Efficacy of selected control agents against the black bean aphid (*Aphis fabae*) in Najaf governorate, Iraq

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Abstract

This study was conducted to evaluate the efficacy of selected control agents against the black bean aphid (*Aphis fabae*), with the experimental design comprising two primary components. The first involved a laboratory assessment of three control agents: the insect growth regulator Match, the botanical insecticide Oxymatrine, and the Entomopathogenic fungus *Metarhizium anisopliae*, each tested at concentrations of 0%, 50%, and 100% with three replicates per treatment. Based on laboratory findings, the optimal concentration (100%) for each agent was selected for subsequent field application. In the field experiment, treatments included individual applications of each agent and a combined application (Match + Oxymatrine + *M. anisopliae*) as an integrated spray treatment.

The experimental design also incorporated an evaluation of the effect of bean cultivar on aphid mortality. Laboratory results demonstrated a marked efficacy of the 100% concentration for all agents tested. Match induced mortality rates of 88.44% and 83.55% for nymphal and adult stages, respectively; Oxymatrine achieved 96.67% and 97.78%, while *M. anisopliae* resulted in 63.75% and 62.39% mortality among nymphs and adults, respectively.

Field experiment outcomes revealed a significant increase in adult aphid mortality following application of the combined treatment (Match + Oxymatrine + *M. anisopliae*), which yielded the highest mortality rate 122.12 adults per plant. In contrast, the control treatment exhibited no mortality throughout the study.

Keywords: *Aphis fabae,* Match, Oxymatrine, *Metarhizium anisopliae*.

Introduction

Broad bean (*Vicia faba* L.), a member of the Fabaceae family, is a widely cultivated leguminous crop valued for its green pods and seeds used either fresh or dried in diverse culinary applications. In addition to their nutritional and economic importance, broad bean plays a vital role in crop rotation systems due to its ability to enhance soil quality. This crop contributes to soil fertility by biologically fixing atmospheric nitrogen through symbiotic associations with root nodules [1].

According to the Central Statistical Organization [2], Iraq produced 451 tons of dry broad bean in 2021 across a cultivated area of 247 dunums. Despite its agronomic significance, the crop is vulnerable to various phytopathogenic fungi and insect pests, with the black bean aphid (*Aphis fabae* Scop.) being among the most prevalent and damaging. This species, a member of the Aphididae family and the order Homoptera, poses considerable threats to crop health and productivity [3].

The black bean aphid (*Aphis fabae* Scopoli; Homoptera: Aphididae) is recognized as the most damaging pest affecting broad bean cultivation worldwide. It is one of 14 aphid species of considerable agronomic relevance. While *A. fabae* is highly polyphagous, its specific host range, especially among populations infesting beans and sugar beet, remains uncertain due to its inclusion in a complex assemblage of closely related taxa, several of which exhibit broad host adaptability [4]. The species is widely distributed across Europe, Western Asia, Africa, and South America. Moreover, *A. fabae* acts as a vector for over 30 phytopathogenic viruses. These include non-persistent viruses that affect legumes such as beans and peas, as well as beets, crucifers, cucurbits, dahlia, potato, tomato, and tulip. It also transmits persistent viruses like Beet Yellow Net Virus (BYNV) and Potato Leaf Roll Virus (PLRV) [4].

Chemical pesticides remain a critical and widely employed component of pest management strategies, particularly in regions with high population densities. Despite their associated environmental and health risks to humans, animals, and plants, they offer rapid and effective suppression of insect populations and mitigation of crop damage. Aphid control relies on various pesticide classes, notably pyrethroids, which are particularly effective against the black bean aphid (*Aphis fabae*), along with neonicotinoids such as Thiacloprid and Fenvalerate [5]. Additional compounds, including Chlorpyrifos methyl, Cypermethrin, Oxydemeton methyl, imidacloprid, and Thiometon, have also demonstrated efficacy in curbing *A. fabae* populations [6].

Due to *A. fabae* prolific reproduction and its ability to transmit plant viruses, farmers often resort to elevated pesticide dosages to manage infestations [7]. In a laboratory study, Hussein [8] evaluated the comparative efficacy of four fungal Biopesticide formulations *Lecanicillium muscarium*, *Isaria fumosorosea*, *Beauveria bassiana*, and *Metarhizium anisopliae*, against *A. fabae* and *A. gossypii*. The *L. muscarium* preparation was the most effective, yielding corrected mortality rates of 92.85% in *A. gossypii* nymphs and 88.78% in adults, and 87.32% and 83.65% in *A. fabae* nymphs and adults, respectively. These results were comparable to those achieved with the botanical insecticide oxymatrine, which produced corrected mortality rates of 95.92% and 92.85% for *A. gossypii*, and 95.84% and 89.43% for *A. fabae*.

Aphids, as globally significant crop pests with intricate life cycles, necessitate effective control measures. Agrochemical pesticides have proven successful in reducing their populations [9, 10]. Among these, neonicotinoids are considered less detrimental to human and animal health [11]. Sétamou *et al*. [12] found that soil application of imidacloprid significantly reduced grapefruit tree infestation and citrus leaf miner density within one week of treatment. Similarly, Ammar and Hajar [13] demonstrated that thiamethoxam was more effective than acetamiprid, achieving 100% mortality of leaf miner larvae within 24 hours compared to 75% under acetamiprid treatment via leaf immersion.

Materials and Methods

Source of Black Fabaceae Insects

To establish a stable insect colony for research purposes, Faba beans were cultivated over an area of 100 m² in the Al-Suhailiya region of the Kufa District. These plants served as a nutritional host for black bean aphids, which were subsequently utilized in both laboratory and field investigations. Cultivation commenced in October 2024, with the crops receiving regular care through fertilization, pruning, and pest and disease management to ensure optimal plant health. In February 2025, the Faba bean plants were deliberately infested with black bean aphids, providing a continuous and controlled source of specimens for subsequent experimental procedures.

Source of Faba bean varieties

Two Faba bean (*Vicia faba*) cultivars were selected for comparative analysis of their susceptibility to insect infestation and their response to the applied control measures. The first cultivar (Albu Faris) represents a locally adapted variety traditionally cultivated by farmers in the region. The second (Fito) is a Spanish cultivar developed by Luz de Otono and introduced for evaluation. Both varieties were procured from the Al-Nour Engineering Office located in the Najaf Governorate. Subsequent laboratory experiments were conducted to assess their performance under controlled conditions.

A- Laboratory Experiments

The Effect of the Growth Regulator Match on the Mortality of Aphis fabae

Three concentrations of Match were prepared to evaluate its efficacy against third-instar *Aphis fabae* nymphs. The recommended concentration (100%) was obtained by diluting 3 mL of *Match* in a 1-liter graduated cylinder to the calibration mark. A 50% concentration was prepared by transferring 500 ml of the 100% solution into a second 1-liter cylinder and diluting to volume. This dilution process was repeated to produce a 25% concentration.

Twelve Petri dishes (9 cm diameter) were used in the bioassay. Each dish was lined with filter paper and contained a broad bean (*Vicia faba*) leaf infested with 10 third-instar nymphs. The dishes were arranged in triplicate for each concentration, with 1 ml of the respective solution applied to each. An additional three dishes served as untreated controls, receiving 1 ml of distilled water. All dishes were incubated at 25 ± 2°C, and insect mortality and behavioural responses were recorded at two-day intervals over six days.

Effect of the growth regulator Match on the mortality of the adult stage of Aphis fabae

The exact steps of the previous experiment were repeated, with the difference being that 10 black bean aphid adults were placed in each dish.

The effect of the insecticide Oxymatrine on the mortality of the nymphal stage of the black bean aphid

To evaluate the efficiency of Oxymatrine against third-instar nymphs of the black bean aphid (*Aphis fabae*), three concentrations of the insecticide were prepared through serial dilution. Initially, 1.5 ml of oxymatrine solution was diluted to 1 liter with distilled water to obtain the recommended 100% concentration. Subsequently, 500 ml of this solution was diluted to 1 liter to yield a 50% concentration, and the same procedure was repeated to produce a 25% concentration.

Twelve Petri dishes (9 cm diameter) were prepared, each lined with filter paper and containing a faba bean (*Vicia faba*) leaf infested with 10 third-instar aphid nymphs. The dishes were arranged in triplicate for each concentration, with 1 ml of the respective solution applied to each. An additional three dishes served as untreated controls, receiving 1 ml of distilled water. This setup facilitated a comparative assessment of insecticidal activity across treatment levels.

The treated dishes were transferred to an incubator, and the temperature was set at 25 ± 2 °C. The insects were monitored, and results were recorded every two days for six days.

Effect of the pesticide Oxymatrine on the mortality of the black bean aphid

The exact steps of the previous experiment were repeated, with the difference being that 10 black bean aphid adults were placed in each dish.

The effect of the fungicide *Metarhizium anisopliae* on the mortality of the nymphal stage of the black bean aphid.

To assess the efficiency of *Metarhizium anisopliae* against third-instar nymphs of the black bean aphid (*Aphis fabae*), three concentrations of the fungal formulation were prepared via serial dilution. The recommended concentration (100%) was obtained by dissolving 5 grams of *M. anisopliae* in distilled water to a final volume of 1 liter. A 50% concentration was prepared by diluting 500 ml of the 100% solution to 1 liter, and the same procedure was repeated to produce a 25% concentration.

Twelve Petri dishes (9 cm diameter) were used in the bioassay. Each dish was lined with filter paper and a faba bean (*Vicia faba*) leaf infested with 10 third-instar aphid nymphs. The dishes were arranged in triplicate for each concentration, with 1 ml of the respective solution applied to each. An additional three dishes served as untreated controls, receiving 1 ml of distilled water. This setup enabled comparative evaluation of fungal pathogenicity across treatment levels.

The treated dishes were transferred to an incubator, and the temperature was set at 25 ± 2°C. The insects were monitored, and results were recorded every two days for six days.

Effect of the fungicide Metarhizium anisopliae on the mortality of the adult stage of the black bean aphid

The same steps of the previous experiment were repeated, with the difference being that 10 black bean aphid adults were placed in each dish.

B. Field Experiment

In October 2024, a field experiment was established using two *Vicia faba* cultivars, Albu Faris (local) and Fito (Spanish) to evaluate their response to various pest control treatments. The experimental site was divided into 30 plots, each measuring 1 m² and containing five faba bean plants. Treatments were arranged in a completely randomized block design with three replicates per condition.

The following treatments were applied:

- **Control**: Three plots per variety were sprayed with distilled water.
- **Match (growth regulator)**: Three plots per variety received the recommended concentration.
- **Oxymatrine (fungicide)**: Three plots per variety were treated at the recommended concentration.
- **Metarhizium anisopliae (entomopathogenic fungus)**: Three plots per variety were treated at the recommended concentration.
- **Combined treatment**: Three plots per variety were sprayed with Match, Oxymatrine, and *M. anisopliae* simultaneously at their respective recommended concentrations.

Spraying was conducted in April 2025. Post-treatment monitoring involved counting third-instar aphid nymphs and recording adult mortality at daily intervals over seven days.

Statistical Analysis

All the data from the experiment were analysed using the GenStat program version 12. A completely randomized design (C.R.D.) was used in laboratory experiments, and field experiments were conducted using factorial experiments using a randomized complete block design (R.C.B.D.) at a probability level of 0.05 ($P \leq 0.05$). Means were compared using the least significant difference (L.S.D.) test at the same previous probability level [14].

Results and discussion

The data presented in Table 1 revealed statistically significant differences in the efficiency of various concentrations of the insect growth regulator Match in managing third instar black bean aphid nymphs. The highest nymphal mortality rate, recorded at 88.44%, occurred at the recommended concentration (100%), whereas the control group exhibited no mortality. Additionally, the duration of exposure to the growth regulator had a notable impact, with nymphal mortality increasing over time. The greatest mortality rate, 67.48%, was observed after six days of treatment, compared to the lowest rate of 29% recorded at two days post-treatment.

Table 1 further illustrates that the interaction between concentration and exposure duration significantly influenced nymphal mortality. Specifically, complete (100%) mortality was achieved at both the four-day and six-day intervals when treated with the full-strength concentration. In contrast, no mortality was observed in the control group across all time points.

Regarding adult Aphis fabae, the recommended 100% concentration of Match also yielded the highest mortality rate at 83.55%, while no adult mortality was recorded in the control treatment. As with the nymphs, exposure duration significantly affected adult mortality, with the six-day post-treatment interval yielding a 67.48% mortality rate, compared to 29% at two days post-treatment. The combined influence of concentration and exposure time was again significant, with the highest adult mortality (100%) observed under the 100% concentration following six days of exposure. At the same time, the control treatment consistently showed no effect.

Table (1): Effect of different concentrations of Match on the mortality percentage of *A. fabae* nymph and adult instar under laboratory conditions

Nymphs				
Concentration (%)	**Exposure time (days)**			**Mean of Concentration**
	2	**4**	**6**	
Control	0.00	0.00	0.00	0.00
25	15.33	44.67	79.33	46.44
50	35.33	76.62	90.58	67.51
100	65.33	100	100	88.44
Mean of Time	29.00	55.32	67.48	
L.S.D. 0.05 Concentration = 5.329, Time = 3.591, Interaction = 7.227				
Adults				
Concentration (%)	**Exposure time (days)**			**Mean of Concentration**
	2	**4**	**6**	
Control	0.00	0.00	0.00	0.00
25	12.33	25.15	61.73	33.07
50	30.75	75.33	90.69	65.59
100	60.33	90.33	100	83.55
Mean of Time	21.08	38.96	51.68	
L.S.D. 0.05 Concentration = 6.876, Time = 4.009, Interaction = 8.132				

Analysis of the data in Table 2 demonstrated statistically significant differences in the mortality of third instar Aphis fabae nymphs following treatment with varying concentrations of Oxymatrine. The 100% concentration yielded the highest mortality rate (96.67%), while no mortality was observed in the control group (0%). Furthermore,

post-treatment time significantly influenced mortality, with the lowest rate (41.58%) recorded at two days and the highest (70.32%) after six days of exposure.
The interaction between concentration and exposure duration also produced notable effects. The highest nymphal mortality rates (100%) were observed under the following interaction conditions: 50% concentration after six days, 100% concentration after four days, and 100% concentration after six days. In contrast, no mortality occurred in the control treatment across all time intervals.

For adult A. fabae, similar trends were evident. The 100% concentration resulted in the highest mortality rate (97.78%), with no adult mortality detected in the control group. The timing of post-treatment exposure also significantly affected adult mortality, reaching 69.89% after six days compared to 46.48% at two days. As with nymphs, the interaction treatments (50% after six days, 100% after four days, and 100% after six days) produced complete adult mortality (100%), while no mortality occurred in the control across all durations.

Table (2): Effect of different concentrations of Oxymatrine on mortality percentage of *A. fabae* nymph and adult instar under laboratory conditions

Nymphs				
Concentration (%)	**Exposure time (days)**			**Mean of Concentration**
	2	**4**	**6**	
Control	0.00	0.00	0.00	0.00
25	25.97	67.32	81.26	58.18
50	50.33	75.00	100	75.11
100	90.00	100	100	96.67
Mean of Time	41.58	60.58	70.32	
L.S.D. 0.05 Concentration = 6.884, Time = 4.067, Interaction = 8.106				
Adults				
Concentration (%)	**Exposure time (days)**			**Mean of Concentration**
	2	**4**	**6**	
Control	0.00	0.00	0.00	0.00
25	32.36	59.33	79.57	57.09
50	60.23	80.67	100	80.30
100	93.33	100	100	97.78
Mean of Time	46.48	60.00	69.89	
L.S.D. 0.05 Concentration = 7.099, Time = 5.129, Interaction = 8.997				

Table 3 presents data evaluating the impact of the fungal biocide *Metarhizium anisopliae* on mortality rates of both nymphal and adult stages of the black bean aphid (*Aphis fabae*). The results indicate a statistically significant influence of biocide concentration, with the highest mortality rates observed at the 100% concentration, 63.75% for nymphs and 62.39% for adults whereas the control group exhibited no mortality.

The post-treatment interval also significantly affected mortality outcomes. Six days after application, the highest mortality rates were recorded 47.83% for nymphs and 39.84% for adults—contrasting with the lowest rates observed at two days post-treatment, measuring 11.59% and 10.35%, respectively.

Analysis of the interaction between biocide concentration and exposure duration reveals a pronounced synergistic effect. The treatment combining 100% concentration with a six-day exposure period resulted in the maximum mortality rates for both developmental stages, reaching 90.21% for nymphs and 90.00% for adults. In contrast, the control treatments, regardless of the duration, showed no mortality across any examined time points.

Table (3): Effect of different concentrations of *Metarhizium anisopliae* on morality percentage of *A. fabae* nymph and adult instar under laboratory conditions

Nymph				
Concentration (%)	**Exposure time (days)**			**Mean of Concentration**
	2	**4**	**6**	
Control	0.00	0.00	0.00	0.00
25	5.23	20.33	40.26	21.94
50	10.29	25.99	60.84	32.37
100	30.84	70.21	90.21	63.75
Mean of Time	11.59	29.13	47.83	
L.S.D. 0.05 Concentration = 3.158, Time = 1.527, Interaction = 5.972				
Adults				
Concentration (%)	**Exposure time (days)**			**Mean of Concentration**
	2	**4**	**6**	
Control	0.00	0.00	0.00	0.00
25	3.29	15.94	35.15	18.13
50	8.26	20.53	34.19	20.99
100	29.84	67.33	90.00	62.39
Mean of Time	10.35	25.95	39.84	
L.S.D. 0.05 Concentration = 5.031, Time = 3.726, Interaction = 6.999				

Table 4 presents the outcomes of a field experiment conducted to assess the effectiveness of various control strategies against adult black bean aphids (*Aphis fabae*). The analysis considered three primary factors: bean cultivar, type of control agent, and post-treatment duration. The Spanish bean variety exhibited superior performance in aphid suppression, with an average mortality of 63.36 adults per plant, surpassing the local variety, which recorded 48.68 dead adults per plant.

Regarding treatment type, the combined application of all three control agents Match, Oxymatrine, and *Metarhizium anisopliae* resulted in the highest observed mortality (122.12 adults/plant). In contrast, the control treatment produced no aphid mortality.

Post-treatment duration also significantly influenced outcomes, with the most significat mortality (80.77 adults/plant) observed at seven days post-application, in contrast to the lowest value (29.12 adults/plant) recorded one day after treatment. Interaction effects between the studied factors were statistically significant. The control treatments within both bean cultivars, across all time intervals, exhibited no aphid mortality. In contrast, the most effective treatment combining all three control agents applied to the Spanish variety and evaluated seven days post-treatment yielded the highest recorded mortality rate of 188.24 adults per plant.

Table (4): Effect of type of variety and control methods on the number of adults of *A. fabae* under field conditions

Variety	**Treatments**	**Time (days)**				**Mean of Treatment**	**Mean of Variety**
		1	**3**	**5**	**7**		
Local	**Control**	0.00	0.00	0.00	0.00	0.00	48.68
	Match	9.26	15.21	50.01	70.33		
	Oxymatrine	10.33	19.67	55.61	82.33	46.15	
	M. anisopliae	5.32	12.65	49.26	66.33		
	Match + Oxymatrine + *M. anisopliae*	89.26	99.33	109.35	129.28	58.60	
Spanish	**Control**	0.00	0.00	0.00	0.00		63.36
	Match	20.62	45.94	68.71	89.12	40.72	
	Oxymatrine	50.15	70.57	77.62	102.51		
	M. anisopliae	15.92	35.92	60.74	79.59	122.12	
	Match + Oxymatrine + *M. anisopliae*	90.29	112.59	158.64	188.24		
Mean of Time		29.12	41.19	62.99	80.77		
L.S.D. 0.05 Variety = 3.159, Time = 5.007, Treatments = 7.068, Interaction = 9.828							

The findings presented in Tables 1 through 4 underscore the significant efficiency of the applied control agents in both laboratory and field settings. These agents markedly enhanced the mortality rates of the targeted developmental stages of *Aphis fabae*.

Notably, the botanical insecticide Oxymatrine yielded auspicious results. Extracted from the ancient Chinese herb known as Kushen, which contains substantial quantities of alkaloids, Oxymatrine is recognized as a broad-spectrum pesticide with a favourable safety profile when compared to conventional chemical insecticides [15]. Its mode of action involves contact-based absorption, leading to feeding inhibition and repellency, while also promoting vegetative plant growth [16].

Research by Rabea et al. [17] reported minimal toxicity of Oxymatrine towards honeybees and negligible effects on acetylcholinesterase (AChE) and adenosine triphosphatase (ATPase) activities. Similarly, Abdul Razzaq et al. [18] demonstrated the insecticidal potential of a 2.4% Oxymatrine extract, achieving an 88% mortality rate in *Ommatissus lybicus* nymphs seven days post aerial application. Al-Shaibani [19] confirmed Oxymatrine effectiveness against third and fourth-instar larvae of *Tuta absoluta*, as formulated by the Australian company Agrichem.

The insect growth regulator Match also showed substantial bioactivity, likely through its inhibition of cuticle synthesis during ecdysone. This disruption not only leads to mortality but also suppresses successive generations. Match is further effective against freshly laid eggs by impairing proper nymphal hatching. It is utilized for managing larval and nymphal stages across insect orders such as Coleoptera, Lepidoptera, Diptera (e.g., leaf miners and fruit flies), Hemiptera, and rust mites from Eriophyidae. Its applications extend to diverse crops including sugar beets, potatoes, cotton, maize, grapes, citrus, ornamentals, and other vegetables and fruits [20].

Furthermore, *Metarhizium anisopliae* demonstrated potent pesticidal properties attributed to its ability to secrete multiple bioactive secondary metabolites. Among these, destruxins A, B, and C, cytochalasin, swainsonine, and citrinin have been identified as key toxins [21]. HPLC analysis of the fungal filtrate quantified these compounds at concentrations of 0.34, 0.23, 0.41, 0.35, 0.72, and 1.41 µg per 0.5 ml, respectively.

Aphis fabae inflicts damage by penetrating plant tissues and extracting sap, causing leaf deformation and tissue collapse [22]. Salivary secretions introduced during feeding further exacerbate damage, inducing tumor-like formations and localized necrosis [23, 24] (Miles, 1989; Otha et al., 2000). Host plant defense mechanisms, such as glandular trichomes, offer physical barriers against aphid colonization, ultimately reducing infestation severity and associated harm [25, 26].

The findings indicate that the Spanish bean cultivar exhibits greater resistance to *Aphis fabae* infestation compared to the local variety, thus its cultivation is recommended for enhanced pest suppression. Furthermore, the integrated application of multiple control agents significantly increased insect mortality and effectively minimized pest-induced damage. The combined use of Oxymatrine, a plant-derived insecticide, and the growth regulator Match yielded rapid and effective aphid control. Following their initial impact, the application of the biological agent *Metarhizium anisopliae* demonstrated efficiency in targeting residual aphid populations and subsequent developmental stages. This sequential strategy ensured sustained suppression of the pest community, ultimately leading to a substantial reduction in infestation levels and associated crop damage.

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