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BIOCONTROL POTENTIAL OF PESTICIDAL METABOLITE PRODUCED BY POTENTIAL FUNGAL BIOPESTICIDE *NOMURAEA RILEYI* (F.) SAMSON AGAINST GROUNDNUT DEFOLIATOR *SPODOPTERA LITURA* (F.) (LEPIDOPTERA : NOCTUIDAE) UNDER MICROPLOT CONDITION

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Abstract

Biocontrol agents based on fungi play a vital role in insect pest management against economic important pests. Among the fungi, *Nomuraea rileyi* (Farlow) Samson is an extensively used in agriculture sector as biopesticidal agents. In the present study, pesticidal activity of metabolite extracted from *N. rileyi* against *Spodoptera litura* (Fab.) (Lepidoptera : Noctuidae) was studied under microplot condition. Pesticidal peptide was isolated from ethyl acetate extract of the culture filtrate of *N. rileyi* and the extract was chromatographically separated using sephadex column chromatography and the isolated metabolite was characterized by Fourier transform infrared spectroscopy (FTIR) and UV visible spectroscopy. FTIR and UV visible spectra data coincided with those expected for the protein The purified protein thus acquired was evaluated at different concentrations against *S.litura*. Pesticidal activity of the isolated peptide revealed all the tested concentration caused distinct effect on the larval instars as dose dependent manner. Microplot evaluation was carried out with pesticidal peptide with the various adjuvants. Among the adjuvants, maximum mortality was recorded against all the tested instars in the metabolite formulated with tween 80. Distinct difference in mortality was not observed in starch powder and charcoal treatment.

Key words : *Nomuraea rileyi, Spodoptera litura*, pesticidal metabolite, microplot.

Introduction

The management of insect pests rarely relies on a single management practice, usually a variety of tactics are integrated that are safe to non-target organisms to maintain pests at economic threshold levels (Varma *et al*., 2013). The goal of the Integrated Pest Management (IPM) is not to eradicate the pests but to control or manage the pest population. Since the availability of pests below the ETL is essential to maintain the natural enemy population the control tactics used in integrated pest management include pest resistance to talent plants and cultural, physical, chemical, mechanical and biological control in a compatible manner. Biological control is an important tactic in IPM system (Padmaja, 2005). Biological control plays a pro-vital role in the pest management programme. In several situations, parasitoids (trichogrammatids, encyolids, tochinids and others), predators [coccinelids lacewings, reduviids, spiders] and entomopathogonic microorganisms (bacteria, fungi, virus, protozoa and nematode) have been used to keep the population of pests below the damaging level (Sharma, 2004).

The development of pest control measures using microorganisms especially entomopathogens has received increasing attention in recent years (Brar *et al*., 2001 and Enkerli *et al*., 2004). Entomopathogens such as bacteria, virus, fungi, protozoa and nematode, which play a major role in the natural regulation of pest population and if properly utilized can be useful augmentative bio-control agents, their relative specificity to target pest groups, safety to non-target beneficial organisms including host plants, animals and their ability to cause epizootics make them alternative candidates in sustainable pest management (Butt *et al*., 2001 and Noris *et al*., 2002).

Several fungal species have potential as microbial insecticides and in some countries they are commercially

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available in formulations that can be applied using conventional spray equipment (Gopalakrishnan and Mohan, 2000). Entomopathogenic fungi are currently attracting attention as potential biological control agents against coleopteran, lepidopteran and acridid pests. Fungal bio agents have been used for controlling pests all over the world such as *Beauveria bassiana, Metarhizium anisopliae, Nomureae rileyi, Verticillium lecani (*Shin *et al*., 2007). Entomopathogenic fungi are known to produce a wide range of metabolites known to affect the insects. The toxins, beauverucin produced by the fungi of the genus *Beauveria* were shown to be cyclodepsipeptides by Onafre *et al.* (2002) and destruxins of *Metarhizium* species were also subsequently found to be cyclodepsipeptide (Richard *et al.*, 1995). Apart from the fungal biomass and their conidia which were conventionally used as entomopathogenic biopesticides to penetrate, grow and kill the insect pests, the metabolites they produce also hold good for the same purpose (Karthick Raja Namasivayam *et al.*, 2014). The utility of such metabolites for pest control has not yet been attempted in the Indian context. Biocontrol potential under microplot and field condition of metabolites produced by the fungal biocontrol agents against insect pests is an important criteria of sustainable ecofriendly agriculture The present study was undertaken to evaluate the biocontrol potential of soil isolate of *Nomuraea rileyi* mediated metabolite against *Spodoptera litura* (Fab.) (Lepidoptera : Noctuidae) under microplot condition.

Materials and Methods

Soil sampling

N. rileyi was isolated from the soil sample obtained from groundnut field, Chengalpet, Kanchipuram district, Tamil Nadu and processed for the isolation of fungi (Asensio *et al.*, 2005). 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 Tamil Nadu, India

Isolation of *N. rileyi*

N. rileyi SSK 7 strain was isolated from the processed soil sample by the modified method of Clark (1997) 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 on CTC agar slant. Fungal morphology was confirmed by lacto phenol blue staining (Humber, 1997).

Inocula preparation

Fungal inoculum was prepared from 7 days old SMYA slant 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 haemocytometer (Germany) and used as the source of inocula.

Extraction of pesticidal metabolites

Sabouraud maltose yeast extract broth (4% maltose, 1% peptone, 0.5% yeast extract and pH 6.0) was selected for the crude extraction of metabolites. SMYB media was prepared in one litre of distilled water with components as mentioned earlier and sterilized by autoclaving. After sterilization, 2.5 mL of respective fungal spore suspensions was inoculated and incubated at 25ºC for 14 days under shaking conditions. After incubation period the cultured broth was filtered through cheese cloth and the collected filtrate was extracted with double the volume of ethyl acetate. The resulting organic layer was evaporated under rotary evaporator and the concentrated extract was separated using Sephadex Column with chloroform, methanol and ethyl acetate at 10:1:1 ratio. The final fraction collected was qualitatively identified by TLC, lyophilized and used for further studies. Characterization of the metabolites thus obtained was characterized by UV visible spectroscopy and Fourier transform infrared spectroscopy (FTIR).

Screening of pesticidal activity against *Spodoptera litura*

Pesticidal activity of the metabolites was studied against 2nd and 3rd instars larvae of *S.litura* under *in vitro* condition. Lyophilized formulation of pesticidal metabolite was reconstituted in millipore water at the concentrations of 1.0, 0.1, 0.01 and 0.001 mg/ml and the pesticidal activity was carried out by cumulative mortality, lethal time 50 (LT50), lethal concentration 50 LC_{50} (Blever and Hostetter, 1971 and Finney, 1966).

Laboratory bio-assay on *Spodoptera litura*

The 2nd and 3rd instars larvae of S.litura selected for bio-assay studies. 20 larvae in each instars (2nd and 3rd) were sprayed with respective concentration using ULV (Ultra Low Volume) sprayer. The treated larvae were introduced into the plastic container (34mm × 21mm) 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

Fig. 1a : *N. rileyi* on CTC media.

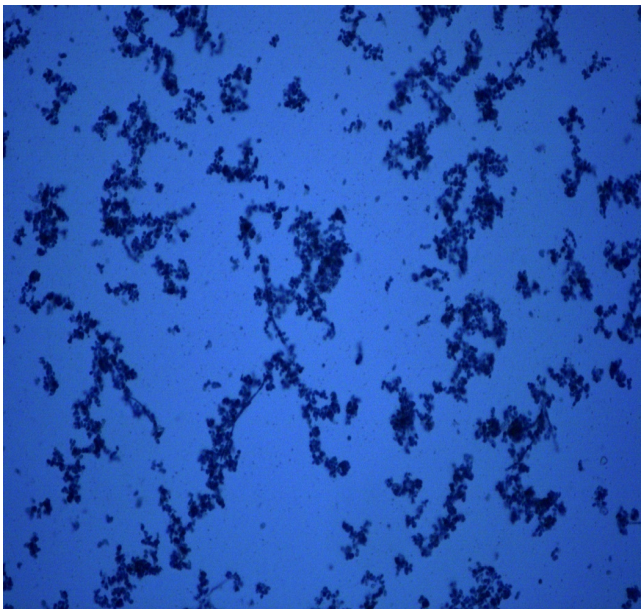

Fig. 1b : Microscopic examination of *N. rileyi* spore by lactophenol otton blue.

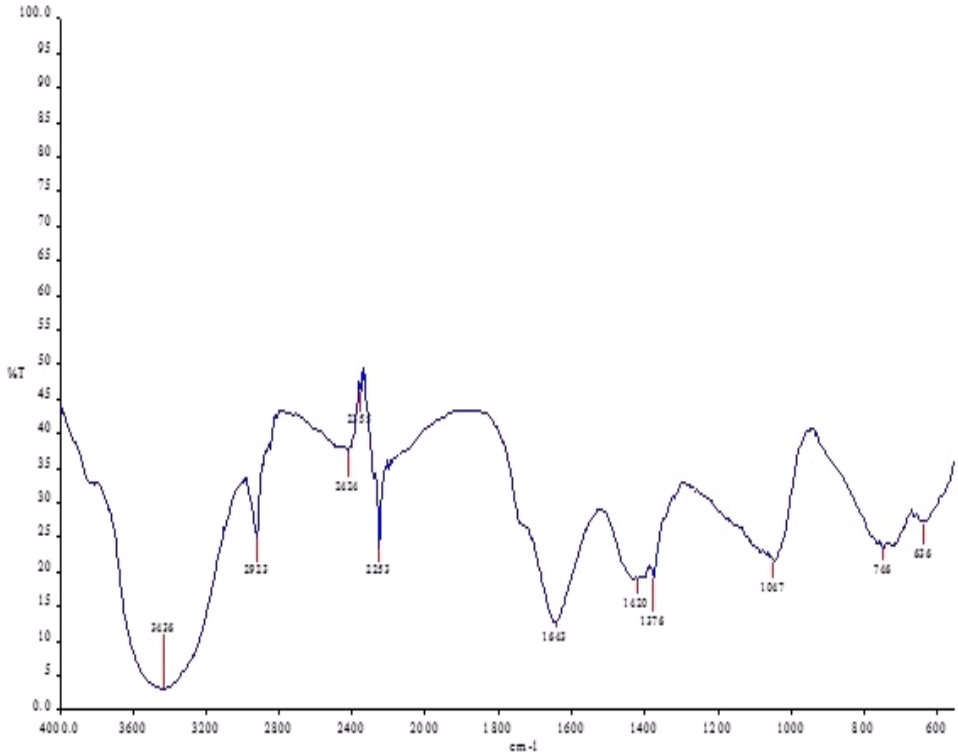

Fig. 3 : FTIR spectra of isolated peptide.

larvae of each instars 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. Lethal time 50 (LT50) and lethal concentration 50 (LC50) was also studied.

Formulation

Formulation was carried out using starch, tween 20 and charcoal. Lyophilized metabolite (1 mg/ml final concentration) and respective formulating agent (0.1% concentration) were suspended in 100 ml of deionized water, kept under magnetic stirrer at room temperature for three hours. Homogenous slurry thus obtained was

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 : Mortality of *Spodoptera litura* larval instars treated metabolites.

S. no.	Concentration (µg/ml)	Mortality (%)	
		2nd instar	3rd instar
1.	0.001	21.5	7.0
2.	0.01	43.0	21.0
3.	0.1	100.0 ab	54.0
4.	1.0	100.0 ab	76.0 ab
5.	Control	0.0	0.0

In column, mean values carries alphabets is statistically significant at 0.5% level by DMRT.

lyophilized and used for microplot assay.

Microplot assay

Microplot trail was carried out in Sathyabama University, Chennai, Tamil Nadu during June to August 2014. The trail was laid out with 6 treatments (T_1 to T_6 including untreated control having three replication each. The plot size of 7 × 4 m leaving a gangway of one meter all around the plot has been maintained for this experiment. The groundnut cultivar TMV-7 was sown during 1st week of June 2014 at a spacing of 30 × 10 cm. After 20 DASE, egg masses of *S.litura* were introduced. After 10 days of inoculation, the treatments were carried out with knapsack sprayer with *N.rileyi* spores (X1 × 10^8 spores/ ml), lyophilized metabolite alone and metabolite with respective formulating agent (1 mg/ml concentration). To compare the efficacy of treatments, untreated check (control) and cypermethrin treatment (standard) were maintained. Pre treatment count of populations. *S. litura*

Table 3 : Effect of metabolites on LT 50 of *Spodoptera litura*.

S. no.	Concentration (µg/ml)	LT 50 (Days)	
		2nd instar	3rd instar
1.	1.0	1.01	2.35
2.	0.1	2.31	4.01
3.	0.01	4.21 ab	6.72 ab
4.	0.001	6.21 ab	8.12 ab
5.	Control	0.0	0.0

In column, mean values carries alphabets is statistically significant at 0.5% level by DMRT.

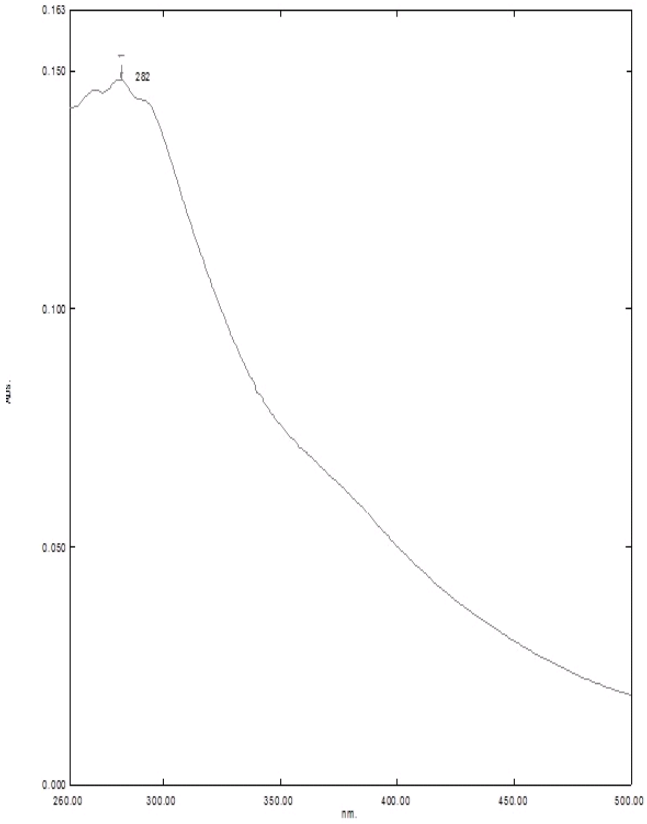

Fig. 4 : UV visible spectra of isolated peptide.

were made 2 days prior to the application of fungi and subsequently larval population was observed 5 days after spraying of respective formulation.

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 (Fig. 1a) and the microscopic examination of fungal spore by lactophenol cotton blue showed spherical conidia (Fig. 1b). Soil physico-chemical parameters highly influenced the natural occurrence of *Nomurea rileyi*.

Table 4 : Effect of metabolites on LC_{50} against *Spodoptera litura* 2nd and 3 d instar larvae.

LC_{50} (mg)	95% Confidence limit		LC_{90} (mg)	95% Confidence limit		Chi square value
	Lower	Upper		Lower	Upper	
71.01 (2 nd instar)	31.12	184.59	423.5	228.90	638.54	0.036*
236.9(3 d instar)	2273.39	25468.00	1247.8	944.65	3212.00	0.810*

LC_{50} and LC_{90} values are expressed as percentage (n=24). * χ^2 values are significant at $P \leq 0.05$ levels.

Table 5 : Effect of formulation of pesticidal peptide on biocontrol potential against S.litura under miroplot condition.

Treatment	Instars	Mortality (%) at different time period (Days)			
		5	10	15	20
Control	I	0.0	0.0	0.0	0.0
Cypermethrin		90.0	100.0	100.0	100.0
Pesticidal peptide		16.0	39.0	67.0	80.7 ab
Pesticidal peptide + Starch		11.0	34.0	60.0	0.0
Pesticidal peptide + tween 80		23.0	45.0	76.0 ab	92.0ab
Pesticidal peptide + charcoal		7.0	15.0	0.0	0.0
Control	II	0.0	0.0	0.0	0.0
Cypermethrin		90.0	100.0		
Pesticidal peptide		13.0	30.0	61.0	70.7ab
Pesticidal peptide + Starch		10.0	31.0	58.0	0.0
Pesticidal peptide + tween 80		21.4	41.2	73.4 ab	89.0ab
Pesticidal peptide + charcoal		6.0	12.0	0.0	0.0
Control	III	0.0	0.0	0.0	0.0
Cypermethrin		90.0	100.0		
Pesticidal peptide		14.0	25.0	47.0	56.0
Pesticidal peptide + Starch		10.2	28.0	51.2	0.0
Pesticidal peptide + tween 80		17.0	31.5	60.0	71.3 ab
Pesticidal peptide + charcoal		0.0	0.0	0.0	0.0
Control	IV	0.0	0.0	0.0	
Cypermethrin		85.0	94.0	100.0	
Pesticidal peptide		7.0	21.0	47.0	53.0
Pesticidal peptide + Starch		3.0	12.0	23.0	0.0
Pesticidal peptide + tween 80		15.0	29.0	58.4	67.0ab
Pesticidal peptide + charcoal		0.0	0.0	0.0	0.0
Control	V	0.0	0.0	0.0	0.0
Cypermethrin		62.0	74.0	89.0	0.0
Pesticidal peptide		4.0	18.0	37.0	43.0
Pesticidal peptide + Starch		3.0	12.0	23.0	0.0
Pesticidal peptide + tween 80		12.0	23.4	51.2	60.2 ab
Pesticidal peptide + charcoal		0.0	0.0	0.0	0.0
Control	VI	0.0	0.0	0.0	0.0
Cypermethrin		57.0	64.0	74.0	0.0
Pesticidal peptide		4.0	14.0	29.0	34.0
Pesticidal peptide + Starch		3.0	9.0	12.0	0.0
Pesticidal peptide + tween 80		8.0	19.0	32.0	51.2 ab
Pesticidal peptide + charcoal		0.0	0.0	0.0	0.0

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*. The principle responsible for the pesticidal activity of the culture free filtrate of *N. rileyi* grown in SMYB media was readily extracted into organic solvents ethyl acetate at pH 4 to 5. Extract thus obtained was concentrated, partially purified by sephadex column and the collected fractions were lyophilized and stored at -2^{0}C. Characterization of the metabolite was primarily carried out by TLC, which showed a positive ninhidrin reaction and soluble in methanol, chloroform and water.

FTIR and UV visible spectra data coincided with those expected for the protein (figs. 2 and 3).

Pesticidal activity was studied against second and third instars larvae of *S. litura*. Both the instars were susceptible to metabolite treatment as dose dependent manner (table 2). In the case of second instars, complete mortality was recorded in 1.0 and 0.1 mg/ml concentration. 43.0 and 21.5 mortality was recorded in 0.01 and 0.001mg/ml concentration 76.0,54.0,21.0 and 7.0% of mortality was observed in third instars at the respective concentration. The LT_{50} increased as the larvae grow older as well as the increase in the concentration. As the instars advanced, a increased in time was recorded (table 3). In the case of 2nd instar, LT_{50} was found to be 1.01, 2.31, 4.21 and 6.21 days at 1.0, 0.1, 0.01 and 0.001mg/ml concentration. Similarly, dose dependent variation in mortality was also recorded in 3d instar, which revealed 2.35, 4.01, 6.72 and 8.12 days. Influence of dosage of plant and microbial based metabolites has been reported (Karthick Raja Namasivayam *et al.* (2014), Karthick Raja Namasivayam and Arvind Bharani (2014). The result of LC_{50} value determination through probity analysis was presented in table. Among the various estimate of regression based probity analysis, 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, it is very clear that the LC_{50} values of different larval instar of *Spodoptera litura* in response to the metabolites shown an increased trend in the LC_{50} value, when the age of larva advanced concentration. The medium lethal concentration of 2nd and 3rd instar of *Spodoptera litura* was 423.5mg and 1247..8 mg in 3rd instars (table 4).

Microplot study was carried out using different formulation of metabolites as described above. Significant difference was observed ($P < 0.05$, $P = 0.006$) in mortality rate in the respective formulation (table 5). Distinct reduction in *S. litura* population could be observed in metabolite with tween 80. In general, mortality rate was found to be maximum in early instars. Enhanced reduction of pest population was observed in fungal metabolites with tween 80. 23.0, 45.0, 76.0 and 92.0%, 21.4, 41.2, 73.4 and 89.4% of mortality was recorded in first, second instar of *S. litura* at 5,10,15 and 20 after treatment. Third and fourth instars larval reduction showed 17.0, 31.5, 60.0, 71.3%, 15.0, 29.0, 58.4 and 67.0% of mortality at 5, 10, 15 and 20 after treatment. 12.0, 23.4, 51.2, 60.2% and 8.0, 19.0, 32.0, 51.2% of mortality was reported in the case of fifth and sixth instars. Charcoal and starch formulation did not cause any distinct effect on biocontrol potential. Formulation of the biocontrol agents and their metabolites with materials act as wetting agents or spreaders. Spreaders or wetting agents are added to the water diluent to ensure "wetting" of the surface to the sprayed. Many material have been used as wetting agents which including dried milk, powdered casein, gelatin, saponins, oils, soaps etc. In the present study, pesticidal metabolite with tween 80 caused enhanced pest reduction may be due to the complete the emulsifying activity of tween 80 and thus dispersion of the metabolites on the leaf surface, which in turn caused prolong exposure of the metabolite to *S. litura*. Metabolite with starch and charcoal did not cause any effect in pest reduction. Formulation of fungal biocontrol agents against major pests reported by Vimala Devi *et al.* (2000), Sahayaraj and Karthick Raja Namasivayam (2007), Karthick Raja Namasivayam *et al.* (2014). Further study will helpful to use the formulated pesticidal metabolite of *N. rileyi* for the control *S. litura.*

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