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Intraguild interaction between the entomopathogenic fungus *Beauveria bassiana* and two aphid predators

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ABSTRACT

Entomopathogenic fungi and aphid predators can independently manage *Aphis craccivora*, Koch (Hemiptera: Aphididae) populations. It is important to assess the risk of possible fungal infections in predators when integrated in Integrated Pest Management (IPM). The virulence of two entomopathogenic fungi isolates, *Beauveria bassiana* (Balsamo) and *Paecilomyces lilacinus* (Thom) Samson have been tested against *A. craccivora*. A laboratory experiment was conducted to study the direct spray effect of different spores' concentrations on the aphid adult stage. The obtained results indicated that *B. bassiana*is was more efficient against *A. craccivora* according to LC_{50},1.2×10^7spores/ml. LC_{50} of *B. bassiana* isolate was applied against the second larval instars of both the green lacewing, *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) and the seven spotted lady beetles, *Coccinella septempunctata* (Linnaeus) (Coleoptera: Coccinellidae). The bioassay indicated that *B. bassiana* was slightly harmful against *Ch. carnea* and significantly injurious against *C. septempunctata*. The total mortality was 13.3 and 43.3% for *Ch. carnea* and *C. septempunctata*, respectively. According to the high pathogenic efficiency of *B. bassiana* on aphids and the low efficiency on the *Ch. carnea*, the finding suggested that *B. bassiana* has the potential to be utilized as highly efficient entomopathogenic fungi in IPM programs, in conjunction with the predator *Ch. carnea*, against *A. craccivora*.

Keywords: Aphid, Entomopathogenic fungi, *Beauveria bassiana*, predator, *Chrysoperla carnea* and *Coccinella septempunctata*

INTRODUCTION

The cowpea aphid, *Aphis craccivora* Koch (Hemiptera: Aphididae) infests leaves, pods, and flowers of the cowpea plants. Various legume species experience yield losses of up to 100% due to the impact of this aphid species (Das, 2002). Moreover, (Thottappilly and Rossel, 1985; Ignacimuthu, 2002) stated that *A. craccivora* is responsible for transmitting around nonpersistent plant viruses across various regions worldwide. The conventional insecticides synthetic was used to control this aphid, but most of these chemicals are hazardous to human health and have toxic effects on beneficial organisms, especially natural enemies. Research on alternative strategies for aphid management has been prompted by the detrimental impacts caused by these destructive effects.

The larvae of the green lacewing, *Chrysoperla carnea* (Steph.) (Neuroptera: Chrysopidae) are voracious predators. They have a wide prey range such as aphids, whiteflies, mites, and eggs of lepidopteron insects so, *Ch. carnea* is used in IPM programs (El-Gantiry *et al*., 2007; Gaber *et al*., 2012). *Coccinella septempunctata***,** (L.) (Coleoptera: Coccinellidae) commonly known as the seven spotted lady beetles, are the majority of ladybird beetles that work as predators of aphids, whiteflies, jassids, thrips, mealybugs, leafhoppers, scale insects and mites (Elheneidy *et al.,* 2008)**,** both adults and larvae of *C. septempunctata* have a great potential to feed on aphid population. To use *Ch. carnea* and *C. septempunctata* as biocontrol agents in integrated pest management programs, their susceptibility to the pesticides used must be taken into consideration. So, selective agents against insect pests must preserve the natural enemies (El Arnaouty *et al*., 2007).

The introduction of biocontrol agents, such as entomopathogenic fungi (EPF) and predators, has been driven by the findings of researchers aiming to achieve sustainable agricultural management. All that lead the researchers to introduce biocontrol agents such as entomopathogenic fungi (EPF) and predators for sustainable agricultural management (Lacey *et al*., 2015; Awad *et al*., 2022; Nouh *et al*., 2022; Ashraf *al et.*, 2024). Fungal biocontrol agents possess a distinct method of infection that sets them apart from viruses and bacteria which rely on ingestion, while fungi directly invade their hosts through the cuticle. The infection rate is determined by the specific type of fungus and the number of spores that successfully infect the host. Once the target organism is infected and dies, the fungus persists in producing new spores on the deceased host's body. These spores then spread and perpetuate their life cycle on new hosts.

It is imperative to choose entomopathogenic microorganisms that are safe and can coexist harmoniously with natural enemies (Rimoldi, *et al.,* 2012). To control various insect pests, a variety of fungal species, including

Paecilomyces fumosoroseus, Lecanicillium lecanii, Metarhizium anisopliae, Isaria fumosoroseus, and *Beauveria bassiana*, are utilized as biocontrol agents (Draganova and Simova, 2010, Gehan *et al*., 2022). *B. bassiana* possesses numerous advantageous characteristics, including the ability to potentially infect up to 80% of pest populations, a wide range of genetic variation among different isolates, the potential to cause mortality in all stages of the targeted pest, a high capacity for both vertical and horizontal dispersal, and the absence of any significant environmental implications. (Groden and Lockwood 1991; Jaronski, 2014).

Due to importance of protecting natural enemies in integrated control programs, the possible action of entomopathogenic fungi as a biocontrol agent in an agroecosystem and its effect on beneficial insects must be considered. It has been found that some species of entomopathogenic fungi have harmful effects on some predators, whereas others show little or no effects on the predators. Intraguild predation refers to the situation where two species coexist by sharing a common host or prey, leading to potential competition or a trophic interaction such as parasitism or predation. This interaction can have significant effects on the population dynamics of the target pests and their natural enemies, making the combination of fungi and predators a potential catalyst for enhancing insect control rates. (Rosenheim *et al*., 1995; Baverstock *et al*., 2009; Barahona *et al*., 2018; Abbas, 2020; Sayed *et al*., 2021). The equilibrium density of the pest is reduced by positive intraguild interactions, which in turn contribute to the overall control. Conversely, negative intraguild interactions have the opposite effect. Hindering control measures (Pell, 2007). In an effort to explore and propose environmentally friendly solutions for managing the cowpea aphid, *A. craccivora*, this study was conducted. The objective was to incorporate green agents into the Integrated Pest Management (IPM) approach**.** The plan of the study involved two purposes, the first one: Assessing the level of toxicity of two isolates of entomopathogenic fungi, *B. bassiana* (Blas.) and *P. lilacinus* (Thom) Samson, whereas the second compatibility interaction between the predators (*Ch. carnea*, *C. septempunctata*) and the entomopathogenic fungus *B. bassiana*, where they are important biological control agents of aphids.

MATERIALS AND METHODS

Rearing of the cowpea aphid, *Aphis craccivora* Koch (Hemiptera: Aphididae):

The laboratory strain of *A. craccivora* was obtained from a colony cultured at the Piercing-sucking Insect Research Department, Plant Protection Research Institute, ARC, Dokki, Giza, Egypt.

Rearing of the predators:

a. *Chrysoperla carnea* (Stephens) **(Neuroptera:Chrysopidae):**

The predator, *Ch. carnea* maintained from laboratory rearing at 25±1°C and photoperiod of 16:8h (L:D). The adults were placed in plastic boxes (25x20x10 cm) covered with black muslin for deposited eggs and changed every day. Semi-artificial diet (5g yeast extract, 10 g honey and 10 ml distilled water) drops were provided on wax paper for adult's nutrition on the muslin. The deposited eggs were collected daily and kept in plexiglass boxes until hatching. The hatched larvae were supplied by eggs of the grain moth, *Sitotroga cerealella* as food source. Eggs of *S. cerealella* were obtained from the Mass Production Unite of *S. cerealella* eggs, bollworm Research Department, Plant Protection Research Institute, ARC, Dokki, Giza, Egypt.

b. *Coccinella septempunctata* (L.) (**Coleoptera: Coccinellidae**):

Fifty couples (males and females) of, *C. septempunctata* were reared in plastic boxes (30 x 30 x 20 cm) with an opening in the cover (20 cm in diameter) and covered by white muslin for proper ventilation. Both larvae and adults of *C. septempunctata* fed on *A. craccivora*, the adults laid their eggs in clusters on black rumpled papers. Eggs were transferred to plastic boxes (20x 20 x 10cm), and reared till pupation under the constant conditions of 23 ± 2ºC, 60 ± 5 RH%, and photoperiod of 16:8h (L:D).

Obtaining Fungi:

Isolates of entomopathogenic fungi were employed in this investigation, *Beauveria bassiana* (Balsamo) and *Paecilomyces lilacinus* (Thom) Samson were previously isolated from soil (Ali *et al*., 2020). Isolates were grown into autoclaved Czapeck's Dox's agar mediumat 25± 1°C for 14 days. To obtain the stock spore suspension, the developed spores were washed with distilled water mixed with 0.01% Tween 80. The spores were then harvested by rinsing with distilled water containing 0.01% (v/v) Tween 80 and filtered through cheesecloth to reduce mycelium clumping. The diluted spores were counted using a hemocytometer (Neubauer improved HBG, Germany). Four concentrations were prepared: $5x10^6$, 10^7, $5x10^7$, and 10^8spores/ml. (Nada, 1999).

Test pathogenicity of the *Beauveria bassiana* and *Paecilomyces lilacinus* against:

a. The cowpea aphid, *A. craccivora* Koch:

Each treatment had ten faba bean leaves (as 10 replicates). Ten aphid individuals were added to every faba bean leaf (a replicate), faba bean leaves were sprayed by a glass atomizer (at 30cm high) with 1ml spore

suspension for each treatment. Leaves were sprayed with 0.01%(v/v) Tween 80 distilled water used as control. All replicates (treatments and control) were maintained under laboratory conditions, 25±1°C and 70% R.H. After 24h from treatment, Percentages mortality was calculated daily and corrected according to Abbott's formula (Abbott, 1925) as follow:$Corrected\%ofmortality =$

$$\frac{\%tested\ mortality - \%control\ mortality}{100 - \%controlmortality} x100$$

LC_{50}, LC_{90} and slope values were calculated according to (Finney 1971), using "Ldp line" software by (Bakr 2005). There lative efficiency of the tested fungi was determined according (Sun, 1950) as follows:

$Toxicityindex = \frac{LC_{50}ofthecompound(A)}{LC_{50}ofthecompound(B)} x100$Where:

(A) =is the highest effective compound.

(B) = is the lowest effective compound.

b. The aphid predators:

This part of the investigation was undertaken to evaluate the direct effect by using more effective entomopathogenic fungus, *B. bassiana*, on the predators according to the tested LC_{50} concentration (1.2×10^7 spores/ml). The second larval instars of both *Ch. carnea* and *C. septempunctata* were treated with spray application, while the control insects were sprayed with 1ml of 0.01% (v/v) Tween 80 distilled water. Either treatment or control consisted of 3 replicates. Each replicate (10 individuals, 1 day old or less, i.e., molted in the 24 h before the experiment) sprayed with 1mL suspension of *B. bassiana*. After treatment, all instars were kept individually in plastic boxes (3.5 cm diameter and 2.5 cm height). Sufficient number of preys was provided, larvae of *Ch. carnea* were supplied with eggs of *S. cerealella*, while larvae *C. septempunctata* were fed on *A. craccivora*. The mortality was recorded daily until the larvae to cocoon in *Ch. carnea* or pupa in *C. septempunctata*. In order to confirm fungal infection, dead individuals were kept under controlled conditions of 25 ± 1°C, and 70 ± 5% relative humidity (R.H.), and observed till whether appearance of mycosis signs.

Scan electronic microscope (SEM):

This work was carried out in the electronic microscope unit, Central Laboratory, National Research Centre, Cairo, Egypt. Cadavers of *C. septempunctata* treated with *B. bassiana* LC_{50} were scanned.

Statistical analysis:

LC_{50}, LC_{90}, and slope values were calculated according to Finney (1971), using “Ldp Line” software (Bakr 2005).

All experiments contained up to five replicates and data were analyzed by one–way analysis of variance (ANOVA) using SPSS 17.0 statistical software. When the ANOVA statistics were significant (P <0.05), means were compared by Duncan’s multiple range test.

RESULTS

Pathogenicity of entomopathogenic fungi on:

a. ***Aphis craccivora*:**

After seven days of treatment, an increase in the total mortality rate was observed due to the exposure of *A. craccivora* adult to a range of concentrations of both entomopathogenic fungi, 5×10^6, 10^7, 5×10^7 and 10^8 spores/ml (Fig. 1). The pathogenicity of fungus increased with the time and concentration increment. Death might be occurring as a consequence of extensive tissue damage, toxicosis, cellular dehydration, and the inability to absorb essential nutrients.

According to the data presented in Table (1), *Beauveria bassiana* and *Paecilomyces lilacinus* exhibited 83.45% and 45.03% mortality rates, respectively at the highest concentration, 1×10^8 spores / ml. The LC_{50} for *B. bassiana* and *P. lilacinus* were 1.21×10^7 and 1.91×10^8 spores/mL, respectively. LC_{50} and LC_{90} values after treatment were presented in Table (2). The effectiveness of *B. bassiana* than *P. lilacinus* in controlling adult aphids was demonstrated in Figs (1 and 2).

The One-way ANOVA analysis provided evidence of significant differences between the effects of fungal isolates. A significant difference between the isolates effect (F 11.39, P≤0.05) is presented in Table (2) where the means of mortality were 60.14 and 30.00% for *B. bassiana* and *P. lilacinus*, respectively. It was noticed that *B. bassiana* was more effective.

Table 1. The mortality rates of adult *A. craccivora*, after seven days of treatment by *B. bassiana* and *P. lilacinus*.

Conc. spores/mL	**Mortality%**	
	B. bassiana	***P. lilacinus***
$5x10^6$	28.55	6.00
10^7	54.8	23.73
5×10^7	73.69	34.72
10^8	83.45	45.03

Table 2. Pathogenicity of *B. bassiana* (B) and *P. lilacinus* (P) on *A. craccivora* adult.

Isolate	**LC_{50} spores/ml**	**Lower limit**	**Upper limit**	**Slope**	**LC_{90}**	**Index**	**Mean ±SE**
B	1.2×10^7	7.97×10^6	1.69×10^7	1.09	1.81×10^8	100	60.14 ± 12.1^a
P	**1.**91×10^8	7.5×10^7	3.31×10^{10}	0.57	3.26×10^{10}	7.49×10^7	30.34 ± 6.01^b

Index compared with *B. bassiana* Resistance Ratio (RR) compared with *B. bassiana*, where B (*B. bassiana*) and P (*P. lilacinus*).

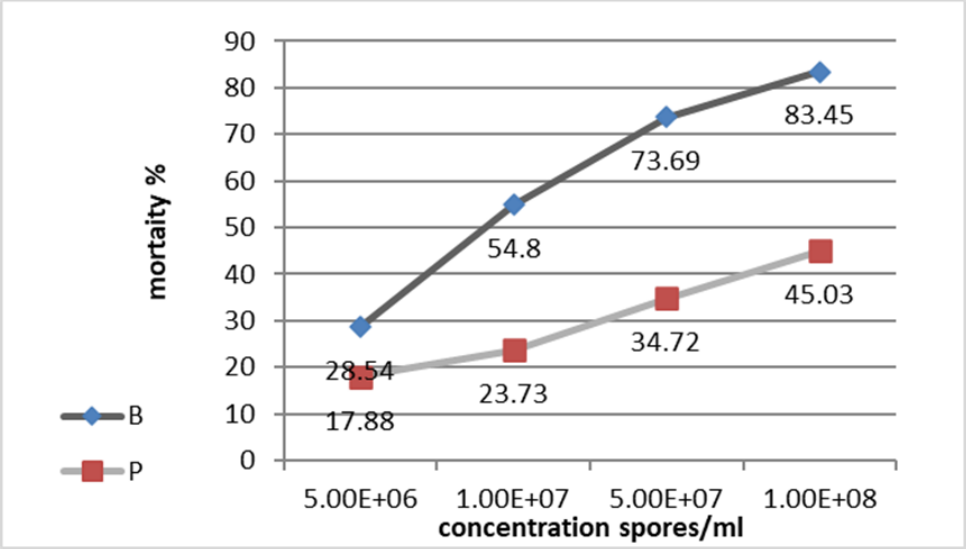

Fig. 1. Mortality % rate of *A. craccivora* adult treated with concentrations of *B. bassiana* (B) and *P. lilacinus* (P) after 7 days of treatments.

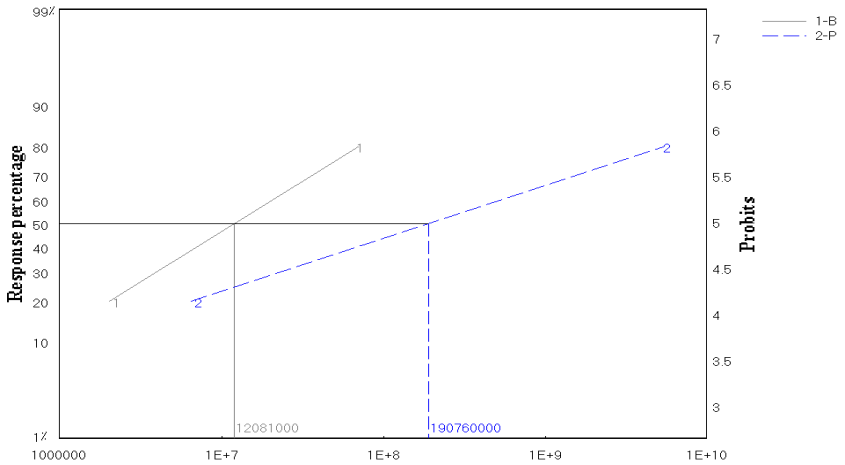

Fig. 2. Ldp lines for *B. bassiana* (B) and *P. lilacinus* (P) on adults *A. craccivora*

b. Pathogenicity of entomopathogenic fungi on second larval instars of *Chrysoperla carnea* and *Coccinella septempunctata*

It became apparent that *C. septempunctata* has been more influenced by the entomopathogen than *Ch carnea*. In the tested concentration, the percentage mortality of *C. septempunctata* was 43.3% after nine days of exposure, whereas it was 13.3%, for *Ch. carnea* after seven days of exposure (Fig. 3). All living *Ch. carnea* larvae entered the cocoon stage after seven days for treated and control, also all living *C. septempunctata* larvae pupated after nine and seven days for treated and control, respectively. Fig. (4) shows mycelium and spores of *B. bassiana* covered cadavers of C. *septempunctata*.

Statistical analysis indicated significant difference (T=2.92; P≤0.05) at LC_{50} treatment between both predators presented. Means were 1.4 and, 0.27 for *C. septempunctata* and *Ch. carnea*, respectively. From these finding it could be concluded that compatible effect between natural enemies in integrated control programs, the possible action of entomopathogenic fungi as a biocontrol agent in an agroecosystem and its effect on beneficial insects must be considered.

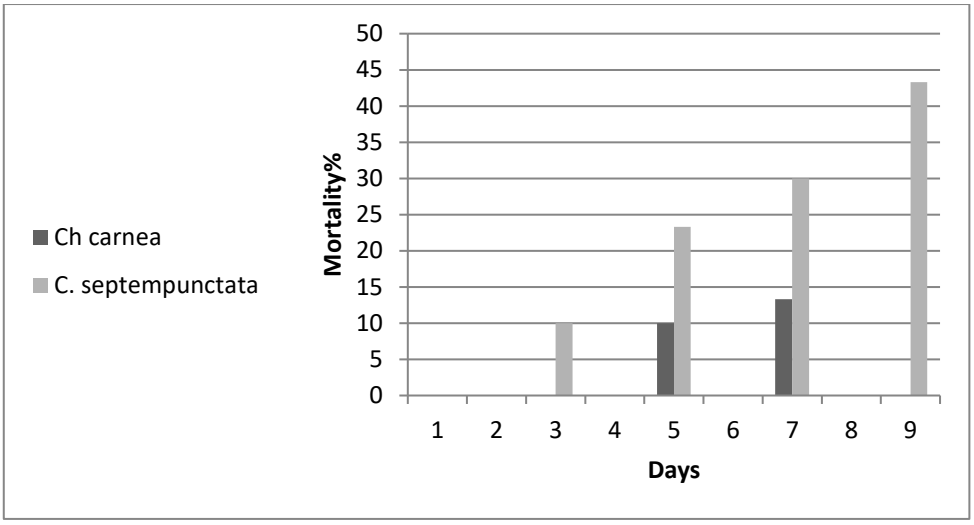

Fig. 3. Mortality percentages of the 2nd larvae *Ch.carnea* and *C. septempunctata* during the period of treatment with *B. bassiana*.

Fig. 4. Cadavers of *C. septempunctata* covered by white mycelium of *B. bassiana*

Scanning electron microscopy of entomopathogenic:

SEM analysis of immature *C. septempunctata* treated with LC_{50} concentration of the fungus, *B. bassiana.* SEM observations indicated the death of *C. septempunctata* due to infection with *B. bassiana*. After incubating the cadaver, the observation of degradation of the cuticle and presence of aged spores of fungus was noted.

Figures (5 and 6) showed the colonization, germination and dislocation of the chitin plates on surface of cadavers treated by *B. bassiana,* while cadavers of untreated one was infection free (Fig 7). This finding supports the side effect of entomopathogenic fungi on some predators as *C. septempunctata*

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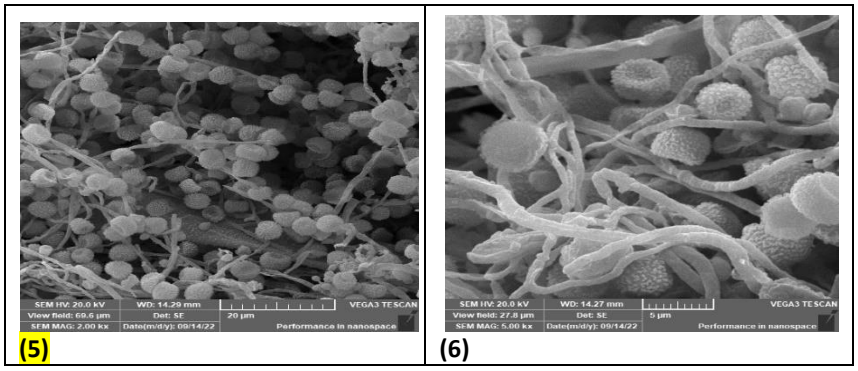

Fig. (5 and 6): Dead *C. septempunctata* adult showed degraded cuticle and old conidia (2000 and 5000X).

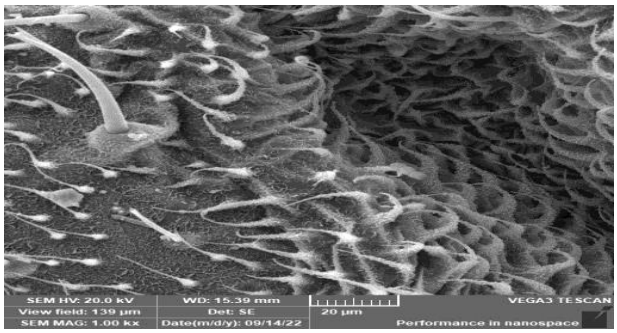

Fig. 7. SEM showing untreated cadaver of *C. septempunctata* adult (1000X)

DISCUSSION

Our results concluded that entomopathogenic fungi; *B. bassiana* was pathogenic to adult *A. craccivora* (lc_{50} was 1.21×10^7 spores/ml). The results are compatible with the findings of multiple researchers Ashraf *et al.* (2024) concluded that *B. bassiana* at a concentration of 1.05×10^{13} conidia per milliliter exhibited the highest mortality rate against *R. padi* (83.39%) compared to *S. graminum* (79.36%) in controlled laboratory conditions after a 7-day period. Likewise, in field conditions, the mortality rates for *R. padi* and *S. graminum* were recorded as 69.33% and 66.34% respectively after the third application of the spray treatment. Also, Khanal *et al.* (2023) evaluate the effect of *V. lecanii*, *M. anisopliae*, *B. bassiana* and Neem oil on Mustard aphid. Mentioned that the aphid population decreased significantly after each treatment compared with the control. Wang *et al*. (2023) suggested that *Cordyceps javanica* IJ-tg19 possesses numerous favorable characteristics to serve as a potent tool for managing aphids through biological means. Biryol *et al*, (2022) revealed that 15 EPF isolates had>50% effectiveness against *Myzus persicae*a 1×10^7 conidia/ ml. Tang *et al*. (2019) also explained the idea that the fungus *M. anisopliae* can be effectively utilized as a biocontrol agent against *Nilaparvata lugens* (Stål) and *Sogatella furcifera*. As an alternative method for pest control, *M. anisopliae* was occasionally combined with insecticides to enhance its efficacy. Sahar *et al*. (2016) also studied four distinct concentrations ($1x10^6$, $1x10^7$, $1x10^8$, $1x10^9$ spores/ml) of the fungi species: *B. bassiana*, *P. lilacinus*, *M. anisopliae*, and *Lecanicillium antillanum* on *A. caraccivora*, and reported that all isolates have pathogenicity effect on aphid. Similarly, Aker and Abacı (2016), demonstrated that the application of *M. anisopliae* can effectively safeguard hazelnuts against aphid infestation. This research highlights the potential of *M. anisopliae* as a promising biocontrol agent for *Monocesta coryli*. In the same line, Maketon *et al.* (2013) examined the efficacy of *B. bassiana* against *A. craccivora.* The results indicated that the LC_{50} was 6.69×10^7spores/ml for the nymphs and 8.25×10^7 spores/ml for the adults.

The pathogenicity of fungus increased with the time and concentration increment. Death might be occurring because of extensive tissue damage, toxicosis, cellular dehydration, and the inability to absorb essential nutrients. The hyphae may grow out of the insect's body, releasing spores and initiating a new cycle of infection (Perez *et al*., 2014).

In this study *B. bassiana* was more effective than *P. lilacinus***.** Similar finding was also found by Haron *et al*. (2020), who examined the pathogenicity of two isolates of *B. bassiana* (B1, B2) and the pathogenicity of two isolates of *Metarhizium anisopliae* (M1, M2), was investigated in relation to *Schizaphis graminum* (Rond.) and reported that B1 exhibited the highest efficacy based on LC_{50} values. . Also, Saranya *et al*. (2010), who studied the efficiency of *Verticillium lecanii*, *Hirsutella thompsonii*, M. *anisopliae*, *B. bassiana,* and *Cladosporium oxysporum* on the *A. craccivora* adults in the laboratory. They examined six concentrations, a 100 % mortality percentage was recorded in the highest concentration (10^8spores/ ml), with *V. lecanii* and *H. thompsonii* followed by *B. bassiana* (96.6 %), *M. anisopliae* (80.76 %) and *C. oxysporum(77.5 %).*

Our results indicated that *Coccinella septempunctata* was significantly more affected by the entomopathogen fungi compared to *Chrysoperla carnea* whereas the mortality rate of C. *septempunctata* reached 43.3%, while *Ch. carnea* only had a mortality rate of 13.3%. Similar results were found by Sayed *et al*. (2021) who studied the side effects of *B. bassiana* against *C. undecimpunctata* and *Hippodamia variegate* and reported that *C. undecimpunctata* and *H. variegata* remain unaffected by the presence of *B. bassiana*, as no significant changes have been documented. Also, Dias *et al.* (2020) noted a low mortality rate among *Ch*. *externa* larvae treated with Buvariae. They indicated that certain *B. bassiana* isolates exhibit selectivity or compatibility with green *Ch. carnea.* In addition, Scorsetti *et al*. (2017) tested the pathogenicity of *B. bassiana* against *Eriopis connexa* (Coleoptera: Coccinellidae) and proved that the susceptibility to mortality varied significantly among different developmental stages of larvae. The first instar experienced the highest mortality rate (38.8± 3.51%) . Conversely, the pupal stage demonstrated the lowest mortality, with only (2.22 ±1.47%) of pupae succumbing to death before reaching adulthood. Hassan *et al*. (2017) tested the effect of the *B. bassiana* against the adult of two predators, *Neoseiulus californicus* and *Phytoseiulus persimilis*. The results indicated that the isolated *B. bassiana* was slightly affected by *N. californicus* and no effect against *P. persimilis* was noted. The pre-imaginal stage of *Ch*. *exotera* remained unaffected following exposure to high dosage of *B. bassiana* (Leyva *et al*. 2011). Similarly, Thungrabeab, and Tongma (2007), conducted a study to examine the impact of entomopathogenic fungi *B. bassiana* and *M. anisopliae*, on non-target insects. Their findings revealed that *B. bassiana* did not exhibit pathogenic properties towards various natural enemies, including *C. septempunctata*.

Studying the side effect of *B. bassiana* on *Ch*. *carnea* and *C. septempunctata* explains how to include it within the IPM. One of the most intriguing findings relies on the observation that the concentration1.2×10^7spores/ml caused 50% mortality of the aphid adults after the 7^{th} day of exposure. However, the same concentration caused little effect on *Ch*. *carnea* (13 % mortality) and 43% mortality on *C. septempunctata*. It can be inferred that the entomopathogenic fungus *B. bassiana* intraguild interaction could be used with consideration in IPM programs for aphid control**.** Similarly, Hassan *et al.* (2023) stated that *B. bassinae* has the potential to be employed with certain precautions in an integrated pest management approach to control the *Tetranychus urticae*. Abbas (2020) recorded that the application of some isolated entomopathogenic fungi did not yield significant outcomes on the targeted predators, whereas other species have detrimental effects on some predator species. The pathogenicity of the entomopathogenic fungi concerning *Ch*. *carnea* raises the potential for biological control by the synergism and conservation of natural enemies in the agroecosystem. Pell, (2007) stated that Positive intraguild interactions have been found to play a crucial role in reducing the density of pests and contributing to the overall control of pest populations, on the other hand, negative intraguild interactions can have detrimental effects on pest control efforts, The utilization of both control agents in combination would not yield compatible results in the suppression of pests. In contrast, the application of multiple species can potentially work together in reducing the population of pests, especially when there is minimal or no interference between these species (Roy and Pell, 2000). Due to the importance of protecting natural enemies in integrated control programs, the possible action of entomopathogenic fungi as a biocontrol agent in an agro-ecosystem and its effect on beneficial insects must be considered. Sixteen genera of Coccinellidae were shown to be susceptible to *B. bassiana* (Goettel *et al.* 1989).

CONCLUSION

Laboratory experiments have demonstrated that the application of fungi to predators can result in varying effects on insects, either weak or detrimental, depending on the specific fungus species and its concentration. Based on the significant pathogenicity of *B. bassiana* towards aphids and its limited effectiveness on *Ch. carnea*, the discovery implies that *B. bassiana* holds promise as a remarkably effective entomopathogenic fungus in integrated pest management (IPM) initiatives. This potential can be harnessed by combining it with the predator *Ch. carnea* to combat *A. craccivora*.

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