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Laboratory evaluation on the potential of entomopathogenic fungi, *Nomuraea rileyi* against Tobacco caterpillar, *Spodoptera litura* Fabricius (Noctuidae: Lepidoptera) and its safety to *Trichogramma* sp.

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ABSTRACT

Tobacco caterpillar, *Spodoptera litura* was reared in semi-synthetic diet and the different stages were maintained for bioassay. Entomopathogenic fungi, *Nomuraea rileyi* was sub-cultured using Potato Dextrose Agar (PDA). Spore suspensions of four different concentrations (2.4×10^7, 2.4×10^6 and 2.4×10^5, 2.4×10^4 conidia/ml) were prepared from the 15 day old culture of the fungi for evaluation. A preliminary study on *N. rileyi* against *S. litura* larvae was done. Cypermethrin 25 EC, Neem Plus and untreated were used as controls. Though the pupation was not suppressed as expected, the pupal weight, length and duration were severely affected. The malformed adults were the highest (96.7%) in pupae treated with 2.4×10^6 spore concentration. Subsequently, the fecundity was completely arrested in *N. rileyi* at 2.4×10^7, 2.4×10^6 and 2.4×10^5 spore conc. The biosafety of *N. rileyi* against *Trichogramma japonicum* and *Trichogramma chilonis* was confirmed by monitoring the parasitization and male-female ratio and proved that the *N. rileyi* was safe to these egg parasitoids.

Keywords: *Nomuraea rileyi*, *Spodoptera litura*, fecundity, biosafety

INTRODUCTION

Spodoptera litura devastates a large host range of more than 120 host plants including crops, vegetables, weeds and ornamental plants (Ramana *et al*., 1988). It feeds gregariously on leaves leaving behind only the midrib veins resulting in great yield loss. Environmental concerns and health risks associated with the use of synthetic chemicals have stimulated the efforts to use biocontrol agents. Among them, entomopathogens namely, virus, bacteria, protozoa and fungi are widely used against lepidopteran pests (David, 2008).

Nomuraea rileyi is a dimorphic hyphomycete that causes epizootic in a wide range of insect pests namely *Heliothis zea*, *Pseudoplusia includes, Trichoplusia ni* and *S. litura*. Its host specificity and ecofriendly nature has encouraged its use in pest management. But there are a few studies in relation to the use of this organism as a biological control agent (Pornpoj Srisukchayakul *et al.*, 2005). Hence, the present study is aimed at exploring the insecticidal activity of this fungus against the *S. litura* by screening and analysis of life table parameters after treatment and its biosafety to *Trichogramma. chilonis* and *T. japonicum*.

MATERIALS AND METHODS

Collection and rearing of *Spodoptera litura*

Spodoptera. litura larvae were collected and maintained in the laboratory at 22 ± 2°C and 70 – 75 % relative humidity (RH). The larvae were reared both on castor and semi-synthetic diet in individual containers to prevent contamination.

Fungal source and Preparation of spore suspension

Nomuraea rileyi cultures were obtained from Project Directorate of Biological Control (PDBC), Bangalore. Spore suspension was prepared from 15 day old cultures of *N. rileyi* on PDA medium. The fungal surface was scraped using a sterile loop with 10 ml of sterile distilled water having 0.02% Tween 80 as a wetting agent (Rombach *et al*., 1986). The suspension was then filtered through sterile muslin cloth to eliminate the medium (Sasidharan and Varma, 2005). Spore concentration of the filtrate was determined using a Neubauer Hemocytometer. This served as the stock suspension. Different spore concentration was prepared by adding sterile 0.02% Tween 80 in distilled water. Spore suspension of *N. rileyi* at four different concentrations, 2.4×10^7, 2.4×10^6, 2.4×10^5 and 2.4×10^4

spores/ml was prepared and tested for its efficacy on third instar larvae, pupae and adults of *S. litura*.

Growth inhibition of larvae

Nine ml of different spore concentrations of *N. rileyi* was sprayed against *S. litura* larvae. Ten larvae were used per replication. Larvae treated with sterile distilled water, Neem Plus (3 %) and cypermethrin (0.006 %) (v/v) served as positive control. After treatment, the larvae were allowed to feed on semi-synthetic diet. Each treatment was replicated thrice. Growth parameters *viz*., larval duration (days), larval length, larval weight and pupation (%) were recorded. (Hafez *et al.,* 1994).

Growth inhibition of pupae

Four different spore concentrations of the test fungus, *N. rileyi* with three replications each were used for infecting the pupa of *S. litura*. The pupae were sprayed with 10 ml of respective fungal spore suspensions using hand atomizer. The pupae treated with sterile distilled water, 3% Neem Plus and 0.006 % (v/v) cypermethrin served as positive control. The pupa after the treatments was kept up to adult emergence and observed for the parameters, pupal duration (days), pupal weight (mg), pupal length (cm) and adult emergence (%). (Hafez *et al.,* 1994).

Adult longevity, fecundity and egg hatchability

Healthy adults were released into mud pots at 1:1 male-female ratio. Cotton swabs dipped in 10% honey treated with one ml of the test fungi served as treatment. The experiment was performed using four different spore concentrations of the test fungi, for an indepth study. Cypermethrin, Neem Plus and untreated served as controls. Adult longevity (days), fecundity (numbers) and hatchability (%) were recorded (Malarvannan, 2004). Triplicates were maintained for each treatment. The data were analysed statistically using Agres package version 4 and SPSS version 9.

Biosafety of *N. rileyi* against *Trichogramma* spp

Preparation of egg cards

Fresh *C. cephalonica* eggs (12 hrs old) collected from the insectary was cleaned and taken in glass Petri dishes (15-20 cm diameter). Eggs were sterilized with UV light (156W) in a closed chamber for a half-an-hour duration so as to kill the embryo without damaging other egg contents. The UV sterilized eggs were sprinkled on thick cards (5 x 2 cm) smeared with thin layer of diluted gum at 100 ± 5 eggs/ card. Four different concentrations of fungal spore suspensions and controls (Neem Plus®, cypermethrin and untreated) were sprayed on the *C. cephalonica* eggs using manually operated atomizer. Untreated check was also maintained. Honey solution (10 %) was streaked on the smooth side with a camlin brush. This sheet was folded and stapled in such a way that honey surface was inside and the adults fed on the honey through the holes from the eruptive surface. The experiment was conducted at room temperature of 25 ± 2º C and at 60 % R. H.

Inoculation of parasitoids

The fungal suspensions of *N. rileyi* at four concentration levels (2.4×10^4, 2.4×10^5, 2.4×10^6 and 2.4×10^7) were tested for their safety on egg parasitoids of *T. japonicum* and *T. chilonis* and observed for the percent parasitization and male and female wasps emergence in numbers. The pre-conditioned and fungal treated *C. cephalonica* egg cards were dried under fan and placed inside the glass vials and a pair of *Trichogramma chilonis* and *T. japonicum* was segregated from the pool culture and introduced into the vial for parasitization. The adult wasps started to parasitize the *Corcyra* eggs. Three days after inoculation, daily observation for blackening of eggs was made till adult emergence to check the percent parasitization. Parasitoids under above mentioned room temperature and relative humidity emerged on the 7th and the 8th day. Percent parasitism, number of progeny adults emerged, sex ratio, and percent emergence of adult in each vial were assessed and recorded.

RESULTS AND DISCUSSION

Effect of *N. rileyi* on different stages of *S. litura*

The percent pupation varied in different treatments. The least pupation of 83.3% with fungal treatment was observed in 2.4×10^6 spore conc. In untreated control it was 100% (Table 1). Larval mortality was less in the fungal treatments. Tang *et al.* (1999) earlier reported that *N. rileyi* was less virulent against younger instars of corn earworm, *Helicoverpa armigera.* Variation in the growth parameters was evident among the different treatments. Incomplete metamorphosis and larval pupal intermediates were evident in the highest conc. while larval duration was prolonged up to 7.1 days in 2.4×10^4.

The larval length showed variation (2.8-3.5 cm) between the treatments. In the fungal treatments, the shorter larvae (2.8 cm) were observed in 2.4×10^5 spore concentration. (Table 1). It was different in the case of larval weight. The larval weight ranged from 350.0–372.1 mg. It was least (350 mg) in 2.4×10^4 spore conc. whereas in the untreated, the larval weight was 372.1 mg (Table 1).

Pupal growth

Among the fungal concentrations, lighter larvae (167.9 mg) was recorded with 2.4×10^4 compared to heavier ones (195.3 mg) in untreated control (Table 2). The pupal length showed

Table 1. Effect of *N. rileyi* on growth of *S. litura* larvae

Treatments		Larval growth parameters			
		length (cm)	weight (mg)	duration (days)	Pupation (%)
Control	Untreated	3.5^{c}	372.1^{d}	7.1^{b}	100.0^{d}
	Cypermethrin (0.006 %)	3.0^{b}	349.4^{a}	6.8 ^{a}	73.33^{a}
	Neem Plus (3%)	3.3^{b}	356.1^{c}	7.1 ^{b}	66.67^{a}
N. rileyi (spores /ml)	2.4 x 10^4	3.0^{b}	350.0^{a}	7.1^{b}	90.00^{c}
	2.4 x10^5	2.8^{a}	354.1^{b}	6.7^{a}	93.33^{c}
	2.4 x 10^6	3.0^{b}	360.3^{c}	6.9^{b}	83.33^{b}
	2.4 x 10^7	3.1^{b}	362.4^{c}	6.7^{a}	90.00^{c}
CD (P = 0.05)		0.4	42.4	0.7	19.0

Each value mean of triplicate, Different letters in each column differ significantly (5%) by LSD

a significant difference between the treatments. It ranged from 1.31 to 1.51 cm, with 2.4×10^4 superior among the fungal treatments (1.31 cm) (Table 2 and Plate 1.b). In addition, pupal discolouration, damage and larval-pupal interme diates were observed. The pupal duration was 3.0 days in 2.4×10^6 as against 9.3 days in untreated (Table 2). More number of malformed moths emerged from 2.4×10^6 (96.7%) as against highest healthy moth emergence in untreated (90.0%). These results are in agreement with those put forward by Hafez *et al*. (1994) who also reported that *B. bassiana* treated pupae of potato tuber moth, *P. operculella* resulted in a highly progressive decrease in the percentage of moth emergence with increasing concentration.

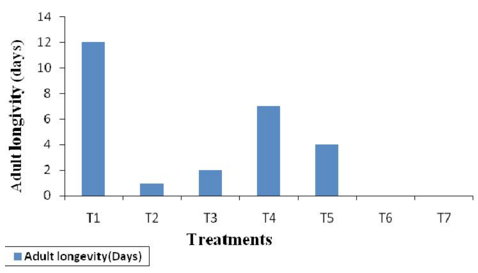

Figure 1. Adult longevity of *S.litura*

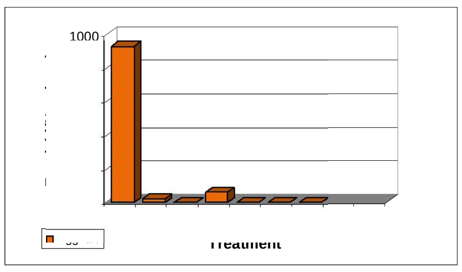

Figure 2. Fecundity of *S.litura*

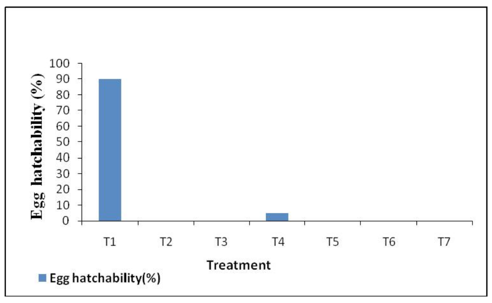

Figure 3. Egg hatchability (%) of *S.litura*

Figures: Effect of *Nomuraea rileyi* on growth of *S. litura* adults

T1- Control (10% honey solution); T2- cypermethrin; T3- Neem Plus; T4- 2.4×10^4;T5 – 2.4×10^5;T6- 2.4×10^6; T7- 2.4×10^7

Adults for longevity, fecundity and egg hatchability

Adult longevity varied from 0 to 12 days between treatments (Fig 1). With the honey solution the adults lived longer (12 days). Knock down effect was observed in three hours and mortality was also observed within 12 hours in adults treated with Neem Plus. In fungal treatments, an early adult mortality was observed with 2.4×10^7 and 2.4×10^6 followed by cypermethrin and Neem Plus (1 and 2 days). There was a wide variation in the fecundity among different treatments. Few treatments *viz.*, 2.4×10^5, 2.4×10^6, 2.4×10^7 and Neem Plus arrested the fecundity completely, which was followed by Cypermethrin The egg hatchability was suppressed in most of the treatments. The highest percentage 90% was recorded with untreated concentration, whereas it was nil in 2.4×10^7, 2.4×10^6 2.4×10^5, cypermethrin and Neem Plus. This result was in conformity with the earlier report

Table 2. Effect of *N. rileyi* on growth of *S. litura* pupae

Treatments		Pupal growth parameters		Adult emergence		
		Weight (mg)	Length (cm)	Dura- tion	Healthy (%)	Mal- formed / Dead Pupa %
Control	Untreated	195.3^{d}	1.51^{d}	9.3^{d}	90.0^{e}	10.0^{c}
	Cypermethrin	181.3^{c}	1.35^{a}	6.3^{c}	8.9^{d}	91.1^{b}
	Neem Plus	170.1^{a}	1.32^{a}	6.7^{c}	8.9^{d}	91.1.0^{b}
N. rileyi (spores /ml)	2.4x10^4	167.9^{a}	1.31^{a}	6.3^{c}	8.0^{c}	92.0 ^{b}
	2.4 x10^5	186.5^{c}	1.37^{b}	6.5^{c}	7.4^{b}	92.6^{b}
	2.4 x 10^6	169.9^{a}	1.35^{b}	3.0^{a}	3.3^{a}	96.7^{a}
	2.4 x 10^7	176.7^{b}	1.39^{c}	5.7^{b}	7.5^{b}	92.5^{b}
CD (P =	0.05)	23.2	0.06	9.1	11.9	11.9

Each value mean of triplicate, Different letters in each column differ significantly (5%) by LSD

Table 3. Biosafety of *N. rileyi* to egg parasitoid, *Trichogramma chilonis* and *Trichogramma japonicum*

Treatments		Parasitization (%)			
		Trichogramma chilonis		*Trichogramma japonicum*	
		Parasi-tized but un-hatched	Parasi tized and hatched	Parasi tized but un-hatched	Parasi tized and hatched
Control	Untreated Neem Plus Cypermethrin	8.8[a] 29.0[b] 29.5[b]	91.2[a] 71.0[b] 70.5[b]	8.8[a] 29.0[c] 29.5[c]	91.2[a] 71.0b[c] 70.5[c]
N. rileyi (spores /ml)	2.4 x 10^4 2.4 x10^5 2.4 x 10^6 2.4 x 10^7	10.5[a] 16.3[a] 11.5[a] 10.3[a]	89.5[a] 83.7[a] 88.5[a] 89.7[a]	20.4[b] 21.9[b] 20.8[b] 18.3[b]	79.6[b] 78.1[b] 79.2[b] 81.7[b]
CD (P = 0.05)		9.4	10.9	9.5	11.1

Each value mean of triplicate, Different letters in each column differ significantly (5%) by LSD

from Vimala Devi (1994) who has tested the efficacy of the *N. rileyi* against *S. litura* in a lab, net house, and field. Dosage of 2×10^{11} conidia/litre of spray solution was found to bring about effective control of late second to early third instar *S. litura* larvae on *Ricinus communis,* the highest cumulative mortality of 88-97% was observed.

Plate 1 a. Normal pupa untreated, b. Pupal shrinkage-fungal treated.

Plate 2. Growth of *N. rileyi* on *S. litura* pupa (arrow).

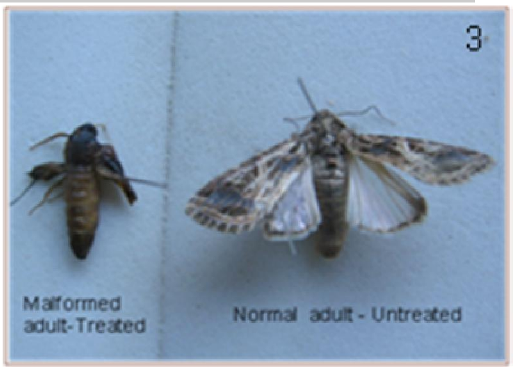

Plate 3. Moth emerged from Fungal treated pupa (left), Normal moth emerged from untreated (right).

Biosafety of *N. rileyi* against beneficial insects
Trichogramma japonicum

Parasitization and adult emergence was reduced in the highest concentration *viz.,* 2.4 × 10^7 (81.7%) as against untreated control (91.2%) (Table 3). The parasitization was affected by the Neem Plus and cypermethrin treatments. According to Greathead and Prior (1990) there have been no signs of intolerance adverse effects by *Metarhizium flavoviride* on non-target species. However, Goettel *et al.* (1990) postulated that side effects can be expected in a wide range of non-target arthropods. Peveling and Demba (1997) tested blastospores of *M. flavoviride* on *Pharoscymnus anchorago* L. (Coleoptera: Coccinellidae) and recorded no adverse effects of the entomopathogens on this West African lady bird beetle. However, Ball *et al.* (1994) in their laboratory experiments with *M. flavoviride* on *Apis mellifera* found that bees can be infected if kept under stress. The male female ratio was slightly irregular. The maximum number of female wasps was recorded in fungal treatment 2.4 × 10^7(14.0) (Fig 4) on a par with untreated (15) proving that this fungus would not cause any adverse effect against the parasitoid.

Trichogramma chilonis

Among the different treatments tested, even at the highest concentration *viz.,* 2.4 × $10^7$the parasitization was 89.7% compared to 91.2 per cent in untreated control (Table 3). It is proved that entomopathogenic fungus could be quite specific and probably it might infect only certain type of insect host. The results were supported by Broza *et al.,* 2001, Dromph and Vestergaard (2002) who noted that *Beauveria bassiana, Beauveria brongniartii, Hirsutella spp, Metarhizium anisopliae and Verticillium lecanii* did not affect three Collembolan species. The balanced sex ratio observed in the fungal treatments, on a par with the untreated control, clearly showed that the adult emergence is not affected by the fungus. The female emergence was highest in 2.4 × 10^6 (12.0) (Fig 5) as against least in Neem Plus and cypermethrin (4.0).

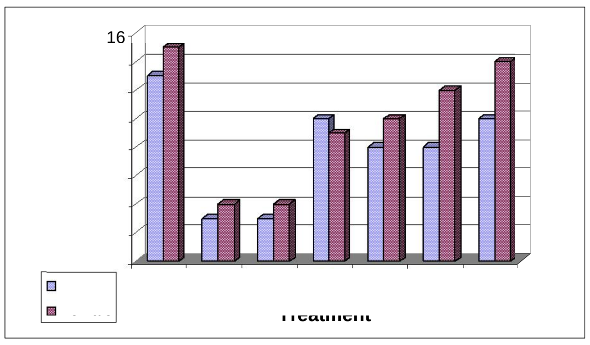

Figure 4. Number of *Trichogramma japonicum* adult emerged after the treatment

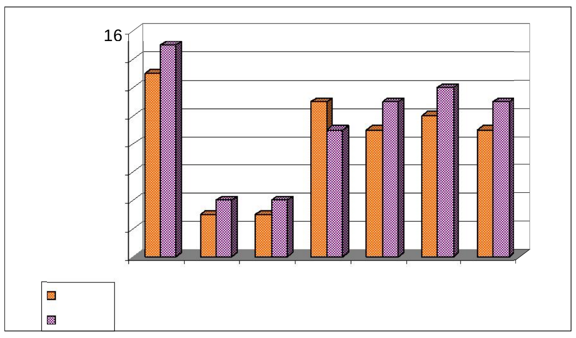

Figure 5. Number of *Trichogramma chilonis* adult emerged after the treatment

T1- Control (10% honey solution); T2- Neem Plus; T3 cypermethrin; T4- 2.4×10^4; T5 – 2.4×10^5; T6- 2.4×10^6; T7- 2.4×10^7

The experimental results proved that *N. rileyi* is a promising biocontrol agent. Its wide application as a biological pesticide could be taken up after exploring its toxicity and field trials.

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