

Original Research Article

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Virulence of Local isolates of Entomopathogenic Fungi on the common agricultural pest *Spodopteralitura* (Fabricius) Lepidoptera:Noctuidae

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ABSTRACT

The polyphagous insect pest *Spodoptera litura* is known to cause huge losses to agriculture all over the world. Its increasing resistance to chemical insecticides necessitates the use of biological options such as Entomopathogenic fungi. Strains of Entomopathogenic fungi, *Beauveria bassiana*, *Metarhizium anisopliae* and *Lecanicillium lecanii* are known to control the incidence of the pest. Laboratory bioassay was conducted to evaluate the effect of different local isolates of *M. anisopliae* and *L. lecanii* on the growth and development of *S. litura*. Results indicate that the susceptibility of the insect pest depends on various factors like the fungal isolate, dosage of the infective propagule, age of the insect larvae and the developmental stage tested. Among the isolates studied, *M. anisopliae* isolate PaCo4 was found to be most virulent on *S. litura* neonates and the isolate ArCo3, the least virulent. In a subsequent study on the 3rd instar larvae of insect pest, the isolates were found to prolong the larval duration, lower the pupation but the pupal period remained unchanged. A lowered adult emergence and longevity was also observed. Further study of the field efficacy of the Entomopathogenic fungal isolates are needed so that they can be successfully used for controlling the pest on the field.

Keywords

Entomopathogenic
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Introduction

Spodoptera litura Fabricius (Lepidoptera: Noctuidae) is a polyphagous chewing insect pest of subtropical and tropical agricultural crops causing heavy losses to the farmers (Brown and Dewhurst 1975; Holloway and Jeremy, 1989). It is widely distributed

throughout the world (Anand *et al.*, 2009) and has been reported from nearly 120 food crops all over the world of which 40 are grown in India. Indiscriminate and non-judicious use of insecticides to control the pest has resulted in the development of resistance to as many as 20 different active ingredients of insecticides (Armes *et al.*, 1997) besides the loss of

natural enemies of the pest. Hence, there is a need to explore ecologically sustainable options to control this devastating pest.

One such option is the use of biological control involving the use of natural enemies like predators, parasitic wasps and flies as well as pathogens like Bacteria, fungi and viruses to suppress the pest. Myco-biocontrol strategies involve the environmentally sound and effective use of a group of fungi termed Entomopathogenic fungi (EPF) in mitigating insect pests.

EPFs are a large polyphyletic group of insect pathogens mainly from the orders of Entomophthorales and Hypocreales. Only few genera among them are well characterized with respect to their pathogenicity to different insects and thus are of agricultural importance. Some are been used commercially as biological control agents worldwide (de Faria and Wraight, 2007).

The mode of entry of the fungal pathogen into the insect body is either by ingestion or by contact of the fungal conidia with the host cuticle. Upon adhesion of the cuticle surface, the conidia of the Entomopathogenic fungi germinate and the germ tubes penetrate the cuticle and the epidermal layers by the consorted action of mechanical pressure and secretion of enzymes like proteases, chitinases, and lipases (Cho *et al.*, 2006b).

In the Haemocoel of the insect, the fungus proliferates into yeast like hyphal bodies called Blastospores. Death of the insect is the result of starvation or physiological /biochemical disruption brought about by the fungus (Feng *et al.*, 1994).

Virulent strains produce toxic proteins such as cyclic peptides, cyclosporins and dextrins which are known to enhance the virulence of the pathogen. Fungal mycelia eventually emerge from the cuticle of the dead cadaver

of the insect and produce conidiophores. However, under suitable humidity conditions, aerial conidia are formed externally dead insect aiding the spread the fungus.

Metarhizium anisopliae was first isolated near Odessa, Ukraine from infected larvae of the wheat cockchafer *Anisopliae austriaca* in 1879 and was initially named *Entomophthora anisopliae*. It causes green muscardine disease and is pathogenic to a large number of insect species, many of which are agricultural and forest insects (Ferron, 1978).

It was the first known fungus to be used for bio control of an insect pest in 1888 and represents about 33.9 percent of the total Myco insecticides developed for Biological control use (de Faria and Wraight, 2007).

Lecanicillium lecanii is a Deuteuromycete (imperfect) fungal species widely distributed in warm and humid environments (Nunez *et al.*, 2008). It was first observed by Zimmermann in 1898 on *Lecanium viride* on coffee in Indonesia. *L. lecanii* is proven to be very effective against scale insects like Aphids, whiteflies (Wang *et al.*, 2007; Van *et al.*, 2007) and Thrips (Vestergaard *et al.*, 1995). Few strains of *L. lecanii* are also found to be effective against some plant pathogenic fungi (Askary *et al.*, 1998; Alavo, 2015) and Nematodes (Shinya *et al.*, 2008) also.

In the present study, local strains of Entomopathogenic fungi isolated from different crop rhizospheres of the semi-arid region of Andhra Pradesh and Telangana were tested for their virulence against the polyphagous agricultural pest *Spodoptera litura* in a laboratory bioassay.

Materials and Methods

Preparation of conidial suspensions of the fungal isolates

A total of seven fungal isolates were tested in

a laboratory bioassay for their virulence against the pest *Spodoptera litura*. Five isolates were isolated from different crop rhizospheres of Telangana and Andhra Pradesh using semi selective medium and were morphologically identified as *Metarhizium* and *Lecanicillium* spp using standard identification key. Later molecular characterization of the ITS region of the isolates using specific primers (White *et al.*, 1990) identified three isolates among them, KoGn5, LaMa1 and PaCo4 as *Metarhizium anisopliae* (Chandra teja and Rahman, 2016) and two isolates, ArCo3 and PRg4 as *Aphanoas custerreus*.

One strain each of *Metarhizium anisopliae* and *Lecanicillium lecanii* i.e., MaAICRP and LIAICRP were obtained from the All India Coordinated Research Programme (AICRP) on Biological control of Crop Pests and Weeds, Hyderabad. Sterile Tween 80 solution (0.02%) was poured on 15 day old culture plates of the fungi. The mycelia and conidia were scrapped using a sterile spatula and taken in a conical flask. The suspension was passed through double layered muslin cloth to obtain a conidial suspension free of mycelial fragments and media. The concentration of suspension was determined using a haemocytometer and further serially diluted to obtain spore suspension of concentration 3.2×10^8 , 10^7 , 10^6 and 10^5 /ml

Rearing of *Spodoptera litura* insect culture

Eggs of *Spodoptera litura* (accession number: NBAII-MP-NOC-02: *S.litura*) were obtained from the insect rearing facility of The National Bureau of Agricultural Insect Resources (NBAIR), Bengaluru. The emerging neonate larvae were fed on tender castor leaves. To maintain the turgidity of the leaves the cut ends of the petiole were kept in moist cotton.

All the instars of the larvae were cultured in plastic trays covered with muslin cloth for aeration. The trays were cleaned daily and the fresh feed was added. The pupae were separated into a glass jars filled with sterile sand till one inch from the bottom. The jars were covered with black paper to maintain dark conditions. The emerging adult moths were transferred to glass jars of size 15 X 20 cm covered with muslin cloth. Around twenty adult moths were kept per jar to maintain the optimum sex ratio. The adults were fed with 10% honey solution. Folded cheese paper was placed in the jars to facilitate laying of egg masses. The egg masses laid were transferred to petriplates lined with moistened filter paper and kept at $28 \pm 2^\circ\text{C}$ for hatching. Uniform sized 3rd instar larvae were used in the Bioassay study.

Insect Bioassays

The virulence of the Entomopathogenic fungal isolates on the larvae, pupae and adults of the *S. litura* was tested using leaf dip assay method. Tender castor leaves used for the bioassay were first cleaned with running tap water to remove dirt particles & other contaminants and air-dried. Leaves were dipped in fungal spore suspension for 30 sec and air dried in a laminar air flow hood. Single leaf was placed in a perforated plastic container of the size 9cm diameter and 9cm height. Ten larvae per container were released on the leaf for feeding. Five replications were maintained for each of the seven fungal isolates and four conidial concentrations. A negative control was maintained with 0.02% sterile Tween80[®] in sterile distilled water. The mortality of the larvae was recorded first on the second day after treatment and from then on daily till the seventh day after treatment. For the effect on the different developmental stages of the insect, observations were recorded till the death of the adults. Feeding was changed daily from

second day with fresh untreated castor leaves. Mortality in the treatments was corrected by removing the natural mortality in the control (Abbott, 1925) and arcsine transformed to normalize the data before the analysis of variance (ANOVA).

Results and Discussion

Virulence of the Fungal isolates at different conidial concentrations

The fungal isolates were evaluated for their Mortality of *Spodoptera litura* larvae at four different conidial concentrations. Three day old larvae were fed on castor leaves treated with conidial suspensions of four different concentrations (3.2×10^5 , 10^6 , 10^7 and 10^8 conidia/ml) and the effect of the isolate as well as the conidial concentration on the mortality of the larvae was evaluated after 48 hrs till the seventh day from the treatment.

The results indicated a variation in the susceptibility of the *S. litura* larvae to isolates studied (Table-1). Significant variation in mortality of the insect pest at different conidial concentrations was also noted for few isolates. The mortality caused by the isolates ranged from 10.82% for ArCo3 at 10^5 /ml to 63.73% for PaCo4 at 10^8 /ml. An increase in the mortality was observed with the increase in concentration of the conidial suspension from 10^5 to 10^8 conidia/ml. However, for the *L. lecanii* isolate LIAICRP and *A. terreus* isolate ArCo3, there was no significant increase in the mortality percentages from the lowest to the highest conidial concentration studied. Among them, *M. anisopliae* isolate PaCo4 showed the highest mortality at concentrations 10^5 and 10^8 conidia/ml and KoGn5 showed the highest mortality at 10^6 and 10^7 conidia/ml concentrations. The isolate PaCo4 is the most virulent of all the isolates as its mortality percentage was higher than the others at the

lowest conidial concentration studied. However, *M. anisopliae* isolate MaAICRP showed second highest mortality (27.89%) at 10^5 conidia/ml concentration which was same with that shown by the most virulent isolate (PaCo4). Of the *A. terreus* isolates, PRg4 at 10^8 /ml conidial concentration showed mortality of *S. litura* larvae equal to that of the *M. anisopliae* isolate MaAICRP (48.51%). However, isolate ArCo3 showed the least virulence of all the isolates.

In terms of the least concentration for 50% mortality (LC50), *M. anisopliae* isolates KoGn5 and PaCo4 had the least LC50 (3×10^6 conidia/ml) at followed by LaMal (1×10^7 conidia/ml) and MaAICRP (4×10^7 conidia/ml). The highest LC50 was observed for *A. terreus* isolate ArCo3 at 7×10^{14} /ml. The other *A. terreus* isolate PRg4 had an LC50 of 6×10^7 /ml and the *Lecanicillium* isolate LIAICRP had 3×10^{11} /ml.

Effect of different strains of Entomopathogenic fungi on various developmental stages of *Spodoptera litura* (Table-2)

Larval Duration and Percent Mortality

There was only little variation observed between the larval durations of the isolates which ranged from 3.67 to 4.67 days. Among the treatments, *L. lecanii* isolate LIAICRP (4.67) prolonged the larval period of 4.67 days followed by KoGn5 (4.33 days) which are significantly longer than the untreated control. The percentage of mortality caused by the treatment of Entomopathogenic fungal isolates ranged from 10.33 to 66.67 percent. The isolates, *L. lecanii* LIAICRP (66.67), *A. terreus* ArCo3 (46.67) and *M. anisopliae* KoGn5 (43.33) had significantly higher larval mortality when compared to that of untreated control (10.33). The least mortality among the treatments was observed for *M. anisopliae*

isolate MaAICRP isolate (10.33%) which is same as untreated control. The mortality caused by *M. anisopliae* isolates, LaMa1, PaCo4 and the *A. terreus* isolate PRg4 though higher than the control did not show much variation.

Pupation percentage and pupal period

The mean percentage of pupation of the isolates ranged from 59.70 percent in *M. anisopliae* isolate PaCo4 to 90 percent in the untreated control. Except PaCo4, all the other isolates showed non-significant difference in percentage of pupation than the control. The pupal period in *L. lecanii* isolate LIAICRP was 7.67 days and *A. terreus* isolates ArCo3 and PRg4 was 7.67 and 8.67 days respectively. There was no significant difference in the mean pupal period between the isolates.

Percentage of Adult emergence and longevity

No adults emerged from the pupae infected with *M. anisopliae* isolate KoGn5. The percentages of adult emergence of the remaining isolates ranged from 61.67 to 83.33% compared to that of the control (90%). The adult emergence of the larvae treated with *A. terreus* isolate PRg4 (46.69) and *M. anisopliae*, LaMa1 (52.54) are significantly lower than the control. A wide variation was observed among the isolates in their effect of the longevity of *Spodoptera litura* adults. The *L. lecanii* isolate LIAICRP (5 days) significantly reduced the life span of *S. litura* adults compared to the control (10 days).

It was observed that each isolate had a varied effect on the different stages of insect

development. For instance, LIAICRP isolate caused high larval mortality among the isolates but had little effect on the pupation and adult emergence percentages. It again reduced the longevity of the adults significantly. *M. anisopliae* isolate Ma AICRP though was unable to kill the insect larvae, significantly lowered pupal formation and adult emergence.

The isolate, KoGn5 had significant effect on all the developmental stages of the insect pest. It caused 43.33 percent mortality of larvae which was significantly higher than the control mortality. Though there was 83.33 percent pupation in the remaining larvae, none emerged into adults. *M. anisopliae* isolate Ma AICRP had the least virulence among the isolates and its effect on the different stages of development of insect pest was similar to that of untreated control. It showed the least mortality of the *S. litura* larvae, without any reduction in percent pupation and percent adult emergence which are comparable with the untreated control.

Entomopathogenic fungi have been proven to be effective in controlling different species of *Spodoptera* both in laboratory bioassay and in field conditions. Vinayagamoorthi *et al.*, (2011) tested the efficacy of three local isolates of *B. bassiana* on the larvae of *S. litura* and found isolate Bb₁₀ to be most virulent in terms of percent mortality, LC50 and LT50.

In another study, they also tested the potential of three isolates of *M. anisopliae* against *S. litura* and *Euproctis fraterna* (Castor hairy caterpillar) and noted that Ma2 isolate was more effective on both the insects than the other two (Vinayagamoorthi *et al.*, 2011).

Table.1 Evaluation of different isolates of Entomopathogenic Fungi at different concentrations on mortality of *S. litura*

Isolates	Percent mortality of <i>S. litura</i> neonates larvae at 7 Days after Treatment				LSD @ 0.05	LC50
	1 x 10 ⁵	1 x 10 ⁶	1 x 10 ⁷	1 x 10 ⁸		
MaAICRP	27.89 ^a ± 2.97 (22)	33.09 ^{bc} ± 4.48 (30)	41.54 ^{abc} ± 3.16 (44)	48.46 ^c ± 3.16 (56)	9.17	4 x 10 ⁷
KoGn5	21.69 ^b ± 4.45 (14)	41.54 ^a ± 3.16 (44)	48.46 ^a ± 3.15 (56)	60.78 ^{ab} ± 3.64 (76)	4.88	3 x 10 ⁶
LaMa1	21.69 ^b ± 4.45 (14)	27.89 ^{cd} ± 2.97 (22)	40.33 ^{abcd} ± 4.92 (42)	58.37 ^{ab} ± 6.94 (72)	6.73	1 x 10 ⁷
PaCo4	30.43 ^a ± 5.70 (26)	36.82 ^{ab} ± 3.30 (36)	47.31 ^{ab} ± 3.16 (54)	63.73 ^a ± 5.24 (80)	6.02	3 x 10 ⁶
LI AICRP	21.68 ^b ± 4.45 (14)	23.01 ^{de} ± 6.70 (16)	27.17 ^f ± 9.15 (22)	31.28 ^d ± 9.78 (28)	10.47	3 x 10 ¹¹
ArCo3	10.82 ^c ± 6.85 (4)	12.44 ^f ± 9.62 (6)	14.98 ^g ± 10.23 (8)	19.14 ^e ± 8.52 (12)	11.53	7 x 10 ¹⁴
PRg4	17.51 ^{bc} ± 7.47 (10)	20.77 ^{de} ± 9.11 (14)	37.75 ^{cde} ± 9.85 (38)	48.51 ^c ± 6.65 (56)	11.22	6 x 10 ⁷
LSD @ 0.05	7.01	8.04	8.72	8.64		

Values are arc sine transformed mean mortality percentage ± SD (α = 0.05). Values in parentheses are actual mean mortality percentages. The figures denoted by same alphabet in a column are not significantly different by DMRT (p = 0.05)

100% mortality of the 3rd instar larvae of *S. litura* seven days after treatment was reported for some strains of *M. anisopliae* (Petlumal and Prasertsan, 2012). Freed *et al.*, (2012) tested several isolates of *M. anisopliae* isolated from different crops on *Spodoptera exigua* and found that the isolates differ in their virulence against the pest. A *M. anisopliae* isolate FT83 was reported to show 100% mortality against *S. exigua* larvae (Han *et al.*, 2014).

Shairra and Noah (2014) tested the efficacy of Entomopathogenic fungi and nematodes against *S. littoralis* and found *M. anisopliae* to be very potent both individually and in combination with nematodes. Similarly, in the

present study, different isolates of Entomopathogenic fungi differ in their virulence against pest. The highest virulence against the three day old larvae was shown by the *M. anisopliae* isolate PaCo4 and *A. terreus* isolate ArCo3 was the least virulent.

Apart from the fungal strain, the susceptibility of an insect pest depends on various factors like dosage, method of application and the temperature of the environment. Boucias *et al.*, (1988) noted that direct spraying of the conidia on the insect surface enhanced the infectivity of the pathogenic fungi due to the easy cuticular adhesion and germination of the fungal conidia.

Table.2 Evaluation of different isolates of Entomopathogenic Fungi on growth and development of 3rd instar larvae of *S. litura*

Isolates	Larval duration (days)	Larval mortality (per cent)	Pupation (per cent)	Pupal Period (days)	Adult emergence (per cent)	Adult longevity (days)
MaAICRP	4.00 ± 0 ^{ab}	14.20 ^c ± 7.33 (6.67)	55.89 ^{ab} ± 13.11 (67.10)	8.33 ^a ± 1.15	56.92 ^{abc} ± 30.47 (61.67)	8.67 ^{abcd} ± 2.89
Ma KoGn5	4.33 ± 0.58 ^{bc}	36.14 ^{abc} ± 19.34 (36.67)	75 ^{ab} ± 25.98 (83.33)	Pupae failed to emerge	Pupae failed to emerge	Pupae failed to emerge
Ma LaMa1	4.00 ± 0 ^{ab}	21.83 ^{bc} ± 14.33 (16.67)	76.36 ^{ab} ± 23.62 (85.70)	8.00 ^a ± 0	52.54 ^{ab} ± 10.25 (62.60)	9.00 ^{bcde} ± 2.65
Ma PaCo4	4.00 ± 0 ^{ab}	26.07 ^{bc} ± 7.40 (20)	50.64 ^a ± 5.04 (59.70)	8.00 ^a ± 0	66.92 ^{abc} ± 20.51 (78.33)	7.33 ^{ab} ± 0.58
LI AICRP	4.67 ± 0.58 ^c	51.14 ^a ± 10.64 (60)	70 ^{ab} ± 34.64 (75.00)	7.67 ^a ± 0.58	75 ^{abc} ± 25.98 (83.33)	5.00 ^a ± 2.65
VI ArCo3	4.00 ± 0 ^{ab}	38.85 ^{ab} ± 21.29 (40)	75 ^{ab} ± 25.98 (83.33)	7.67 ^a ± 0.58	68.44 ^{abc} ± 18.67 (80.93)	7.67 ^{abc} ± 0.58
VI PRg4	4.00 ± 0 ^{ab}	32.71 ^{abc} ± 10.64 (30)	77.41 ^{ab} ± 21.80 (87.50)	8.67 ^a ± 0.58	46.69 ^a ± 11.55 (52.60)	7.33 ^{ab} ± 3.51
Control T8	3.67 ± 0.58 ^a	--	90 ^b ± 0 (100.00)	8.00 ^a ± 0	90 ^c ± 0 (100.00)	10.00 ^{bcde} ± 0
LSD @ 5%	0.61	24.48	37.63	1.01	33.82	3.93

The values in percentages are arc sine transformed ± SD.

Values in parentheses are means of actual percentage.

The figures denoted by same alphabet in a column are not significantly different by DMRT ($\alpha = 0.05$)

The mode of infection employed in the present study though showed less mortality percentage of the insect larvae compared to some other reports closely mimics the mode of application in the field and thus is more of practical use.

Vinayagamoorthi *et al.*, (2011) noted that the mortality of the pest increases with the increase in the conidial concentration. Results in the present study also indicate that the mortality percentage of the *S. litura* larvae increased with the increase in the conidial concentration of the isolates. The decrease in the mortality percentage from the 1st instar larvae to the 3rd instar larvae in the present study is in agreement with findings in the previous studies that the susceptibility of an insect pest to the pathogenic fungi decreases with the increase in the age of the larvae (Osborne *et al.*, 1990). The body size and the increased immunity of the higher instar larvae may affect the infection, multiplication and mycosis of the fungi on the insect.

The unique mode of action of Entomopathogenic fungi involving contact and penetration of the fungal conidia to the host cuticle makes them to be able to infect sucking pests, such as aphids and mosquitoes (Wang and Feng, 2014) as well as the non-feeding stages of the insect pests (Anand *et al.*, 2009). Anand *et al.*, (2009) treated the pupae of *Spodoptera litura* (Fab.), (Lepidoptera: Noctuidae) with conidial suspensions of different species of entomopathogenic fungi and found *M. anisopliae* to be most virulent among them followed by *L. muscarium*. Asi *et al.*, (2013) studied the effect of different Entomopathogenic fungi against all life stages of *Spodoptera litura* and found that while pupae were less susceptible to fungal infection, eggs and larvae are more susceptible to the fungal infection. In the present study, a laboratory bioassay was

carried out on the 3rd instar larvae to evaluate the effect of fungal infection on the different developmental stages of the *S. litura* such as mortality, larval duration, percentage and duration of pupae, adult emergence percentage and longevity. It was found that the isolates increased the larval duration and decreased the longevity of the adults of the insect pest but did not affect the pupal period. Similar result was reported by Malarvannan *et al.*, (2010) but Hafez *et al.*, (1997) reported prolonged pupal duration in *Phthorimaea operculella* treated with *B. bassiana*.

It was found that each isolate in the study affected the stages of insect development differently. For instance, while *L. lecanii* isolate VI AICRP showed prolonged larval duration, highest mortality and lowest adult longevity, *M. anisopliae* isolate PaCo4 had the lowest percent pupation. It was also found that all the pupae of the KoGn5 treated larvae failed to emerge into adults. The metamorphosis of an insect is known to be greatly influenced by the juvenile hormone titre as its changes can cause disturbances in the storage proteins and fats necessary for moulting (Palli and Locke, 1987; Koul and Isman, 1991). Larval-Pupal intermediates like deformed pupae such as those reported by Malarnannan *et al.*, (2010) were also seen in the present study. Asi *et al.*, (2013) noted that the susceptibility of an insect pest to the fungal pathogen decrease as the larvae grows. However, in the present study, it was interesting to note that *L. lecanii* isolate VI AICRP which had only 28 percent mortality against 1st instar larvae of *S. litura* at 10⁸ conidia/ml had a mortality percentage of 66.67% on the 3rd instar larvae even at a lower concentration of 10⁶ conidia/ml.

Eventhough, *Aphanoascus* spp are not known to be Entomopathogenic in nature, it was found in the present study that *A. terreus* isolate PRg4 caused mortality of *S. litura*

larvae similar to that of the AICRP strain of *M. anisopliae* in the laboratory conditions. *Aphanoascus scusterreus* belongs to the family onygenaceae of Ascomycetes. Many species in the genus are known to be keratinolytic saprotroph mostly found in keratin rich soils. *Chrysosporium*, a teleomorph of the *Aphanoascus* is reported to control mosquitos (Preeti and Prakash, 2010). Entomopathogenic fungi are important components of Biological control of insect pests. Wide variations occur in the effectivity of different strains and selection of virulent ones is critical in their success. Few isolates in the present study were promising candidates for further investigation on their field efficacy before their successful use in agriculture. Moreover, the efficient mass multiplication of the virulent isolates also plays a key role in their employability as Mycopenesticides

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