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EFFICACY OF AQUEOUS AND ETHANOLIC EXTRACTS OF *MYRTUS COMMUNIS* AGAINST *APHIS FABAE* SCOPOLI ON FABA BEAN

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ABSTRACT

The efficicacy of the ethanolic and the cold aqueous extracts of the *Myrtus communis* plant was evaluated against the nymphs of been aphid *Aphis fabae.* The results showed the superiority of the cold aqueous extract over the ethanolic extract. The mortality rate of nymphs reached 100% at a concentration of 20 mg/ ml for the periods 24, 48, and 72 hr, with the ethanolic extract, the highest mortality was at a concentration of 20 mg/ ml (70, 90, and 100%) for the periods of 24, 48, and 72 hr, respectively. Thus, cold aqueous and ethanolic extract of *M. communis* can be used as safe and ecofriendly insecticide to control *Aphis fable*.

Key words: Aqueous extract, *Aphis fabae,* ethanolic extract, Faba bean, *Myrtus communis,* nymphs, plant extracts

Faba beans are an essential source of protein for most of the population of the Middle East, besides being a source of feed for farm animals (Dhull et al., 2022). China, North Africa, Europe, Ethiopia, Egypt, India, and Afghanistan are its largest producers (Rahate et al., 2021). It is now widely used as food and medicine in most countries, being a rich source of a range of nutritional components such as proteins, fats, carbohydrates, minerals, and vitamins (Abdalla et al., 2019). In addition, they enter the crop rotation which improve soil fertility (Newall, 1998; Barnes et al., 2003). The bean aphid *Aphis fabae* has been observed in Iraq and neighboring countries and is widespread worldwide (Wamonje et al., 2020), and it is serious pest affecting many leguminous crops (Calin et al., 2020; AL-Sabie and Ali., 2023). The prevalence of this insect increases in winter and decreases at high temperatures, and the more it numbers, the lower the productivity (Pålsson et al., 2020); its numbers on a single plant can reach between 150 and 1030 (Calin., 2022), and can cause death of some plants (Shannag, 2007; Maulion et al., 2021). Damage is increased by the role of aphids as vectors of many plant viruses (Aldryhim and Khalil, 1993; Smith and Boyko, 2007), such as faba bean necrotic yellow virus, broad bean yellow mosaic virus, and bean leaf roll virus (Weigand and Bishara, 1991). The control of this insect has relied on the use of chemical pesticides, and despite their rapid effectiveness, their incorrect use in high concentrations has led to environmental pollution and negative effects like resistance (Peterson et al., 2000; Boulogne et al., 2012). Plant extracts provide an alternative to pesticides (Raja et al., 2001; Okwute, 2012), as they are considered safe and ecofriendly (Bläske and Hertel, 2001). These extracts have insect-repellent substances or insecticides, feeding inhibitors, or obstacles to laying eggs (Abd El-Azlz, 1998). *Myrtus communis* L. contains secondary compounds such as volatile oils, phenolic acids, tanning acids, and resins (El Haddad et al., 2022), and also contains basic compounds such as carbohydrates, proteins, fatty acids, and effective secondary compounds such as alkaloids, turpentines, flavonoids and glycosides, the latter of which play an important role in medicine. This aromatic plant is widespread in the Mediterranean basin (Hassiotis and Lazari, 2010; Hagos et al., 2017; Khan, 2017; Mouterde et al., 1983; Lefta, 2017). This study aims to evaluate the efficacy of plant extracts and their use in the control of *A fabae*.

MATERIALS AND METHODS

This research was completed at the end of December 2023, in the research laboratory of the Department of Biological Control Technologies at Al Mussaib Technical College, Al-Furat Al-Awsat Technical University, Iraq. *A. fabae,* samples were collected from one field of F. bean in the Al-Husseiniyah area in Karbala Governorate. Samples were taken from plants infected with the insect and placed in plastic boxes containing small holes for ventilation. The insect was then bred by infecting healthy plants that had been previously planted for laboratory experiments in the pots. Some plants that had been infected were also

transferred to the laboratory inside the insect breeding box and thus rearing was carried out.

The leaves of the *M. communis* were collected from a garden located in the Husseiniya area in Karbala on 13.12.2023, the leaves were separated from the branches, cleaned, and washed directly to remove the dust stuck on them, then were dried in a well-ventilated place and the shade at laboratory temperature and away from sunlight for 8 days. Then the dried leaves were placed in an electric oven at a temperature of 40 °C for 2 hr, to exclude any moisture in the leaves and facilitate grinding. Then it was ground and placed in opaque glass bottles and the name of a plant, the date and place from which it was taken, was recorded and kept in the refrigerator until use. The cold aqueous and ethanolic extract were prepared according to the method of Nair et al. (2005) and Amensour et al. (2010), adapted from (Harborne, 1984).

From the aqueous extract the stock solution was prepared by dissolving 5 g of dry matter in 100 ml distilled water, so the concentration of the solution became (50) mg/ ml, then the solution was placed in a vibrating device at 130 shakes/minute at laboratory temperature to mix it. From this solution, preparations were made with concentrations of 5, 10, and 20 mg/ ml, while the control treatment consisted solely of distilled water. Ethanolic extract, A stock solution was prepared by taking 5 g of extract and dissolved in 5 ml of ethanol concentration (99.7-100) and the volume was completed to 100 ml of distilled water, thus the concentration of the solution became 5%, equivalent to 50 mg/ ml, and from it the concentrations (5, 10, 20) mg/ ml were prepared, either the control treatment was distilled water with solvent (ethanol), where 5 ml of ethanol was taken and completed the volume to 100 ml of distilled water.

Effect of aqueous and ethanolic extracts on nymphs of *A. fabae* was studied in laboratory. The leaves of the bean plant were taken clean and free of any infection and placed inside plastic cans size (8x7x3) cm containing a perforated cover with small holes for ventilation, then 10 nymphs were placed on each leaf, where 12 cans were used for each extract and divided by 3 concentrations are 5, 10, 20 and each concentration has 3 repetitions besides the comparison treatment. Each can be sprayed with (3) sprays of each concentration of plant extracts by a hand sprayer with a capacity of height (15 cm) approximately while the control treatment was sprayed with sterile distilled water only for the aqueous extract, while ethanolic and distilled water were used. The cans were left (22-25 °C; 45-60% RH). The mortality was observed after 24, 48, and 72 hr of treatment and these were corrected according to the equation (Abbott, 1987). Statistical analysis was done according to the completely randomized design (CRD) with the least significant difference (LSD) significantly of ($p>0.05$) using the statistical analysis program GenStat Release 2009.

RESULTS AND DISCUSSION

The effect of cold aqueous extract and ethanolic extract of *M. communis* on the mortality of *A. fabae* are given in Table 1. The cold aqueous extract of *M. communis* was found superior over the ethanolic extract. The highest mortality rate for nymphs was at concentration was 20 mg/ ml, which amounted to (100, 100, 100)%, respectively after 24, 48 and 72 hr; while the lowest mortality rate for nymphs was recorded at a concentration of 5 mg/ ml (20.10.7). The mortality rate increased with concentration and time period. Table 2 shows the efficacy of ethanolic extract. The highest mortality for nymphs was at a concentration of 20 mg/ ml 100, 90, and 70% for periods 24, 48, and 72 hr,

Table 1. The effect of cold aqueous extract of *M. communis* on the mortality of *A. fabae* nymphs

Concentrations mg/ ml	Mortality/ hr			Concentrations
	24	48	72	
Control	0	0	0	0
5	7	10	20	12.3
10	27	60	93	60
20	100	100	100	100
Time period	33.5	42.5	53.25	
LSD ($p<0.05$) for concentrations = 8.43	For periods = 7.30			For interaction = 14.59

Table 2. The effect of ethanolic extract of *M. communis* on the mortality of *A. fabae nymphs*

Concentrations mg/ ml	Mortality/ hr			Concentrations
	24	48	72	
Control	0	0	10	3
5	4	7	20	10
10	10	40	80	44
20	70	90	100	80
Time period	21	34.25	52.5	
LSD ($p<0.05$) for concentrations = 8.11	For periods = 7.02			For interaction = 14.04

respectively compared to the results of the concentration of 5 mg/ ml, which amounted to (4,7, 20) % for the same periods. The toxicity of *M. communis* is due to toxic substances, including saponins. These substances work by binding with fats in the gut, preventing their absorption by the insect's body. This causes significant harm to the insect, and its interaction with cholesterol also affects other functions.

Al-Khafaji (2016) showed the efficacy of boiled, cold, and ethanolic aqueous extract of *M. communis* on *Musca domestica* adults, and the highest rate of expulsion rates was 62.5% when adults were exposed to cold water extract at a concentration of 10 mg/ ml after 48 hr, while the lowest expulsion rates were recorded at 10% when adults were exposed to ethanolic extract at a concentration of 2.5 mg/ ml after 24 and 48 hr of treatment. The highest attraction rate was 33.3% when adults were exposed to ethanolic extract at a concentration of 10 mg/ ml after 24 hr of treatment, and the lowest attraction rate was recorded at 2.5% when adults were exposed to boiled water extract and ethanolic at concentrations of 2.5 and 7.5 mg/ ml after 48 hr of treatment, respectively. Lefta (2017) showed the superiority of the aqueous extract of *M. communis* on the nymphs of the first phase of *A. fabae* at a concentration of 15 mg/ ml after 24 and 48 hr of treatment; for adults, the treatment at the highest concentration caused maximum mortality of 80, 70 and 56.6%. Nayak et al. (2004) observed 82% mortality for *A. fabae* first-stage nymphs when exposed to the aqueous extract of *M. communis* at a concentration of 205 mg/ ml after 24 hr of treatment. This might be due to proteins or enzymes causing poisoning in the gut (Mann et al., 2001). Shalmoon and Mahmoud (2009), evaluated the effect of eight aquatic plant extracts on the growth and development of the ovaries and ovarian follicles. The two best extracts, were that of *M. communis* and the fruits of *Thuja orientalis*. Accordingly, it is preferable to use aqueous plant extracts instead of alcoholic and acetone ones, because they are easier to prepare, less expensive, and have a high morale inhibitory effect. Al-Rahimi (2022) studied the effect of *M. communis* in combating *Culex molestus* larvae and showed its superiority. The current study evidences the efficiency of *M. communis* plant extracts in controlling *A. fabae* nymphs, varying according to the extraction method. The results also showed the superiority of the cold aqueous extract over the ethanolic extract.

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AUTHOR CONTRIBUTION STATEMENT

MUA, AAH and MHH conceived of the original idea. AAH and MHH developed the theoretical and performed the statistical analysis for experimental data. MUA and MHH verified the analytical methods. MUA, AAH and MHH worked for lab analysis and supervises the project. MUA, AAH and MHH discussed the results and contributed to write the manuscript.

CONFLICT OF INTEREST

No conflict of interest.

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