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POTENTIAL OF ENTOMOPATHOGENIC FUNGI FOR BIOCONTROL OF *SPODOPTERA LITURA* FABRICIUS (LEPIDOPTERA: NOCTUIDAE)

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ABSTRACT

Susceptibility of different biological stages of *Spodoptera litura* to various strains of entomopathogenic fungi was evaluated under laboratory conditions at Department of Entomology University of Agriculture, Faisalabad using the insect immersion method. Virulence potential of the entomopathogenic fungi varied with different biological stages of the insect pest. Eggs and larvae were comparatively more susceptible to infections by entomopathogenic fungi, while pupae were less susceptible. The susceptibility of the insect to entomopathogenic fungi decreased with the advancement in age of larvae of the insect. The LC_{50} values for eggs were 1.13×10^6, 4.82×10^6 and 2.45×10^7conidia ml^{-1} in *M. anisopliae* L6, *I. fumosorosea* 32 and *B. bassiana* 25, respectively. The median lethal concentration (LC_{50}) for 3rd instar larvae was 1.11×10^7conidia ml^{-1} in *B. bassiana* 25 and 2.17×10^7conidia ml^{-1} in *I. fumosorosea* 32. Mortality of the larvae increased with increase in conidial concentrations and time elapsed after treatment.

Key words: Bioassays, entomopathogenic fungi, eggs, larvae, mortality, pupae, *Spodoptera litura.*

INTRODUCTION

Spodoptera litura Fabricius (Lepidoptera: Noctuidae) is the most notorious chewing insect pest that causes heavy losses in cotton, thus, deprives the farmers from getting high yield. It is difficult to control because of its cryptic habitat and high rate of infestation post-heavy rains (Chen, 1979). Plant protection measures with frequent applications of synthetic chemical insecticides to protect cotton crop from this pest are typical of intensive cultivation. Insecticides of synthetic origin have been used to manage insect pests for more than 50 years (Charnley and Collins, 2007). However, due to adverse effects of insecticides on environment, their rational use is being advocated.

The biological plant protection with entomopathogenic fungi has key role in sustainable pest management program. Entomopathogens as biocontrol agents have several advantages when compared with conventional insecticides. These include low cost, high efficiency, safety for beneficial organisms, reduction of residues in environment, and increased biodiversity in human managed ecosystems (Lacey *et al*., 2001). Fungal biocontrol agents have unique mode of infection. In contrast to bacteria and viruses, they do not need to be ingested and can invade their host directly through the cuticle. That is why entomopathogenic fungi are capable of infecting non feeding mesh like eggs (Ujian and Shahzad, 2007; Anand and Tiwary, 2009) and pupae of insects (Nguyen *et al*., 2007; Anand *et al*., 2008).

It is estimated that 750 (Samson *et al*., 1988) to over 800 (Thackar, 2002) fungal species from more than 90 genera have been described as pathogenic against different insect species. But only a dozen of entomopathogenic fungus species are available for pest management at grower level (Hajek and St. Leger, 1994). Fungal biological control agents have demonstrated efficacy against a wide range of insect pests including *Spodoptera* species (Purwar and Sachan, 2005; Lin *et al*., 2007; Amer *et al*., 2008).

The potential of entomopathogenic fungi often vary among fungal species and strains. Therefore, highly virulent fungal genotypes against particular insect can be identified and manipulated. The objective of present study was to critically evaluate the potential of entomopathgenic fungi, *Isaria fumosorosea (=Pacilomyces fumosoroseus* (Wize) Brown and Smith), *Beauveria bassiana*, *Metarhizium anisopliae* (Metsch.) Sorokin and *Lecanicillium lecanii* (Zimmerman) (= *Verticillium lecanii*) for biocontrol of various life stages of *S. litura* on cotton.

MATERIALS AND METHODS

Fungus culture: Different isolates of the entomopathogenic fungi; *Isaria fumosorosea* and *Lecanicillium lecanii* were obtained from Plant protection institute, South China University, China. *M. anisopliae* L6 and *Beauveria bassiana* 25 were of local origin. These entomopathogenic fungi were cultured on potato dextrose agar medium containing 20g glucose, 20g starch, 20g

agar, and 1000 ml of distilled water in test tubes. The test tubes containing PDA medium were autoclaved at 121°C (15 Psi) for 15-20 minutes and incubated at 27±1°C, 80±5% relative humidity and photophase of 12 hours for 15 days. The relative humidity was measured using Huger Hygrometer.

The conidia were harvested by scraping the surface of 14-15 days old culture gently with inoculation needle. The conidia were suspended in distilled water containing 0.1% Tween-80. The mixture was stirred with a magnetic shaker for ten minutes. The hyphal debris was removed by filtering the mixture through fine mesh sieve. The conidial concentration of final suspension was determined by direct count using Haemocytometer. Serial dilutions were prepared in distilled water containing 0.1% Tween-80 and were preserved at 5°C until used in bioassay.

Rearing of army worm: Larvae of *Spodoptera litura* were reared in plastic containers which were ethanol (90%) washed and UV-sterilized. These plastic containers were placed in incubator maintained at 27±1°C and photophase of 12 hours. First two larval instars were provided with tender cotton (Neelem 121) leaves surface sterilized with aqueous solution of sodium hypochlorite (0.5%v/v) followed by washing twice with distilled water daily. From third instar onwards, larvae were provided with surface sterilized mature leaves. The larvae rearing containers were cleaned daily. In the final instar the number of larvae was kept to six or less in each container to avoid crowding. Final instar larvae and pupae were covered with sterilized leaves. Adults were transferred to plastic containers (120 x 116 x 95 mm) immediately after emergence for egg laying. Cotton swab soaked in 5% honey solution was kept suspended as adult food. The egg masses were again transferred to larvae rearing containers.

Bioefficacy of entomopathogenic fungi against eggs of *S. litura*: Bioassay was conducted with fresh eggs dipped into four different spore concentrations ($1x10^5$, $1x10^6$, $1x10^7$, and $1x10^8$ conidia ml^{-1}) of the fungal isolates for two minutes. The eggs in control were treated with distilled water containing 0.1% Tween-80. The eggs were transferred to Petri plates with a layer of UV irradiated filter paper at the bottom. Each Petri plate was provided with fresh tender cotton leaves daily for hatched larvae. The Petri plates were placed in incubator maintained at 27±1°C, 80±5% relative humidity and photophase of 12 hours. There were four replicates of 50 eggs for each treatment. The larvae emerging from treated eggs were removed from the Petri plates at an interval of 12 hours. Data for hatched/ un-hatched eggs was recorded up to 18 days (Anand and Tiwary, 2009). Un-hatched eggs after 18 days of treatments were regarded as dead.

Bioefficacy of entomopathogenic fungi against various larval stages of *S. litura:* Second to fourth (L2-L4) instar larvae of *S. litura* were immersed individually for 30 seconds into a fungal suspension containing $1x10^7$ conidia ml^{-1}. For the control treatment, larvae were dipped into a 0.1% Tween 80 solution. Treated larvae were allowed to crawl freely on tissue paper in a Petri dish to remove excess moisture. Then treated larvae were placed individually in small plastic containers (3.5x1.3 cm). These containers were placed in crispers having wet towel paper to maintain humidity. All treated larvae were incubated at 27±1°C, 80±5% relative humidity and photophase of 12 hours. Excised parts of fresh cotton leaves surface sterilized with aqueous solution of sodium hypochlorite (0.5%v/v) followed by washing twice with distilled water were provided as a food source for the larvae. Leaves were regularly replaced with fresh ones at an interval of 24 hours. Each treatment, having batch of 20 larvae, was replicated four times. Mortality data was recorded up to 10 days.

Dose-mortality effect of entomopathogenic fungi against 3rd instar Larvae of *S. litura:* Third instar larvae of *S. litura* were immersed individually for 30 seconds into different spore concentrations ($1x10^5$, $1x10^6$, $1x10^7$ and $1x10^8$ conidia ml^{-1}) of the fungal isolates. For the control treatment, larvae were dipped into a 0.1% Tween 80 solution. Each treatment, having batch of 20 larvae, was replicated four times. Insect mortality was assessed daily up to ten days. The median lethal concentration and time were determined.

Effect of entomopathogenic fungi on pupae of *S. litura*: Bioassay was conducted with 1-2 days old pupae, surface sterilized with 0.5% (v/v) sodium hypochlorite followed by washing with two changes of distilled water, dipped in spore concentration of $1x10^8$ conidia ml^{-1} of the fungal isolates for two minutes with gentle shaking. Pupae were transferred into bioassay vials. They were placed in incubator maintained at 27±1°C, 80±5% relative humidity and photophase of 12 hours. For each treatment, 20 pupae were selected. The pupae in control treatment were treated with distilled water containing 0.1% Tween-80. Treated pupae were kept in dark & were examined for emergence of adults at interval of 24 hours up to 18 days post inoculation (Anand *et al.*, 2008). Un-emerged pupae were regarded as dead after 18 days.

Statistical analysis: Mortalities were corrected using Abbot (1925) formula. The final data were subjected to analysis of variance (SAS System 2004) and means were compared by the Tukey HSD test at P = 0.05. The data was subjected to Probit analysis (Mini tab English 15) for calculation of LC_{50} and LT_{50} values.

RESULTS AND DISCUSSION

Bioefficacy of entomopathogenic fungi against eggs of *S. litura*: The entomopathogenic fungi used in the bioassay were all infective to freshly laid eggs of *S. litura*. Significantly different (P<0.001) mortalities were caused by the entomopathogenic fungi at each conidial concentration tested. *Metarhizium anisopliae* L6, *I. fumosorosea* 32 and *B. bassiana* 25 resulted in 48.19-71.56% egg mortality (Table 1) above $1x10^6$ conidia ml^{-1}. *L. lecanii* 17 did not caused egg mortality beyond 35% at the highest concentration tested. *Metarhizium anisopliae* L6, *I. fumosorosea* 32 and *B. bassiana* 25 resulted in statistically equal egg mortalities that were significantly different from egg mortality caused by *L. lecanii* 17 at the highest concentration tested. Egg infection rate was lower at lower conidial concentration in all fungal isolates and increased proportionately to the conidial concentration. The LC_{50} values of the entomopathogenic fungi also varied (Table 2). Based on LC_{50} values of three promising fungal species tested, *M. anisopliae* L6 with LC_{50} value of 1.13×10^6 conidia ml^{-1} was found the most virulent followed by *I. fumosorosea* 32. On the other hand, *B. bassiana* 25 was found to be comparatively less virulence with LC_{50} value of 2.45×10^7 conidia ml^{-1}.

Rodriguez-Rueda and Fargues (1980) found that *I. fumosorosea* was highly virulent against eggs of *Mamestra brassicae* and *Spodoptera littoralis*. Lezama-Gutierrez *et al*., (1996) observed that eggs of fall armyworm, *Spodoptera frugiperda*, were highly susceptible to insect pathogenic fungi; *M. anisopliae*, *I. fumosorosea* and *Isaria javanicus*. The fungal infection rate of eggs varies depending upon the conidial concentration where egg mortality increases with increase in the conidial concentration (Al-Deghairi, 2008; Anand and Tiwary, 2009). On the other hand, some studies suggest that eggs are more resistant to fungal infections than other life stages of insects (Abdel-Baky *et al*., 1998; Gopalakrishanan and Narrayanan, 1989: Mochi *et al*., 2009).

Bioefficacy of entomopathogenic fungi against different larval instars of *S. litura*: All entomopathogenic fungi tested caused significantly different mortalities against different larval stages of the *S. litura* (F = 15.3, P < 0.001 for 2nd instar; F = 16.3, P < 0.001 for 3rd instar; F = 20.3, P < 0.001 for 4th instar) at conidial concentration of $1x10^7$ conidia ml^{-1} ten days post treatment. *Beauveria bassiana* 25 and *I. fumosorosea* 32 caused comparatively higher mortalities (Table 3) followed by *M. anisopliae* and *L. lecanii*. Mortalities by *I. fumosorosea* 32 and *B. bassiana* 25 were statistically equal but significantly higher than mortalities caused by *L. lecanii*. Young larvae were more susceptible to fungal infection than older larvae.

Virulence of entomopathogenic fungi varies from species to species and strain to strain against *S. litura* (Dayakar and Kanaujia, 2003; Lin *et al*., 2007). Young instars succumb faster to the pathogenic fungi than older ones. Susceptibility of the insect to entomopathogenic fungi decreases with advancement in age of larvae of the target host (Purwar and Sachan, 2005; Amer *et al*., 2008). Chemical constituents of insect cuticle change gradually with advancement in age of larvae resulting in hardening of the cuticle and increased humoral immune to the microbial infections (Boman, 1980).

Dose-mortality effect of entomopathogenic fungi against 3rd instar larvae of *S. litura*: Third instar larvae of *S. litura* were susceptible to all entomopathogenic fungal isolates used in the bioassay in a dose dependent-manner (Table 4). Mortality caused by each fungus was low at lower conidial concentrations. It increased with increase in conidial concentration. Mortalities caused by the entomopathogenic fungi also varied significantly (P < 0.01) at different conidial concentrations against 3rd instar larvae of the insect. The highest mortality was caused by *B. bassiana* 25 followed by *I. fumosorosea* 32 and *M. anisopliae* L6. The median lethal concentration (LC_{50}) value for 3rd instar larvae was 1.11×10^7 conidia ml^{-1} in *B. bassiana* 25 (Table 5) and 2.17×10^7 conidia ml^{-1} in *I. fumosorosea* 32. The median lethal time (LT_{50}) for 3rd instar larvae was 187 hours (Table 6) in *B. bassiana* 25 and 192 hours in *I. fumosorosea* 32 at concentration of $1x10^8$ conidia ml^{-1}.

Anand and Tiwary (2009) observed the highest morality of 2nd instar larvae of *S. litura* at the highest conidial concentration of fungal isolates. Median lethal time (LT_{50}) of *S. littoralis* showed prolongation with decrease in tested concentrations of fungi, *M. anisopliae* (Abou-Bakar, 1997). Similarly, Amer *et al* (2008) investigated that mortality of *S. littoralis* larvae increased with increase in conidial concentrations and time elapsed after treatment.

Effect of entomopathogenic fungi on pupae of *S. litura*: Pupae were found less susceptible to fungal isolates as compared with other biological stages of *S. litura*. It was observed that adult emergence was delayed in fungal treated pupae. Effect of different fungal isolates on pupae of *S. litura* varied significantly (F = 6.01, df = 4, P < 0.01) fourteen days post treatment. There was no statistical difference (Table 7) in adult emergence in *L. lecanii* 17, *B. bassiana* 25, and *M. anisopliae* L6 as compared with control 14 days post treatment. Adult emergence in *I. fumosorosea* was statistically different from that of control. Effect of different fungal isolates on pupae of *S. litura* also varied significantly (F = 6.49, df = 4, P < 0.01) 18 days post treatment. Adult emergence in *I. fumosorosea* (85.00%) was statistically different from that of control 18 days post treatment. All other fungal

treatments were statistically at par with control 18 days post treatment. So, pupal stage was found highly resistant to the entomopathogenic fungi among all developmental stages of the insect tested.

Table 1- Bioefficacy of entomopathogenic fungi against eggs of *S. litura*.

	Concentration of conidia ml-1	Infection of eggs (%± Std. Error of Means)		
Fungal isolate	≈10^5	≈10^6	≈10^7	≈10^8
M. anisopliae L6	35.32 ± 4.89a	49.20 ± 3.59 a	67.29 ± 4.09 a	71.56± 3.08 a
I. fumosorosea 32	24.39 ±2.24ab	41.70 ± 3.16 ab	56.79 ± 4.18ab	66.70 ± 4.74 a
B. bassiana 25	19.55 ±2.34bc	34.55 ± 2.85 bc	48.19 ± 4.29 b	55.43 ± 4.62 a
L. lecanii 17	8.93± 1.31 c	13.79 ± 2.40 c	22.43 ± 3.18 c	34.85 ± 5.13 b

The values given are mean of four replicates ± Standard error of means at a given concentration. Means in columns followed by different letters are significantly different from each other according to Tukey HSD Test at P = 0.05.

Table 2- LC_{50} values of fungal isolates against eggs of *S. litura*.

Isolate	LC_{50} (conidia ml^{-1})	LFL	UFL	Regression Equation: y = a + bx	Chi Square	P value
M. anisopliae L6	1.13×10^6	3.63×10^5	2.74×10^6	0.1433 x –1.9976	1.5	0.47
I. fumosorosus 32	4.82×10^6	2.21×10^6	1.13×10^7	0.1650 x -2.5390	0.86	0.65
B. bassiana 25	2.45×10^7	9.51×10^6	1.07×10^8	0.1428 x –2.4291	1.46	0.48

Table 3- Mortality of different larval instars of *S. litura* 10 days after exposed to fungal isolates (1 x 10^7 conidia ml^{-1}).

Treatment	Mean Larval mortality (%± Std. Error of Means)		
	L2	L3	L4
B. bassiana 25	59.06 ± 5.12 a	53.22 ± 6.13 a	27.30 ± 2.59 a
I. fumosorosea 32	53.5± 6.67 ab	50.79 ± 5.26 a	36.32 ± 3.50 a
M. anisopliae L6	41.16 ± 2.59 bc	35.8 ± 3.26 a	14.28 ± 3.29 b
L. lecanii 17	25.26 ± 3.16 c	13.25± 2.58 b	7.83 ± 1.55 b

The values given are mean of four replicates ± Standard error of means at a given concentration. Means in columns followed by different letters are significantly different from each other according to Tukey HSD Test at P = 0.05.

Table 4- Dose-mortality effect of fungal isolates against 3rd instar larvae of *S. litura* ten day post treatment.

	Concentration of conidia (ml^{-1})	Mean Larval mortality (%± Std. Error of Means)		
Fungal isolate	≈10^5	≈10^6	≈10^7	≈10^8
B. bassiana 25	21.16 ± 2.17 a	28.29 ± 2.99 a	56.32 ± 2.9 a	63.26 ± 3.51 a
I. fumosorosus 32	14.80 ± 1.07 ab	21.33 ± 3.30 ab	52.63 ± 2.49 a	57.82 ± 3.62 a
M. anisopliae L6	9.31 ± 2.31 b	11.95 ± 2.27 b	36.21 ± 2.94 b	43.03 ± 2.65 b

The values given are mean of four replicates ± Standard error of means at a given concentration. Means in columns followed by different letters are significantly different from each other according to Tukey HSD Test at P = 0.05.

Table 5- LC_{50} values of fungal isolates against 3rd instar larvae of *S. litura*.

Isolate	LC_{50} (conidia ml^{-1})	LFL	UFL	Regression Equation: y = a + bx	Chi Square	P value
B. bassiana 25	1.11×10^7	5.51×10^6	2.67×10^7	0.1856x-3.0108	3.27	0.19
I. fumosorosea 32	2.17×10^7	1.08×10^7	5.51×10^7	0.1702x-2.9571	5.93	0.05

The published results on the efficacy of entomopathogenic fungi against pupae are contradictory. Some studies suggest that pupae are resistant to fungal infection. Pupae have seldom been attacked by fungal pathogens because thick and sclerotised cuticle of pupae offers an effective barrier to fungal infection (Hajek and St. Leger, 1994; De La Rosa *et al*, 2002). It was observed that adult emergence was delayed in fungal treated pupae.

Some of emerged adults were also malformed with reduced wings or reduced body size. They were unable to fly and died with out mating. It has been investigated that pupae treated with fungal pathogens often result reduction in the adult emergence (Ekesi *et al.*, 2002), increase in pupal duration and malformed adults (Hafeez *et al*., 1994). Our findings are contradictory to findings of Abou- Bakar *et al* (1997) and Anand *et al* (2008) who reported high level of fungal infectivity on pupae of *Spodoptera*.

Present investigations displayed that all biological stages of *S. litura* were not equally susceptible to infection by the entomopathogenic fungi corroborating previous findings (Angel-Sahagun *et al*., 2005; Anand *et al*., 2008; Anand and Tiwary, 2009). Eggs and larvae were found more susceptible to infections by entomopathogenic fungi, while pupae were less susceptible. High susceptibility of eggs to insect pathogenic fungi could be due to lack of humoral defence systems (Bulet *et al*., 2003). The larvae and pupae having humoral immune reactions are less susceptible to infections by microbial pathogens (Gorman *et al*., 2004).

Our results suggest that entomopathogenic fungi, particularly *B. bassiana* 25 and *I. fumorososeus* 32 and *M. anisopliae* L6, are effective against eggs and different larval instars of *S. litura*. Application of entomopathogenic fungi can be carried out in presence of eggs and larvae at different developmental stages; however, for their effective control, sufficient concentrations of the pathogens are required.

Table 6- LT_{50} values of fungal isolates at conidial concentration of $1x10^8$ conidial ml^{-1} against 3rd instar larvae of *S. litura*.

Isolate	LT_{50} (Hrs)	LFL	UFL	Regression Equation: Y = a + bx	Chi Square	P-value
B. bassiana 25	187	181.15	193.95	0.1673 x -3.1333	45.67	0.00
I. fumosorosea 32	192	185.63	198.77	0.0167 x- 3.2141	39.76	0.00

Table 7- Effect of fungal isolates against pupae of *S. litura* at conidial concentration of 1 x 10^8 conidia ml^{-1}.

Sr. no.	Fungal isolates	Adult emergence(%± Std. Error of Means)	
		14 days post treatment	18 days post treatment
1	*I. fumosorosea* 32	76.255 ± 3.75 b	85.00± 2.04 c
2	*M. anisopiae* L6	82.50± 4.33 ab	88.75± 2.39 bc
3	*B. bassiana* 25	83.75 ± 4.27 ab	90± 2.04 abc
4	*L. lecanii* 17	92.50±2.5 a	93.75± 1.25 ab
5	Control	97.50± 1.44 a	97.50± 1.44 a

The values given are mean of four replicates ± Standard error of means at a given concentration. Means in columns followed by different letters are significantly different from each other according to Tukey HSD Test at P = 0.05.

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