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Studies on effect of *Metarhizium* (*Nomuraea) rileyi* (Farlow) against *H. armigera* (Hubner)

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Abstract
Metarhizium (Nomuraea) rileyi is an effective entomopathogenic fungus having a specific host range for many insect pests. *Metarhizium (Nomuraea rileyi)* (NR-DHA) strain of entomopathogenic fungus was tested against the cotton bollworm, *Helicoverpa armigera* (Hubner) larvae reared on artificial diet. Increased spore concentration 2×10^3, 2×10^4, 2×10^5, 2×10^6, 2×10^7 and 2×10^8 were tested against larvae of first instar to sixth instar. Mortality of larvae was increased as increasing concentration of fungal spores. The larvae became dead within one week after the treatment of *N. rileyi*. The growth of fungus mycelium was observed on the outer surface of larval cadavers on the fourth day. LC_{50} 1.01×10^3(I^{st} instar), 5×10^4(II^{nd} instar), 2.9×10^5 (III^{rd} instar), 1.97×10^6 (IV^{th} instar), 2.5×10^7 (V^{th} instar), 8.2×10^8 (VI^{th} instar) spores/ml was evaluated. Suppression of larval populations was seen in the current studies.

Keywords: *Helicoverpa armigera*, *Metarhizium (Nomuraea) rileyi*, LC_{50}, insect pest, biopesticide

Introduction

H. armigera is the most economically significant pest because of its host range that encompasses over 300 plant species which includes a variety of fruit crops and vegetables and tree species in along with other important cotton, legumes, sunflower, wheat, sorghum, field beans, tomatoes and tobacco (Rajapakse and Walter, 2007) [13]. *Helicoverpa armigera* Hübner commonly known as the cotton bollworm or legume pod borer is a significant global agricultural production obstacle. It is the dominant insect pest in the worldwide due to its adaptability, strong polyphagy, short life cycle and fast regeneration rate (Lawo *et al.*, 2008) [9].

Nowadays, the utilization of chemical insecticides around world has risen destruction to the environment, pest rejuvenation, lethal effect on non- target organisms and pest resistance to the insecticides (Abudulai *et al.*, 2001) [1]. As a matter of choice, there is a natural "eco-friendly" option in utilizing bacteria, viruses, and fungi as a biological agent for the management of insect pest (Dhakal and Singh 2019) [2]. Biological pesticides provide a more natural, beneficial to environment approach to pest control than synthetic insecticide. Among various biological controlling agents, use of entamopathogenic fungi has considerable potential for efficacious suppression of variety of arthropod pest.

Metarhizium (Nomuraea) rileyi as a biocontrolling agent has numerous advantages over conventional insecticides including cheap cost, high effectiveness, safety for beneficial species and decrease of harmful residues in the environment which results in greater biodiversity in human-managed environments. The fungus is effective against caterpillar pests of vegetable crops like cabbage, potato, oil seed plants like soybean, groundnut, castor and cotton etc. *Metarhizium (Nomuraea) rileyi* is a cosmopolitan fungal pathogen. It causes infection to several lepidopteran species of important agricultural pests. There are at least 44 insect species susceptible to *N. rileyi* infection; 38 them are lepidopteran (Alves, 1998; Ignoffo, 1981; Vimala Devi *et al.*, 2003) [7, 17]. Hence, current studies were done to evaluate the concentrations of *N. rileyi* under laboratory condition bioassay methods for evaluation of *N. rileyi* efficacy against various larval instars of *H. armigera*.

Material and Methods

1. Collection and Maintenance of *H. armigera* under laboratory conditions

Larvae of *Helicoverpa armigera* from chickpea field were collected from Satara district of Maharashtra during November to April month in 2021 and their rearing carried out in laboratory conditions. Laboratory temperature was maintained at 25±2℃, 75±5% RH and 14-10 (L:D) h photoperiod. Larvae were reared individually in sterile plastic bottles including pieces of artificial diet (Sharma *et al.*, 2014) with slight modification in the method of preparation (Fig. 1).

2. *Metarhizium (Nomuraea) rileyi* (biopesticide)

The culture of *N. rileyi* was obtained from University of Agricultural Sciences, Dharwad. *N. rileyi* (NR-DHA) was grown at 28 ℃ for 14 days on SMAY medium (Fig. 1). Conidia were withdrawn from the culture medium and a suspension of the conidia was produced using sterile distilled water having 0.1% Tween-80. To disperse clumps of the conidial suspension was vortexed for 15 minutes. The concentrations of conidia were evaluated using a Neubauer haemocytometer chamber by counting under a 40X microscope (Optika-Fluoseries B-600TiFL, Italy). The concentrations were adjusted to 2×10^3, 2×10^4, 2×10^5, 2×10^6, 2×10^7 and 2×10^8 spores/ml with sterile water. The growth of *N. rileyi* was obtained by periodically sub culturing on Sabouraud's Maltose Agar with Yeast extract (SMAY) medium (Morrow *et al.*, 1989) [11].

3. Determination of LC_{50} value of *N. rileyi* against developmental stages of *H. armigera*

In order to investigate biopesticide activity the *Metarhizium (Nomuraea) rileyi* was sprayed topically on *H. armigera* larvae. The concentration at 2×10^3 to 2×10^8 spores/ml of *N. rileyi* was applied to *H. armigera* larvae with small sprayer. Each concentration replicated three times. After this treatment, larvae were transferred to separate Petri dishes containing larval diet. For control set, 1 ml of distilled water was sprayed on the larvae. Both Petri dishes with larvae were maintained at temperatures 25 ± 2°C and 75±5 % RH. Larval mortality of different instars were observed and recorded from 24 hours to 7 days after treatment. LC_{50} values were calculated by analysing data using Probit analysis (Ingale *et al.*, 2015).

Results

Effect of *N. rileyi* on larval stage of *H. armigera*

N. rileyi at different concentration were able to cause death of larvae after treatment. The *N. rileyi* treated larvae were found less active and stopped feeding. The alteration in larval body colouration was observed. The outer integument was bleached, remained immotile slowly and died finally. The larvae became dead within one week after the treatment of *N. rileyi*. The growth of fungus mycelium was observed on the outer surface of larval cadavers on the fourth day. The white coloured growth of mycelium was distinct from 7 day after treatment. The mycelium which is whitish then turned to olive green indicated onset of sporulation from 10^{th} day onwards. Phenotypic observations of *N. rileyi* treated *H. armigera* larvae were displayed in Fig. 2

In bioassay studies, concentrations at 2×10^3 to 2×10^8 were applied to each instar larvae and recorded mortality for each concentration. In the 1^{st} instar larvae 52 to 93.33% mortality were observed and the LC_{50} (1×10^3 spores/ml) value was determined. Mortality percentage of 1^{st} instar larvae was changed with different concentrations represented in Table 1. The differences between mortality readings were statistically significant ($P < 0.001$). The LC_{50} value along with other associated values such as fiducial limit, chi-square were given in Table 1 For 2^{nd} instar larvae 41 to 77% mortality was observed. The differences between larval mortality readings of the 2^{nd} instar larvae were statistically significant ($P < 0.001$). The LC_{50} value and other related values such as slope value, fiducial limit and chi-square value were mentioned in Table 2. As the total percentage mortality was changed with increasing concentrations; the concentration dependent mortality was observed in all instar larvae,

Similarly, in bioassay studies the concentrations 2×10^3 to 2×10^8 spores/ml were applied to other 3^{rd}, 4^{th}, 5^{th} and 6^{th} instar larvae and recorded 39 to 65%, 28.5 to 69.3%, 12 to 60 % and 18 to 53.3% mortality respectively. The LC_{50} value and other related values such as slope values, fiducial limits and chi-square values were mentioned in the Table 3 (3^{rd} instar), Table 4 (4^{th} instar), Table 5 (5^{th} instar) and Table 6 (6^{th}instar). It is very clear that the LC_{50} values of different instars of *H. armigera* larvae on action to *N. rileyi* showed a rising trend in the LC_{50} value as the larval age advanced.

Fig 1: 1) Rearing of *Helicoverpa armigera* on artificial diet 2) Maintenance of culture of *Metarhizium (Nomuraea) rileyi*

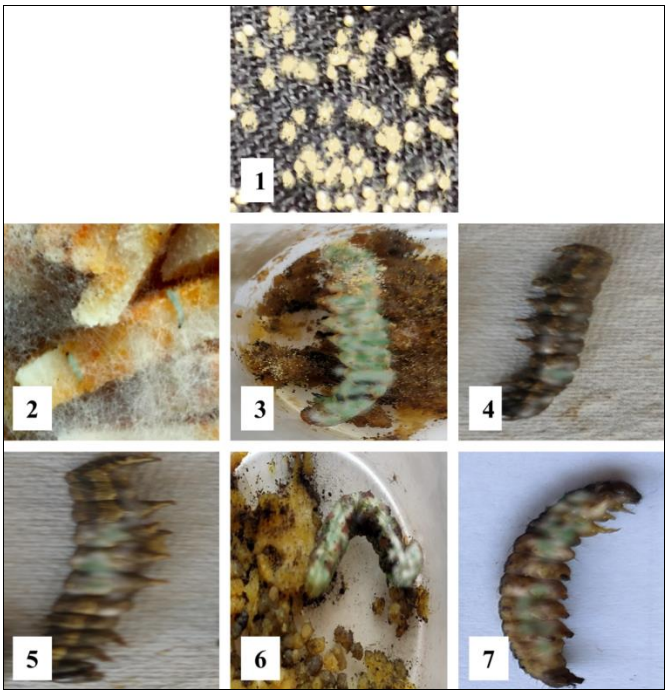

Fig 2: Development of mycosis indicated as flocculent whitish growth of fungus along with sporulation (greenish) on dead larvae 1) Eggs mortality after treatment of *N. rileyi* 2) First instar larval mortality along with fungus growth 3) Fungal growth (mycosis) on second instar larvae 4) Fungal growth **(**mycosis) on third instar larvae 5) Fungal growth (mycosis) on forth instar larvae 6) Fungal growth (mycosis) on fifth instar larvae 7) Fungal growth (mycosis) on sixth instar larva

Table 1: Effect of concentration of N. rileyi spores on 1st instar larvae of H. armigera

Concentration *N. rileyi* spores/ml	Log of concentration	Mean larval mortality (%) *	Probit mortality
2×10^3	3.30103	52± 2.16	5.05
2×10^4	4.30103	63.3± 3.8	5.33
2×10^5	5.30103	76.6± 4.7	5.75
2×10^6	6.30103	83.03± 1.64	5.97
2×10^7	7.30103	86.06± 3.59	6.1
2×10^8	8.30103	93.33± 2.40	6.5
LC_{50}	1.01×10^3		
Fiducial limit	Lower		Upper
	49.67		20650.99
Slope ± SE	0.27 ± 0.66		
x^2 value	10.87		

*Avg. of three replications, SE = Standard error
Larval mortality recorded up to 10 days

Table 2: Effect of concentration of *N. rileyi* spores on 2nd instar larvae of *H. armigera*

Concentration *N. rileyi* spores/ml	Log of concentration	Mean larval mortality (%) *	Probit mortality
2×10^3	3.30103	41± 3.2	4.77
2×10^4	4.30103	45± 2.8	4.87
2×10^5	5.30103	53± 3.6	5.07
2×10^6	6.30103	64± 1.5	5.35
2×10^7	7.30103	69± 3.57	5.49
2×10^8	8.30103	77± 4.4	5.73
LC_{50}	5×10^4		
Fiducial limit	Lower	Upper	
	1159.72	2180473.30	
Slope ± SE	0.19 ± 0.8		
x^2 value	10.56		

*Avg. of three replications, SE = Standard error
Larval mortality recorded up to 10 days

Table 3: Effect of concentration of *N. rileyi* spores on 3rd instar larvae of *H. armigera*

Concentration *N. rileyi* spores/ml	Log of concentration	Mean larval mortality (%) *	Probit mortality
2×10^3	3.30103	39 ± 1.6	4.72
2×10^4	4.30103	45± 4.2	4.87
2×10^5	5.30103	48± 3.6	4.94
2×10^6	6.30103	55± 2.4	5.12
2×10^7	7.30103	58± 3.37	5.2
2×10^8	8.30103	65± 3.40	5.38
LC_{50}	2.9×10^5		
Fiducial limits	Lower	Upper	
	926.21	92198438.49	
Slope ± SE	0.12 ± 1.2		
x^2 value	10.43		

*Avg. of three replications, SE = Standard error
Larval mortality recorded up to 10 days

Table 4: Effect of concentration of *N. rileyi* spores on 4th instar larvae of *H. armigera*

Concentration *N. rileyi* spores/ml	Log of concentration	Mean larval mortality (%) *	Probit mortality
2×10^3	3.30103	28.5± 5.6	4.43
2×10^4	4.30103	36± 2.5	4.64
2×10^5	5.30103	48.5± 3.45	4.96
2×10^6	6.30103	56± 5.3	5.15
2×10^7	7.30103	60± 2.37	5.25
2×10^8	8.30103	69.3± 1.97	5.5
LC_{50}	1.97×10^6		
Fiducial limits	Lower	Upper	
	20254.40	24156656.02	
Slope ± SE	0.21 ± 0.7		
x^2 value	11.49		

*Avg. of three replications, SE = Standard error
Larval mortality recorded up to 10 days

Table 5: Effect of concentration of *N. rileyi* spores on 5th instar larvae of *H. armigera*

Concentration *N. rileyi* spores/ml	Log of concentration	Mean larval mortality (%) *	Probit mortality
2×10^3	3.30103	12± 1.8	3.82
2×10^4	4.30103	20± 2.6	4.16
2×10^5	5.30103	30± 1.64	4.48
2×10^6	6.30103	40± 2.32	4.75
2×10^7	7.30103	46± 3.56	4.9
2×10^8	8.30103	60± 4.3	5.25
LC_{50}	2.5×10^7		
Fiducial limit	Lower	Upper	
	1458347.60	431702554.37	
Slope ± SE	0.27 ± 0.6		
value	12.79		

*Avg. of three replications, SE = Standard error
Larval mortality recorded up to 10 days

Table 6: Effect of concentration of *N. rileyi* spores on 6th instar larvae of *H. armigera*

Concentration *N. rileyi* spores/ml	Log of concentration	Mean larval mortality (%) *	Probit mortality
2×10^3	3.30103	18± 5.6	4.08
2×10^4	4.30103	20± 4.5	4.15
2×10^5	5.30103	29± 3.45	4.44
2×10^6	6.30103	36± 4.3	4.64
2×10^7	7.30103	46± 2.7	4.89
2×10^8	8.30103	53.3± 1.43	5.08
LC_{50}	8.2×10^8		
Fiducial limits	Lower	Upper	
	2142165.72	3188962340.20	
Slope ± SE	0.21 ± 0.8		
x^2 value	12.49		

*Avg. of three replications, SE = Standard error
Larval mortality recorded up to 10 days

Discussion

Larval mortality recorded up to 10 days *N. rileyi* at various concentrations were able to cause death of all stages of *H. armigera* followed by growth of fungus mycelium on integument of the larvae. The larvae became inactive, stop feeding to cause mortality. The fungal growth (mycosis) was observed on cuticle of insect host after treatment, displayed in the fig. 2. The bioassay with all 6 instar larvae of *H. armigera* concluded that *N. rileyi* was extensively virulent. The study also revealed that the mortality percentage was more at highest concentration of *N. rileyi* (2×10^8 spores/ml) than other lower concentrations *viz.* 2×10^3, 2×10^4, 2×10^5, 2×10^6, 2×10^7 spores/ml against *H. armigera*. The concentration dependant mortality was observed in all instars of *H. armigera* larvae (Table 1 to Table 6). It was also reported that the highest mortality of all larval instars (I-VI instars) of *H. armigera* due to *N. rileyi* at the highest concentration 2×10^8 spores/ml. There are various laboratory bioassay studies using EPF to denote as an ideal source of biopesticide has been the *N. rileyi.* From considering above results are corroborate with the results of Hazarika *et al.* (2016) [6] who studied the effect of entamopathogenic fungus, *N. rileyi* (Farlow) a local isolate against *Helicoverpa armigera*. The authors concluded that the *N. rileyi* was highly virulent caused dose dependant mortality. A concentration at highest level 1×10^9 spores/ml of *N. rileyi*, 17.40% mortality was observed in 4 day and 85.92 % at 10 day after treatment. The conidial concentration at 1×10^7, 1×10^6, 1×10^5 conidia /ml resulted in 71.85%, 68.15% and 39.25% mortality respectively. The concentration at 1×10^2 conidia/ml the lowest mortality was 25.14%. The forth instar larvae of *H. armigera* treated with *N. rileyi* was discovered to be LC_{50} at 2×10^4 conidia/ml after 10 days. Gable *et al.* 2014 conducted bioassay of scarab pests, *Schizoncha affinis* and *Tenebrio molitor* against *Beauveria brongniartii* and *B. bassiana*. This strain showed a wide range of virulence toward *T. molitor* (39-74% mortality) and *S. affinis* (50.1 to 95% mortality). To kill half of the adult *S. affinis* test insect, a median lethal concentration (LC_{50}) of 7.65×10^6 conidia/ml was required.

These findings were also matched with those of Gundaanavar *et al.* (2005) evaluated the percentage mortality of larval instars of *H. armigera* treated with *N. rileyi*. The concentration dependant mortality percentage was observed. Sharmila and Manjula (2015) [15] determined the effectiveness of *M. rileyi* formulation on *Spodoptera litura* and *Helicoverpa armigera* larvae. The authors

evaluated 65.35% decrease in *S. litura* larvae using a talc-based *M. rileyi* formulation. Mortality was seen to rise with increasing concentration of formulation probable of high spore load. Tang and Hou *et al.* (1998) [16] studied the effect of *N. rileyi* on 4th instar larvae of corn earworm, *H. armigera* caused 90.5 to 100% mortality. The *N. rileyi* conidial solution when applied the early stage larvae in field were observed to be effective in than other chemical insecticide. Therefore, the authors suggested that *N. rileyi* may be an efficient microbial pest controlling agent for this insect. Manjula and Krishnamurthy (2005) [10] studied the bioefficacy of *N. rileyi* against *Spodoptera litura* and *Helicoverpa armigera*. The author observed that early instar larvae of these insect pests were more susceptible to *N. rileyi* infection. Mortality of the pest was higher at higher concentrations. It was indicated from studies the later instars of *H. armigera* were more susceptible than that of *S. litura*. Padanad and Krishnaraj *et al.* (2018) [12] studied the pathogenicity of *N. rileyi* isolates against 3rd instar of *S. litura* with spore concentration of 10^8conidia/ml. The all ten isolates of *N. rileyi* were resulted in 85 to 97% mortality.

Conclusion

According to the findings of this study, *N. rileyi* used as bipesticide that infects *H. armigera* and affects all larval stages at various incubation times and concentrations. NR-DHA is highly infective fungal strain that further be exploitation as mycoinsecticide.

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