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Pathogenicity of a Native Isolate of *Nomuraea rileyi* (Farlow) Samson Against Tobacco Caterpillar, *Spodoptera litura* (Fabricius)

R Dhanapal, D V Sai Ram Kumar, C Sandhya Rani, V Manoj Kumar and R Lakshmipathy
Department of Entomology, Agricultural College, Bapatla 522 101, A P

ABSTRACT

The median lethal concentration (LC_{50}) and time (LT_{50}) of *Nomuraea rileyi* (Farlow) Samson were determined against the second instar larvae of *Spodoptera litura* (Fab.) by dipping the larvae in fungal spore suspension concentrations varying from 1×10^4, 1×10^5, 1×10^6, 1×10^7 and 1×10^8 spores ml^{-1}. The LC_{50} recorded with *N. rileyi* against the second instar larvae of *S. litura* was recorded as 5.55×10^4 spores ml^{-1} whereas LT_{50} value was 190.09 hours post infection.

Key words : LC_{50}, LT_{50}, Native isolate, *Nomuraea rileyi, Spodoptera litura.*

Tobacco caterpillar, *Spodoptera litura* (F.), is one of the most destructive insect 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) including tobacco, tomato, cotton, chillies, okra, cauliflower, castor, groundnut, soybean, maize and blackgram. The control of *S. litura* using insecticides has become difficult due to the development of resistance. Biological control of insect pests is one of the most important components of integrated pest management (IPM), wherein entomopathogens play a pivotal role in suppression of pests. Several pathogens like nucleo polyhedro virus, *Beauveria* spp, etc. (Pandey and Kanujia, 2005) have been isolated from *S. litura* and found to be highly effective. *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 that *N. rileyi* has a lot of potential as a mycoinsecticide (Vimala Devi *et al*., 2002). Hence, the present study was taken up to evaluate the pathogenicity of a local isolate of a *N. rileyi* lawae against second instar of *S. litura* under laboratory conditions.

MATERIAL AND METHODS

Local isolate of *N. rileyi* was isolated from soil sample of Coastal Andhra Pradesh. The fungus was isolated from soil by using *Galleria* bait method. The infected *Galleria* larval cadaver was removed from soil. The infected cadavers were surface sterilized with 2% sodium hypochloride (NaOCl) solution for two minutes and serially washed with sterile distilled water for three times before placing on a sterilized blotter paper for removing residual water. The surface sterilized cadavers were then transferred to Potato Dextrose Agar (PDA) medium. The fungus was purified by single hyphal tip method (Rangaswami, 1972). The pure culture of entomopathogenic fungus was maintained on PDA slants at 4^0C in a refrigerator throughout the study period.

The spores were harvested aseptically using 10 ml of sterile water fortified with 0.02 % Tween 80 with the help of camel hair brush. After thorough shaking, the suspension was filtered through double layered muslin cloth to filter the conidia and the suspension was used for bioassay. The number of spores in the initial suspension was counted by using improved Neubauer Haemocytometer under binocular research microscope at 40X. This served as stock solution and different working concentrations were prepared from the stock solution by serial dilution method (Sabita, 2005).

Table 1. Mean Per cent Mortality of *S. litura* by *Nomuraea rileyi* isolate (N-1).

Treat ments	No of insects released	Per cent mortality	LC_{50} (spores ml^{-1})	LT_{50}(hpi) at 1×10^8 spores ml^{-1}	Heterogeneity ($\chi2$)		Fiducial limits	
					LC_{50}	LT_{50}	LC_{50}	LT_{50}
1×10^4	30	38.89						
1×10^5	30	54.44	5.55×10^4	190.09	0.715	18.879	8.12×10^3– 3.79×10^5	173.15-208.52
1×10^6	30	65.56						
1×10^7	30	73.33						
1×10^8	30	83.33						
Control	30	3.33						

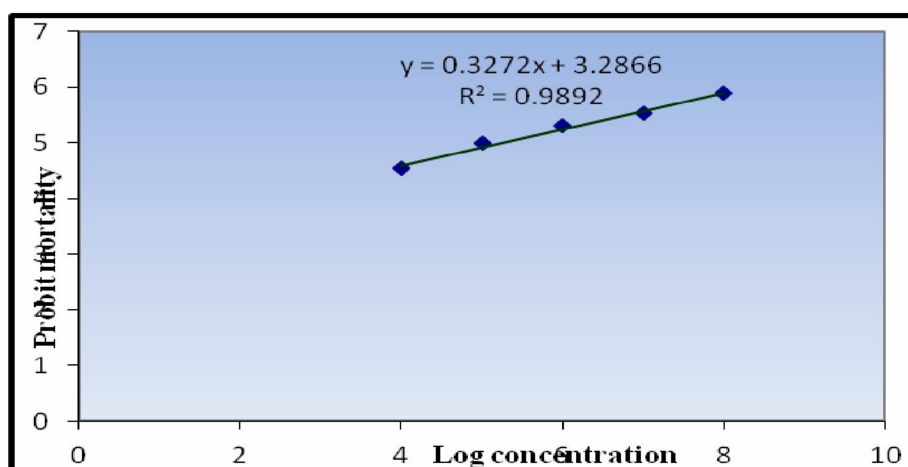

Fig. 1. Spore concentration mortality response of *Spodoptera litura* to *Nomuraea rileyi* (N-1).

The egg masses of tobacco caterpillar, *S. litura* collected from the field were initially surface sterilized with 2% NaOCl and were kept for hatching between two young tender castor leaves in a glass jar (25 cm height and 15 cm diameter). The open ends of the jars were closed with white muslin cloth. Every day, the hatched larvae were changed to new containers with fresh leaves and the used containers were cleaned and sterilized regularly. As the larvae grew larger, the number of larvae per glass jar (15 x 15 cm) was limited to 20 to 25 to provide sufficient spacing. The grown up larvae were fed twice a day with fresh and clean castor leaves. After three to four days of pupation, pupae were taken out and kept in an oviposition cage lined inside with black paper. The emerged adults were transferred to fresh cage with butter paper placed along the inner walls and they were fed with 4% honey solution fortified with Vitamin-E through a cotton swab.

The second instar larvae of *S. litura* were immersed individually for 30 seconds in the different concentrations (1×10^4, 1×10^5, 1×10^6, 1×10^7, 1×10^8 spores ml^{-1}) of fungal spore suspensions of isolated entomopathogenic fungus prepared by serial dilution method (Asi *et al.*, 2013). In control, the larvae were treated only with 0.02% Tween 80 which was mixed with water. Treated larvae were allowed to crawl freely on tissue paper in a petri dish to remove excess moisture. After air drying, the treated larvae were fed with fresh and clean castor leaves in the laboratory (27 ± 2^{0}C and 60 to 70% RH). Leaves were washed with water twice and air dried for 15 minutes. Every day, the moribund larvae were removed from the Petri plates. Thirty larvae per treatment with three replications were maintained in each experiment. The data on larval mortalities were recorded daily until 14 Days After Treatment (DAT). Mortality was recorded in the control category also for Abbott's correction.

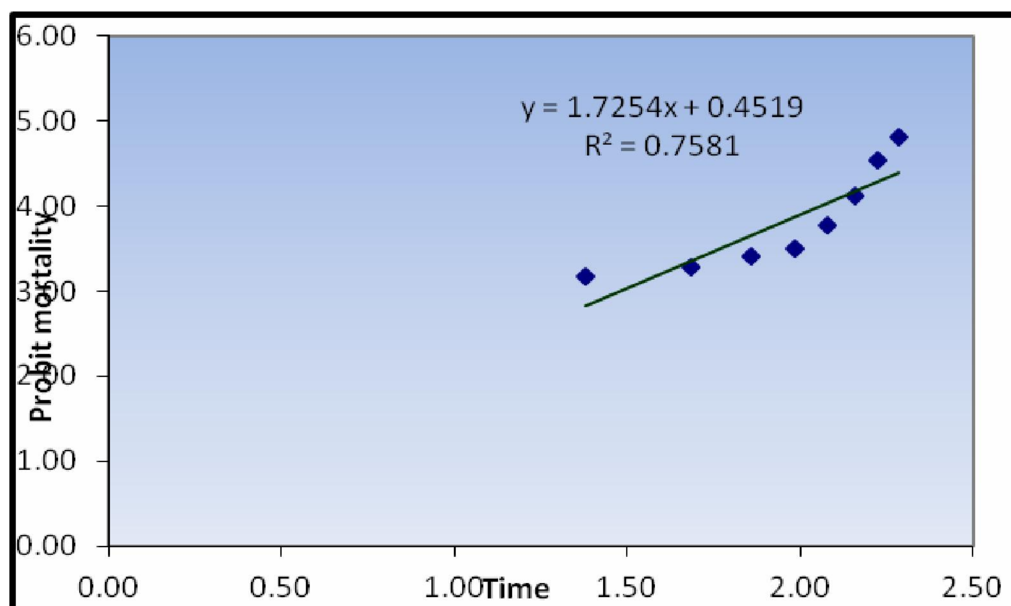

Fig. 2 Time mortality response of *Spodoptera litura* to *Nomuraea rileyi* (N-1)

RESULTS AND DISCUSSION

The median lethal concentrations (LC_{50}) and median lethal times (LT_{50}) of entomopathogenic fungus were determined with reference to 50 per cent mortality of the second instar *S. litura* larvae. Second instar larvae of *S. litura* were susceptible to all entomopathogenic fungal isolates used in the bioassay in a dose dependent manner. Statistical analysis was done by using probit analysis (Finney, 1984).

In the bioassay study, *N. rileyi* had shown its median lethal concentration for 2nd instar of *S. litura* at 5.55×10^4 spores ml^{-1}. The LT_{50} value for *N. rileyi* against 2nd instar of *S.litura* were calculated for a higher dose of 1×10^8 spores ml^{-1}. The LT_{50} value was observed as 190.09 hours post infection (hpi). The values of X^2, per cent mortality and LC_{50} and LT_{50} values of *N. rileyi* were presented in the Table 1, Fig. 1 and Fig.2.

The test for the goodness of fit indicated that line was significantly a good fit @ $P \leq 0.05$. For bioassay, the corrected mortality was transferred to probit unit (y) then regressed against the log10-transformed spore concentrations (x), yielding a well fitted linear relationship, which indicated that the tested fungal pathogen caused the increased mortality of *S. litura* with increase in concentration and time.

In the present study, larval dipping method was used for bioassay of the entomopathogenic fungi against *S. litura*. Since the entomopathogenic fungi act by contact mode of action, the larvae when dipped in the fungal solutions, spores adhere to the cuticle of larvae and thus exposed to different treatments. Several researchers used the same method for assessing the pathogenicity of entomopathogenic fungi (Asi *et al.,* 2013, Kaur *et al.,* 2011, Elizabeth Roy *et al.,* 2008).

The larval mortality was observed from 4th day onwards which was supported by Pokhrel *et al.* (2014) in which during the first four days of treatment, there was no death of silkworm larvae due to the *M. anisopliae* in either of the treatments. Similar results have been earlier reported in different insects (Steinhaus, 1949). In the present experimentation, 1×10^8 concentration gave 83.33 per cent mortality. Tang and Hou (2001) found that the infection of *H. armigera* by *N. rileyi* resulted in approximately 95 % mortality of 4th stage larvae, and LT_{50} of only 5.8 days, at the inoculum concentration of 5 x 10^6. LC50 was between 10^5 and 10^6 spore ml^{-1} for the native isolates of *N. rileyi* such as, ARSEF 7794, ARSEF 7793 and ARSEF 7791 while, it was more than 10^6 for the isolate ARSEF 7792 (Iqtiat *et al.*, 2009). In case of fungal pathogenesis, dose responsive mortalities were also documented earlier by Steinhaus (1949). Anand and Tiwary (2009) also observed the highest morality of 2nd instar larvae of *S. litura* at the highest conidial concentration of fungal isolates. Median

lethal time (LT_{50}) of *S. littoralis* prolonged with decrease in concentrations of fungi, *M. anisopliae* (Abou-Bakar, 1997).

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