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RESEARCH

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Using predatory species and entomopathogenic fungi as alternatives to chemical pesticides in green bean field

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Abstract

Background Green bean, *Phaseolus vulgaris* L. (Fam.: Leguminosae) is a widely consumed grain legume prized for its edible seeds and pods. It is susceptible to infestations with various pests as insects and mites throughout the growing season. In this study, the efficacy of the predatory species, *Chrysoperla carnea* (Stephens), *Phytoseiulus persimilis* Athias-Henriot and the entomopathogenic fungus (EPF) *Metarhizium anisopliae* (Hypocreales: Clavicipitaceae), as well as conventional pesticides, Mospilan and Vertimec, were evaluated against the most important pests, mainly the whitefly, *Bemisia tabaci* (Genn.) and the two-spotted spider mite, *Tetranychus urticae* Koch, infesting the green beans cultivated at two locations Giza and El-Menoufia Governorates in Egypt.

Results The findings demonstrate that treatments using *C. carnea* and *M. anisopliae* effectively reduced the whitefly population, while pesticide treatments were comparatively less effective. In the Giza plots, at the end of the experiments, the use of both *M. anisopliae* and *C. carnea* showed high reductions in whitefly population (85.57 and 84.87%), respectively, while in El-Menoufia, *C. carnea* (97.74%) was the most effective treatment followed by *M. anisopliae* (90.32%). Pesticide treatment in this case yielded a reduction rate of (22.76 and 59.67%) in Giza and El-Menoufia plots, respectively. However, for spider mite control, *P. persimilis* proved to be the most effective treatment in Giza and El-Menoufia plots, reducing the spider mite population to 98.44 and 96.14%, respectively. *Metarhizium anisopliae* treatment also displayed moderate effectiveness, with reduction rates of 75.62 and 75.37% in Giza and El-Menoufia plots, respectively. In comparison, pesticide treatment showed low effectiveness, with reduction rates of only 23.92 and 53.16% in the two locations, respectively.

Conclusion Applications of the predator, *C. carnea* and the EPF, *M. anisopliae* were highly effective in reducing the population of whitefly, while the predator mite *P. persimilis* proved to be the most effective for controlling the spider mites. Overall, the study suggests that biocontrol agents, such as the predators and the EPF, can be considered as alternatives to synthetic chemical pesticides for controlling pests infesting green beans.

Keywords *Phaseolus vulgaris*, Pests, *Chrysoperla carnea*, *Phytoseiulus persimilis*, *Metarhizium anisopliae*, Pesticides

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Background

Green bean (*Phaseolus vulgaris* L., Leguminosae) is a major grain legume that is widely consumed as edible seeds and pods. Common beans are a valuable source of protein, minerals such as iron and zinc, and vitamins for numerous human populations (Beebe et al. 2000). Immature pods are consumed fresh and can be easily preserved by freezing, canning, or dehydrating. Mature pods and seeds are typically dried and can be eaten boiled, baked, fried, or ground into flour. Additionally, crop residues such as dried pods and stems (straw) and processing by-products like discarded pods and pod extremities can be used as fodder for livestock (Wortmann 2006). Overall, green beans are a versatile and nutritious crop with a range of uses for both human consumption and animal feed. However, the use of pesticides in bean cultivation can pose risks to human health, animal health and the environment. Therefore, it is essential to ensure that bean crops are grown and processed, using sustainable and environmentally friendly practices, such as integrated pest management and organic farming methods.

Green bean is usually infested with a variety of pests throughout its growing season, including the two-spotted mite, whitefly, aphids, leaf miners, leafhoppers and thrips. These insect pests and mites can cause an extensive damage to the bean pods, affecting both their quantity and quality. As a result, the infestation often leads to reduced yields, which can have a significant impact on the overall productivity of the crop (El-lakwah et al. 2010).

Use of synthetic pesticides has been the primary method for managing pests, but they had led to challenges in controlling the whitefly, *Bemisia tabaci* Genn. (Hemiptera: Aleyrodidae). This main pest poses a significant threat to the vegetable industry due to its resistance to many pesticides and its role as a vector for numerous plant viruses (Gerling 1990). Unfortunately, the extensive use of synthetic insecticides has resulted in *B. tabaci* developing resistance to a wide range of insecticides, making it difficult to manage (Patra and Hath 2022). Similarly, the two-spotted spider mite, *Tetranychus urticae* Koch, has also developed resistance to various insecticides and acaricides, including organophosphates (OPs), carbamates, synthetic pyrethroids and even some recently developed compounds (Xu et al. 2018).

Mixed infestations of insects and mites are commonly occurring, and the traditional method of controlling them involves the use of large amounts of insecticides and acaricides. It is essential to adopt effective non-chemical control measures of both pests. One promising alternative is the use of biocontrol agents, such as predators, parasitoids and microbial entomopathogenics. These agents can be effective in reducing the pest population and minimizing the harmful side effects of pesticides (Batta 2003).

Tetranychus urticae and *B. tabaci* are significant pests that can cause substantial damage to green bean crops if not managed effectively. Due to avoid potential harm to human health and the environment from conventional pesticide treatments, this study aimed to assess the efficacy of EPF, *M. anisopliae* and two predatory species, *P. persimilis* and *C. carnea*, for controlling major pests in green bean fields, and to compare their performance with conventional pesticide treatments. Therefore, this study's findings will be of great value to farmers and agricultural practitioners seeking to implement safe sustainable and effective pest management strategies.

Materials

Sources of bioagents

Larvae of the predatory *C. carnea* were obtained from the Center of Bio-Organic Agricultural Services (CBAS) in Aswan, Egypt. In this study, eggs of the angoumois grain moth, *Sitotroga cerealella* (Oliver), were used, as prey, for *C. carnea* larvae. The cocoons of *C. carnea* were collected daily and transferred to a plastic jar. The adults were placed in a 2-l transparent plastic jar covered with black muslin cloth fixed with rubber bands, for egg laying. The predatory adults were fed on artificial diet containing yeast, honey, pollen and water (1:1:1:1) and pasted on horizontal plastic strips placed in an adult rearing cage. Wet-soaked cotton was placed inside the jar to provide moisture. The rearing was carried out under the controlled conditions of 25 ± 2 °C, 60% R.H. and 16:8 photoperiods (L/D). The rearing was continued throughout the experimental period to ensure that second instar larvae of *C. carnea* were readily available for release in the field.

The formulation of *M. anisopliae* (Bio-Magic®) which was used against *T. urticae* and *B. tabaci* was manufactured by Gaara Establishment for Import and Export, Egypt. It was available as a Powder package containing spores and mycelial fragments (1×10^9 CFU's/gm).

Pesticides

The pesticides used were the synthetic neonicotinoid Mospilan 25% SP and the acaricide-insecticide Vertimec 1.8% EC.

Experimental design

The study was conducted at two farms located in the Giza and El-Menoufia governorates of Egypt. The farms were planted with green bean seedlings of the Almonte and Paulista varieties on November 24th and December 25th, 2021, in the Giza and El-Menoufia governorates, respectively. The daily weather conditions, including minimum and maximum temperatures and relative humidity, were provided by the Central Laboratory for Agricultural Climate (CLAC) located in Dokki, Egypt. A randomized complete block design with five replications was used for each treatment in the experiment. Each experimental unit (plot) was 10 m^2 to accommodate 30 plants spaced at 0.5 m × 0.5 m. A two-meter-wide walkway was used to separate the plots to prevent any treatments from drifting.

Treatments

Four treatments: T1 predators (*C. carnea* and *P. persimilis*), T2 fungi (*M. anisopliae*), T3 pesticides and T4 control were applied.

To control *B. tabaci*, five-second larval instars of *C. carnea* per plant were released in the field, while for *T. urticae*, ten adults of *P. persimilis* were released per plant. The *M. anisopliae* formulation was applied as a foliar spray (6 gm/liter) to control both pests. The pesticides used were synthetic neonicotinoid Mospilan 25% SP at 25gm/100l and the acaricide-insecticide Vertimec 1.8% EC at 75cm/150l.

Metarhizium anisopliae and pesticides were applied using a separate Pomsan sprayer 10L for each treatment (model: K-93), with a nozzle size of 1 mm. Once the pests' infestations were detected, the treatments were applied 3 times at 14-day intervals to control the infestations.

Assessment of the effectiveness of treatments

To evaluate the effectiveness of all treatments, data were collected by inspecting 25 plants chosen at random from each treatment (five plants from each replicate) weekly started from seedling stage until harvest. Population density of pests was determined by counting the number of pests on three leaves that were randomly selected from the top, middle and lower levels of each plant. Square inch lens with 10X magnification was used for inspection. The total number of *B. tabaci* nymphs and pupae and the mobile stages of *T. urticae* was recorded.

Statistical analysis

Collected data were coded and entered into the statistical package SPSS version 22. Quantitative variables were described in terms of mean and standard deviation. To test significant differences among treatments, Analysis of variance (ANOVA) was conducted, followed by post-hoc Tukey's (HSD) with a significance level of $p < 0.05$. This was done to reject the null hypothesis and confirm the presence of significant variance among treatments' groups.

The percentage of reduction in pests' populations was calculated per plant leaf as a mean from each plant compared to the control. These percentages were estimated according to Henderson and Tilton (1955):

$$\text{Reduction } \% = \left(1 - \frac{\text{no. in co. before treatment X no. in T after treatment}}{\text{no. in Co. after treatment X no. in T before treatment}}\right) * 100$$

where no. = pest population, Co. = control, and T = treated.

This formula allowed for a standardized method of calculating the reduction in pest populations and enabled comparison among different treatments. The data were statistically analyzed by correlation analysis between weather parameters and pest populations.

Results

The study recorded weather data during field applications, including maximum temperature ranged from 37.53 to 11.76 °C, minimum temperatures of 19.9–4.1 °C and a relative humidity ranged from 82.81 to 53.83% in the Giza Governorate from November 2021 until March 2022, while in the El-Menoufia Governorate the maximum temperature ranged from 42.72 to 15.14 °C), minimum temperatures of 19.78–8 °C and relative humidity of 81.62–57.92% from December 2021 until April 2022. These data indicated that the conditions seem to be similar in both locations.

The data underwent statistical analysis involving correlation analysis between weather parameters and pest populations. In the Giza plots, a correlation was observed between maximum temperature ($r = 0.0175$ and 0.215), minimum temperature ($r = 0.0023$ and 0.219) and relative humidity ($r = 0.654$ and 0.392) with whitefly and mite populations, respectively.

In El-Menoufia plots, a correlation was identified between maximum temperature ($r = 0.34$ and 0.29) and minimum temperature ($r = 0.284$ and 0.229) with whitefly and mite populations, respectively. Furthermore, a negative correlation was observed between relative humidity ($r = -0.058$ and $-$ 0.115) and whitefly and mite populations, respectively.

In Giza plots, the whitefly was occurred first in small numbers during the 4th week after planting, and its population gradually increased over time. Prior to the beginning of treatments, the whitefly population varied between 6.48 ± 2.1, 5.96 ± 1.4, 5.96 ± 2.4 and 6.1 ± 1.9 nymphs and pupae/leaf in control, pesticides, *M. anisopliae* and *C. carnea* treated plots, respectively. Three applications of pesticides (Mospilan 25% SP), *M. anisopliae* and *C. carnea* were applied on the 5th, 7th and 9th weeks after planting. On the 12th week after planting, the whitefly population reached (50.8 ± 1.6, 36.99 ± 2.3, 14.1 ± 1.7 and 9.44 ± 1.2 nymphs and pupae/leaf) in the control, pesticides, *M. anisopliae* and *C. carnea* plots, respectively. After the third application, the whitefly population in *M. anisopliae* and *C. carnea* plots had reduced to 9 ± 4.1 and 8.95 ± 3.1 nymphs and pupae/leaf, respectively. However, in pesticides and control plots, they increased to 48.2 ± 15.74 and 62.4 ± 20.7 nymphs and pupae/leaf, respectively (Fig. 1).

Significant differences were recorded among different treatments ($F = 8.31$, df = (3), $p < 0.05$). Non-significant differences were found between control and pesticides plots and *M. anisopliae* and *C. carnea* plots at $p < 0.05$. However, there was a significant difference between pesticides and *M. anisopliae* plots and pesticides and *C. carnea* plots at $p < 0.05$.

The pesticides treatment had the lowest reduction in the whitefly population after the third application, reaching (22.76%). The whitefly population reduction was the highest in *M. anisopliae* and *C. carnea* plots, with their proportions being close to each other, reaching (85.57 and 84.87%), respectively (Fig. 2).

In El-Menoufia plots, also, the whitefly was first observed in small numbers during the 4th week after planting and varied between 0.5 ± 0.2, 0.56 ± 0.4, 0.7 ± 0.3 and 0.62 ± 0.3 nymphs and pupae/leaf in control, pesticides, *M. anisopliae* and *C. carnea* plots, respectively (Fig. 3). A total of three applications of Mospilan 25% SP, *M. anisopliae* and *C. carnea* in the 5th, 7th and 9th weeks after planting were applied.

By the 12th week after planting, the whitefly population reached 7.35 ± 2.5, 3.3 ± 1.8, 1.3 ± 0.5 and 1.26 ± 1.4 nymphs and pupae/leaf in control, pesticides, *M. anisopliae* and *C. carnea* plots, respectively. After the third application, the whitefly population in *M. anisopliae* and *C. carnea* plots reduced to 1.2 ± 0.47 and 0.28 ± 0.53 nymphs and pupae/leaf, respectively. However, in pesticides and control plots, it increased to 12.4 ± 4 and 5 ± 0.68 nymphs and pupae/leaf, respectively (Fig. 3). There was a significant difference among treatments ($F = 13.72$, df = (3), $p < 0.05$).

Data analysis revealed significant differences between control and each treatment's group (pesticides, *M. anisopliae* and *C. carnea* at $p < 0.05$. Non-significant difference was found between pesticides and *M. anisopliae*, between *M. anisopliae* and *C. carnea*, and between pesticides and *C. carnea* at $p < 0.05$.

The highest reduction in whitefly population was observed in *C. carnea* treatment (97.74%) followed by *M. anisopliae* treatment (90.32%) and pesticides treatment (59.67%) (Fig. 4). Overall, the results of these findings suggested that biological control agents such as *M.*

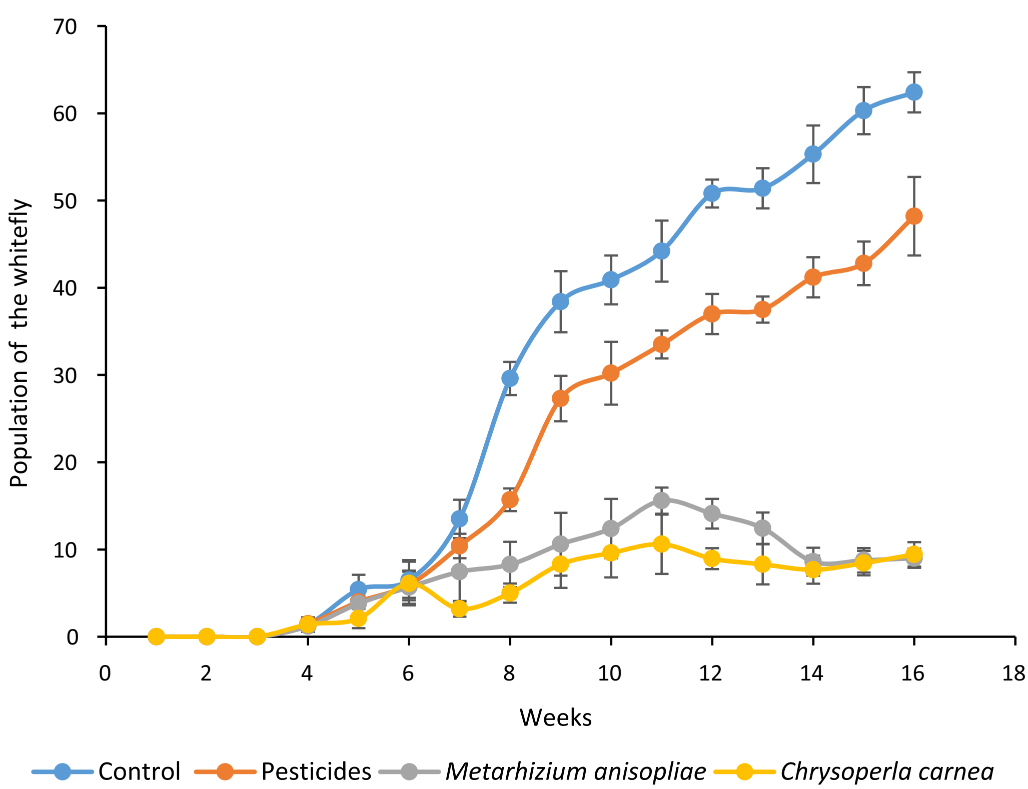

Fig. 1 Impact of the treatments on weekly average number of whitefly per leaf during the green bean season in Giza plots

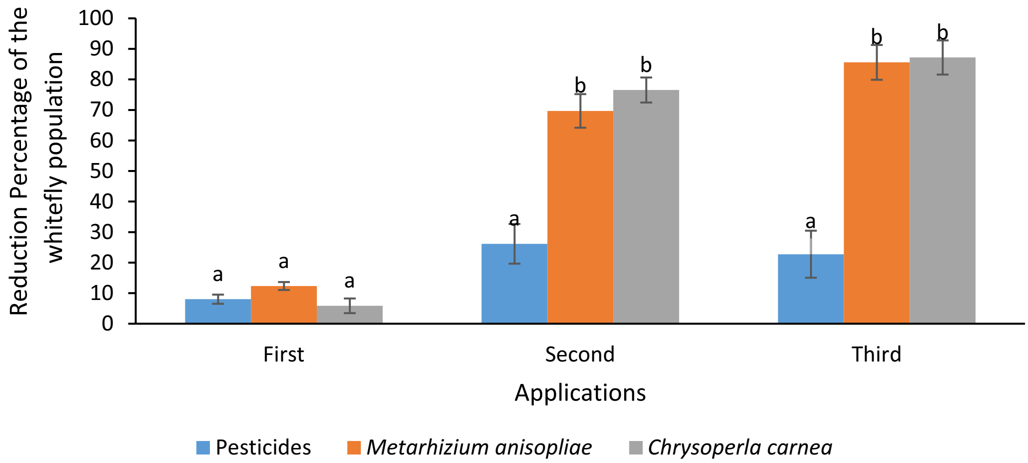

Fig. 2 Percentage of reduction in whitefly population after applied pesticides, *Metarhizium anisopliae* and *Chrysoperla carnea* during the green bean season in Giza plots

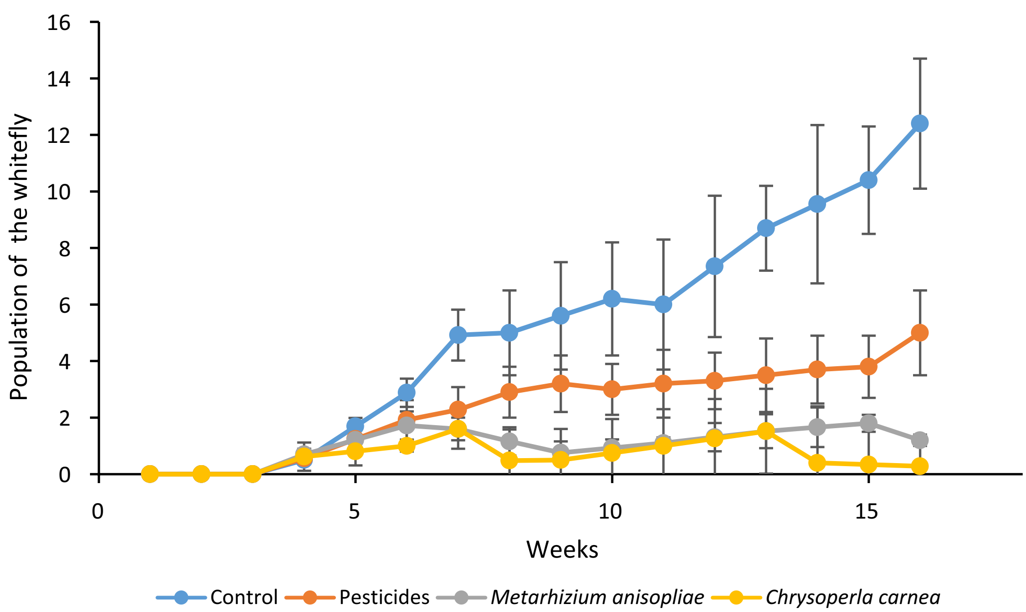

Fig. 3 Impact of the treatments on weekly average number of whitefly per leaf during the green bean season in El-Menoufia plots

anisopliae and *C. carnea* can be effective alternatives to chemical pesticides for whitefly control.

This study investigated the effectiveness of different treatments for controlling mite populations in plots at Giza and El-Menoufia. The initial spider mite population in Giza plots was observed in the 4th week after planting, and its density varied between 0.08 ± 0.2, 0.16 ± 0.2, 0.2 ± 0.3 and 0.08 ± 0.3 mites/leaf in control, pesticides, *M. anisopliae* and *P. persimilis* plots, respectively (Fig. 5). Three applications of pesticides (Vertimec 1.8% EC), *M. anisopliae* and *P. persimilis* in the 7th, 9th and 11th weeks after planting were applied. The spider mite population gradually increased, reaching 9.3 ± 1.1, 8.4 ± 2.2, 8.6 ± 0.3 and 8.62 ± 0.5 mites/leaf in control, pesticides, *M. anisopliae* and *P. persimilis* plots, respectively, by the 8th week after planting (Fig. 5). After the second application, the spider mite population in control, pesticides and *M. anisopliae* plots increased to 54.9 ± 7, 36.4 ± 8.1 and 22.36 ± 5.1 mites/leaf, respectively. After the third application, the spider mite population decreased to 2.6 ± 1.1 mites/plant in *P. persimilis* plots and increased significantly to 127.4 ± 26.8, 40.7 ± 11.3 and 167.4 ± 33.8 mites/leaf in pesticides, *M. anisopliae* and control plots, respectively (Fig. 5). Significant differences were observed

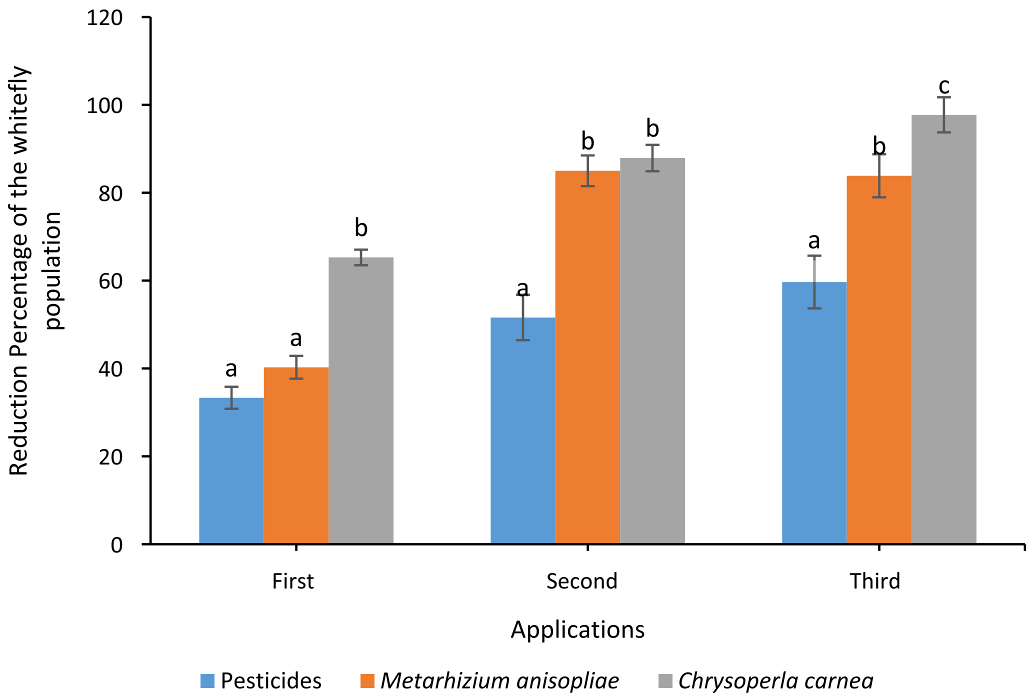

Fig. 4 Percentage of reduction in whitefly population after applied pesticides, *Metarhizium anisopliae* and *Chrysoperla carnea* during the green bean season in El-Menoufia plots

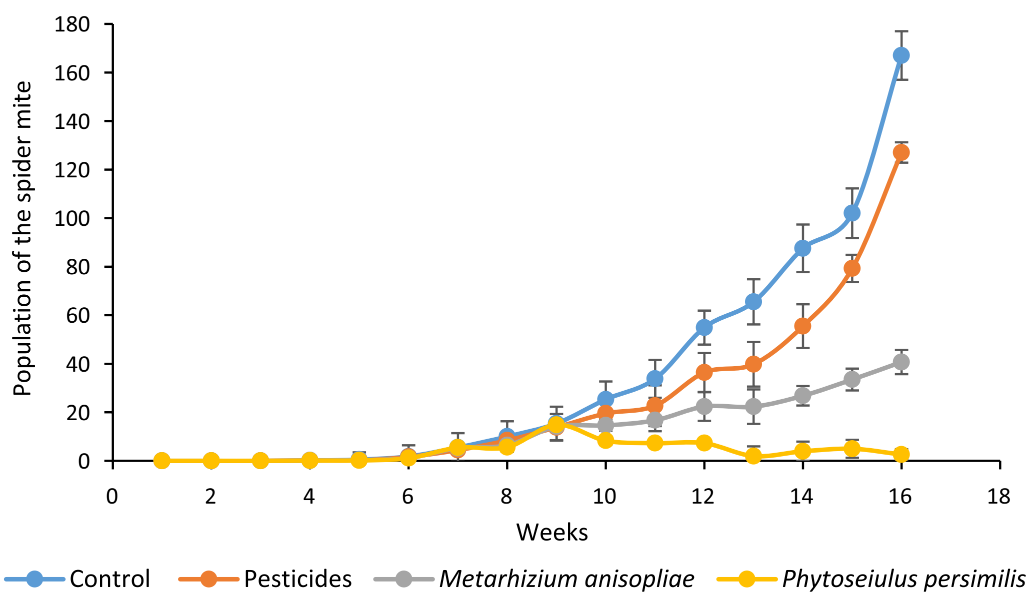

Fig. 5 Impact of the treatments on weekly average number of spider mite per leaf during the green bean season in Giza Plots

among treatments ($F=3.16$, df $=(3)$, $p<0.05$). There were non-significant differences between control and pesticides treatments, at $p<0.05$. However, a significant difference was found between control and *P. persimilis* treatments at $p<0.05$. The predator mite *P. persimilis* treatment resulted in the highest reduction in spider mite population (98.44%), followed by *M. anisopliae* treatment (75.62%) and then pesticides treatment (23.92%) (Fig. 6).

At El-Menoufia plots, the spider mite population was monitored for eight weeks prior to treatment.

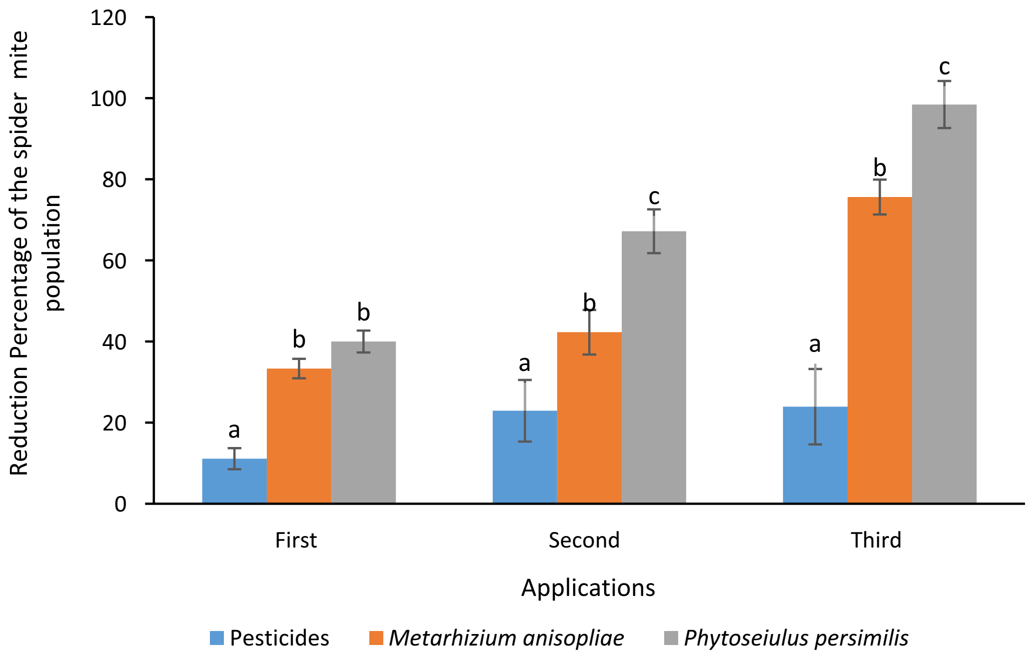

Fig. 6 Percentage of reduction in spider mite population after applied pesticides, *Metarhizium anisopliae* and *Phytoseiulus persimilis* during the green bean season in Giza plots

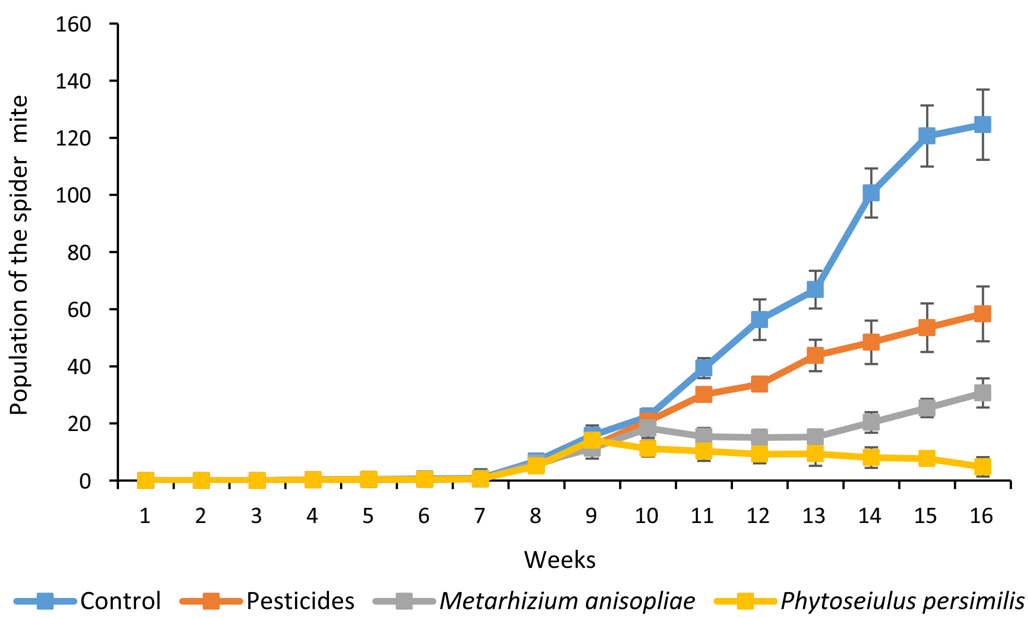

Fig. 7 Impact of the treatments on weekly average number of spider mite per leaf during the green bean season in El-Menoufia Plots

The initial mite population varied between 6.84 ± 2.3, 5.44 ± 1.5, 6.04 ± 2.6 and 5.08 ± 2.5 mites/leaf in control, pesticides, *M. anisopliae* and *P. persimilis* plots, respectively (Fig. 7). Three applications of pesticides (Vertimec 1.8% EC), *M. anisopliae* and *P. persimilis* in the 9th, 11th and 13th weeks after planting were applied. After the second application, the spider mite population in control, pesticides, *M. anisopliae* and *P. persimilis* plots reached 56.32 ± 7.1, 33.7 ± 2.3, 15.08 ± 2.7 and 9.24 ± 3.2 mites/leaf, respectively.

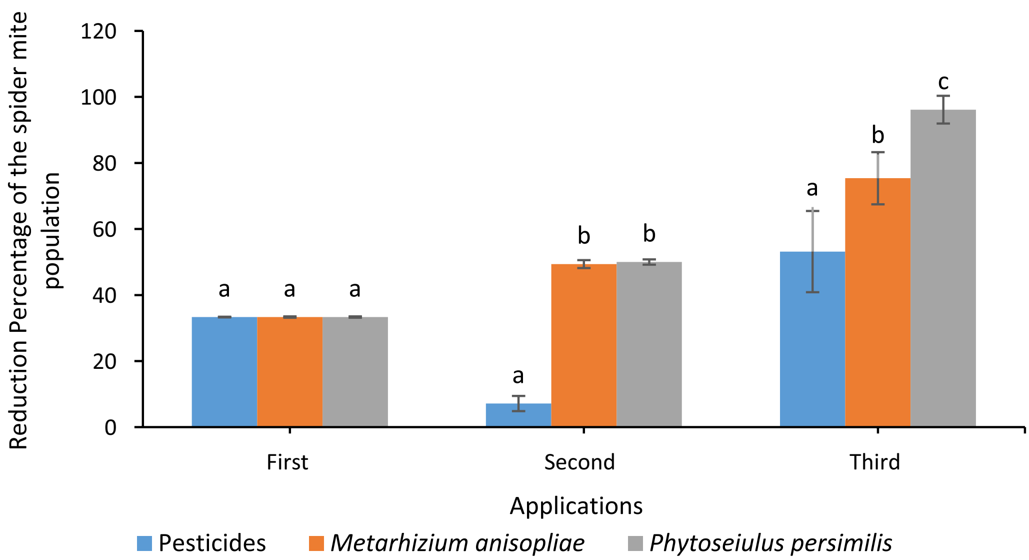

Fig. 8 Percentage of reduction in spider mite population after applied pesticides, *Metarhizium anisopliae* and *Phytoseiulus persimilis* during the green bean season in El-Menoufia plots

After the third application, the spider mite population further decreased to (4.8 ± 0.3 mites/leaf) in *P. persimilis* plots. In contrast, it increased to (58.36 ± 10.7, 30.68 ± 9.2 mites/leaf) in pesticides and *M. anisopliae*, respectively. Interestingly, the spider mite population in the control plots increased significantly to (124.6 ± 28.6 mites/leaf) (Fig. 7). The results of the statistical analysis revealed a significant difference among treatments ($F = 4.07$, df = (3), $p < 0.05$).

Data analysis revealed significant differences between control and *M. anisopliae* and control and *P. persimilis* at $p < 0.05$. Non-significant difference was found between control and pesticides and pesticides and *M. anisopliae* at $p < 0.05$.

The treatment with *P. persimilis* resulted in the highest reduction in spider mite population (96.14%) followed by *M. anisopliae* treatment (75.37%) and then the pesticides treatment (53.16%) (Fig. 8). The results indicated that the biocontrol agent *P. persimilis* was the most promising treatment for spider mite control, as it resulted in the highest reduction in mite populations.

Discussion

The findings of this study suggest that using the predator *C. carnea* and the EPF *M. anisopliae* can be effective tools for reducing whitefly populations in green bean fields, providing a promising alternative to synthetic chemical pesticides.

Several studies have reported the effectiveness of *M. anisopliae* in controlling whitefly populations in the field. Abdel-Raheem and Al-Keridis (2017) observed that *Beauveria bassiana*, *M. anisopliae* and *Lecanicillium lecani* isolates were promising fungal biocontrol agent for the whitefly control in the field. Similarly, Flores et al. (2012) reported that *M. anisopliae* was significantly more effective against eggs, first, second and third nymphal instars and pupae of the whitefly *B. tabaci*. Mixed applications of *M. anisopliae* and *B. bassiana* were found to maximize the likelihood of control of all stages of *B. tabaci*. Additionally, Alghamdi et al. (2018) found successfully suppressed of the aphid, *Aphis gossypii* Glov and the whitefly *B. tabaci* populations on sweet pepper and squash plants in open fields, through the releases of *C. carnea*. Also, Zaki et al. (1999) observed that different releasing rates of *C. carnea* induced highly significant reduction of *A. gossypii* and *B. tabaci* on various vegetable crops. These studies provided further support for the use of *C. carnea* and *M. anisopliae* as effective biocontrol agents for managing whitefly populations in agricultural fields.

The results of the present study also demonstrated the potential effectiveness of *P. persimilis* and *M. anisopliae* as treatments for managing spider mite populations in green bean fields. Notably, *P. persimilis* was found to be more effective in reducing the population of *T. urticae* than *M. anisopliae* These findings are consistent with previous researches that have shown the efficacy of these natural enemies in controlling mite infestations in various crops. Abdel-Aziz (2016) found that releasing six individuals of *P. persimilis* per plant can be an effective approach for controlling populations of *T. urticae*. Similarly, Tiftikçi et al. (2020) reported that *P.*

persimilis could be released for the effective control of *T. urticae* on tomato plant from the mid-August to the beginning of September in the Çanakkale province of Northwest Turkey.

Abdallah et al. (2014) compared the effectiveness of *P. persimilis*, *Typhlodromips swirskii*, entomopathogen *B. bassiana* and the biochemical compound Abamectin (Vapcomic) in reducing the population of *T. urticae* on kidney beans and sugar snap peas. It was showed that *P. persimilis* was the most effective treatment followed by Vapcomic, *B. bassiana* and *Typhlodromips swirskii*.

Bugeme et al. (2015) reported that *M. anisopliae* isolate ICIPE78 could be an alternative to acaricides for managing *T. urticae* on common bean in the screen house and field experiments. Shaef Ullah and Lim (2017) found that a single application of *B. bassiana* was effective in controlling *T. urticae* and reduced the egg and adult populations of initially, but mite populations rebounded again after few days. *Phytoseiulus persimilis* at the highest release rate eliminated the mite population completely, while the lowest release rate failed to control the spider mites. The combined application of *B. bassiana* and low release rate of *P. persimilis* also successfully controlled *T. urticae* population, with the lowest corrected leaf damage (1.5%).

Batta (2003) reported that *M. anisopliae* had a great potential for controlling whitefly *B. tabaci* and the spider mite *T. cinnabarinus*, particularly when applied in an invert emulsion formulation. However, further studies are necessary to clarify the effect of the fungus on the mite predator to ensure safe application of these treatments together.

Based on the results of this study, both the combination of the mite predator *P. persimilis* and the EPF *M. anisopliae* can be used for controlling the whitefly and mites, but the necessary studies have to be done to clarify the effect of the fungus on the mite predator so that they can be applied together safely.

Conclusion

The findings of this study demonstrated the potential of using the predator *C. carnea*, the predatory mite *P. persimilis* and the EPF *M. anisopliae* for controlling some of the main pests in green bean fields. These natural enemies offered a promising alternative to synthetic chemical pesticides for managing pests' infestations in the crop. Further research is needed to determine the optimal application rates and conditions for these treatments, as well as their compatibility when used together.

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Author contributions
Dalia Adly conceived research. Hamzah M. Kamel, Aziza E. Eid, Gehan M. Nouh and Dalia Adly conducted experiments. Hamzah M. Kamel, Aziza E. Eid, Gehan M. Nouh and Dalia Adly contributed material. Gehan M. Nouh and Dalia Adly analyzed data and conducted statistical analyses. Gehan M. Nouh and Dalia Adly wrote the manuscript. Hamzah M. Kamel, Aziza E. Eid, Gehan M. Nouh and Dalia Adly secured funding. All authors read and approved the manuscript.

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Availability of data and materials
All data and materials are available.

Declarations

Ethics approval and consent to participate
Not applicable—the study was conducted on insect species that are abundant in the ecosystem and does not require ethical approval.

Consent for publication
The manuscript has not been published in completely or in part elsewhere.

Competing interests
The authors declare that they have no competing interests.

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