Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2016; 4(3): 167-169

Received: 16-03-2016
Accepted: 17-04-2016

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Efficacy of local isolate of *Nomuraea rileyi* (Farlow) Sampson against *Helicoverpa armigera* (Hubner)

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Abstract
A laboratory experiment was conducted to determine the efficacy of local isolate of entomopathogenic fungi *Nomuraea rileyi* (Farlow) Sampson against *Helicoverpa armigera* Hubner. The present investigation was carried out at Mycological Research Section, Department of Plant Pathology, Assam Agricultural University, Jorhat, Assam, India during the period 2013-2014. Different spore concentrations were adjusted and comparison was made with Malathion 50EC @ 1.5ml/l. Observations on larval mortality of 4th instars *H. armigera* were recorded at an interval of 24hrs upto 10 days of post treatment. The results revealed that *N. rileyi* was highly virulent and infected the larvae in a dose dependent manner. At the highest concentration of 1×10^9 spores/ml, 17.40 % of the larvae were killed in 4 days and subsequently observed to be 85.92% at 10 days after treatment. At the next lower dose (1×10^8) total mortality was recorded to be 78.89%. The conidial concentration at 1×10^7, 1×10^6 and 1×10^4 conidia per ml caused 71.85%, 68.15% and 39.25% mortality respectively, with a minimum mortality of 25.14% at 1×10^2 concentration. The LC_{50} value of *N. rileyi* tested on 4th instar larvae of *H. armigera* was found to be 2×10^4 conidia/ml at 10 days. Thus, field studies on combine application of the *N. rileyi* along with the selective pesticides will be a useful strategy for integrated pest management of *H. armigera.*

Keywords: Efficacy, *Helicoverpa armigera, Nomuraea rileyi*

1. Introduction
Helicoverpa armigera Hubner (Noctuidae; Lepidoptera) is a major pest that attacks a range of different vegetable crops and causes serious economic damage and yield loss. It is a polyphagous insect-pest and considered as an important insect-pest in different geographical regions. The major characteristics which contribute to *H. armigera* a pest status includes polyphagy, high mobility, high fecundity and a facultative diapauses that makes it particularly well adapted to different agro-ecosystems [1]. Management tactics for *H. armigera* relies heavily on insecticides and the extensive use of chemical pesticides resulted in insecticides resistance. The role of fungal pathogens as natural enemies for vegetable insect pests has been explored and many isolates have been identified [2]. *N. rileyi* (Farlow) Sampson is one of the naturally occurring potent entomopathogen pathogenic to a majority of the noctuids. Earlier, the natural occurrence of *N. rileyi* on *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae) have been documented from Assam [3] and in most localities where it occurs, it affects the insect pest complex. Hence, the present study was initiated to test the efficacy of local isolate of *N. rileyi* against *H. armigera* under laboratory condition.

2. Materials and Method
The present investigation was carried out at Mycological Research Section, Department of Plant Pathology, Assam Agricultural University, Jorhat, Assam, India during the period 2013-2014.

2.1 Culture of *Helicoverpa armigera*
H. armigera cultures were established from a primary colony obtained from the Department of Agricultural Biotechnology, AAU, Jorhat, Assam. The culture of *H. armigera* was maintained in the laboratory on chickpea flour based semi synthetic diet [4] at a temperature of 27±1°C and 60±10 per cent relative humidity. The neonate larvae were fed individually on an artificial diet and healthy 4th instars were used for the experiment.

2.2 Source of *N. rileyi*

Local isolates of *N. rileyi* was obtained from the culture collection of the Mycological Research Section, Department of Plant Pathology, AAU, Jorhat, Assam. Pure cultures of *N. rileyi* were maintained on Saboraud's Maltose Agar with Yeast extract (SMAY) slants throughout the period of experimentation by periodical sub-culturing on fresh medium and storing at 4°C in refrigerator.

2.3 Efficacy of *N. rileyi* on *H. armigera*

To test the efficacy of *N. rileyi* against *H. armigera*, different spore concentrations from nutrient media plates were mixed with distilled water and 0.2 per cent Tween-80 to get the suspension. The spore count of this stock suspension was estimated with an improved Neubaur haemocytometer and a homogenous spore suspension were obtained by proper agitation and adjusted at 1 X 10^2, 1 X 10^4, 1 X 10^6, 1 X 10^7, 1 X 10^8, 1 X 10^9 spore/ml of water. Newly molted 4th instar of *H. armigera* were bioassayed for their susceptibility to *N. rileyi*. Ten larvae were taken in a Petri plate lined by a filter paper at the bottom for absorbing excess moisture spore suspension (5ml) at required concentration mixed with Tween **@** 0.23 per cent was sprayed with an atomizer no. 600 (Holmspray T.J. Holmes. Co. Inc. Charley, Mass). The larvae were then carefully transferred and reared in the containers by providing fresh food daily. Three replicates of ten larvae were used in each case. Control set was sprayed sterile water mixed with Tween 80 @ 0.23 per cent and comparison was made with Malathion 50EC @ 1.5ml/l. Observations on larval mortality were recorded at an interval of 24hrs upto 10 days of treatment. Dead larvae were placed in a Petri plate containing sterilized Whatman No. 1 Filter paper moistened with sterile distilled water and kept in moist chamber at 25±1°C, and 85±1 per cent relative humidity with 12hr light and dark period (ORBITEK, Scigenics Biotech). Corrected mortality from the treatments was calculated [5]. The mortality data so obtained were analyzed using Fisher's method of analysis of variance for comparison among different concentrations. Significance of variance among data were calculated out by calculating the 't' at 5 per cent probability level. Regression analysis was used to determine the median lethal concentrations of the fungal isolates using probit analysis [6].

3. Results and Discussion

The bioassay with 4th instar larvae of *H. armigera* revealed that *N. rileyi* was highly virulent. The data presented in table 1, indicated that at the highest concentration of 1 × 10^9 conidia/ml, 17.40 per cent of the larvae were killed at a minimum period of 4 days after treatment with highest mortality percent of 85.92% at 10th day after treatment. This was followed by conidial suspension of 1 × 10^8 conidia/m with 78.89% mortality. The mortality rates recorded at 1 × 10^7, 1 × 10^6, 1 X 10^4 and 1 X 10^2 conidia/ml were 71.85%, 68.15%, 39.25% and 25.14% respectively. The dead larvae became hard and stiff followed by external sporulation. However, the highest mortality of 100% was observed with Malathion 50EC @ 1.5ml/L at 4th day after treatment against 3.33% mortality in control. The fungus *N. rileyi* performed better at higher concentration (10^8 conidia/ml) compared to lower concentrations *viz.*, 10^7, 10^5, 10^3 and 10^2 conidia/ml against *H. armigera* [7]. They also reported that the mortality of all larval instars (I-V instars) of *H. armigera* due to *N. rileyi* in aqueous spore suspension at the highest concentration (10^8 conidial/ml) was marginal on fifth day after treatment and cumulative mortality steadily increased to reach maximum of 47.5–100 per cent within 10 days. It was also reported that the highest mortality (91.2%) was obtained in the first instar of *Spodoptera litura* and 95 per cent in the second instar larvae of *H. armigera* with the highest concentration of 1 x 10^9 of *N. rileyi* spore/ml [8].

The mean LC_{50} value of *N. rileyi* required to kill 50 % of tested 4th instar larvae of *H. armigera* determined from the probit transformed dose- response graph was log inverse of 4.31 (2 × 10^4 conidia/ml) at 10 days with a lower and upper fudicial limit of 1093.0 to 149926.8, conidia/ml, respectively (Table 2). In this respect, Sonai Rajan *e* [9] reported with LC_{50} values at 3.18 x 10^7, 3.97 x 10^7 and 4.50 x 10^7 spores/ ml, respectively for PDBC, DOR and local isolates of *N. rileyi* against the first instar larvae of *S. litura*. However the doses causing 50% mortality on third instar larvae were 15.00 x 10^7, 16.68 x 10^7 and 18.27 x 10^7 spores/ml for PDBC, DOR and a local isolates, respectively. Earlier, Iqtiat *et al.*, [10] also reported that the LC_{50} value of *N. rileyi* against *H. armigera* could range between 10^7 and 10^8 spore/ml. This variation in lethal concentrations of *N. rileyi* could be due to isolates genetic variation in virulence and efficacy in laboratory bioassays.

4. Conclusion

The present studies have shown that insecticide application was more effective compared to the application of conidial concentration of *N. rileyi*, however, when applied in high conidial concentration, *N.* rileyi also caused substantial mortality of *H. armigera.* Thus, field studies on combine application of the *N. rileyi* along with the lower dosage of selective pesticides will be a useful strategy for integrated pest management of *H. armigera* contributing towards an eco friendly IPM strategy.

5. Acknowledgement

The author is thankful to the head, Department of Plant Pathology, Assam Agricultural University, Jorhat-13 for providing the necessary facilities.

Table 1: Efficacy of *Nomuraea rileyi* (conidia/ml) against *Helicoverpa armigera*

Treatments (Conidia/ml of water)	Corrected mortality %									
	1 DAT	2 DAT	3 DAT	4 DAT	5 DAT	6 DAT	7 DAT	8 DAT	9 DAT	10 DAT
1 × 10^2	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	7.40 (15.75)	14.07 (21.98)	25.14 (30.24)
1 × 10^4	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	7.40 (15.75)	14.07 (21.98)	21.85 (27.94)	32.22 (34.36)	39.25 (38.79)
1 × 10^6	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	6.67 (14.85)	17.77 (24.90)	31.85 (34.39)	46.29 (42.85)	53.70 (47.65)	57.41 (49.29)	68.15 (55.77)
1 × 10^7	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	11.11 (19.74)	28.88 (32.49)	42.96 (40.97)	57.41 (49.29)	61.11 (51.38)	64.81 (53.85)	71.85 (58.15)
1 × 10^8	0.00	0.00	0.00	13.33	36.29	46.66	64.44	68.15	75.18	78.89

	(0.00)	(0.00)	(0.00)	(21.70)	(37.09)	(43.06)	(53.59)	(55.88)	(60.15)	(63.14)
1×10^9	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	17.40 (24.59)	42.96 (40.87)	57.04 (49.03)	67.78 (55.39)	71.11 (57.47)	78.52 (62.37)	85.92 (68.13)
Malathion (50 %EC)	40.00 (39.84)	60.00 (50.83)	76.66 (61.19)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
Control (water spray)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	3.33 (10.49)	6.66 (14.88)	6.66 (14.88)	6.66 (14.88)	6.66 (14.88)	6.66 (14.88)	6.66 (14.88)
S.Ed(±)	-	-	-	7.29	5.31	4.20	6.08	4.86	4.85	3.49
$CD_{0.05}$	-	-	-	15.58	11.35	8.99	13.01	10.39	10.38	7.46

DAT = Days after treatment

Data are mean of 3 replication

Figure in the parentheses are angular transformed value

Table 2: Effect of concentration of spores of *Nomuraea rileyi* on larval mortality of *Helicoverpa armigera*

Concentration (Spore/ml)	Mean Larval Mortality (%)	LC_{50} (Spore/ml)	Fiducial Limit (95%)		Slope (± S.E)
			Lower	Upper	
1×10^2	25.14	2.0×10^4	1093.0	149926.8	0.23 (0.04)
1×10^4	39.25				
1×10^6	68.15				
1×10^7	71.85				
1×10^8	78.89				
1×10^9	85.92				

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