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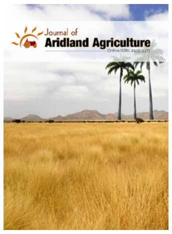

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Predation potential of flower fly *Eupeodes nuba* (Wiedemann, 1830) (Diptera: Syrphidae) on black bean aphid *Aphis fabae* Scopoli (Hemiptera: Aphididae) and the parasitoids species on its host in the field

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ABSTRACT

The flower fly species, *Eupeodes nuba* (Wiedemann, 1830) (Diptera: Syrphidae) is crucial for agroecosystems as it provides various ecosystem services such as pest control through the use of predatory larvae and crop pollination by adults. In this research, the consumption rate of different instars of *Aphis fabae* (Scopoli, 1763) by *E. nuba* larvae has been studied under laboratory conditions at 25±2 °C, 60±10% humidity (RH). Fifty aphids and one larva of flower fly were present in each replicate. The results showed that the maximum number of *A. fabae* consumed by the larvae of *E. nuba* was 49.13 individuals at the third instar, followed by 35.73 individuals of aphids at the second instar and 16.26 individuals for the first instar of the predator. The highest daily consumption of aphids occurred during the third instar of the predator. *A. fabae* was heavily parasitized by different aphid parasitoids, *Binodoxys acalephae* (Marshall, 1896), *Lysiphlebus fabarum* (Marshall, 1896), and *Aphelinus asychis* (Walker, 1839) on the broad bean. Parasitization rates of these parasitoids increased during the broad bean plant season. The first recording of *A. fabae* as a new host for *A. psychic*. The survey of three parasitoids indicated that *L. fabarum* had the highest population in December, the parasitoid *B. acalephae* was the highest in January, and the highest population of *A. asychis* was recorded in February. It seems that biological control can be used as an alternative to chemical pesticides to control *A. fabae*.

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INTRODUCTION

Vegetables, sugar beetroot, beans, potatoes, sunflowers, and tomatoes are among the crops the black bean aphids are most severely affected. More than 200 different cultivable and wild plant species suffer damage. *Aphis fabae* can cause direct and indirect damage to plants and be involved in spreading plant viruses (Adhab & Schoelz, 2015; Adhab *et al.*, 2019; Khalaf *et al.*, 2023). Flower flies, commonly known as hoverflies *Eupeodes* sp. (Diptera: Syrphidae), play a crucial role in agroecosystems because they offer a variety of ecosystem services, such as crop pollination with adults and pest control using predatory larvae (Wotton *et al.*, 2019; Dunn *et al.*, 2020; Pekas *et al.*, 2020). Most predatory syrphinae larvae are considered generalist predators since they eat aphids and other soft-bodied insects (Rojo *et al.*, 2003; Rotheray & Gilbert, 2011). Many studies have highlighted the potential of syrphid larvae as biocontrol agents, particularly for species that feed on aphids (Fidelis *et al.*, 2018). Syrphid larvae have a history of being used as biocontrol agents for aphidophagous species (Fidelis *et al.*, 2018). *E. corollae* has been reported to prey on at least 64 Aphididae species, various Lepidoptera and Thysanoptera insect species (Rojo *et al.*, 2003). In addition, flower fly behavior is suggested as a biocontrol agent because, even if it does not completely utilize the available biomass, it attacks as many pests as possible during its predatory stage (Scott & Barlow, 1986). However, there is still a significant chance of predators in the biological control agent of aphids. Alkhafaji *et al.* (2022, 2023) reported that the larvae of *E. corollae* consumed prey species at different percentages of the provided prey number. By combining its influence with that of other natural enemies and their impact on aphid population growth, Chambers (1986) showed how hoverfly species could be a useful part of the integrated management of aphid pests resistant to chemical control on chrysanthemum and cucumber plants. Due to its economic impact, it is essential to understand the natural enemies to create effective biocontrol programs.

Parasitoids, of which numerous species are commercially available to manage aphids in various crops, are one of the most critical agents in the biological management of aphids (Ahmed *et al.*, 2022). However, some factors can influence the parasitoids; for example, the host plant is one of the most critical factors affecting their presence. A*phelinus asychis* Walker (Hymenoptera: Aphelinidae) is an endoparasite of different species of aphids worldwide that can parasitize over 40 distinct aphid species on various crops (Shirley *et al.*, 2017). Studies on changed host aphids show that A. *asychis* can adapt to a new host in just two generations (Li *et al.*, 2022). In agroecosystems, *Lysiphlebus fabarum* (Marshall) (Hymenoptera: Braconidae) is one of the most frequent parasitoids of A*phis fabae* Scopoli (Hemiptera: Aphididae), spreading mostly globally and attacking A. *fabae* on diverse crops and weeds (Rasekh *et al.*, 2010). Additionally, the parasitoid wasp genus *Binodoxys* is documented as an aphid parasite and a member of the subfamily Aphidiinae (Lazarević *et al.*, 2017). The main goal of this study was to investigate the predatory efficiency rates of flower fly *E. nuba* using the nymphs and adults of the black bean aphid A. *fabae* as prey and identify the species of the aphid parasitoid on broad bean fields in Baghdad, Iraq.

MATERIAL AND METHODS

Location, Agriculture, and Sampling

The study was conducted at the College of Agricultural Engineering Sciences, University of Baghdad, located in the Al-Jadriya area, on 150 m^2 of land. All necessary agricultural operations, including tillage, fertilization irrigation, and weed control, were carried out according to the approved recommendations for agriculture. The agricultural area was divided into three lines with a 12 m length, divided into three blocks, with a distance of 1.5 m between lines. Broad bean (local variety) was planted on both sides of each line with a distance of 20 cm between plants. After the germination of broad bean seeds, the seedlings were left to the natural infestation by black bean aphids A*phis fabae*.

Breeding of Predator *Eupeodes Nuba*

Adult flower flies of *E. nuba* were collected from the College of Agricultural Engineering Sciences/Al-Jadriya campus fields in January 2022. *E. nuba* was caught directly using a trapping net made of a lightweight bag with fine mesh attached to a frame with circle-shaped attached to a handle of different lengths. A net was utilized to capture *E. nuba* insects, which were then placed in transparent plastic tubes measuring 3 cm in width and 6 cm in height. The samples were brought to the laboratory, and the flower fly predator colony was prepared to obtain the required number of predators necessary for s the experiments. The experiments on the efficacy of the predator were conducted after the identification of *E. nuba* and confirmed by morphological diagnosis at the Museum of Natural History and Research Center, University of Baghdad.

To breed *E. nuba*, the second generation of females and males were isolated in pairs and placed in wooden cages measuring 40 × 40 × 40 cm, covered with a fine mesh cloth to prevent the escape of flower fly adults. The cages were provided with zippers on one side for easy handling of the adults. For food, a combination of pollen (approximately 5 g) and a sugar nutrient solution (7-10 g of sugar mixed with distilled water) was provided inside the cages for the adult flower flies (Almohamad *et al.*, 2010). Fresh acacia tree flowers were also provided inside the cage to acclimate and feed on their nectar. A sugar solution was provided by immersing a small piece of cotton in a sugar solution and placing it in a transparent plastic dish (diameter 9 cm and height 1.5 cm) (Alkhafaji *et al.*, 2023).

For the experiment, broad bean seeds were planted in plastic pots measuring 6×12 cm and placed in the open field. The seedlings were covered with fine mesh to prevent parasitoids and predators from accessing the plants. Then, the broad bean plants were artificially infested with black bean aphids A. *fabae* when they reached 10 cm in height. The black bean aphids were obtained by collecting from the field of broad bean plants and identified by specialists at the Museum of Natural History and Research Center, University of Baghdad. Different stages of black bean aphids were developed on the broad bean plants for studying the predatory efficiency of the predator flower fly *E. nuba*.

Infested broad bean branches with black bean aphids (a mix of adults and nymphs) were collected from the open field mentioned above and placed in a petri dish measuring 20 cm in width and 3.5 cm in height. Then, the petri dish was placed in reared flower fly cages to provide a suitable place for egg laying. The eggs were collected daily, isolated, and then placed in a petri dish measuring 9 × 1.5 cm to prevent cannibalism. The Petri dishes that contained flower fly eggs were monitored daily until the eggs hatched. Fifty Petri dishes were treated as five replicates and fifty black bean aphids were provided for each flower fly larva in each petri dish. The Petri dishes were covered and tightened by a rubber band. Each replicate contained one larva and 50 individuals of aphids (10 larvae and 500 individuals of black bean aphids/daily). All Petri dishes were placed in an incubator at the temperature of 24±1 °C, 70±10% humidity (RH), and a photoperiod of 12 light: 12 dark.

Predatory Efficiency

The daily consumption of black bean aphids by the predator larvae *E. nuba* was calculated for each age of the larvae instar. Moreover, the period of each larvae development and the average of black bean aphids consumed for each larvae instar were also calculated. The predatory efficiency values were calculated using the following equations (Al-Dahwi *et al.*, 2012):

$$\text{Daily Predatory efficiency} = \frac{\text{Number of consumed aphids by larvae instar}}{\text{Duration of the larvae instar}}$$

$$\% of\ Number\ consumed = \frac{Whole\ instar\ consuming\ number}{Total\ number\ consumed\ by\ all\ instars} \times 100$$

Identification and Investigation of Population Fluctuations of Bean Aphid Parasitoids

To investigate the population fluctuations of parasitoids in the field, 150 m^2 of the area was planted with broad beans. The planted area was divided into 3 blocks, and each block contained 60 plants of broad beans. After infestation of plants with *A. fabae*, 10 samples of infested broad bean leaves with black bean aphids and mummified aphids were collected from each block with three replications, placed in plastic bags (size 1 kg), and transported to the laboratory for weekly calculation using a light microscope, from 15/10/2021 to 30/2/2022 for the autumn season. Ten black bean aphid individuals were randomly selected, taken as a sample, isolated, and placed in a petri dish (9 cm) with five replications to identify the aphid parasitoids. They were monitored until the adult aphid's parasitoids emerged. The parasitoids were then divided into groups depending on parasitoid species and identified morphologically by the taxonomical expert at the Museum of Natural History and Research Center, University of Baghdad. The average number of parasitoids was calculated for each individual.

Statistical Analysis

For statistical analysis, the data were analyzed using SAS statistical analysis software version 9.6 (2018) following a complete random design (CRD). Using the least significant difference (LSD) test with a significance level of 0.05, the variance between treatments was compared (SAS, 2018).

RESULTS AND DISCUSSION

Consumption and Development Period of the Predator *E. nuba* Larval Stage on Black Bean aphid *A. fabae*

The daily consumption rate of black bean aphids by the flower fly *E. nuba* larvae was studied under incubator conditions across different instars stages. The mean daily consumption of black bean aphids by first, second, and third was 16.26, 35.73, and 49.13 individuals, respectively (Table 1). In comparison, the total prey consumed during the development of each predator stage was 55.93 individuals out of the 500 aphids provided, representing 15.33% of the prey consumed by the first instar of *E. nuba*. For the second instar, the mean total prey consumed was 102.86 individuals, which accounted for 25.76% of the total number of real aphid individuals consumed during the entire instar. The third instar consumed 208.12 black bean aphids, representing 56.96% of the total aphid consumption during the lifespan development of the third instar of *E. nuba* larvae.

The results indicated that the development period of the first instar larvae of *E. nuba* was 3.33 days, followed by 3.00 days for the second instar to transition to the third instar. The metamorphosis of the third instar to the pupal stage took 4.53 days.

The study found that the third instar of syrphid larvae, *E. nuba*, consumed the most black bean aphids, followed by the second and the first instars. At the same time, the third instar consumed more of aphids overall than the first and second instars. The findings are supported by Sood *et al.* (2007), who stated that the success of a predator is determined by the relative numbers and sizes of the prey (aphids) and the predator larvae's instar. These findings align with those of Alkhafaji *et al.* (2023), who discovered that 50 cotton aphid insects provided the most significant daily and total consumption of *E. corollae* predators, with a shorter development time of predator larvae at the same density. The average number of insects consumed by the predator's larvae increased with aphid population density (25, 50, 75, and 100), reaching 84.64, 143.16, 170.9, and 217.93 for cotton aphids and 56.23, 110.67, 124.78, and 148.16 for oleander aphids, respectively. Sood *et al.* (2007) reported that the larvae of *E. nuba* frequently fed on 289.4 aphids throughout their lifetime. Other studies have shown that the third larval instar of the aphid-preying syrphid flies consumes more than 80% of the aphids consumed during the period of complete larval development because it is more capable and effective at attacking aphids than other larval instars (Joshi & Ballal, 2013; Adhab & Alkuwaiti, 2022; Jiang *et al.*, 2022). Our results are consistent with the study conducted by Al-Rawy and Abdulhay (2012), who found that the third larval instar of the predator, *Chrysoperla carnea* Stephens, was the most voracious, and predation efficiency increased as the larvae developed. The larvae of the predator consumed 47, 80, and 181.67 nymphs of the first instar with predation rates of 14.71%, 23.75%, and 50.13% for the first, second, and third larval instars, respectively.

The Parasitoids Species on *A. fabae*

The results showed that three parasitoids from two families parasitize *A. fabae* in the broad black bean field, namely Braconidae (*Binodoxys acalephae* and *Lysiophelbus fabarum* Marshall) and Aphilinidae (*Aphelinus asychis* Walker) from the order of Hymenoptera (Table 2). The average number of *B. acalephae*

Table 1: Predation efficiency of flower fly *E. nuba* larvae on black bean aphid *A. fabae* under incubator conditions acorss different larval durations

Larval Instars	Mean±SE			
	Daily Prey consumption	Total Prey consumption	% Prey consumption	Instar duration/day
Instar 1	16.26±2.13c	55.93±4.52c	15.33±1.28c	3.33±0.16a
Instar 2	35.73±2.34ab	102.86±10.95ab	25.76±2.45ab	3.00±0.36a
Instar 3	49.13±5.34ab	208.12±20.73a	56.96±5.26ab	4.53±0.46a
LSD	12.41 *	47.71 *	10.28 *	1.224 *

*Indicates statistical significance at $P \leq 0.05$

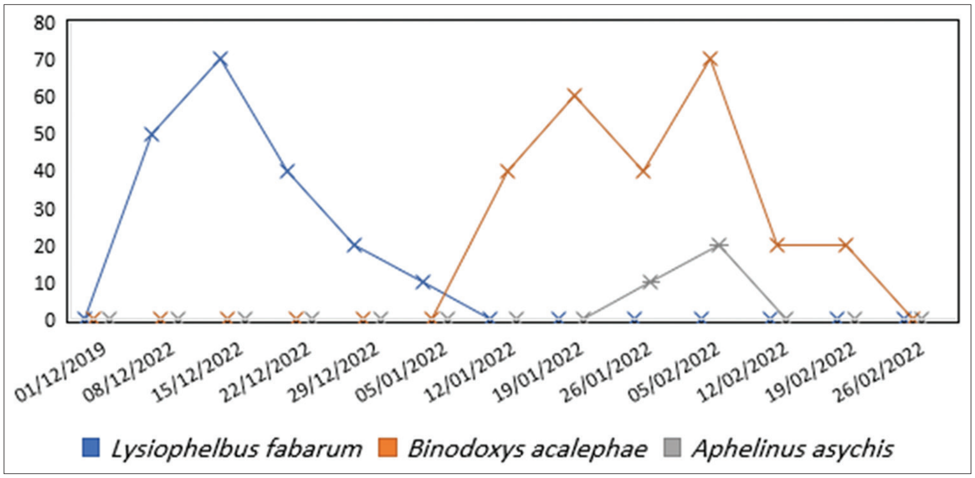

Figure 1: Percentage of parasitoid species on the black bean aphid *A. fabae* that infested broad bean plants in the field during the season of 2021-2022

Table 2: Percentage of different parasitoid species found on black bean aphid *A. fabae* that infested broad bean plants in the field during the season of 2021-2022

Parasitoid name	Order	Family	Average±SE
Aphelinus asychis	Hymenoptera	Aphilinidae	2±0.12
Lysiophelbus fabarum	Hymenoptera	Braconidae	4±0.10
Binodoxys acalephae	Hymenoptera	Braconidae	6±0.18

and *L. fabarum* parasitoids collected was six and four individuals, respectively, while the average for *A. asychis* was two individuals. Accordingly, among them, *B. acalephae* was the dominant species. Furthermore, the black bean aphid *A. fabae* was identified as a new host for *A. asychis*. All parasitoid species mentioned above were identified by the taxonomical expert at the Museum of Natural History and Research Center, University of Baghdad.

Moreover, the results indicated that three parasitoid surveys were conducted in the broad black bean field on a different collection date (Figure 1). *L. fabarum* was recorded on 8/12/2021 with a population of 50%, and the parasitoid population decreased to 10% from 5/1/2022. At the same time, the highest population of *L. fabarum* was observed on 15/12/2021 and recorded at 70% of *the L. fabarum* population. Followed by the parasitoid of *B. acalephae* appeared on 12/1/2022 on the infested black bean plants with *A. fabae* in 40% of individuals, and the presence of *B. acalephae* decreased to 20% by 19/2/2022. The parasitoids of *B. acalephae* disappeared on 26/2/2022 with a percentage of 10% of individuals and the highest population of *A. asychis* was recorded on 19/2/2022 with 20% of individuals (Figure 1).

Aphid parasitoids (Hymenoptera: Braconidae: Aphilinidae) are among the most significant biological control agents. In this study, the presence of aphid parasitoids was surveyed in a broad bean field during the winter and spring seasons of 2021 and 2022. A total of three species from three genera and two families were recorded, namely Braconidae (*B. acalephae* and *L. fabarum*) and Aphilinidae (*A. asychis*), which were collected from parasitized black bean aphids. Results indicated that *L. fabarum* was the first parasitoid to appear in the broad bean field and was followed by *B. acalephae* and *A. asychis*. The sequential appearance of parasitoids may be attributed to environmental factors, aphid hosts, and plant types. It was revealed that the North of Iraq had the most observed parasitoids and aphids. Generally, there were fewer parasitoids than aphids belonging to the families Braconidae and Aphelinidae, which have many parasitoid species (Bandyan *et al.*, 2021; Hassan, 2021). Our research suggests that aphid parasitoids may be found on the broad bean plants because of the aphid infestation that locates damaged plants and identifies aphid insects on the infested plant by detecting the host plants. This is because most aphid parasitoids effectively search for damaged plants where aphids will be present before searching for infested plants to find aphids (Ahmed *et al.*, 2021; Adhab, 2021).

CONCLUSION

E. nuba (Diptera: Syrphidae) is an important predator for controlling various species of aphids by consuming them and reducing their population on host plants. The adults of this species also contribute to crop pollination, while the predatory larvae aid in pest control. In a laboratory study, we investigated the consumption of different aphid stages by *E. nuba* larvae. The results showed that the third-instar aphid was the most frequently consumed by the predator's larvae, followed by the first and second instars. However, syrphid larvae can effectively control the number of this aphid species in the early stages of colonization. In agricultural crops, such as broad beans, the black bean aphid *A. fabae* was significantly parasitized by the aphid parasitoids *B. acalephae*, *L. fabarum*, and *A. asychis*. Throughout the season, broad bean plants showed an increase in parasitization rates. *A. asychis* was discovered to have a new host in the black bean aphid *A. fabae*. Biological control can be used as an alternative to chemical pesticides to manage *A. fabae* infestation effectively.

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