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Pathogenicity of *Nomuraea rileyi* (Farlow) Samson isolates against *Spodoptera litura* (Fabricius)

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ABSTRACT: Investigations were carried out to assess the pathogenicity of some isolates of the entomopathogenic fungus, *Nomuraea rileyi,* against different instars of *Spodoptera litura* (F.) under *in vitro* conditions. Bioassays on *S. litura* with *N. rileyi* isolates revealed that PDBC isolate was most virulent against *S. litura* and also had lower LC_{50} and LT_{50} values than DOR and local isolates of *N. rileyi,* indicating that it was more effective than DOR and local isolates against *S. litura*.

KEY WORDS: *Nomuraea rileyi,* pathogenicity, *Spodoptera litura.*

INTRODUCTION

Tobacco caterpillar, *Spodoptera litura* (F.), is one of the most destructive pests of various crops and is more or less of universal occurrence except in regions where extremes of climate prevail. It has been reported to feed on 112 cultivated food plants all over the world (Mousa *et al*., 1980), of which 40 are grown in India (Basu, 1981; Muthukrishnan *et al.,* 2005) including tobacco, tomato, cotton, chillies, okra, cauliflower, castor, groundnut, soybean, maize and black gram. Control of *S. litura* using insecticides has become difficult because of the development of resistance. Biological control of insect pests is one of the most important components of integrated pest management (IPM), wherein entomopathogens are exploited against pests. Several pathogens like nuclear polyhedrosis virus, *Beauveria*, etc. (Pandey and Kanujia, 2005) have been isolated from *S. litura* and found to be effective. The green muscardine fungus, *Nomuraea rileyi* (Farlow) Samson is a deuteromycetous fungus of cosmopolitan nature. *N. rileyi* infects mainly Lepidoptera, particularly economically important and polyphagous noctuid pests. Progress of research on *N. rileyi* in India is slow though the results of a few studies have revealed *N. rileyi* as a potential mycoinsecticide (Vimala Devi *et al*., 2002). Hence, the present study was taken up to evaluate the pathogenicity of *N. rileyi* isolates against different instars of *S. litura* under *in vitro* conditions.

MATERIALS AND METHODS

Sources of *N. rileyi*

Pure cultures of *N. rileyi* were obtained from Project Directorate of Biological Control (PDBC), Bangalore, and Directorate of Oilseeds Research (DOR), Hyderabad. Surveys were also conducted in cotton, tomato, castor and pulse cropping areas of Coimbatore District (Thondamuthur) and *N. rileyi* infected cadavers were collected and maintained as local isolates. The three fungal isolates of *N. rileyi* (PDBC, DOR and LOCAL) were maintained at the Department of Agricultural Entomology, Agricultural College and Research Institute, Madurai, Tamil Nadu.

Isolation from infected cadavers

Sabouraud's Maltose Agar medium supplemented with 1% Yeast extract (SMAY) medium was used to isolate the fungus. Conidia of *N. rileyi* formed on the cadavers were taken by a mycological loop and streaked on SMAY medium. After incubation at room temperature (25 ± 1ºC) for a week, the colonies obtained were subcultured on SMAY slants for preservation. The isolates were identified by microscopic observation of the conidia forming mycelia for conidiogenous structure and conidial morphology (Samson *et al*., 1988; Aoki, 1989). *N. rileyi* isolates were refrigerated at 4ºC.

Mass culturing of *S. litura*

Mass culturing of S. *litura* was carried out with a laboratory stock which was supplemented with field collected larvae from castor periodically. Mass rearing was done according to the methodology of Britto (1980). The egg masses of *S. litura* were collected from the field and the larvae were maintained on castor leaves kept fresh in a conical flask containing water. The whole set up was kept in a plastic bucket (10 litres capacity) and the mouth was secured with cloth. Fresh castor leaves were provided

every day as larval feed. Adequate care was taken to avoid disease incidence. During pre-pupal stage, the larvae were transferred to a container provided with sawdust and placed in an adult emergence cage. The emerging adults were used for the maintenance of subsequent cultures. Honey mixed with Vitamin E (Tocopherol acetate) was kept inside the cage as food for adults. Nerium leaves inserted in conical flaks containing water were kept inside the cage for oviposition by adults.

Pathogenicity of *N. rileyi* against *S. litura*

For the pathogenicity test, castor leaves treated with conidial suspensions ($2x10^{10}$ to $2x10^{5}$) were provided to starved larvae of different instars (I–VI) for each concentration. Five replications were maintained with 10 larvae per replication. Untreated control was maintained simultaneously with 0.02% Tween 80 in sterile distilled water. The larvae were carefully transferred and reared in the containers by providing fresh food daily. The larval mortality was recorded at an interval of 24 hours until pupation / death. This study was conducted at a room temperature of 26ºC ± 2ºC and RH of 91–95%.

RESULTS AND DISCUSSION

Nomuraea rileyi is a cosmopolitan species and reported to be pathogenic to several economically important lepidopterous insect pests. In the present investigation, *N. rileyi* isolates showed variation in their pathogenicity against *S. litura.* Among the three isolates, PDBC isolate showed higher pathogenicity (83.40 %) against first instar larvae of *S. litura* followed by DOR isolate (80.00%) and local isolate (73.40%). In the case of second instar larvae of *S. litura,* the highest pathogenicity was observed to PDBC isolate (76.60%) followed by DOR and local isolates (70.60%), which were significantly on par in their efficacy. Similarly, PDBC isolate showed higher pathogenicity (70.60 %) against third instar larvae of *S. litura* and the least mortality was observed in local isolate (63.40%) (Table 1, Fig.1).

The data on dose-mortality and time-mortality response of *S. litura* to *N. rileyi* isolates showed significant differences in the LC_{50} and LT_{50} values. LC_{50} values were 3.18-15.00 x 10^7 spores ml^{-1} for PDBC isolate, 3.97-16.68 x 10^7 spores ml^{-1} for DOR isolate and 4.50–18.27 x 10^7 spores ml for local isolate, respectively (Table 2). In all the isolates, lower LC_{50} doses caused 50% mortality of first instar larvae. LC_{50} values on first instar larvae of *S. litura* were 3.18 x 10^7, 3.97 x 10^7 and 4.50 x 10^7 spores ml^{-1} for PDBC, DOR and local isolates, respectively. However, LC_{50} levels of second instar larvae were found to be higher for PDBC isolate (5.15 x 10^7 spores ml^{-1}), DOR isolate (5.89 x 10^7 spores ml^{-1}) and local isolate (5.68 x 10^7 spores ml^{-1}). The doses causing 50% mortality on third instar larvae were much higher at 15.00 x 10^7, 16.68 x 10^7 and 18.27 x 10^7 spores ml^{-1} for PDBC, DOR and local isolates, respectively (Table 2).

Table 1. Pathogenicity of *N. rileyi* isolates against *S. litura* under *in vitro* conditions

N. rileyi	Pathogenicity of *S. litura* (% mortality)		
	I instar	II instar	III instar
PDBC isolate	83.40[a] (58.90)	76.60[a] (57.20)	70.60[a] (54.73)
DOR isolate	80.00[b] (56.99)	70.60[b] (56.79)	66.60[b] (52.73)
Local isolate	73.40[c] (56.79)	70.60[b] (54.73)	63.40[c] (52.73)
CD (P = 0.05)	0.0117	0.0111	0.1372
SEd	0.0479	0.0454	0.0561

Values are means of five replications and figures in parentheses represent arcsine transformations; means in a column followed by the same superscript letters are not significantly different according to Duncan's multiple range test at P = 0.05.

Time–mortality response analysis revealed that the LT_{50} was 61.41 h for PDBC isolate, 62.95 h for DOR isolate and 64.52 h for local isolate against first instar larvae of *S. litura* (Table 3). In case of second instar larvae, the LT_{50} values were 74.43 h, 81.77 h and 85.72 h for PDBC, DOR and local isolates, respectively. On third instar larvae, the LT_{50} was 103.84 h for PDBC isolate, 106.86 h for DOR isolate and 110.9 h for local isolate. Marginal increase in the LT_{50} values was noted against second and third instar larvae of *S. litura.*

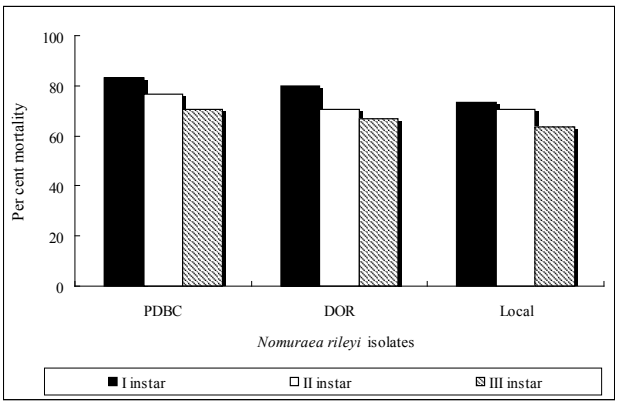

Fig. 1. Pathogenicity of *Nomuraea rileyi* isolates against *Spodoptera litura*

Table 2. Dose-mortality response of *S. litura* to *N. rileyi* isolates

N. rileyi	Characteristics	Dose-mortality response of *S. litura* larvae		
		I instar	II instar	III instar
PDBC isolate	LC_{50} (Spore^{-ml})	3.18 x 10^7	5.15 x 10^7	15.00 x 10^7
	χ^2 (n-2) **	0.899	0.771	0.604
	Slope‘ b’ ± SE	0.82 ± 0.37	0.921 ± 0.36	0.931 ± 0.36
	Fiducial limit	0.001 – 7.20	0.11 – 9.27	7.06 – 31.56
DOR isolate	LC_{50} (Spore^{-ml})	3.97 x 10^7	5.89 x 10^7	16.68 x 10^7
	χ^2 (n-2) **	0.597	0.504	0.426
	Slope ‘ b’ ± SE	0.85 ± 0.37	1.02 ± 0.37	1.03 ± 0.37
	Fiducial limit	0.001 – 8.11	0.59 – 9.80	9.3 – 38.86
Local isolate	LC_{50} (Spore^{-ml})	4.50 x 10^7	5.68 x 10^7	18.27 x 10^7
	χ^2 (n-2) **	0.246	0.218	0.191
	Slope ‘ b’ ± SE	0.81 ± 0.37	0.835 ± 0.37	0.843 ± 0.37
	Fiducial limit	0.006 – 8.94	0.009 – 10.57	19.61 – 96.40

** No. of larvae per treatment = 50; *** All lines significantly good fits (P<0.05).

Only limited reports are available on the use of *N. rileyi* on *S. litura*. In the present study, *N. rileyi* was found to be highly infective to early instars of *S. litura* than later instars. The present finding is in conformity with Manjula and Krishna Murthy (2005) who reported that the highest larval mortality of 91.2 per cent was obtained in the first instar of *S. litura* and 95 per cent in the second instar of *H. armigera* with the highest concentration of 1 x 10^9 of *N. rileyi* spores ml^{-1}.

Vimala Devi and Prasad (1994) conducted field studies and found that *N. rileyi* was effective against *S. litura* as foliar spray and soil application. Likewise, Sridhar and Prasad (1996) recorded up to 36.9 per cent infection of *N. rileyi* on *S. litura* in groundnut fields in Andhra Pradesh. *N. rileyi* was a key natural mortality factor of *S. litura* populations in coastal Andhra Pradesh (Sridhar and Prasad, 1996). Vimala Devi *et al*. (2002) reported larval mortality when *S. litura* was reared on castor leaves inoculated with 2 x 10^8 spores ml^{-1}.

Navi *et al.* (2006) indicated that *N. rileyi* caused higher mortality of *S. litura* under field conditions. In India, it is frequently observed in tomato, cabbage, field bean, banana

Table 3. Time-mortality response of *S. litura* to *N. rileyi* isolates

N. rileyi	Characteristics	Time-mortality response of *S. litura* larvae		
		I instar	II instar	III instar
PDBC isolate	LT_{50}	61.41 h	74.43 h	103.84 h
	χ^2 (n-2) **	1.17	1.48	3.46
	Slope‘ b’ ± SE	1.62 ± 0.43	1.39 ± 0.43	1.16 ± 0.43
	Fiducial limit	44.42 – 88.60	57.79 – 154.99	76.82 – 199.78
DOR isolate	LT_{50}	62.95 h	81.77 h	106.86 h
	χ^2 (n-2) **	0.708	1.631	3.985
	Slope ‘ b’ ± SE	1.64 ± 0.43	1.45 ± 0.43	1.13 ± 0.43
	Fiducial limit	43.13 – 85.34	52.62 – 123.53	78.27 – 217.33
Local isolate	LT_{50}	64.52 h	85.72 h	110.9 h
	χ^2 (n-2) **	1.06	3.488	4.26
	Slope ‘ b’ ± SE	1.74 ± 0.44	1.44 ± 0.44	1.09 ± 0.44
	Fiducial limit	46.96 – 89.37	61.65 – 162.62	79.97 – 147.99

** No. of larvae per treatment = 50; *** All lines significantly good fits (P<0.05).

and pigeon pea ecosystems as a natural epizootic on *Helicoverpa armigera*, *S. litura* and *Tricoplusia ni* (Gopalakrishnan and Mohan, 1997).

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