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Biocontrol potential of entomopathogenic fungi *Nomuraea rileyi* (f.) Samson against major groundnut defoliator *Spodoptera litura (fab.) Lepidoptera; Noctuidae*

Abstract

Biopesticides based on bacteria, viruses, entomopathogenic fungi and nematodes are often-considerable scope as plant protection agents against several insects. Use of entomopathogenic fungi as biological control agents for insect species has increased the global attention during the last few decades in the present study, biocontrol potential of entomopathogenic fungi Nomuraea rileyi (F.) Samson against groundnut defoliator Spodoptera litura (Fab.) (lepidoptera;Noctuidae) was investigated. Nomuraea rileyi SSK 07 isolate was isolated from local groundnut field soil adopting soil dilution method and the isolate was identified based on the cultural and morphological characteristics. Effect of N.rileyi on the cumulative larval mortality, lethal concentration 50(LC50), lethal time 50 (LT50), total larval and pupal period, pupal, adult emergence and adult longevity were studied. All the life stages of S.litura were susceptible to N.rileyi. In general, the concentration, duration and life stage dependent mortality could be observed. The LC50 and LT50 values were increased as the larvae grew older. As the instars advanced, a decrease in mortality and an increase in time for the mortality were recorded. Distinct effect on the development revealed short larval and pupal period. Adult emergence and adult longevity was highly influenced.

Keywords: *spodoptera litura, nomuraea Rileyi*, developmental period; mortality; LT50; LC50

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Introduction

Groundnut (*Arachis hypogaea*, L.) (Fam: Leguminaceae) is an important oil seed crop in India. It occupies 8.6 million hectares, of which 85% is rainfed and 15% is irrigated. The edible oil economy of the country primarily depends upon the groundnut production.[1] Insect pests are the major constraints in groundnut production.[2] More than 360 species of insects and mites were reported to attack the groundnut crop in the field and the pods in storage all over the world.[3] Among the various pests defoliator *Spodoptera litura (Fab.) (Lepidoptera; Noctuidae)* is the most serious pest causes yield loss in high magnitude throughout the world.[4] *S. litura* is widely distributed throughout Asia and the Pacific islands.

It is an important polyphagous pest reported to feed on 112 species of plants belonging to 44 different families. Dhin have reported that one *S. litura* larva per plant at seedling stage reduced the pod yield by 25.8 percent. The early larval stages of *S. litura* feed on the leaves, flowers and pods of groundnut and reduce the production. Whereas, the late larval stages feeds on the pods in addition to the above mentioned parts. The defoliator population in groundnut ecosystem has been found to increase in number and intensity both during rainy and post rainy season, due to the destruction of natural control system, especially in fields where insecticides have been applied.[5] The management of this pest using chemical insecticides is unsuccessful because of its insecticide resistance.[6]

Even though chemical pesticides are used to control the pest, the indiscriminate use of these chemical pesticides leads to various health hazards and insecticide resistance.[7] The development of pest control measures using microorganisms' especially entomopathogens has received increasing attention in recent year.[8–10] Biopesticides offer several advantages over the chemical pesticides viz. safety, targeted activity to the desired pests, effective in lower quantities thereby offering lower exposure and quick decomposition to leave no residues and allowing field re-entry immediately after application and amenability to use in rotation with chemical pesticides as part of IPM programs.

Hence research interest in augmentation and application of biopesticides has also been growing with the ultimate objective of improving commercial production and sustainable utilization of the biopesticides. In the last decade, extensive and systematic research has enhanced the effectiveness of biopesticides while the techniques for their mass production, storage, transport and application have vastly improved.[4,10] Usage of entomopathogenic fungi as biological control agents of insect pests has been increasing during the last few decades. More than 750 species of fungi mostly deuteromycetes and entomophthorales are pathogenic to insects.[11,12] Species that have been most intensively investigated as mycoinsecticides in the crop pest control includes *Beauveria bassiana, B. brongniartrii, Metarhizium anisopliae, Nomuraea rileyi, Paecilomyces fumosoroseus, P. farinosus, Entomophthora* sp, *Fusarium* sp, *Aspergillus* sp.[5]Entomopathogenic fungi are associated with insects living in

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diverse habitats such as freshwater, soil and aerial location. They are very specific to insects and do not infect host plants. Among the different fungi, *Nomuraea rileyi* is a cosmopolitan species infecting many noctuids such as *Helicoverpa armigera*, *Spodoptera litura, Tricoplusiani, Anticarsia gammatalis, Pseudoplusia* includes and has a potential for development into mycoinsecticide and occurs in soils of various agro ecosystem. The present study undertaken to evaluate the bioefficacy of *Nomuraea rileyi* (F.) Samson on major groundnut defoliator *Spodoptera litura* (Fab.) (*Lepidoptera; Noctuidae*) under laboratory condition.

Material and methods

Soil sampling

Entomopathogenic fungi were isolated from the soil sample obtained from groundnut field, Chengalpet, Kanchipuram district, Tamil Nadu and processed for the isolation of fungi. Approximately 2kg of soil was collected from four points a few meters apart by digging to a depth of 10-15cm with a small spade. The soil samples were put in plastic bags and taken to the laboratory and stored at 25°C. For processing, the soil was thoroughly mixed and passed through a 0.4mm mesh sieve to break or separate any coarse lumps of soil or litter. Before microbial analysis, soil aggregates were broken by hands, trays with soil were kept open until moisture was at equilibrium.[13] Soil texture pH electrical conductivity organic matter nitrate, phosphorous, potassium, calcium, magnesium sulphur, sodium, zinc, iron, copper were determined for all soil collected. These measurements were determined in national agro foundation at Taramani TamilNadu, India.

Isolation of N.rileyi

N. rileyi SSK 7 strain was isolated from the processed soil sample by the modified method of Clark[14] using CTC (Chloramphenicol Thiobenzodazole Cyclohexamine) media. The organism was identified based on the morphological and cultural characteristics adopting standard methods and the pure culture was maintained sabouraud dextrose agar slant. Fungal morphology was confirmed by lacto phenol blue staining.[15]

Preparation of conidial suspensions

The re-isolated conidia were sub cultured in SMYA medium. Fungal conidia were collected from 15 days old culture by scrapping off with a sterilized glass rod. A homogenous conidial suspension was prepared in sterile distilled water by adding a few drops of the wetting agent Tween 80 (0.01%). The conidial concentration of the suspension was determined using an improved Neubauer hemocytometer (Germany). Serial dilutions were made from the stock solution to obtain different conidial concentration 10^{10}, 10^{9}, 10^{8}, 10^{7}, 10^{6}, 10^{5}, 10^{4} and 10^{3}spore/ml.

Laboratory bioassay on Spodoptera lituraLarva

The egg masses and larval instars of *S. litura* were collected from the groundnut field in an area around Kanchipuram and Thiruvallur district, Tamil Nadu, India. Collected larvae were maintained on groundnut leaves (TMV-7 variety). Twenty larvae in each in stars separately were sprayed with eight dose *viz*. $1x10^{10}$, $1x10^{9}$, $1x10^{8}$, $1x10^{7}$, $1x10^{6}$, $1x10^{5}$, $1x10^{4}$ and $1x10^{3}$ spores/ml using hand sprayer. The treated larvae were introduced into the plastic container (34mmx21mm) provided with moist cotton swap covered with tissue paper at the bottom of the container to provide humidity. The containers were covered with meshed lid to provide aeration to the larvae. For control category, another 20 larvae of each in star treated with distilled water only. The containers were incubated at room temperature 28±0.5°C in a incubator (Remi BOD incubator, Mumbai, India). Daily observation on larval mortality was recorded for a period of 4days. The dead larvae that showed mycelial growth were considered as dead due to the fungal infection. After 96hours of the conidinal treatment, all the surviving larvae from each treatment were transferred to another container of the same size for further development. The total larval and pupal durations, adult longevity and the adult emergence were recorded.

The LT_{50} of the dose of fungi to kill the different larval instars was assessed in hours following Blever & Hostetter.[16]

$LT50=a+e(c-b)D$

Where, a=the number of hours from the initiation of the test until the reading made just before the 50% value was recorded; b=the total number of larvae dead at the reading just before 50% value was reached; c=50% of the total number tested; D=the number of larvae dying in 24hours period during which the 50% mortality was reached and e=the number of hours between mortality counts. The dose mortality data were subjected to profit analysis[17] for LC_{50}.

Pupa and adult

About 300gram of finely sieved soil was taken in 500 ml capacity bottle and autoclaved at 15 psi pressure for 30minutes. After the sterilization this soil was transferred in to a clean surface sterilized 500ml capacity plastic container (65x32mm) and the soil moisture was maintained by adding 5ml of sterilze-distilled water. Different concentrations of conidia ranged from 10^{10} to 10^{3}spore/ml were added separately. Pupa of *S. litura* was placed individually. Each treatment was replicated 10 times. Another set was maintained by adding only distilled water as control. Observations on the pupal mortality and adult emergence were recorded.

Statistical analysis

The data (between concentration of the fungal spores and instars) was subjected to ANOVA using 'STATISTICA' computer statistical package.

Results and discussion

N.rileyi was isolated from the groundnut field soil adopting culture dependent method and the isolated fungi was identified based on the cultural characteristics on the CTC media which revealed brilliant green aerial mycelia and the microscopic examination of fungal spore by lactophenol cotton blue showed spherical conidia. Soil physico-chemical parameters highly influenced the natural occurrence of *Nomurea rileyi*. *Nomurea rileyi* isolated from the respective soil sample reveals high organic matter, available nitrogen and phosphorous (Table 1). This may favour the viability of the fungal spore and thus improved the natural occurrence of *Nomurea rileyi*.

Impact of N. rileyi on the development of S. litura

Biocontrol study revealed all the life stages of *Spodoptera litura* susceptible to the *N. rileyi* as dose dependent manner. Concentration dependent variation on mortality was observed in all the life stages (Table 2). In the case of larval development the total larval period was statistically significant at 5% level in higher concentrations when compared to the control. Larval period was not recorded in

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the 10^{10},10^{9},10^{8} and 10^{7} spores/ml. 10^{6},10^{5},10^{4},10^{3} and 10^{2} spores/ml recorded 12.4,13.4,14.1,15.0 and 15.4days .But in control ,the total larval period was 17.1days.

Pupal emergence was not recorded in all the tested concentration of fungal spores. Adult emergence was not recorded at the spore concentration of 10^{10} to 10^{4}. The percentage of adult emergence was 12.0 and 10.0 at the spore concentration of 10^{3} to 10^{2} and the same concentration revealed 4.1 and 5.0hours of adult longevity. Effect of fungal pathogens on development of insect pests has been reported. Hafez et al.,[18] has studied the effect of *Beauveria.bassiana* on the developmental parameters of potato tuber moth *Phthorimaea operculella* (Seller).

LT 50 and LC 50

All the tested concentration was capable of infecting *S. litura* larvae. Among the different concentration tested, high concentrationwas found to be significant over the least concentration ($P<0.05$, $P=0.006$). The LT_{50} increased as the larvae grew older as well as with the increase in the concentration of the spore used. As the instar advanced, a decrease in mortality and an increase in time for initial mortality were recorded. Furthermore, the first and second, third instars larvae were highly susceptible to the fungus with 10^{10},10^{9} and 10^{8} spores/ml which recorded the total larval mortality of 100% followed by fourth (93.4%), fifth and sixth instar larvae (86.2 and 74.3%) at the dosage of 10^{10}, 10^{9} and 10^{8} spores/ml. LT_{50} value of 10^{10} for the fourth, fifth and sixth instars was found to be 0.12, 0.91 and 1.54days (Table 3).

A gradual increase in LT 50 was recorded in spore concentration dose and stage of the instar. Similar observation was also recorded in remaining concentration. The results of LC_{50} values determined through probit analysis were presented in Table 4. Among the various estimate of the regression based probit analyses, the chi-square test of the bioassay showed homogeneity of the test population which is a reflection of a good fit of the observed and expected response. From the Table 3 it is very clear that the LC_{50} values of different larval instars of *S. litura* in response to fungal pathogen showed an increased trend in the LC_{50} value when the age of the larva advanced. The present study clearly reveals that all the tested fungi were found infecting life stages of *S.litura* and distinct effect could be observed in higher spore concentration. Field study is now progress to evaluate the fungal activity in groundnut field.

Table 1 Physico chemical parameters of soil samples collected from Chengalpet groundnut field

S. No	Parameters	
1	pH	7.95
2	Electrical conductivity(ms/cm)	0.600
3	Organic matter (%)	2.33
4	Nitrate nitrogen (ppm)	24.9
5	Available phosphorous(ppm)	237.7
6	Potassium exchangeable k(ppm)	93
7	Calcium exchangeable (ppm)	1932
8	Magnesium exchangeable (ppm)	511
9	Sulphur available s as so4 ((ppm)	49.3
10	Sodium exchangeable Na((ppm)	302
11	Zinc available Zn (ppm)	2.15
12	Manganese available Mn (ppm)	4.72
13	Iron available Fe (ppm)	1.36
14	Copper available	1.84

Table 2 *N.rileyi* spore concentration on larval, pupal period, adult emergence (%) and adult longevity (in hours) of *Spodoptera litura*

Dosage Spores/ml	Larval Period	Pupal Period	Pupal Mortality	Adult Emergence	Adult Longevity
10^{10}	-	-	100.0	0.0	-
10^{9}	-	-	100.0	0.0	-
10^{8}	-	-	100.0	0.0	-
10^{7}	-	-	100.0	0.0	-
10^{6}	12.4*	-	100.0	0.0	-
10^{5}	13.4*	-	100.0	0.0	-
10^{4}	14.1*	-	100.0	0.0	-
10^{3}	15.0	-	100.0	12.0	4.1
10^{2}	15.4	-	100.0	10.0	5.0
Control	17.1	9.3±0.7	0.0	100.0	96.7±0.2

*Indicates significant between control with doses at 5% level.

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Table 3 Effect of *N.rileyi* on LT 50 (days) on *S.litura*

Dosage spores/ml	LT 50 (Days)						Mortality (%)					
	I	II	III	IV	V	VI	I	II	III	IV	V	VI
10^{10}				0.12	0.91	1.54	100	100	100	93.4	86.2	74.3
10^{9}				0.13	0.94	1.57	100	100	100	89	82.1	70.1
10^{8}				0.15	0.96	1.58	100	100	100	79.1	70.2	65.2
10^{7}	0.21	0.99	1.62	1.7	1.91	2.01	90.2	87.2	78	69	60.2	55.4
10^{6}	0.34	0.82	1.97	2.12	2.32	2.71	86.1	79	69	59.2	48.1	40.3
10^{5}	0.51	1.21	2.21	2.93	3.21	4.23	78.1	69	54	41.2	30.2	21
10^{4}	0.71	2.02	2.91	3.11	4.03	5.11	69.2	60.1	50	37.2	25.2	11.2
10^{3}	1.11	3.01	3.97	4.11	5.1	6.01	51.2	40.3	28	19	10	1.21
10^{2}	2.11	4.32	5.1	5.5	6.11	7.13	35.4	24	11	3.1	0	0
Control	0	0	0	0	0	0	0	0	0	0	0	0

Table 4 LC50 parameters of S.litura

Instars	regression equation (Y=a+bx)	LC_{50} µg/ml	Variance	chi-square value	95% confidence limit	
					Lower limit	Upper limit
I	–0.44+1.280x	$1.67X10^{2}$	0.4516	1.59	1.35	1.76
II	– 3.48+1.698x	$1.85X10^{2}$ $XX10^{2}X10^{2}$	0.5018	1.61	1.55	1.98
III	– 0.52+0.897x	$1.98X10^{3}$	0.3801	2.92	1.87	2.10
IV	0.16+0.509	$2.45X10^{4}$	1.5362	4.93	2.22	2.95
V	0.26+0.609 x	$7.12X10^{4}$	2.011	5.01	3.10	3.92
VI	0.56+ 0.709	$2.12X10^{5}$	3.0.213	6.12	3.90	4.34

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None.

Conflict of interest

The author declares no conflict of interest.

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