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NATURAL INCIDENCE OF *NOMURAEA RILEYI*, AN ENTOMOPATHOGENIC FUNGUS ON *SPODOPTERA LITURA* INFESTING GROUNDNUT IN EASTERN REGION OF INDIA

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ABSTRACT
An entomopathogenic fungus, *Nomuraea rileyi* on *Spodoptera litura* larvae infesting groundnut was observed during 32-37th Standard Meteorological Week (SMW) of 2014 in Eastern Region of India on 2nd and 3rd instar larvae of *Spodoptera*. Maximum larval mortality (93.67%) was observed on 37th SMW and high larval mortality positively relates to rainfall and relative humidity. Pathogenicity of *N. rileyi* (3.4X 10^{-9}cfu) on 2nd and 3rd larval instars revealed high mortality within 3-4 days and visible fungal growth were observed on 5th and 6th day of treatment. *N. rileyi* can be easily isolated and could be incorporated in biological management modules for wide area management of Lepidopteran insect-pests especially against the second and third instars larvae of *S. litura*

INTRODUCTION

Indiscriminate use of synthetic/chemical pesticides causes deleterious effects on flora and fauna *viz.*, earthworms, predatory mites, centipedes and predatory beetles as well as soil microenvironment. To tackle these problems, thousands of researches are in search of ecofriendly and novel entomopathogenic fungi and bacteria for the management of harmful insect pests. Entomopathogenic fungi are widely distributed in the tropical and subtropical agro ecosystem especially in forest, agricultural, pasture, desert and urban areas (Sánchez-Peña 1990). They have the potential ability to regulate insect-pest populations in tropical and temperate habitats (Meyling and Eilenberg 2007, Choudhary *et al.*, 2014). Soil is considered as an excellent environmental shelter for entomopathogenic fungi because it is protected from UV radiation and other abiotic and biotic stresses. Weather parameters *viz.*, temperature, humidity and rainfall play a crucial role in the distribution, prevalence and antagonistic efficacy of entomopathogenic fungi (Maurya *et al.*, 2013, Choudhary *et al.*, 2012). It is also reported that the weather parameters temperature, humidity and rainfall directly influence the growth and development in *Vigna radiata*. Several entomopathogenic fungal genera *viz.*, *Beauveria*, *Conidiobolus*, *Metarhizium* and *Isaria* (*Paecilomyces*) are commonly found in soil (Kellar and Zimmermann1989)

S. litura commonly known as tobacco caterpillar, is a serious and generalist agricultural pest which can infest over 290 plant species in different parts of the world including India, Japan, China, and other countries of Southeast Asia. In India, *S. litura* caused huge 26-100% yield losses in groundnut at peak incidence, by infesting new tender shoots, new flushes in the crop plants during the cropping season of groundnut (Dhir*et al.*, 1992). In eastern region of India, incidence of *S. litura* prevails more or less throughout the year on their suitable hosts but major incidence was observed in the rainy and early winter season during July to October, 2014 (Fig. I e). This high incidence of *S. litura* might be due to emergence of new tender shoots, new flushes in the crops. *S. Litura* has been reported as one of the major pest of *Flemengia semialata*, a host plant for lac cultivation (Meena *et al.*, 2014).

Among existing entomogenous fungi, *Nomuraea rileyi* (Farlow) Samson is a widely distributed entomopathogenic fungal species which can infect many noctuid defoliators *viz.*, *Helicoverpa armigera* Hubner, *Spodoptera litura* Fabricius, *Trichoplusiani* (Hubner), *Anticarsia gemmatalis* Hubner, *Chrysodeixis* (=*Pseudoplusia*) *includes* (Walker)(Kellar and Zimmermann 1989). *S. Litura* Fabricius (Lepidoptera: Noctuidae). *N. rileyi* has been reported as one of the most common entomopathogenic fungus on *S. Litura* (Rao and Phadke 1977). It is also widely exploited as myco-insecticides in the form of grain based/carrier formulations (talc based) for eco-friendly management of defoliator lepidopterous insect-pests. Several reports of entomopathogenic fungi occurrence have been reported from different parts of India (Kulkarni and Lingappa 2002) and (Namasivaya *et al.*, 2013, Krishnaveni *et al.*, 2016) from Southern part (Vimla Devi1994, Thakre *et al.* 2011, Ingle 2014, Kachhadiya *et al.*, 2014) and (Patiland Abhilash 2014) from North-Western except Eastern India where no documented information is available so far in the best of our knowledge. Keeping these views in mind, experiments were design to see the impact of weather parameters on the distribution, prevalence, pathogenicity, growth and development and possibility in the formulation development of *N. rileyi* for wide area management of Lepidopterans in the Eastern Region of India.

MATERIALS AND METHODS

Infected cadaver of *S. litura* caterpillars (Fig. 1a) were collected in individual vials from the ground nut experiment field of ICAR Research complex for Eastern Region, Research Centre, Ranchi, Jharkhand (23° 45′ N, 85° 30′ E, Elevation 620m amsl) in August, 2014 and further identification was done on the basis of morphological characteristics in the laboratory of Plant Pathology of the centre. Observations on natural incidence of *N. rileyi* in respect to *S. litura* larval mortality were also recorded under field conditions. Cadavers were examined microscopically by preparing temporary slides with the help of Lactophenol amended with cotton blue. Slides were observed on a Phase Contrast Microscope (type 020-519.503 LB 30T equipped with Photographic camera, Leica, Germany) and diagnostic features were recorded and confirmation was done on the basis of available literatures (Kulkarni and Lingappa 2002, Choudhary *et al*., 2012, Maurya *et al*., 2013). The entomopathogenic fungus was isolated on potato dextrose agar medium and purified by hyphal tip isolation techniques (Fig. 1b).

Test of pathogenicity of the isolated entomopathogenic fungus was assayed in the laboratory by brushing the conidial suspension of *N. rileyi* (3.4X 10^{-9} cfu) on 2nd and 3rd instar larvae of S. *litura*. Twelve-fifteen healthy larvae of both the larval stages, collected from the field were kept separately on Petri plates before treating with *N. rileyi*. Disinfected fresh leaves of groundnut were offered to larvae for feeding during experimentation.(Fig. I f, g) and a similar experiment was also maintained without the fungal treatment. Mortality of the larvae of S. *litura* were recorded and compared with the control. Data on seasonal incidence of entomopathogenic fungus and weather parameters were recorded in the field conditions on weekly basis. Dead and mummified cadavers were collected to avoid the repetition of data during the observations.

RESULTS AND DISCUSSION

Weather parameters especially Temperature (Min., Max.), humidity and rainfall showed positive attributes in mycelial growth and development of *N. rileyi*. Due to high humidity and rainfall induced the parasitism of the entomopathogenic fungus against *S. litura* which covered the entire surface of IInd and IIIrd instar larvae of spodoptera within 48-72 hours. The mycelium/hyphae of *N. rileyi* and their key characteristics observed through binocular light microscope revealed that the fungal mycelia of the entomopathogenic fungi *N. rileyi* were septate, white, hyaline, smooth-walled with flocculent overgrowth, sparse in culture but dense on insects (often completely covered the host). Conidiophores arise single or (rarely) synnematous (with a sterile base) from the conidiogenous cells, erect, septate, smooth walled, bearing whorls of short and blocky branches (metulae) with clusters and divergent flask-shaped 4.0-7.5 × 2.8-4.6μm in size, mostly swollen phialides with a distinct neck and conidia were produced in heads. Phialides usually sub-globose to short cylindrical, or ampliform with a globose base, 2.5-3.75 × 2.5-4.0 μm, with necks short or absent, smooth-walled, hyaline. Conidiophores emerged from conidiogenous cells were dense with individual distinct whorls. Conidiogenous cells were short, blocky, with no distinct neck and conidia were single celled in short divergent chains (Samson 1974, Samson *et al*., 1988, Sosa-Gómez *et al*., 2009,Kepler *et al*., 2012, Maurya *et al*., 2013). Conidiophores bears conidia which were hyaline, aseptate, smooth round to ovoid or elongate and size varied from 2.2-3.75 X 2.5-3.0 μm(Fig. I c, d). Test of pathogenicity of *N. rileyi* (3.4X 10^{-9} cfu) on 2ndand 3rd instar larvae of S. *litura* showed mortality (Fig I-f,g).As per the results of the test of pathogenicity, all the treated larvae (of both the stages) were found dead within 3to 4 days of treatment and visible fungal growth were observed on 5th to 6th days, whereas larvae without treatment continued to feed and moult into their successive instars. Krishnaveni *et al*. (2016) have also tested the virulence of grain formulation of *N. rileyi* against 3 rd instars larvae of *S. litura*. Natural incidence of *N. rileyi* on percent mortality of *S. litura* larvae in field conditions revealed that highest larvae mortality were observed on 37th SMW (93.67 mortality) at 90.8% relative humidity, 84mm rainfall with 28.33°C maximum and 23.67°C minimum temperature followed by 36th SMW (92.85% larval mortality). Even from the previous SMW 70% field mortality was recorded on similar weather conditions (Table1). In rainy season, occurrence of *N. rileyi* incidence was observed to be very high and it reached up to 88-90% mortality on the first instar larvae of *S. litura*. Occurrence of these high incidences might be due to rainfall and high humidity which favours the growth and multiplication

Table 1: Natural incidence of *N. rileyi* on *S. litura* larval mortality (%) in relation to weather variables during 32-37 Standard Meteorological Weeks

Meteorological weeks (SMW)	Temp. Min (°C)	Temp. Max (°C)	Weather parameters Relative Humidity (%)	Rainfall (mm)	Larval mortality (%)
32	24.00	30.00	88.00	66.00	5.67
33	24.20	30.20	88.80	65.00	11.13
34	24.40	31.20	86.20	92.00	33.29
35	24.00	29.17	91.67	83.00	70.06
36	22.67	29.33	91.83	85.00	92.85
37	23.67	28.33	90.83	84.00	93.67
$Y=-1223.9+12.30RH+2.13Rainfall,\ R^2=0.97$					

Figure 1: Natural incidence of *Nomuraea rileyi* on larvae of *Spodoptera litura;* a.*N. rileyi* infected larvae; b. Pure culture of *N. rileyi*; c-d. Microphotograph of *N. rileyi;* e. *S. litura* infestation on groundnut; f-g. Test of pathogenicity of *N. rileyi*

of the fungus and the rain splashes aids in reaching the spores to the leaf surfaces where insects were feeding causing the primary infection. Step wise regression equation also showed that weather variable *viz*., rainfall and relative humidity had significant positive relation with perpetuating the entomopathogenic fungus. In Andhra Pradesh 36.9 per cent infection of *N. rileyi* on *S. litura* in ground nut field was observed by Vimala Devi; (1994), Sridher and Prasad (1996). Vimala Devi *et al*. (2002) reported that the foliar spray and soil application of 2 X10^8 cfu/ml conidial formulation of *N. rileyi* were equally effective against the mortality of the larvae of *S. litura*.

Natural incidence and test of pathogenicity of *N. rileyi* on *S. litura* showed their ability to infect and kill the second and third instar larvae of *S. litura* which can be exploited in the development of eco-friendly, potent bio-insecticide formulation for effective n wide area management of Lepidopteron insect-pests.

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