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Evaluation of the Efficacy of Some Alcoholic Plant Extracts and the Biocide Naturalis-L Against *Aphis fabae* Scopoli (Homoptera: Aphididae) Under Laboratory Conditions

Manar Ahmed Abbas, Ashwaq Hussam Al Husani and Mushtak Talib Mohammadali*
College of Agriculture, Kerbala University, Kerbala, Iraq

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Abstract. This study was conducted to evaluate the efficacy of alcoholic extracts of the bark and leaves of *Eucalyptus* spp., *Mentha virdis*, *Mentha lognifolia*, *Capsicum annuum* and *Dodonaea viscosa*, with the compatibility study between the biocidal Naturalis-L (*Beauveria bassiana*) and the alcoholic extract of Dodonaea against adults of black aphid *Aphis fabae* Scopoli under laboratory conditions. The results showed the superiority of the extract of the Dodonaea plant in causing the highest rates of killing and was significantly superior to the rest of the other treatments, where the concentration of 3000 ppm achieved the highest mortality rates which reached 66.67, 86.67 and 93.33% after 24, 48 and 72 h of treatment under laboratory conditions. The results showed that the extracts of hot pepper plant at the concentration of 3000 ppm caused mortality of 56.67, 83.33 and 91.33% under the same time period. The results of evaluating the compatibility of the commercial preparation Naturalis-L with the alcoholic extract of the Dodonaea plant showed that the concentration (3 mL + 3000 ppm) was caused high mortality 96.33% after 72 h of treatment.

Keywords: *Aphis fabae*, plant extraction, insect control, *Beauveria bassiana*

Introduction

The black bean aphid *A. fabae* is one of the economically important pests with a wide family range, as it infested approximately 37 plant families in Iraq (Zarjis *et al*., 2000; Al-Jassani, 1980). Aphids can caused severe economic damage, as the nymphs and adults of this insect suck the plant sap, as well as secreting the honeydew that covers the infected parts of the plant, collecting dust and growing fungi on it, thus causing a decrease in crop production (Clements *et al*., 2000).

The control programs for this insect relied on the use of chemical pesticides and despite its quick results, their incorrect use and in high concentrations led to polluting the environment in addition to its negative effects on humans and other organisms and the emergence of insect resistance against it (Abu Duka and Mohammadali, 2021). This prompted the interest in plant extracts and their use as alternatives to pesticides, as they are safe natural materials that bio-degrade and environmentally and in a short period, as well as their high effectiveness against insects and low toxicity to humans and beneficial insects (Saira *et al*., 2017). It is known that the effectiveness of plant-based pesticides is due to the secondary metabolic compounds that are produced in plant cells and for the importance of these compounds, studies and research have continued to investigate them. It was found that there are 1005 plant species that have a toxic effect on insects, 389 species with anti-feeding effect, 279 species have a repellent effect, 31 species inhibit growth and 5 species lead to insect sterility (Grainge *et al*., 1980).

It is known that the effectiveness of plant-based pesticides is due to the secondary metabolic compounds that are produced in plant cells and for the importance of these compounds, studies and research have continued to investigate them (Perrino *et al*., 2021; Valerio *et al*., 2021). The biological fungus *Beauveria bassiana* (Bals.) is the most widely marketed species as arthropod pathogens. Most of the strains of this species infect most insect species and it has been used to reduce the number of pests that infested fruit and vegetable trees, thus expanding the range of targets for the products of pathogens Tudi *et al.* (2021).

The current study aimed to evaluate the efficacy of some alcoholic plant extracts of the bark and leaves of Eucalyptus, mint, tuna, pepper, dodonaea and the biocide Naturalis-L (*Beauveria bassiana*) in the resistance of black bean aphid under laboratory conditions.

*Author for correspondence;
E-mail: mushtak.t@uokerbala.edu.iq

Materials and Methods

Collecting and culturing the insect. Different stages of *Aphis fabae* were collected from the research station of the College of Agriculture, University of Karbala 2019. The insects were rared under the laboratory conditions on chard plants that were grown in plastic pots 300 mL for the purpose of per-petuating the colony. The insect was scientifically diagnosed by Professor Ali Abd Alhussain at Department of Plant Protection, College of Agriculture, University of Karbala, using the available taxonomic keys (Blackman and Eastop, 2006).

Preparation and storage of plant samples. The plant samples shown in Table (1) were collected in 2018-2019 from the research station of the College of Agriculture, University of Karbala, Kerbala, Iraq. The plants washed by tap water to remove the dust, dirt and the plant parts were dried using electric oven at a temperature of 25 °C and then grinded by a large electric mill for herbs 5 ppm.

Preparation of alcoholic plant extracts. Absolute ethyl alcoholic was selected as a polar solvent in the extraction process. The method of (Ladd *et al*., 1978) was adopted by taking 50 g of vegetable powder for each 500 mL of ethyl alcohol and placed in an electric vibrator for 24 h, filtering under low pressure twice by Whatman no.1 filter paper. Rotary evapourator was used to evapourate solvent from the plant extracts at 45 °C temperature. At a temperature of 45 °C, then filtered plant extracts was placed in Petri dishes and left to dry. Furthermore, the raw material of the alcoholic extract was kept in the refrigerator until use at a temperature of 5 °C. For the purpose of evaluating the effectiveness of the alcoholic plant extracts in the study, three concentrations of 1000, 2000 and 4000 ppm were prepared in water, while the control treatment was used distilled water only.

1-4 Biocidal Naturalis-L obtained from Fargro Company (UK) for the purpose of evaluating the effectiveness of the biocide on adults of *A. fabae*, three concentrations of 2, 2.5 and 3 mL/liter of water were used. Control was with distilled water only.

Evaluation of the effectiveness of plant alcoholic extracts in the mortality rates of adults of black bean aphid *A. fabae*. Plastic petri dish plates with a diameter of 9 cm were prepared with three replicates for each concentration (1000, 2000 and 3000 ppm). A filter paper was placed at the base of each dish and uninfested small leaf disk of the chard plant placed upon the filter paper. Wrapped the leaf stalk with medical cotton moistened with a sugar solution to prevent wilting of the leaves as long as possible. 10 adults of black bean aphids were transferred for each repeat. Previously, the control treatment sprayed 99% distilled water and 1% alcohol.

The treated dishes were placed in the incubator at a temperature of 25±2 °C and a relative humidity of 60±5%. The mortality rates were recorded after 24, 48 and 72 h of treatment and the results were corrected according to Abbott's equation (1925).

Study evaluating the compatibility between the fungal preparation Naturalis-L and the alcoholic extract of Dodonaea in the mortality rate of black bean. The same method of work above was followed, after preparing three petri dishes and transferring 10 adults of *A. fabae* for each replicate that were treated with three concentrations of Dadonaea extract (1000 ppm, 2000 ppm and 3000 ppm/L water and 2, 2.5 and 3 mL/L) of commercial fungal preparation. Then, a filter paper were placed at the base of each of these dishes to absorb the extract. After that, a small un-infested chard disk was placed in each dish on its lower surface and wrap the leaf stalk with medical cotton moistened with a sugar solution to prevent the leaves from wilting for as long as possible. Moreover, petri dishes were sprayed on the leaves chard with the concentrations mentioned previously and after spraying, (10) adults per replicate of *A. fabae* adults were introduced. As for the comparison treatment, the control

Table 1. Plants and biocides used in the laboratory study.

The scientific name	Common name	Family	Used part	Place of collection
Eucalyptus spp.	Eucalyptus plant	Myrtaceae	bark, leaves	College of Agriculture
Mentha virdis	Mint plant	Labiatae	Fruits	Local market
Mentha lognifolia	Bell pepper	Labiatae	vegetative part	Local market
Capsicum annuum	Pepper	Solanaceae	vegetative part	Local market
Dodonaea viscosa	Dodonaea plant	sapindaceae	Papers	College of Agriculture
Naturalis-L	*Beauveria bassiana*	Biological agent	-	Fargro company

treatment was sprayed with distilled water and the treatment was done with the aforementioned concentrations and using a small hand sprayer. Each treatment was sprayed with extract concentrations from a height of approximately 25 cm. At a temperature of 25±2 °C and a relative humidity of 60±5%, the cumulative mortality rates were recorded after 24, 48 and 72 h of treatment and the results were corrected according to Abbott's equation (Abbott, 1925).

Statistical analysis. The results were analyzed using the Completely Randomized Design (CRD) and the least significant difference (L.S.D) test was used at the probability level (0.05) to test for differences between the transactions. Mortality percentages were corrected according to the Abbott formula (Abbott, 1925). Corrected mortality percentages were calculated according to the following equation:

% corrected mortality = % mortality in treatment / % mortality in control

The results were analyzed using the statistical analysis program SAS (Antar, 2010).

Results and Discussion

Evaluation of the effectiveness of alcoholic plant extracts in the mortality rates of adults of black bean aphids *A. fabae*. The results in Table (2) indicated that there was a significant effect between the tested alcoholic plant extracts after 24 h of treatment on *A. fabae*, while the alcoholic extract of Dadonaea caused high mortality reached to 66.67% at the concentration 3000 ppm, followed by the alcoholic extract of peppermint and hot pepper, with a mortality rate of 60 and 57.67%, respectively, while *Eucalyptus* leaf extract achieved the lowest mortality rate of 20%.

The results of the study of alcoholic plant extracts after 48 h of treatment showed superiority of the extract of the Dodonaea plant with a significant difference over the rest of the plant extracts in the mortality rates at the three concentrations used, where the concentration of 3000 and 2000 ppm achieved the highest mortality rate of 63.66 and 86. 67%, respectively, followed by hot pepper extract, with a 40% and 83.33%, respectively. Eucalyptus leaf extract achieved the lowest mortality rate for adults, at 26.67 and 30% respectively (Table 3).

The superiority of the two plant extracts of Dodonaea and chili pepper continued to cause the highest rates of insect mortality from black bean aphids after 72 h of treatment (Table 4), where they achieved at the concentration 3000 ppm high mortality rate reached 93.33 and 91.33%, respectively, which differed significantly from the rest of the plant extracts. The two extracts of Eucalyptus bark and peppermint followed, with a mortality rate 83.33% and 73.33% respectively.

These results are indicated that treatment of adults of green peach *Myzus persicae* at a concentration of 20 mg/mL of alcoholic alkaloid extract of Dadonaea leaves led to an increase with mortality rates by 90%, while mortality rates were not recorded in the control, as it

Table 2. Effect of concentrations of alcoholic plant extracts on the percentage of adult mortality of *A. fabae* after 24 h of treatment

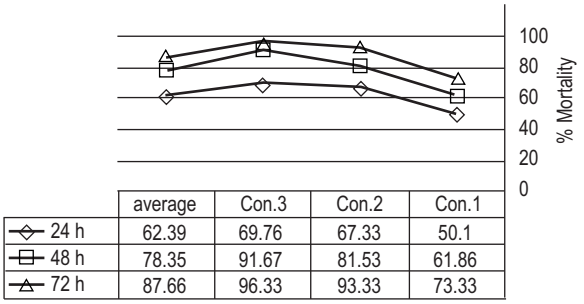
Concentrations/ Extractions	1000 ppm	2000 ppm	3000 ppm
Mentha virdis	13.33	23.33	60.00
Capsicum annuum	33.33	20.00	57.67
Dodonaea viscosa	21.66	30.33	66.67
Eucalyptus bark	23.33	16.67	46.67
Eucalyptus leaf	20.00	13.33	20.00
Mentha lognifolia	16.67	23.33	36.67
Control	0	0	0
L.S.D.	extractions	concentrations	interactions
	16.149	9.8889	27.97
	P. value	P. value	P. value
	0.0001	0.0001	0.0001

Table 3. Effect of concentrations of alcoholic plant extracts on the mortality of adults of *A. fabae* after 48 h of treatment

Concentrations/ Extractions	1000 ppm	2000 ppm	3000 ppm
Mentha virdis	26.67	40.00	63.33
Capsicum annuum	33.67	40.00	83.33
Dodonaea viscosa	43.33	63.66	86.67
Eucalyptus bark	50.00	36.67	66.67
Eucalyptus Leaf	23.33	26.67	30.00
Mentha lognifolia	33.33	20.00	53.33
Control	0	0	0
L.S.D.	extractions	concentrations	interactions
	13.996	8.5709	24.242
	P. value	P. value	P. value
	0.0001	0.0001	0.0001

Table 4. Effect of concentrations of alcoholic plant extracts on the mortality of adults of black bean *A. fabae* 72 h after the transaction

Concentrations/ Extractions	1000 ppm	2000 ppm	3000 ppm
Mentha virdis	46.67	56.67	73.33
Capsicum annuum	51.33	63.33	91.33
Dodonaea viscosa	53.33	73.33	93.33
Eucalyptus bark	63.33	56.67	83.33
Eucalyptus Leaf	36.67	53.33	46.67
Mentha lognifolia	46.67	36.67	63.33
Control	0	0	0
L.S.D.	extractions	concentrations	Interactions
	14.692	8.997	25.447
	P. value	P. value	P. value
	0.0001	0.0001	0.0001



	average	Con.3	Con.2	Con.1
24 h	62.39	69.76	67.33	50.1
48 h	78.35	91.67	81.53	61.86
72 h	87.66	96.33	93.33	73.33

Fig. 1. Shows the compatibility between the fungal preparation Naturalis-L and the alcoholic extract of Dodonaea in the mortality rate of black bean aphids (LSDTime:8.12; Treatment:6.63; concentration:8.17).

was found that there is a direct relationship in the percentage of mortality rates. Destruction of insects by increasing concentrations. Also by AL-Jasman *et al*. (2016) indicated that the cumulative mortality rates of aqueous extracts, including hot pepper extract, increased with the increase in the exposure period from 61.65% insects/dish on the first day and increased to 81.65% insects/dish on the third day. The cause of death may be due to the accumulation of substances present in the extract, which may be phenolic, alkaloid or terpenoid in the digestive tract, which leads to its poisoning or these substances interfere with the work of the endocrine system, which leads to a defect in the growth process (Fast, 1951). From the black bean aphids treated with concentrations of the alcoholic extract of hot pepper to the presence of toxic compounds such as capsaicin, which have a repellent or lethal action, as one fruit of hot pepper contains 0.93-1.34 mg of the active substance (Antonious *et al*., 2007).

Evaluation of the compatibility between the fungal preparation Naturalis-L and the alcoholic extract of Dodonaea in the mortality rate of black bean aphids. The results of the bio-compatibility study between the commercial preparation of the *Beauveria bassiana* with the alcoholic extract of the Dodonaea plant. However, results in Fig. 1 showed the superiority of the treatment after 72 h in the events of the highest killing rates and a rate of 87.66%, which differed significantly from the treatment after 48 h, which recorded a killing rate of 78.35%. The third concentration (biocide + alcoholic Dodonaea extract after 72 h) recorded the highest killing rate of 96.33% and was superior to the rest of the other concentrations.

This is consistent with the findings of Naeem and Mohammadali (2021) who indicated that the commercial preparation Naturalis-L for the *Beauveria bassiana* achieved killing rates of 78.35% against adults of the white scale insect *Parlatoria blancharde* at a concentration of 3 mL/L of water. Ahmed *et al*. (2018) indicated when studying the pathogenicity of three local isolates B2, B3, B4 of the fungus *B. bassiana* in the form of a sporophyte suspension at concentrations of 10/5, 10/6 and 10/7 spores/mL to control *T. urticae* adults under laboratory conditions, achieved death rates of 70, 80 and 84% respectively.

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Conflict of Interest. The authors declare that they have no conflict of interest

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