Evaluation the efficacy of Ethanol Extract Derived from Wild Mint on the Life Parameters of the *Aphis fabae* (Hemiptera: Aphididae) (Scopoli) under laboratory conditions

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Abstract

The black bean aphid, *Aphis fabae* is a significant economic pest with a global presence, impacting a wide range of plant species. This study aimed to assess the effectiveness of ethanol extract derived from wild mint (*Mentha longifolia*) under laboratory conditions on various life performance criteria of *A. fabae*. The evaluation involved measuring mortality rates of nymphs and adults, as well as adult productivity, utilizing three different extract concentrations (1%, 2%, and 3% w/v). The results revealed that the ethanol extract of plant leaves had a substantial effect on all life stages of the aphid, with a more pronounced impact observed at higher concentrations and longer exposure periods. In the first nymphal instar, the mortality rate reached 81% at a concentration of 3%, and in the second nymphal instar, it was 80% at the same concentration. For the third and fourth nymphal instars, the mortality rates were 82% and 82.5%, respectively, at a concentration of 3%. As for adult aphids, the mortality rate was 81.333% at a concentration of 3%. Moreover, the extract significantly reduced the productivity of adult aphids, with rates of 1.68%, 1.82%, and 1% at concentrations of 1%, 2%, and 3%, respectively.

Keywords: Wild mint plant, *M. longifolia*, black bean aphid, *A. fabae*, essential oils, limonene, carvone

Introduction

The *A. fabae*, commonly known as the black bean aphid, has a wide geographical distribution, including Iraq, and it infests more than 200 plant species [1]. The proliferation of aphid populations on plants has been linked to reduced productivity [2]. This pest directly harms plants by consuming substantial amounts of plant sap necessary for their growth, leading to plant weakening, wilting, and even collapse, resulting in significant economic losses for farmers [3]. Moreover, the black bean aphid indirectly causes damage by secreting honeydew on various plant parts, providing a suitable environment for the growth of sooty molds [4]. Additionally, it acts as a vector for plant diseases such as beet yellows virus (BYV) and brome mosaic virus (BMV) [5].

To control these pests, chemical pesticides have been widely employed due to their rapid effectiveness; however, their improper use and high concentrations have adverse effects on both the ecosystem and human health[6]. Consequently, researchers have been actively seeking safer alternatives, including plant extracts[7]. Wild mint (Mentha longifolia) from the *Lamiaceae* family is considered a promising solution due to its broad repellent and anti-feedant effects on insects, along with its capacity to regulate growth and deter egg-laying [8].

The perennial wild mint reproduces mainly through stolons or suckers and can reach a height of 1.5 meters under favorable conditions [9]. It is noteworthy that many species within the Mentha genus, encompassing 20-30 types, are utilized in diverse medical and commercial fields [10,11]. Furthermore, wild mint has been employed in insect control, including against bean aphids like Aphis craccivora and black bean aphids like *A. fabae* [12,13]. This study aims to evaluate the effectiveness of various concentrations of alcohol extract derived from *M. longifolia* leaves in controlling different life stages of *A. fabae* and the extract's indirect influence on the productivity of treated individual adults.

Materials and Methods

Collection and Rearing of *A. fabae*

Several specimens of the black bean aphid, *A. fabae*, were collected from broad bean fields in the Shuwati Farms area, located in Najaf Governorate, for the purpose of rearing them in the Entomology Laboratory, Plant Protection Department, Faculty of Agriculture, University of Kufa. The identification of insects was confirmed by Assistant Professor Dr. Akram Ali Mohammed. The aphids were reared on previously cultivated broad bean (*Vicia faba*) plants in pots measuring 20 * 40 cm. Rectangular wooden cages measuring 50 cm * 100 cm were constructed specifically for the aphid rearing, with six cages used in total. The base of each rearing cage was made of moisture-resistant wood, and the sides were covered with mesh cloth to provide adequate ventilation. The cage had a tightly sealed door through which the broad bean (*V. faba*) seedlings and black bean aphids (*A. fabae*) were introduced for rearing and experimentation. One plant was placed in each cage, along with 50 nymphs and adults of the black bean aphid, for the purpose of rearing and propagating the insects. Continuous monitoring was conducted to ensure suitable conditions for the aphids, including appropriate irrigation and fertilization, to maintain a stable aphid population for subsequent laboratory experiments.

Preparation of Different Nymphal Instars and Adults of *A. fabae* at the same age

Healthy broad bean plants were carefully chosen, and special clip cages designed to accommodate small-sized insects, including black bean aphids, were placed on these plants. The clip cage was constructed with two symmetrical ring-shaped pieces, each measuring 2 cm in diameter and 1.5 cm in depth. One end of the ring was covered with a metal mesh featuring tiny holes for ventilation, while the other end remained open

and was positioned on the surface of the plant's leaf. Upon introducing the black bean aphids onto the leaf, a second clip cage was positioned on the opposite side of the leaf to secure the aphids in place. The two ends of the clip cage were then compressed using a metal clamp to ensure stability and fixation onto the plant leaf. Each clip cage housed 15 adult black bean aphids (*A. fabae*), which were transferred from a permanent insect farm using a brush. After a 24-hour period, the leaves were inspected to confirm the presence of first nymphal instar individuals, and 20 individuals were retained on each leaf, while the adults were removed. To obtain individuals of similar ages from the second, third, and fourth nymphal instars, the same procedure was followed. The first instar nymphs were left for two days to progress to the second instar, and then they were left for 8, 6, and 4 days, respectively, to reach the third, fourth, and fully developed nymphal instars.

Plant Extract Preparation

Wild mint (*Mentha longifolia*) plants were obtained from the fields in Al-Haidariyah, affiliated with Najaf Governorate. The plant was identified in the laboratories of the Faculty of Science, University of Kufa, by Assistant Professor Dr. AbuThar Hatim Majid, as *M. longifolia* (wild mint). The plants were collected, washed, and completely dried. The plants were placed in a perforated container to remove excess water and left to stand for 3 hours with occasional stirring. Subsequently, the plants were spread on newspaper sheets inside the laboratory, away from direct sunlight, and continuously turned to prevent mold formation. Once the plants were fully dried, they were ground into a fine powder using an electric grinder and stored in a refrigerator for future use [14].The plant extract was obtained following the Harborne method (1984) [15] in the Entomology Laboratory, Faculty of Agriculture, University of Kufa. Fifty grams of wild mint powder were placed in an extraction thimble and then inserted into the Soxhlet extractor. Then, 250 ml of 95% ethanol was added [16], and the mixture was allowed to extract for 24 hours. Afterward, the sample was concentrated using a Rotary Evaporator at a temperature not exceeding 50 degrees Celsius and under low pressure. Upon obtaining a gelatinous solution, 5 ml of ethanol was added, and the sample was transferred to a known-weight glass vial and placed in an oven at 50 degrees Celsius to obtain the dried extract. Different concentrations (1%, 2%, and 3%) were prepared by dissolving 1g, 2g, and 3g of the plant extract, respectively, in 2 ml of ethanol, and the volume was completed to 100 ml with sterile distilled water adapted from Harborne, 1984).

Effect of Wild Mint Alcohol Extract on Different Nymphal Instars (First, Second, Third, and Fourth) and Adult Stage of *A. fabae*

Petri dishes with a diameter of 9 cm were prepared, and a filter paper was placed inside each dish. The first nymphal instar of the black bean aphid was sprinkled onto the dishes, and different concentrations (1%, 2%, and 3%) were applied using a manual sprayer at a distance of 15 cm [17]. The control treatment was sprayed with 2 ml of

distilled sterile water and ethanol and left to dry for 30 minutes. Subsequently, the nymphs were transferred to circular plastic containers with a diameter of 6.2 cm, a depth of 5 cm, and a base of 5 cm, filled with Agar-Agar medium according to Milner's method (1981)[18] at a depth of 1.5 cm. The medium was allowed to solidify, and 1-2 drops of distilled sterile water were added to the surface before placing a leaf of the broad bean plant on the surface of the medium inside the plastic container to keep the leaf fresh throughout the experiment. The plastic containers were covered from the top with a piece of mesh cloth for ventilation and to prevent the insects from escaping. The experiment was conducted with four replicates for each concentration, each consisting of 10 individuals. The containers were placed in an incubator at a temperature of 25 ± 2 degrees Celsius and a relative humidity of 60 ± 5. The percentage of mortality was calculated after 1, 2, 3, 4, and 5 days of treatment. The same procedure was followed for the second, third, fourth nymphal instars, and the adult stage after being prepared according to section2.

Statistical analysis

The results were analyzed utilizing the Completely Randomized Design (C.R.D), and the results were compared using the Least Significant Difference (L.S.D) test at a significance level of 5% exploiting GenStat software (version 12). The corrected percentage of mortality was calculated according to Abbott's Formula [19] (Abbott, 1925) (adapted and currently known as Schneider and Orell Formula) [20]. The corrected percentage of mortality was calculated as follows:

$$\text{Corrected Mortality Percentage} = \frac{(\text{Mortality in the Treatment} - \text{Mortality in the Control})}{100 - \text{Mortality in the Control}} * 100$$

Results and Discussion

The results presented in Tables (1), (2), (3), and (4) indicated a significant effect of the ethanol extract of wild mint on the increase in mortality rates of the four nymphal instars of the black bean aphid, *A. fabae*. The impact of the extract increased with higher concentrations and longer exposure periods. The first nymphal instar exhibited a mortality rate of 81% at a concentration of 3%, while the second nymphal instar had an 80% mortality rate at the same concentration. For the third and fourth nymphal instars, the mortality rates were 82% and 82.5%, respectively, at a concentration of 3%. As for the adult stage, the mortality rate reached 81.333% at a concentration of 3%. The interaction effect analysis revealed that all four nymphal instars and the adult stage of *A. fabae* were significantly impacted by the increase in the concentration of the plant extract, resulting in mortality rates reaching 100% at a concentration of 3%.

Table (1): The effect of different concentrations of wild mint extract (*M. longifolia*) on the percentage of mortality of the first nymphal instar of the black bean aphid, *A. fabae*, at various time intervals (days).

Con. of Wild Mint Extract (*M. longifolia*)	Time Interval (Days)					Average Effect of Concentration Factor
1%	22.5	70	85	95	100	74.5
2%	45	70	85	97.5	100	79.5
3%	37.5	75	92.5	100	100	81
Comparison	2.5	5	5	7.5	7.5	5.5
Average Time Effect	26.88	55	66.87	75	76.88	-
LSD 0.05	Concentration	Time	Interaction			
	4.71	6.74	8.42			

Table (2): The effect of varying concentrations of ethanol extract obtained from wild mint (*M. longifolia*) on the mortality percentage of the second nymphal instar of the black bean aphid (*A. fabae*) at different time intervals following the treatment (days)

Con. of Wild Mint Extract (*M. longifolia*)	Time Interval (Days)					Average Effect of Concentration Factor
1%	20	37.5	62.5	92.5	100	62.5
2%	25	55	75	90	100	69
3%	42.5	72.5	90	97.5	100	80.5
Comparison	5	5	5	5	5	5
Average Time Effect	23.125	42.5	58.13	71.25	76.25	
LSD 0.05	Concentration	Time	Interaction			
	6.11	3.68	7.99			

Table (3): Evaluation of the Effect of Various Concentrations of Ethanol Extract Obtained from Wild Mint (Mentha longifolia) on the Mortality Percentage of the Third Nymphal Instar of the Black Bean Aphid (*A. fabae*) at Different Time Points Following Treatment (days)

Con. of Wild Mint Extract (*M. longifolia*)	Time Interval (Days)					Average Effect of Concentration Factor
1%	25	47.5	67.5	85	100	65
2%	25	50	72.5	90	100	67.5
3%	42.5	72.5	95	100	100	82
Comparison	5	10	12.5	12.5	12.5	10.5
Average Time Effect	24.375	45	61.875	71.875	78.125	
LSD 0.05	Concentration	Time	Interaction			
	7.3	5.75	8.66			

Table (4): Assessment of the Impact of Different Concentrations of Ethanol Extract Derived from Wild Mint (Mentha longifolia) on the Mortality Percentage of the Fourth Nymphal Instar of the Black Bean Aphid (*A. fabae*) at Various Time Intervals after Treatment (days)

Con. Wild Mint Extract (*M. longifolia*)	Time Interval (Days)					Average Effect of Concentration Factor
1%	22.5	45	67.5	85	100	64
2%	25	47.5	75	90	100	67.5
3%	42.5	75	95	100	100	82.5
Comparison	5	2.5	10	12.5	12.5	8.5
Average Time Effect	23.75	42.5	61.875	71.875	78.125	
LSD 0.05	Concentration	Time	Interaction			
	5.11	4.88	7.04			

Table (5): Impact of Different Concentrations of Ethanol Extract from Wild Mint (*M. longifolia*) on the Percentage of Mortality of the Adult Stage of the Black Bean Aphid (*A. fabae*) at Different Time Intervals after Treatment (days)

Concentration of Wild Mint Extract (*M. longifolia*)	Time Interval (Days)		Average Effect of Concentration Factor			
1%	20	36.67	53.34	55.84	58.34	44.838
2%	23.33	46.66	79.99	93.32	93.32	67.324
3%	33.33	80	93.33	100	100	81.333
Comparison	3.33	6.66	13.33	13.33	12.33	9.796
Average Time Effect	19.9975	42.4975	59.9975	65.6225	65.9975	
LSD 0.05	Concentration	Time	Interaction			
	5.49	4.22	7.16			

Table (6): Evaluation of the Effect of Ethanol Extract Derived from Wild Mint (Mentha longifolia) on the Reproductive Capacity of Adult Black Bean Aphids (*A. fabae*) after Treatment.

Con. of Wild Mint Extract (*M. longifolia*)	Time Interval (Days)					Average Effect of Concentration Factor
1%	2.4	2.1	1.5	1.3	1.1	1.68
2%	2.3	2	1.8	1.7	1.3	1.82
3%	1.2	1	1.1	1	0.7	1
Comparison	3.5	3.6	3.6	3.8	4	3.7
Average Time Effect	2.35	2.175	2	1.95	1.775	
LSD 0.05	Concentration	Time	Interaction			

	0.19	0.14	0.42			

The results indicated a significant impact of the ethanol extract obtained from *M. longifolia* (wild mint) on the different life stages of the black bean aphid, *A. fabae*, especially at higher concentrations. This effect can be attributed to the chemical compounds present in the plant extract, which have been studied in previous research conducted in Iran, Greece, Tunisia, and South Africa. These studies identified dominant compounds in the essential oils, such as Cicarveol (53-78%), Carvone (55%), Menthol (33%), and Menthone (31-48%) [2,22,23]. Additionally, Pulegone is considered one of the most abundant compounds in the essential oils of wild mint[24][25]. The insecticidal effect of plant oils is attributed to some of their main chemical components [26]. Essential oils extracted from the Mentha genus have shown resistance against various pests[27].

Moreover, wild mint (*M. longifolia*) has demonstrated insect-repellent effects against pests like *Aphis punicae* and the mosquito *Anopheles stephensi*[28,29]. Many essential oils act as neurotoxic agents[30,31], where some of their compounds inhibit the activity of acetylcholinesterase (AChE) enzyme, such as α-pinene, limonene, menthol, menthone, and carvone[32]. Additionally, compounds in essential oils like pulegone have an impact on gamma-amminobutyric acid (GABA) and octopamine receptors in various insects [33,34]. These various effects indicate the presence of different mechanisms in the action of essential oil components [35].

Regarding the effect of the ethanol extract from wild mint (*M. longifolia*) on the fecundity of adult *A. fabae*, the results, as shown in Table (6), revealed a significant reduction in the number of offspring produced by the adults. This reduction can be attributed to the chemical components present in the plant extract, and one example is the compound Limonene, which is a monoterpenoid with toxic effects, either through contact toxicity or by acting as a feeding deterrent, targeting the digestive system or respiratory system of the insect [36]. Limonene also exhibits neurotoxic effects [37] and acts as an inhibitor of reproduction and growth regulator in various insects[38,39].

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