Effects of Entomopathogenic Fungi on the Biology of Spodoptera litura (Lepidoptera: Noctuidae) and its Reduviid Predator, Rhynocoris marginatus (Heteroptera: Reduviidae)

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ABSTRACT: Entomopathogenic fungi (EPFs), *Isaria fumosorosea* and *Beauveria bassiana*, are efficient biological agents in the management of multiple arthropod pests. In this study, the effects of both EPF species on various life stages of *Spodoptera litura* (F.) (Lepidoptera: Noctuidae) and its natural enemy *Rhynocoris marginatus* (Fab.) (Hemiptera: Reduviidae) were determined under laboratory conditions. *I. fumosorosea* significantly ($P < .05$) reduced the growth rate of the third and fourth instar larvae of *S. litura*. For relative consumption rate (RCR), the maximum impact was recorded for *I. fumosorosea*, which reduced the RCR of the larvae. The larvae of *S. litura* treated with *I. fumosorosea* showed significantly lower efficiency of conversion of ingested food (ECI) and the larval mortality rate (58.0%) was also higher compared with *B. bassiana* (33.3%). Similarly, *I. fumosorosea* had a significant effect on the pupal formation of *S. litura*; however, no significant effect was found on adult emergence percentage. To determine the effect of EPF-infected prey on the adult predator, their handling time, predatory rate, consumption rate, and the survival rate were recorded. No significant effect of EPF species on the predation rate was found. Furthermore, no significant difference was found in the survival rate of predators fed on either EPF-infected prey or healthy larvae. The interaction of these EPFs with a reduviid predator suggested that both EPF species, especially *I. fumosorosea*, could be used together with the predator to boost the biological control of *S. litura* in commercial crops.

KEYWORDS: Microbial control, field crop pest, integrated pest management, *Beauveria bassiana*, ***Isaria fumosorosea***, reduviid predator

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Introduction

Armyworm, *Spodoptera litura* (F.) (Lepidoptera: Noctuidae), is an economically important insect pest known to attack various agricultural crops and is widely distributed in the tropical and temperate zones of Asia, Australasia, and Pacific Islands.[1] It is native to India and South-East Asia[2] and is well established in Pakistan.[3,4] It is reported to potentially cause 35% to 55% yield losses at the blossom and vegetative stages of the crops.[5] In south Punjab, Pakistan, it causes damage to the economically important crops[6] like cotton, tomatoes, tobacco, groundnut, soybean, lucerne, cabbage, sunflower, castor, cauliflower, onion, brinjal, and turnip.[7-10] Recently, it has also been reported to feed on citrus cultivars in Sargodha region of Pakistan.[10]

Due to its economic importance and widely known losses to agricultural crops, insecticide application is considered the best method to manage *S. litura*.[11] However, repeated applications and extensive use of insecticides have resulted in ecological imbalances such as toxic effects on natural enemies and humans.[12] Development of insecticide resistance has also been reported for *S. litura*.[13-16] Hence, it is important to explore eco-friendly (ecological and economical) insect pest management (IPM) strategies using natural enemies providing similar efficacy against *S. litura*. The use of microorganisms has achieved a prominent position among different options to control insect pests that cause considerable losses to agroecosystems.[17-19] Use of entomopathogens is one of the management strategies against notorious insect pests. Different entomopathogens, such as *Pseudomonas fluorescens* (Trevisan) Migula, *Metarhizium anisopliae* (Metsch.) Sorokin, and *S. litura* nucleopolyhedrovirus (SpltNPV), have been used against different pests.[19,20] Entomopathogenic fungi (EPFs), namely, *Isaria fumosorosea* and *Beauveria bassiana*, have been reported to effectively reduce the population of lepidopterous insect pests.[21,22]

Furthermore, the release of natural enemies including predators and parasitoids is another strategy to manage insect pests in an eco-friendly way. Previously reported biological control predators of *S. litura* are *Platymeris laevicollis* (Distant),[23] *Zelus renardii* Kolenati,[24] *Rhynocoris marginatus* (Fab.),[25] *Rhynocoris kumarii* Ambrose and Livingstone, *Rhynocoris fuscipes* (Fab.), *Pristhesancus plagipennis* Walker (Reduviidae: Hemiptera), *Acanthaspis pedestris* (Stål), *Catamiarus brevipennis* (Serville), and *Ectomocoris tibialis* Distant[26]; all belong to the family

Reduviidae. A number of insect species from different orders like Lepidoptera, Coleoptera, Hemiptera, Orthoptera, and Isoptera have been controlled by hunter reduviid predators (Reduviidae).[23] These are commonly found in the agricultural fields and suppress the population of many important pests including *Creontiades dilutus* (Stål) (Miridae: Hemiptera), *S. litura, Helicoverpa armigera* (Hubner), *Anomis flava* F. (Noctuidae: Lepidoptera), *Phenacoccus solenopsis* (Tinsley) (Pseudococcidae: Hemiptera), *Dysdercus cingulatus* (Fab.) (Pyrrhocoridae: Hemiptera), and *Aphis gossypii* Glover (Aphididae: Hemiptera).[27,28] *R. marginatus* (Heteroptera: Reduviidae), a polyphagous reduviid predator, feeds on more than 20 economically important insect pests[29] and has paralytic potential using salivary gland extract against its host.[30] Sahayaraj and Ravi[31] reported a 66.6% reduction of *S. litura* population after releasing *R. marginatus* into groundnut field.

Few control strategies focus on limiting the use of insecticides because of their hazardous and non-target effects. Sometimes, insecticides are used synergistically at low doses with microbes to suppress pest populations.[32] However, the non-target effect of these microbial insecticides should be determined before recommendation. Other alternate strategies involve the use of natural enemies entirely.

Due to lack of information, the possible combination of these natural enemies in several pest management programs has not yet been explored. No study has been reported on the reduviid predation against *S. litura* in Pakistan. This study focused on investigating the interaction of EPFs, *B. bassiana* and *I. fumosorosea*, with reduviid predators, especially *R. marginatus* (Reduviidae: Hemiptera), and their combined effect on *S. litura*. Hence, in this study, the effectiveness of EPFs was checked against various life stages of *S. litura* and the performance and survival of its predator, *R. marginatus*, was investigated.

Materials and Methods

To determine the effect of EPFs on *S. litura* and their non-target effect on reduviid predator *R. marginatus*, experiments were conducted in the Entomology Laboratory at College of Agriculture, University of Sargodha, Pakistan.

S. litura culture

Egg batches and larvae of *S. litura* were collected from the lucerne field nearby the University (32°07′42.9″N 72°41′27.2″E). The culture was maintained at 27°C ± 2°C temperature and 75% ± 5% relative humidity (RH) in the laboratory. Newly hatched larvae were provided with artificial diet till pupation. Artificial diet was prepared in accordance with Sorour et al.[33] The adults were shifted into clean plastic cages (120 mm × 116 mm × 95 mm) covered with muslin cloth where they were fed on 10% sugar solution. The cotton wool strips (1 cm wide, 5-10 cm long) were kept in plastic cages as a suitable oviposition substrate to collect eggs. In the experiments, the F_2 generation was used.

Entomopathogenic fungi

The commercial formulations of *B. bassiana* NCIM 1216 ATCC 26851 and *I. fumosorosea* IF-171201 (Agri Life, India) were used and tested at 1×10^8 cfu. Both EPF species were purchased from the Ali Akbar Group of Companies, Lahore, Pakistan. At the time of treatment, spore viability was determined by spraying 1 mL aliquots of suspension on potato dextrose agar (PDA) and incubated at 25°C. The viability of both fungi was more than 90%.

R. marginatus culture

The egg batches of *R. marginatus* were collected from the tobacco fields (32°07′38.8″N 72°40′33.6″E). The eggs were placed in clean glass Petri plates lined with a filter paper. The culture was maintained at 25°C ± 2°C and 70% ± 5% RH. *R. marginatus* was reared for second generation prior to the experiments. Newly hatched nymphs were provided with early instar larvae of *S. litura* and the mature nymphs and adults were provided the later instars of *S. litura*. About 2 larvae of hosts were provided to each predator nymph and adult on a daily basis. *S. litura* larvae were collected from the reared culture in the laboratory.

Effect of EPFs on eggs and larvae of S. litura

Freshly laid egg batches were collected and placed in separate Petri plates lined with a filter paper. The eggs were sprayed with EPFs (*B. bassiana* and *I. fumosorosea*) at 1×10^8 cfu using a hand sprayer (Taizhou Longshixiang Plastic, China). Distilled water was used in control treatment. Each treatment was replicated 3 times and 1 batch of the egg was considered as 1 replication. The total number of eggs was counted per batch before application. The average number of eggs was 130 per batch in each treatment. The eggs were kept in an incubator maintained at 25°C ± 1°C and 70% ± 5% RH. The color changes in eggs were observed daily. After 3, 5, and 7 days of treatment application, egg hatchability was recorded.

The efficacy of EPFs was also tested on the third and fourth instar larvae of *S. litura*. Each treatment was replicated 4 times and 5 larvae of each instar were tested in each treatment. The experiment for both eggs and larvae was repeated thrice. The topical bioassay was performed to test the efficacy of EPFs. About 1-µL drop of each treatment was applied on the thorax of each larva. The treated larvae were shifted into new Petri plates containing sunflower leaves. The leaves were collected from unsprayed field and brought into laboratory. Leaves were washed with water to remove contaminants and dried at room temperature. The leaves were cut into disk size (6 cm) of Petri dish and changed daily. The weights of larvae before and after

24, 72, and 120 hours of the application were recorded. The survival rates of larvae were also recorded at 24-hour interval for a total of 10 days. To determine the consumption rate, the leaves were weighed before and after 24 hours of application. Dead larvae due to the application of EPFs were separated into clean Petri plates and sealed with parafilm. The Petri plates were kept in an incubator at 26°C ± 2°C and 70% ± 5% RH. The pupal formation and adult emergence rate of *S. litura* were also recorded. The digestion, consumption, and use of the third and fourth instar larvae of *S. litura* after infection were calculated using the formulae described by Waldbauer[34]

$$\text{Relative growth rate}\left(\text{RGR}\right)=\frac{\Delta B}{\text{BI}}\times T$$

$$\text{Relative consumption rate}\left(\text{RCR}\right)=\frac{I}{B}\times T$$

$$\text{Effeciency of conversion of ingested food}\left(\text{ECI}\right)=\frac{B}{I}\times 100$$

where ΔB is the change in body weight of the insect (mg), BI is the initial larval weight, T is the duration of the feeding period (days), I is the dry weight of food (mg) consumed, and B is the insect dry weight gain (mg).

Effect of EPFs on the performance of R. marginatus predator

S. litura larvae were treated with EPFs for the reduviid *R. marginatus* predator. Freshly molted third and fourth instar larvae of *S. litura* were starved for 12 hours and then treated with either *B. bassiana* or *I. fumosorosea* on an artificial diet. Larvae for control treatment were fed on diet inoculated with distilled water. Larvae that consumed the entire diet within 24 hours were separated and transferred to clean Petri dishes containing fresh uncontaminated diet and reared under controlled conditions. Larvae that did not eat the diet were discarded. The microbial infection was identified based on their sluggish behavior, food consumption, and later the growth of conidia.

To record the effect of EPFs on the performance of the predator, newly emerged *R. marginatus* adults from the lab culture were selected that were reared on healthy *S. litura* larvae. Throughout their nymphal instar, they were provided the healthy *S. litura* larvae. However, the third and fourth nymphal instars and adults were starved for 12 hours and then EPF-infected third and fourth instar prey were provided separately to each category of *R. marginatus*. For the experimental treatment, 10 nymphs/adult predators (10 replicates/treatment) were provided 5 EPF-infected prey for each category separately. In the case of control treatment, healthy larvae were provided to the predators. Handling time (paralyzing plus sucking), predator rate (number of prey/predator/day), number of preys consumed, and the survival rate were recorded daily for 5 days. A digital video camera (DSC-WX60, 16.2 MP HD, China) was set over the experimental setup for 4 hours daily to estimate the handling time (paralyzing + sucking act).

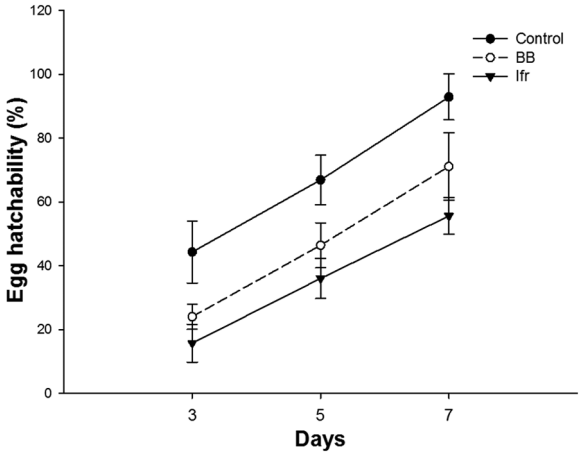

Figure 1. Percent (mean ± *SE*, n = 3) egg hatchability of *Spodoptera litura* after the application of entomopathogenic fungi. BB indicates *Beauveria bassiana*; Ifr, *Isaria fumosorosea*.

Food consumption of *R. marginatus* adults was calculated using the formula[35]

$$\text{FC} = \text{PWB} - \text{PWA}$$

where FC is the food consumption, PWB is the prey weight before providing to predators, and PWA is the prey weight after feeding of predators.

To record the effect of EPF-infected prey on the developmental biology of *R. marginatus*, 10 pairs of newly emerged adults from nymphs were fed on healthy *S. litura* larvae throughout their lives, and then they were selected for further experiment. In total, 10 newly emerged nymphs were fed on EPF-treated prey and 10 were fed on healthy *S. litura* larvae that served as the control treatment. Data on the number of days required for the completion of each stage were recorded.

Data analysis

To check the significance of EPFs on the various stages of *S. litura*, 1-way analysis of variance (ANOVA) was performed. Mortality was analyzed using Kaplan-Meier survival analysis with a log-rank test. Similarly, 1-way ANOVA was also performed to check the effect of EPFs on the handling time, predatory rate, food consumption, and developmental biology of predators. Means were separated with the least significant difference (LSD) test at a probability level of 5%. All the analyses were performed using the Minitab 17.0 software.

Results

Effect of EPFs on eggs and larvae of S. litura

There was a significant effect of EPFs on the hatchability of *S. litura* after 3 ($F = 48.7$, $P < .05$), 5 ($F = 10.6$, $P < .05$), and 7 ($F = 14.1$, $P < .05$) days of exposure. After 7 days, egg hatchability was found lower (55.6%) by the application of *I. fumosorosea* followed by *B. bassiana* (71.1%) compared with the control treatment (92.9%) (Figure 1). A significant effect ($P < .001$) of EPFs was

Table 1. Different nutritional indices: relative growth rate (RGR), efficiency of conversion of ingested food (ECI), and relative consumption rate (RCR) of 2 larval instars of *Spodoptera litura* sprayed with Ifr and BB after exposure to sunflower leaf disks.

TREATMENTS	RGR (MG/MG/DAY)		RCR (MG/MG/DAY)		ECI (%)	
	THIRD INSTAR	FOURTH INSTAR	THIRD INSTAR	FOURTH INSTAR	THIRD INSTAR	FOURTH INSTAR
Control	0.32 ± 0.027^{a}	0.39 ± 0.022^{a}	0.65 ± 0.042^{a}	0.84 ± 0.139^{a}	71.9 ± 2.491^{a}	74.2 ± 3.541^{a}
Ifr	0.07 ± 0.011^{c}	0.13 ± 0.065^{c}	0.23 ± 0.026^{c}	0.27 ± 0.045^{b}	32.9 ± 1.701^{c}	38.6 ± 2.332^{c}
BB	0.20 ± 0.012^{b}	0.26 ± 0.022^{b}	0.35 ± 0.027^{b}	0.52 ± 0.077^{b}	49.7 ± 2.464^{b}	55.0 ± 3.181^{b}
F-value	45.9	10.2	42.6	8.94	75.9	33.8
P-value	<.001	<.001	<.001	<.001	<.001	<.001

Abbreviations: BB, *Beauveria bassiana*; Ifr, *Isaria fumosorosea*.
All values are represented as means ± *SE*, n = 20.
Means sharing similar letters within the column are not significantly different at $P > .05$.

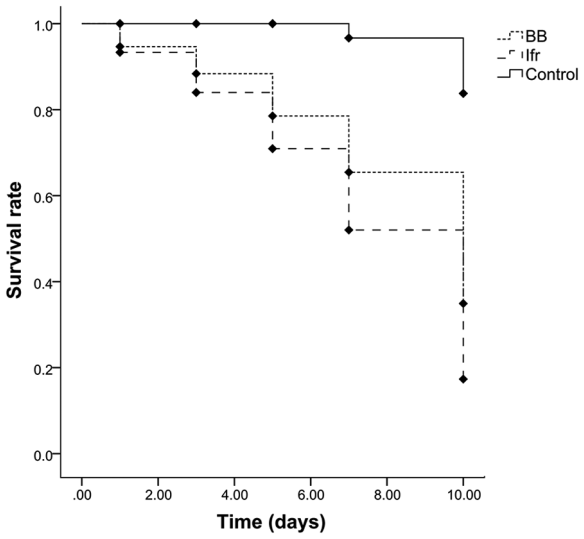

Figure 2. Effect of entomopathogenic fungi on survival rate (%) (mean ± *SE*, n = 20) of *Spodoptera litura* larvae. BB indicates *Beauveria bassiana*; Ifr, *Isaria fumosorosea*.

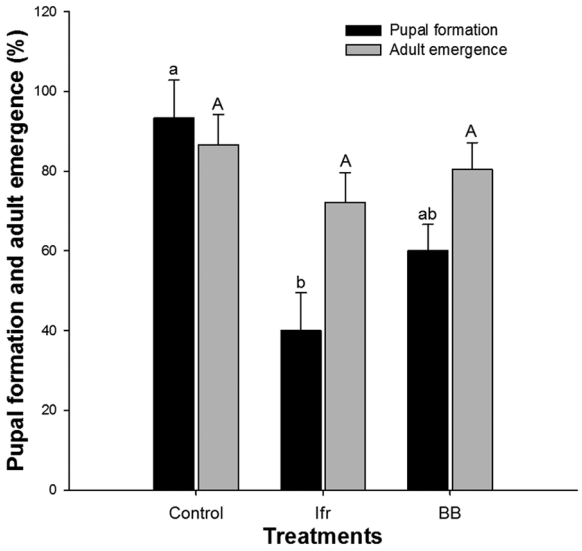

Figure 3. Effect (mean ± *SE*, n = 10) of entomopathogenic fungi on percent pupation and adult emergence of *Spodoptera litura*. Means sharing similar letters are not significantly different at $P > .05$. BB indicates *Beauveria bassiana*; Ifr, *Isaria fumosorosea*.

observed on all nutritional indices in both the third and fourth instar larvae of *S. litura*. The relative growth rates (RGRs) of the third (0.07 mg/mg/day) and fourth (0.13 mg/mg/day) instar larvae were affected more by *I. fumosorosea* compared with *B. bassiana* (0.20 mg/mg/day for the third instar and 0.26 mg/mg/day for the fourth instar). The values of the relative consumption rate (RCR) and efficiency of conversion of ingested food (ECI) indices were also significantly ($P < .001$) lower in the *I. fumosorosea* treatment (Table 1). Both microbial treatments had a similar effect on the third and fourth larval instars of *S. litura* for the different nutritional indices following a general trend: *I. fumosorosea* < *B. bassiana* < control. The survival rate of *S. litura* larvae was significantly (Kaplan-Meier log rank: $df = 2$, $\chi^2 = 32.34$, $P < .001$) affected after the application of EPFs. However, 33.3% of larvae survived after 10-day exposure of *I. fumosorosea* followed by 53.3% in the *B. bassiana* treatment (Figure 2).

Entomopathogenic fungi had a significant effect ($F = 7.00$, $P < .05$) on pupal formation of *S. litura*. However, no significant ($F = 0.44$, $P > .05$) effect of EPFs was observed on adult emergence. *I. fumosorosea* affected the pupal formation and adult emergence of *S. litura* comparatively more than *B. bassiana*. In comparison with control, *I. fumosorosea* lowered 53.3% pupal formation and 14.4% adult emergence rate (Figure 3).

Effect of EPFs on R. marginatus

During predation, *R. marginatus* showed the sequential pattern of behavior such as locating, capturing, sucking, and paralyzing the prey. Food consumption, handling time, and predatory rate of adults of *R. marginatus* fed on EPF-infected *S. litura* prey are presented in Table 2. Food consumption of *R. marginatus* was not significantly ($P > .05$) affected by the application of EPFs. However, a significant ($P < .001$) difference in the handling time of *R. marginatus* on EPF-treated

Table 2. Effect of EPF-infected *Spodoptera litura* larvae (third and fourth instars) on food consumption (FC), handling time (HT), and predatory rate (PR) of the *Rhynocoris marginatus* predator.

TREATMENTS	FC (MG)	HT (MINUTES)	PR (NO. OF PREY/PREDATORS)
Control	20.0 ± 1.457^{a}	172.4 ± 1.077^{a}	1.30 ± 0.133^{a}
BB	24.2 ± 1.218^{a}	72.4 ± 0.690^{c}	1.25 ± 0.133^{a}
Ifr	24.5 ± 2.299^{a}	81.5 ± 0.702^{b}	1.20 ± 0.101^{a}
F-value	2.05	7051.0	0.16
P-value	$.1483^{NS}$	$<.001^{***}$	$.8563^{NS}$

Abbreviations: BB, *Beauveria bassiana*; EPF, entomopathogenic fungus; Ifr, *Isaria fumosorosea*; NS, not significant.
All values are represented as means ± *SE*, n = 10.
Means sharing similar letters within the columns are not significantly different at $P > .05$.
$^{***}P < .001$.

Table 3. Effect of EPF-treated and untreated *Spodoptera litura* prey consumed by *Rhynocoris marginatus* nymphal stages and the total nymphal developmental period (days) and survival rate (%).

TREATMENTS	DEVELOPMENTAL PERIOD (DAYS) OF *RHYNOCORIS MARGINATUS* PREDATOR			
	THIRD INSTAR	FOURTH INSTAR	ADULT LONGEVITY	TOTAL NYMPHAL PERIOD*
Control	11.0 ± 0.422^{b} (100)	12.4 ± 0.401^{b} (95)	23.2 ± 0.573^{b} (95)	61.0 ± 0.667^{b}
BB	13.1 ± 0.585^{a} (85)	14.4 ± 0.618^{a} (90)	26.9 ± 0.481^{a} (80)	64.3 ± 0.760^{a}
Ifr	12.2 ± 0.326^{ab} (90)	12.7 ± 0.495^{b} (90)	22.7 ± 0.701^{b} (100)	62.0 ± 0.699^{b}
F-value	5.30	4.43	15.0	5.68
P-value	<.05	<.05	<.05	<.05

Abbreviations: BB, *Beauveria bassiana*; EPF, entomopathogenic fungus; Ifr, *Isaria fumosorosea*; LSD, least significance difference.
*Data include for the third to fifth nymphal instars, n = 10.
Mean values (±SEM) followed by different letters in a column are significantly different (LSD test, $P < .05$). Values for percent survival rate are presented in parentheses.

and untreated prey was found. The handling time was longer (172.2 minutes) on untreated prey compared with the EPF-treated one. Between the EPF treatments, the handling time of *R. marginatus* fed on *I. fumosorosea*-infected prey was longer. The *R. marginatus* consumed almost the same amount ($P > .05$) of *S. litura* larvae that were either untreated or EPF treated (Table 2).

When *B. bassiana*-infected prey was fed to the third and fourth instar nymphs and adults of *R. marginatus*, the developmental period delayed compared with *I. fumosorosea* and untreated prey. Similarly, the total nymphal period was also affected significantly ($F = 5.68$, $P < .05$) fed on EPF-treated prey. The total nymphal period of *R. marginatus* was 61.0 days in the control treatment followed by 62.0 days in the *I. fumosorosea* and 64.3 days in the *B. bassiana* treatment. The adults lived 23.2 days fed on healthy *S. litura* larvae (control treatment). Total adult longevity of predator was 22.7 days fed on *I. fumosorosea*-treated prey followed by 26.9 days when they fed on *B. bassiana*-treated prey. No significant effect of EPFs was found on the survival rate of the third ($F = 0.82$, $P > .05$), fourth ($F = 0.15$, $P > .05$), and adult longevity ($F = 2.21$, $P > .05$) of *R. marginatus* (Table 3).

Discussion

As this study was based on EPFs and their effects, it was observed that EPFs significantly affected the egg hatchability of *S. litura*. The abnormal reduction in egg hatchability of *S. litura* treated with EPFs was in accordance with Leckie et al[36] where lower egg hatchability rate and delayed development of *Heliothis zea* (Boddie) (Lepidoptera: Noctuidae) larvae fed on diet treated with *B. bassiana* were reported. Similarly, Malarvannan et al[37]observed complete arrest in fecundity of *S. litura* by the application of *B. bassiana* (2.4×10^{7} spores/mL). Gindin et al[38] observed an 80% to 82% reduction in the hatchability rate of EPF-treated red palm weevil adults, *Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae).

In this study, among both EPFs, *I. fumosorosea* proved to be more effective as it reduced the consumption rate and affected the relative growth of *S. litura* larvae compared with *B. bassiana*. Among larval instars, *I. fumosorosea* reduced the consumption rate of *S. litura* by 14.8% and had more effect in reducing the growth rate of small larvae (RGR: 46.1%) compared with large larval instars. Due to the application of *I. fumosorosea*, the values of RGR and RCR remained lower in both larval instars and, consequently, the conversion of ingested food remained low. In

this investigation, *I. fumosorosea*-treated larvae showed a higher reduction in ECI which ranged from 32% to 38% with respect to the third and fourth instar larvae. In a similar study by Moorthi et al,[21] a 46% reduction in ECI in *S. litura* larvae treated with *I. fumosorosea* was observed. This finding is suggestive of the altered digestive activity of *S. litura* after treatment with *I. fumosorosea* which could be used as a suitable biological control agent after testing it in the field. *I. fumosorosea* might have a direct effect on the metabolic process of *S. litura* as a significant decrease in food consumption (FC) and growth rate (GR) was observed (Table 1). It has been reported that entomopathogenic fungi (EPF) degrades the insect cuticle through the enzymes and enters hemocoel where they take on host nutrients and multiply in numbers.[39]

I. fumosorosea reduced the feeding indices and was effective in killing the *S. litura* larvae. The current results were well supported by Moorthi et al,[21] Tefera and Pringle,[40] and Asaff et al[41] who observed more decline in food consumption of *S. litura* after *I. fumosorosea* application in comparison with *B. bassiana* and *Paecilomyces variotii*. A significant effect on the pupal formation of *S. litura* was observed after treatment with EPFs; however, no significant effect was recorded for the adult emergence rate. EPFs reduced the larval weight of *S. litura* during development, due to which the formation of shriveled pupa was observed.[42] Many researchers have reported the effectiveness of *I. fumosorosea* against wide host range, especially lepidopterous insect pests.[43-46]

Before considering the application of these EPFs in field conditions either individually or in combination with other control strategies, such as the release of natural enemies (predators or parasitoids), it is essential to understand the interactions and compatibility of these EPFs with natural enemies. The current results showed that the provision of EPF-infected *S. litura* larvae to *R. marginatus* predator did not affect its food consumption. Furthermore, in the presence of EPFs, the predator took less time to handle the prey compared with untreated prey. This could be due to the sluggish behavior or slow movement of EPF-infected larvae that were easy for predators in locating, capturing, consuming, and digesting the larvae. These findings were well supported by Sahayaraj et al[47] where no significant effect of EPF-infected larvae of *S. litura* on the performance of *R. kumarii* predator was reported. When the EPF-infected prey fed to different life stages of *R. marginatus*, the developmental period was prolonged but did not reduce the survival rate of nymphal and adult *R. marginatus*. Zhang et al[48] have confirmed that *Isaria cateniannulata* fungus has no deleterious effects on the vitality and fertility of the predator *Euseius nicholsi* (Ehara & Lee) (Acari: Phytoselidae). Similarly, Scorsetti et al[49] also reported that *B. bassiana*-infected *Rhopalosiphum padi* L. (Hemiptera: Aphididae) did not affect the development of the predator *Eriopis connexa* (Germar) (Coleoptera: Coccinellidae). The efficacy of these entomopathogens may vary under the field condition where the predator interacts with the pest and environment. However, Nalepa and Weir[50] reported that even if the EPF invades the cuticle of coccinellid predators, there are no known deleterious impacts on the host.

The non-target effect of EPFs on the reduviid predator *R. marginatus* under laboratory conditions was mainly studied to assess the usefulness of the integration of natural predator and FPF. The findings demonstrated that there is a potential for combining *R. marginatus* with other entomopathogenic microbes, especially *I. fumosorosea*. Many researchers have encouraged the integration of commercial formulations of EPFs with other components for IPM.[51-56] Reduviid as generalist predators, abundant in many agroecosystems and distributed worldwide, have been recommended for IPM programs[23] and could be used together with *I. fumosorosea*.

Conclusions

The EPFs, *I. fumosorosea* and *B. bassiana*, proved to be effective in reducing the egg hatchability, food consumption, and growth rate of *S. litura*. However, among both treatments, *I. fumosorosea* significantly proved to affect the growth and development parameters in prey. Moreover, the integration of *I. fumosorosea* with the reduviid predator, *R. marginatus*, also yielded beneficial results as the predator easily handled and captured the EPF-infected prey due to its altered behavior. These findings allude to the consideration of *I. fumosorosea* as an effective eco-friendly mycoinsecticide against *S. litura*. However, limited numbers of studies are available on the interaction of microbes with natural enemies under field conditions. Such studies where more combinations of EPFs and natural enemies are exploited would be helpful in developing effective pest control programs.

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Author Contributions

SM and NA performed the experimental work and analysis in conducting this research study. MIU and MAf contributed in concept and designing of this study and also research supervision. MAr, NM, MR, SA, AA contributed equally in literature search, interpretation of results, manuscript drafting, improvement and critical reviewing of drafted manuscript. All authors approved and agreed for the submission of this article for publication.

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