

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

Authors: Ullah, Muhammad Irfan, Altaf, Nimra, Afzal, Muhammad, Arshad, Muhammad, Mehmood, Naunain, et al.

Source: International Journal of Insect Science, 11(1)

Published By: SAGE Publishing

URL: <https://doi.org/10.1177/1179543319867116>


BioOne Complete (complete.BioOne.org) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.




Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

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

International Journal of Insect Science
Volume 11: 1–7
© The Author(s) 2019
Article reuse guidelines:
sagepub.com/journals-permissions
DOI: 10.1177/1179543319867116


Muhammad Irfan Ullah¹, Nimra Altaf¹, Muhammad Afzal¹,
Muhammad Arshad¹, Naunain Mehmood², Muhammad Riaz³,
Sana Majeed¹, Sajjad Ali⁴ and Asad Abdullah¹

¹Department of Entomology, University of Sargodha, Sargodha, Pakistan. ²Department of Zoology, University of Sargodha, Sargodha, Pakistan. ³Allied Health Sciences, University of Sargodha, Sargodha, Pakistan. ⁴Department of Entomology, The Islamia University of Bahawalpur, Bahawalpur, Pakistan.

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

RECEIVED: April 19, 2019. **ACCEPTED:** July 8, 2019.

TYPE: Original Research

FUNDING: The author(s) received no financial support for the research, authorship, and/or publication of this article.

DECLARATION OF CONFLICTING INTERESTS: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

CORRESPONDING AUTHOR: Muhammad Irfan Ullah, Department of Entomology, University of Sargodha, Sargodha 40100, Punjab, Pakistan.
Email: muhammad.irfanullah@uos.edu.pk

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 Asia² 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 crops⁶ 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 *Platyeris 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), *Catantari brevipennis* (Serville), and *Ectomocoris tibialis* Distant²⁶; all belong to the family



Creative Commons Non Commercial CC BY-NC: This article is distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 License (<http://www.creativecommons.org/licenses/by-nc/4.0/>) which permits non-commercial use, reproduction and distribution of the work without

further permission provided the original work is attributed as specified on the SAGE and Open Access pages (<https://us.sagepub.com/en-us/nam/open-access-at-sage>).
Downloaded From: <https://bioone.org/journals/International-Journal-of-Insect-Science> on 19 Apr 2024
Terms of Use: <https://bioone.org/terms-of-use>

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 pests²⁹ and has paralytic potential using salivary gland extract against its host.³⁰ Sahayaraj and Ravi³¹ 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 F₂ 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 × 10⁸ 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 × 10⁸ 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 $26^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and $70\% \pm 5\%$ 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 Waldbauer³⁴

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

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

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

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).

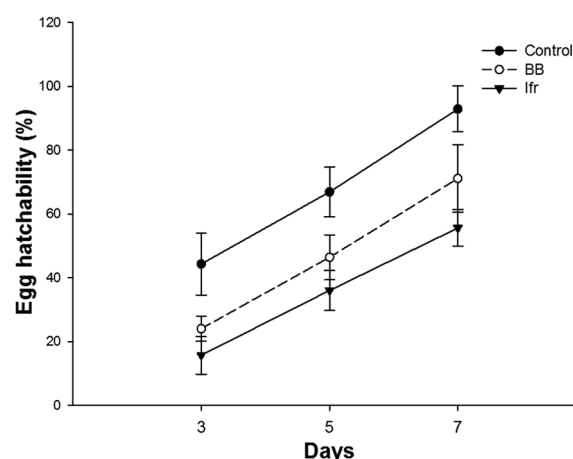


Figure 1. Percent (mean \pm SE, $n=3$) egg hatchability of *Spodoptera litura* after the application of entomopathogenic fungi. BB indicates *Beauveria bassiana*; Ifr, *Isaria fumosorosea*.

Food consumption of *R. marginatus* adults was calculated using the formula³⁵

$$FC = PWB - PWA$$

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 $P > .05$.

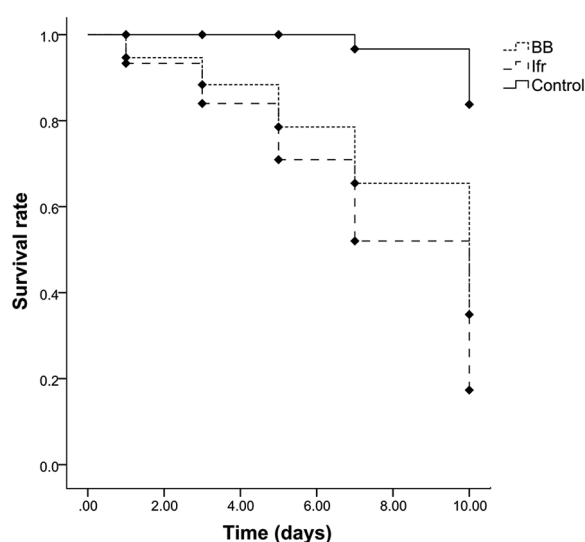


Figure 2. Effect of entomopathogenic fungi on survival rate (%) (mean ± SE, n=20) of *Spodoptera litura* larvae. BB indicates *Beauveria bassiana*; Ifr, *Isaria fumosorosea*.

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

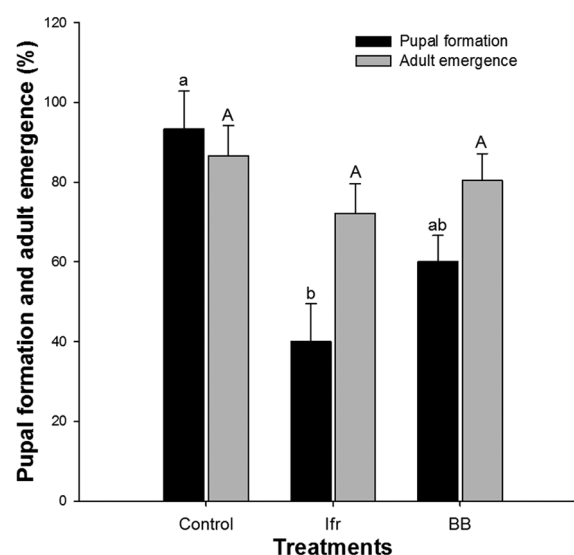


Figure 3. Effect (mean ± SE, n=10) of entomopathogenic fungi on percent pupation and adult emergence of *Spodoptera litura*. Means sharing similar letters are not significantly different at $P > .05$. BB indicates *Beauveria bassiana*; Ifr, *Isaria fumosorosea*.

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 <i>RHYNOCORIS MARGINATUS</i> 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 al³⁶ 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 al³⁷ observed complete arrest in fecundity of *S. litura* by the application of *B. bassiana* (2.4×10^7 spores/mL). Gindin et al³⁸ 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 al⁴¹ 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 al⁴⁷ 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 al⁴⁸ have confirmed that *Isaria catenianulata* fungus has no deleterious effects on the vitality and fertility of the predator *Euseius nicholsi* (Ehara & Lee) (Acari: Phytoseiidae). Similarly, Scorsetti et al⁴⁹ also reported that *B. bassiana*-infected *Rhopalosiphum padi* L. (Hemiptera: Aphididae) did not affect the development of the predator *Eriopsis 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 Weir⁵⁰ 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 programs²³ 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.

Acknowledgements

We are thankful to two anonymous reviewers for useful comments on an earlier version of the manuscript.


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.

ORCID iDs

Muhammad Irfan Ullah  <https://orcid.org/0000-0002-2463-2665>

Naunain Mehmood  <https://orcid.org/0000-0001-7852-9113>

Muhammad Riaz  <https://orcid.org/0000-0002-5524-7735>

REFERENCES

- Ahmad M, Sayyed AH, Saleem MA, Ahmad M. Evidence for field evolved resistance to newer insecticides in *Spodoptera litura* (Lepidoptera: Noctuidae) from Pakistan. *Crop Prot.* 2008;27:1367-1372.
- Waterhouse DF, Norris KR. *Biological Control: Pacific Prospects*. Clayton, VIC, Australia: Inkata Press; 1987.

3. Shad SA, Sayyed AH, Saleem MA. Cross-resistance, mode of inheritance and stability of resistance to emamectin in *Spodoptera litura* (Lepidoptera: Noctuidae). *Pest Manag Sci*. 2010;66:839-846.
4. Ahmad M, Gull S. Susceptibility of armyworm *Spodoptera litura* (Lepidoptera: Noctuidae) to novel insecticides in Pakistan. *Can Entomol*. 2017;149:649-661.
5. Rao MS, Manimanjari D, Rao ACR, Swathi P, Maheswari M. Effect of climate change on *Spodoptera litura* Fab. on peanut: a life table approach. *Crop Prot*. 2014;66:98-106.
6. Ghaffar A, Attique M, Naveed M, Jan M. Host range and population dynamics of beet armyworm, *Spodoptera exigua* (Hubner) (Noctuidae: Lepidoptera) in cotton agroecosystem of Punjab [Pakistan]. *Pak J Zool*. 2002;34:209-213.
7. Ahmad M, Ghaffar A, Rafiq M. Host plants of leaf worm, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) in Pakistan. *Asian J Agric Biol*. 2013;1:23-28.
8. Baskar K, Duraipandian V, Ignacimuthu S. Bioefficacy of the triterpenoid friedelin against *Helicoverpa armigera* (Hub.) and *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae). *Pest Manag Sci*. 2014;70:1877-1883.
9. Suresh U, Murugan K, Panneseelvam C, et al. *Suaeda maritima*-based herbal coils and green nanoparticles as potential biopesticides against the dengue vector *Aedes aegypti* and the tobacco cutworm *Spodoptera litura*. *Physiol Mol Plant Pathol*. 2018;101:225-235.
10. Ullah MI, Arshad M, Afzal M, et al. Incidence of *Spodoptera litura* (Lepidoptera: Noctuidae) and its feeding potential on various citrus (Sapindales: Rutaceae) cultivars in the Sargodha Region of Pakistan. *Fla Entomol*. 2016;99:192-196.
11. Liu Y, Li X, Zhou C, Liu F, Mu W. Toxicity of nine insecticides on four natural enemies of *Spodoptera exigua*. *Sci Rep*. 2016;6:39060.
12. Kranthi K, Jadhav D, Kranthi S, Wanjari R, Ali S, Russell D. Insecticide resistance in five major insect pests of cotton in India. *Crop Prot*. 2002;21:449-460.
13. Armes NJ, Wightman JA, Jadhav DR, Ranga Rao GV. Status of insecticide resistance in *Spodoptera litura* in Andhra Pradesh, India. *Pestic Sci*. 1997;50:240-248.
14. Kranthi K, Jadhav D, Wanjari R, Ali SS, Russell D. Carbamate and organophosphate resistance in cotton pests in India, 1995 to 1999. *Bull Entomol Res*. 2001;91:37-46.
15. Ahmad M, Arif MI, Ahmad M. Occurrence of insecticide resistance in field populations of *Spodoptera litura* (Lepidoptera: Noctuidae) in Pakistan. *Crop Prot*. 2007;26:809-817.
16. Abbas N, Shad SA, Razaq M. Fitness cost, cross resistance and realized heritability of resistance to imidacloprid in *Spodoptera litura* (Lepidoptera: Noctuidae). *Pest Biochem Physiol*. 2012;103:181-188.
17. Sabbour M, Sahab A. Efficacy of some microbial control agents against cabbage pests in Egypt. *Pak J Biol Sci*. 2005;8:1351-1356.
18. Hu QB, Ren SX, An XC, Qian MH. Insecticidal activity influence of destruxins on the pathogenicity of *Paecilomyces javanicus* against *Spodoptera litura*. *J Appl Entomol*. 2007;131:262-268.
19. Yang MM, Li ML, an Zhang Y, et al. Baculoviruses and insect pests control in China. *Afr J Microbiol Res*. 2012;6:214-218.
20. Bhanu Prakash GV, Padmaja V, Jami SK, Kirti PB. Expression of chitinase genes of *Metarhizium anisopliae* isolates in lepidopteran pests and on synthetic media. *J Basic Microbiol*. 2012;52:628-635.
21. Moorthi VP, Balasubramanian C, Selvarani S, Radha A. Efficacy of sub lethal concentration of entomopathogenic fungi on the feeding and reproduction of *Spodoptera litura*. *Springerplus*. 2015;4:681.
22. Gandarilla-Pacheco FL, del Socorro Flores-González M, Morales-Ramos LH, Elías-Santos M, Galán-Wong LJ, Quintero-Zapata I. Effect of native Mexican isolates of *Isaria fumosorosea* (Wize) Brown & Smith on *Spodoptera exigua* (Hübner) and *Helicoverpa zea* (Boddie). *Southwest Entomol*. 2015;40:721-730.
23. Sahayaraj K. Reduviids and their merits in biological control. In Sahayaraj K, ed. *Basic and Applied Aspects of Biopesticides*. New York: Springer; 2014:195-214.
24. Petrakis P, Moulet P. First record of the nearctic *Zelus renardii* (Heteroptera, Reduviidae, Harpactorinae) in Europe. *Entomol Hell*. 2011;20:75-81.
25. Ambrose D, Maran S. Polymorphic diversity in salivary and haemolymph proteins and digestive physiology of assassin bug *Rhynocoris marginatus* (Fab.) (Het., Reduviidae). *J Appl Entomol*. 2000;124:315-317.
26. Ambrose DP. A checklist of Indian assassin bugs (Insecta: Hemiptera: Reduviidae) with taxonomic status, distribution and diagnostic morphological characteristics. *Zoos Print J*. 2006;21:2388-2406.
27. Grundy P, Maelzer D. Assessment of *Pristhesancus plagipennis* (Walker) (Hemiptera: Reduviidae) as an augmented biological control in cotton and soybean crops. *Aust J Entomol*. 2000;39:305-309.
28. Sahayaraj K, Kalidas S, Tomson M. Stage preference and functional response of *Rhynocoris longifrons* (Stål) (Hemiptera: Reduviidae) on three hemipteran cotton pests. *Braz Arch Biol Technol*. 2012;55:733-740.
29. Sahayaraj K. *Pest Control Mechanism of Reduviids*. Jaipur, India: Oxford Book Company; 2007.
30. Maran PM. *Chosen Reduviid Predators—Prey Interaction: Nutritional and Pheromonal Chemical Ecology (Insecta: Heteroptera: Reduviidae)* [PhD thesis]. Chennai, India: Manonmaniam Sundaranar University; 2000.
31. Sahayaraj K, Ravi C. Evaluation of reduviid predators and plant products against chosen groundnut pests. *Arch Phytopathol Plant Prot*. 2007;40:281-290.
32. Guo W, Yan X, Zhao G, Han R. Increased efficacy of entomopathogenic nematode-insecticide combinations against *Holotrichia oblitra* (Coleoptera: Scarabaeidae). *J Econ Entomol*. 2016;110:41-51.
33. Sorour M, Khamiss O, El-Wahab A, El-Sheikh M, Abul-Ela S. An economically modified semi-synthetic diet for mass rearing the Egyptian cotton leaf worm *Spodoptera littoralis*. *Acad J Entomol*. 2011;4:118-123.
34. Waldbauer G. The consumption and utilization of food by insects. *Adv Insect Physiol*. 1968;5:229-288.
35. Tuan SJ, Yeh CC, Atlihan R, Chi H, Tang LC. Demography and consumption of *Spodoptera litura* (Lepidoptera: Noctuidae) reared on cabbage and taro. *J Econ Entomol*. 2015;109:732-739.
36. Leckie BM, Ownley BH, Pereira RM, Klingeman WE, Jones CJ, Gwinn KD. Mycelia and spent fermentation broth of *Beauveria bassiana* incorporated into synthetic diets affect mortality, growth and development of larval *Helicoverpa zea* (Lepidoptera: Noctuidae). *Biocontrol Sci Techn*. 2008;18:697-710.
37. Malarvannan S, Murali P, Shanthakumar S, Prabavathy V, Nair S. Laboratory evaluation of the entomopathogenic fungi, *Beauveria bassiana* against the tobacco caterpillar, *Spodoptera litura* Fabricius (Noctuidae: Lepidoptera). *J. Biopesticides*. 2010;3:126-131.
38. Gindin G, Levski S, Glazer I, Soroker V. Evaluation of the entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana* against the red palm weevil *Rhynchophorus ferrugineus*. *Phytoparasitica*. 2006;34:370-379.
39. Rath S, Prasad B, Sinha B. Food utilization efficiency in fifth instar larvae of *Antheraea mylitta* (Lepidoptera: Saturniidae) infected with *Nosema* sp. and its effect on reproductive potential and silk production. *J Invertebr Pathol*. 2003;83:1-9.
40. Tefera T, Pringle K. Effect of exposure method to *Beauveria bassiana* and conidia concentration on mortality, mycosis, and sporulation in cadavers of *Chilo partellus* (Lepidoptera: Pyralidae). *J Invertebr Pathol*. 2003;84:90-95.
41. Asaff A, Cerda-Garcia-Rojas C, de la Torre M. Isolation of dipicolinic acid as an insecticidal toxin from *Paecilomyces fumosoroseus*. *Appl Microbiol Biotechnol*. 2005;68:542-547.
42. Malarvannan S, Giridharan S, Sekar S, Prabavathy V, Sudha N. Bioefficacy of crude and fraction of *Argemone mexicana* against tobacco caterpillar, *Spodoptera litura* Fab. (Noctuidae: Lepidoptera). *J Biopesticides*. 2008;1:55-62.
43. Asi M, Bashir M, Muhammad A, et al. Potential of entomopathogenic fungi against larvae and eggs of *Spodoptera litura* (Lepidoptera: Noctuidae). *Pak Entomol*. 2012;34:151-156.
44. Hussein H, Zemek R, Habušová S, Prenerová E, Adel MM. Laboratory evaluation of a new strain CCM 8367 of *Isaria fumosorosea* (syn. *Paecilomyces fumosoroseus*) on *Spodoptera littoralis* (Boisd.). *Arch Phytopathol Plant Protect*. 2013;46:1307-1319.
45. Han JH, Jin BR, Kim JJ, Lee SY. Virulence of entomopathogenic fungi *Metarhizium anisopliae* and *Paecilomyces fumosoroseus* for the microbial control of *Spodoptera exigua*. *Mycobiology*. 2014;42:385-390.
46. Zimmermann G. The entomopathogenic fungi *Isaria farinosa* (formerly *Paecilomyces farinosus*) and the *Isaria fumosorosea* species complex (formerly *Paecilomyces fumosoroseus*): biology, ecology and use in biological control. *Biocontrol Sci Techn*. 2008;18:865-901.
47. Sahayaraj K, Subash N, Allingham RW, et al. Lethal and sublethal effects of three microbial biocontrol agents on *Spodoptera litura* and its natural predator *Rhynocoris kumarii*. *Insects*. 2018;9:101.
48. Zhang XN, Jin DC, Zou X, Guo JJ, Qu JJ. Screening of highly virulent strain of *Isaria catenianulata* against *Tetranychus urticae* and its effect to *Euseius nicholsi*. *J Environ Entomol*. 2014;36:372-380.
49. Scorsetti AC, Pelizza SA, Fogel MN, Vianna MF, Schneider MI. Interactions between the entomopathogenic fungus *Beauveria bassiana* and the neotropical predator *Eriopis connexa* (Coleoptera: Coccinellidae): implications in biological control of pest. *J Plant Prot Res*. 2017;57:389-395.
50. Nalepa CA, Weir A. Infection of *Harmonia axyridis* (Coleoptera: Coccinellidae) by *Hesperomyces virescens* (Ascomycetes: Laboulbeniales): role of mating status and aggregation behavior. *J Invertebr Pathol*. 2007;94:196-203.
51. Hajek AE, Butler L. Predicting the host range of entomopathogenic fungi. In Follett PA, Duan JJ, eds. *Nontarget Effects of Biological Control*. New York: Springer; 2000:263-276.
52. Cottrell TE, Shapiro-Ilan DI. Susceptibility of a native and an exotic lady beetle (Coleoptera: Coccinellidae) to *Beauveria bassiana*. *J Invertebr Pathol*. 2003;84:137-144.
53. Bayissa W, Ekesi S, Mohamed SA, et al. Interactions among vegetable-infesting aphids, the fungal pathogen *Metarhizium anisopliae* (Ascomycota: Hypocreales) and the predatory coccinellid *Cheilomenes lunata* (Coleoptera: Coccinellidae). *Biocontrol Sci Techn*. 2016;26:274-290.
54. Gonzalez F, Tkaczuk C, Dinu MM, et al. New opportunities for the integration of microorganisms into biological pest control systems in greenhouse crops. *J Pest Sci*. 2016;89:295-311.
55. Jarrahi A, Safavi SA. Sublethal effects of *Metarhizium anisopliae* on life table parameters of *Habrobracon hebetor* parasitizing *Helicoverpa armigera* larvae at different time intervals. *Biocontrol*. 2016;61:167-175.
56. Jaber LR, Araj SE. Interactions among endophytic fungal entomopathogens (Ascomycota: Hypocreales), the green peach aphid *Myzus persicae* Sulzer (Homoptera: Aphididae), and the aphid endoparasitoid *Aphidius colemani* Viereck (Hymenoptera: Braconidae). *Biol Control*. 2018;116:53-61.