replications for each concentration. As for the control treatment, 5 ml of ethanol was added, 1 ml of liquid paraffin and two drops of Tween 80 were added, and then the volume was completed to 100 ml with distilled water. Vegetable oils were sprayed using a 2-litre hand sprayer. The numbers of adult aphids were calculated before spraying and after 1, 2, 3, and 4 days of treatment and the following equation was used to calculate the relative efficiency of the oils.

$$\% \text{ relative efficiency} = \frac{\textit{The number of pest individuals after treatment x the number of pest individuals in control before treatment}}{\textit{number of pest individuals before treatment x the number of pest individuals in control after the treatment}} \times 100 \quad (1)$$

Preparation of culture media for bio fungi

Medium Potato Dextrose Agar Patagonia Dextrose Agar (PDA))
The use of culture medium prepared in the laboratory for the development of fungi Sterilize the medium with an Autoclave to a degree temperature of 121 °C and pressure 15 pounds / ang for 20 minutes, and after the temperature decreased, the food medium was placed in a refrigerator at 4°C until use.
Medium Potato Dextrose Broth. Prepare the middle without adding agar.
The medium is to obtain the suspension and filtrate of bio fungi.

Biological Control Agents

Isolation of Trichoderma harzianum Biological Diameter by Dilution Method

Random samples were taken from agricultural soils from different areas of Basrah province from the rhizosphere. After mixing it well, it was left in the laboratory to air-dry for 24 hours and sieved in a sieve with a capacity of 2 mm. Dilutions from soil samples (10-6-10-4) Transfer 1 ml of each dilution to sterile Petri dishes with a diameter of 9 cm and add to it. Sterile PD.A food medium, with the addition of the antibiotic Chloramphenicol, at a concentration of 250 mg/liter, with three replicators for each dilution. The plates were moved in a capillary motion to ensure the distribution of the soil sample and its homogeneity with the nutrient medium. The dishes were incubated in the incubator at a temperature of 25 °C for 5 days, then the colonies were re-purified on the same medium, diagnosed according to [6] The diagnosis was confirmed by Dr. Yahya Ashour Saleh, Department of Plant Protection, University of Basra, and the isolates were kept on slant liquid medium at 5 °C in the refrigerator. The two fungi used in the study, Beauveria bassiana and Trichoderma longibrachiatum, were obtained from the Plant Protection Department/University of Basrah.

Storage Isolates

The isolates of T. longibrachiatum and T. longibrachiatum were preserved. harzianum and the fungus B. Bassiana in the center of the PDA. Sterilizer was poured into sterile test tubes diagonally slanted inside the isolation room and sterilized by the autoclave at a temperature of 122° C and a pressure of 15 pounds/inches 2 for 20 minutes. The growing colony of fungi was inoculated with a 0.5 cm diameter disc. The PDA medium was incubated at 25°C for a week and then transferred to the refrigerator for preservation at 5°C.

Preparation of Bacterial Suspension for Fungi

It used 200 ml sterilized glass flasks, and put 250 ml of the medium consisting of each potato extract. Sterile Potato Dextrose broth is prepared as in the above paragraph. Inoculate each beaker by taking one drop tablet. 0,5 from a colony of T. longibrachatum and T. longibrachatum. harzianum and the fungus B. bassiana at the age of five days. The farm was incubated for 14 days in the incubator at a temperature of 25 ° C, taking into account the shaking every 2-3 days during the incubation period. The components of the liquid medium-containing flasks and mushroom colonies were placed in an electric blender for five minutes, and three concentrations of each live fungus 10, 102, and 103 were used to calculate the number of bacteria using a Heaymo cytometer. The number of spores for T. harzianum was (3.2 × 106), (2.7 × 107) and (1.3 × 107) and for Trichoderma. longibrachatum (5.9 x 106), (3.58 x 107) and (1.8 x 107) and for the fungus Beauveria bassiana (5.8 x 106), (4.5 x 107) and (1.9 x 107), respectively.

Effect of The Sporangial Suspension of T. longibrachiatum, T. harzianum and Beauveria bassiana.

In adults of laboratory broad bean bugs, leaves of the broad bean plant were prepared with an area of 2 inches from each plot and 10 individuals of whole adults were placed on it separately and placed in plastic Petri dishes with a diameter of 9 cm. At its base, a layer of sterile cotton was moistened with sterile distilled water, taking into account its moistening to prevent drying and to ensure permanent moisture 7 for fungi and then treated with spore suspension at an amount of 2 ml for each dish and with three concentrations for each fungus and with three replicates for each treatment. As for the control treatment, it was sprayed with sterile distilled water. The method of direct spraying was used by a sterile micro syringe with 2 ml for each dish. The dishes were incubated in the incubator at a temperature of 28 °C and 60 ± 5% humidity. The numbers of dead individuals were recorded after 24, 48 and 72 hours of spraying and were fixed on Temperature and relative humidity. The percentage of loss was calculated and corrected according to the Orell and Schneider equation mentioned in 8 according to the following equation:

Field Effect of The Spore Suspension of T. longibrachiatum, T. harzianum and B. bassiana.

Spore suspension for fungi was used at a concentration of 103 for each fungus and with three replicates at intervals of 1, 3, 5 and 7 days. The percentage of death was calculated according to the equation mentioned in the seventh paragraph.

$$\text{Corrected mortality rate \%} = \frac{\textbf{mortality rate by treatment} - \textbf{mortality rate by control treatment}}{\textbf{100} - \textbf{mortality rate in control}} \times 100 \quad (2)$$

Statistical Analysis

All laboratory experiments were conducted using a completely randomized design (CRD) for three-factor experiments, either as a field experiment. It was implemented by The randomized complete block design (RCBD), and the percentage was angularly converted and compared the means according to the least significant difference method under the probability level of 0.01 in laboratory experiments and 0.05 in field experiments [8]

RESULTS

Effect of Vegetable Oils on The Percentage of Adult Insect Mortality of Black Broad Bean in Vitro

The results shown in Table (2) showed that the highest effect of the oils used on the percentage of adult mortality was the treatment with camphor oil, which amounted to 93.89%, and the least effect was watercress oil, as the percentage of mortality reached 83.88%. There is a direct relationship between the concentration increase and the loss percentage, reaching 94.44, 92.22, and 86.11% for concentrations 6, 4, and 2, respectively. The percentage of mortality increased when the period of exposure to oils increased, as shown in Table (2), and reached 85.56, 96.30% for the first and second day after treatment. The effect of eucalyptus oil is due to the fact that it contains large amounts of safrole, which makes it toxic when it enters the digestive system and causes serious side effects, including death, and its toxicity appears quickly within 5 - 90 minutes. For many insects, including lice, bedbugs, and others [9], The effect of oils as insecticides upon contact with the body of nymphs and adults is due to disrupting gas exchange in the respiratory system and disrupting the function of the cell membrane or structure. It also prevents insects from feeding on oil-covered surfaces. It is anti-nutrition, and its toxic effect is more physical than chemical [7].

oils	Concentrations %	Percentage of mortality/day		Effect of oils	concentration effect
		1	2		
Eruca Sativa	2	73.33	90	83..88	86.11
	4	86.67	93.33		92.22
	6	90.00	96.67		94.44
aloe vera	2	76.67	93.33	90.56	
	4	86.67	96.67		
	6	90.00	100.0		
eucalyptus oil	2	86.67	96.67	93.89	
	4	90	100		
	6	90	100		
Days effect		85.56	96.30		

Table 2. Effect of oils on the percentage of laboratory mortality of black aphid adults.

LSD 0.01 3.90 for the effect of oils, 3.90 for the effect of concentrations, 5.51 for the effect of days and concentrations, 9.55 for the effect of oils, concentrations and days, 5.517 for the effect of oils and days, 3.18 for the effect of days, 6.75 for the effect of oils and concentrations.

Effect of Oils on The Percentage of Dead Black Aphid Adults in The Field

The results shown in Table (3) showed that the highest effect of the oils used on the percentage of adult mortality in the field was the treatment with camphor oil, which amounted to 89.86%, and the least effect was watercress oil, as the percentage of death reached 82. statistical analysis results analysis showed a significant difference in the effect of oils. The results showed a direct relationship between the concentration increase and the percentage of loss, which amounted to 90.42, 89.96, and 80.58 %for concentrations 6, 4, and 2, respectively. The loss percentage increased when the period of exposure to oils increased, as shown in Table (3), and amounted to 83.12, 87.21, 88.63, and 88.99% for days 1, 2, 3, and 4, respectively.

oils	concentration %	mortality rate/day%				Effect of oils rate	concentration-effect rate
		1	2	3	7		
Eruca Sativa	2	61.65	64.90	72.78	79.45		80.58
	4	90.00	90	88	88.17	82.91	89.96
	6	90.00	90	90	90		90.42
aloe vera	2	68.14	90	90	90	88.19	
	4	90	90	90	90		
	6	90	90	90	90		
eucalyptus oil	2	78.14	90	91.78	90	89.86	
	4	90	90	93.34	90		
	6	90	90	91.76	93.33		
Days effect		83.12	87.21	88.63	88.99		

Table 3. Effect of Oils on the percentage of mortality black aphid adults in the field

The Effect of The Sporangial Suspension of The Biological Fungi T. longibrachiatum, T. harzianum and B. bassiana in Laboratory Adults of a Bean Bug

The results in Table (4) showed the excel of T. longibrachiatum on the fungus T. harzianum and B. bassiana caused the highest death rate of adults of black broad bean as the rate was 78.83%, with a significant difference from 60.45 and 64.05%, respectively. The period was also affected, as the killing rate increased with a significant difference, as the rate reached 59.20 and 82.35. The results also showed that the concentrations had a significant effect, as concentration 101 gave the highest killing rate with a significant difference from the other concentrations, as the killing rate reached 81.67, 69.41 and 61.25% for concentrations 101, 102 and 103, respectively. The reason is that the fungus Trichoderma can Analyze the muscles of the insect's body, which makes it non-invasive and able to carry out daily activities such as movement, feeding and breathing, and then dying [11] as the fungus .T. harzianum is affected to its possession of multiple mechanisms of chitinase secretion[12]as well as the ability of mushrooms to produce metabolic compounds Toxic as pyrone and 6-pentlpyrone [8]

fungicide	Concentration	Percentage of mortality /day		fungicide rate	Concentration effect rate
		1	2		
1	1	55.04	90.00	64.05	81.67
	2	53.07	71.56		
	3	43.08	71.56		
2	1	90.00	90.00	60.45	69.41
	2	51.15	83.52		
	3	33.21	68.85		
3	1	75.00	90.00	78.83	61.25
	2	68.85	88.33		
	3	63.44	87.33		
=days :L. S. D.	4.514		=9.57 For fungi and Concentration :L. S. D.		
=to fungus :L. S. D.	5.52		=7.81 For days and fungicide : L. S. D.		
=Concentration : L. S. D.	5.52		13.54 to the three factors :L. S. D.		
=interaction :L. S. D.	7.81				

Table 4. Effect of the sporangial suspension of fungi on the percentage of laboratory mortality of adults of black broad bean bugs.

Effect of The Sporangial Suspension of T. longibrachiatum, T. harzianum and B. bassiana in Adults, an Insect From The Field Black Broad Bean

The results in Table (5) showed that the fungus T. longibrachiatum gave the highest mortality rate to the insect with a significant difference from the fungus B. bassiana, as the mortality rate was 29.12%, while the rate was 19.33% for the fungus B. bassiana. The highest mortality rate was after 7 days of spraying The sporophyte suspension in the field. The longer the time, the higher the killing rate, as the rate was 19.84, 20.73, 28.54 and 34.19% for the period 1, 3, 5 and 7 days after spraying, respectively.

fungicide	motility percentage/day				fungicide rate
	1	3	5	7	
1	7.54	13.65	22.56	33.59	**19.33**
2	24.82	30.51	24.61	36.15	**29.02**
3	27.17	18.02	38.46	32.82	**29.12**
Days effect	19.84	20.73	28.54	34.19	

Table 5. Effect of the sporangial suspension of live fungi on the percentage of mortality adults of field black broad bean

DISCUSSION

The superiority of T. longibrachiatum and T. harzianum over B. bassiana is because T. harzianum contains an enzyme chitinase that breaks down the cuticle and penetrates it, allowing the fungus to enter the body of the insect and cause infection by entering the fungus 5 Also, these fungi secrete toxins that lead to the insect's destruction. 3 mentioned that the ability of the fungal filtrate to influence its containment of toxins leads to killing or destroying some cells as the poison reaches the pest's body through the respiratory stomata or surface coverings. The body of the pest and these toxins are the poison produced by the fungus, such as Trichothenate and Zeralenone, that are secreted from the types of fungi Trichoderma spp. The increase in the concentrations of mycotoxins leads to an increase in the percentage of death after entering these toxins through the mouth and respiratory stomata and contact with the insect's body wall, as these results agree with 2 that after the fungus germ falls on the body of the host, it sticks and germinates the germ tube and penetrates the body wall as it grows rapidly in the tissues of the host insect.

CONCLUSIONS

The study also showed the excel of T. longibrachiatum on the fungus T. harzianum and the fungus B. bassiana in causing the highest mortality rate for adults of black broad bean, where the rate reached 78.83% with a significant difference from 60.45 and 64.05% of the fungi, respectively. The results also showed that the concentrations had a significant effect as they gave Concentration 101 the highest killing rate with a significant difference from the other concentrations, as the killing rate reached 81.67, 69.41 and 61.25% for concentrations 101, 102 and 103, respectively. The fungus T. longibrachiatum gave the highest mortality rate of the insect, with a significant difference from the fungus B. bassiana. The killing rate was 29.12%, while the rate was 19.33% for B. bassiana. The highest mortality rate was after 7 days of spraying the sporophyte suspension in the field.

References

1. Abdul Majeed, ME; Abdul Hamid, Z.H.;Rizk,J.N.;Hegazy, M.F. and Hussein, A. K. Pest Control Technology. Ain Shams University, **2000**.,251pp.
2. Al-Abadi, E. K. M.;Aidan, M. F.; Muhammad, A. H. The effect of killing, attrating and repellent of some oils on identical flour beetle adult. *J. Technical*, **2008**, 11(2):240-250.
3. Al-Allan, M. A.; AL- Menoufy, A.; Hajeej, N. Z.; AL-Amir, G. The effectiveness of some essential vegetable oils on the vitality of the pupae of the Mediterranean sea flour moth. *Syrian Journal of Agricultural Research*, **2007**, 4(3):116- 121.

4. Alfonsina, S. Aphididae (Hemiptera) on ornamental plants in Cordoba (Argentina). *Rev. Soc. Entomol. Argent*, **2008**, 67:49-56.
5. AL- Jubouri, E. E.; Kirkis, M. T. Mycotoxins, their effects and risks, Ibaa. *Centre for Agricultural Research*, **1998**, 243pp.
6. AL-Qudsi, A. A. Allergy of yellow and white corn cultivars to an insect from corn leaves Rapalosiphum maidis Fitch .with reference to some methods of its control .Master Thesis. College of Agriculture,University of Basrah, **2003**, 62pp.
7. AL-Rawi, K. H. .M.; Khalaf Allah, A. Design and analysis of Agricultural experiments. Dar AL-Kutub for printing and publishing .University of Mosul, **1980**, 488.
8. AL-Shuweili, T. S. J. Evolution of efficiency of some biological and chemical factors for broad bean black aphid Aphis fabae Scopolli (Aphididae: Homoptera). Athesis Master, collage of Agriculture, University of Basrah, **2010**, pp73.
9. AL-Youssef, A. A. The efficiency of some fungi in the biogenic resistance of Aphis fabae Scopoli (Homoptera: Aphididae) on Vicia fabae. *Misan Journal of Academic Studies*, **2008**, Vol.7 (13).69-78
10. Ghisalberti, E. L.; Narbey, M.J.; Dewan, M. M; Sivasithamparam, K. Variability among strains of Trichoderma harzianum to reduce take all and produce pyrones. *plant and silo*, **2010**, 121:287-291.
11. Gonzalez; J.; Gutirrez, M.; Ferrero, A.; Band, B. Essential oils nanoformulations for stored-product pest control–Characterization and biological properties. *Chemosphere*, **2014**, 100: 130-138.
12. Gotoh,T. Annual life cycles of the population of two-spotted spider mites Tetranychus urticae (Koth)(Tetranychidae:Acarina) in for Japanese pear orchards. *Appl.Entomo.Zool*, **1997**, 32(1):207-216.
13. Left, H. R. Effect of some plant extract for the Biological aspect of black bean aphid Aphis fabae Scopoli (Homoptera: Aphididae). *Al-Qadisiyah Journal of Pure Science*, **2017**, 22.3: 723-733.
14. Hassan, A. A. Vegetable crop production book712pp, *Arab House for Publishing and Distribution*, **1991**, P.665-662.

Received: Jan 15, 2023/ Accepted: May 10, 2023 / Published: May 15, 2023
Citation: Evaluation of the efficiency of some vegetable oils and bio-fungi in controlling Aphis fabae Scopoli of black bean insect. Revista Bionatura 2023;8 (2) 63. http://dx.doi.org/10.21931/RB/CSS/2023.08.04.90