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Research Note

Testing the pathogenecity of *Nomuraea rileyi* (Farlow) Samson against castor semilooper, *Achaea janata* Linnaeus

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ABSTRACT: The larvae of II, III and IV instars of *Achaea janata* treated with subculture I, II and insect cultures of *Nomuraea rileyi* indicated larval mortalities to be positively correlated with the concentrations of all the three cultures. First subculture and insect culture were almost equally efficacious in causing the disease. Slightly lowered mortality were recorded with subculture II. Reduction in larval mortality was noticed with advancement of the age in *A. janata* larvae. Almost 100 per cent larvae were dead with $1x10^8$ spores ml^{-1} concentration when treated in II intar and it was 5 – 15 per cent when treated in III and IV instars. However, relatively lower concentrations like $1x10^4$ – and $1x10^5$ spores ml^{-1} also recorded mortality of 50 per cent and above. The lowest concentration of $1x10^2$ spores ml^{-1} resulted in 20–30 per cent larval mortality.

KEY WORDS: *Nomuraea rileyi*, subculture, insect cultures, *Achaea janata,* pathogenicity, larval mortality

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The castor semilooper, *Achaea janata* Linnaeus, is one of the key pests and causes severe defoliation in several parts of Andhra Pradesh. The effective management by synthetic chemical insecticides results in environmental hazards. Biological control agents, microbial pathogens, parasitoids, predators etc., can be effectively utilized in the suppression of most of the insects pests. Among the microbes against lepidopteran larvae, the most widely used ones are nuclear polyhedrosis virus, granulosis virus, *Bacillus thuringiensis, Beauveria bassiana* and *Nomuraea rileyi.*

Nomuraea rileyi is an important entomopathogenic fungus which induces epizootics in several lepidopteran larvae throughout the World. In India, natural occurrence of *N. rileyi* has been recorded on many insect pests like *Helicoverpa armigera* Hubner, *Spodoptera litura* Fabricius, *Spodoptera exigua* Hubner, *Trichoplusia ni* (Hubner) etc. The epizootics of *N. rileyi* has been recorded in Andhra Pradesh also, mostly in coastal areas. The occurrence of *N. rileyi* was also recorded in other districts during congenial weather conditions prevailing during November to January.

Studies on the pathogenicity and field evaluation of *N. rileyi* with respect to *S. litura* and *H. armigera* were carried out by several scientists from different places, However, the documented literature against semiloopers particularly in Andhra Pradesh is scanty. Hence, a study was conducted to record the levels of pathogenicity of *N. rileyi* on *A. janata.*

Culturing of test insect and pathogen

Cultures of *A. janata* maintained in the laboratory by using sterilized rearing containers. The eggs of *A. janata* were collected from castor plants from dry land farm, wet land farm and insectary at S. V. Agricultural College, Tirupati. After hatching, larvae were reared on castor leaves in the laboratory. Fresh leaves were provided every time. At pre-pupal stage, the larvae were transferred to troughs containing fine sterile soil for the pupation and kept in the wire cages. The emerged adults were provided with suspended cotton swabs dipped in the solution of water and honey in the ratio of 3:1 for feeding the adults. The four sides of wire cages were covered with butter paper for oviposition. Eggs were collected and freshly hatched neonates were separated into the troughs containing fresh leaves of castor for experimental use.

The *N. rileyi* available in the department was passed through the *S. litura* larvae and then mass produced on

Saboraud's maltose agar fortified with yeast. First, second subcultures and insect culture of *N. rileyi* were used for the bioassay studies.

Stock suspension of $1x10^8$ spores ml^{-1} was prepared in distilled water, measuring the density of spores with Neubaur haemocytometer and a compound microscope. Castor leaves were cleaned with cotton swab and placed into plastic troughs lined with filter paper inside. Seven concentrations of *N. rileyi viz*., $1x10^8$, $1x10^7$, $1x10^6$, $1x10^5$, $1x10^4$, $1x10^3$ and $1x10^2$ spores ml^{-1} were used for infecting the larvae under each culture. For each concentration, 10 uniform sized 2,3 and 4 instars of just moulted larvae were selected. With the help of hand automizer, *N. rileyi* spore suspensions were sprayed on the larvae in Petriplates separately for the treatments. *N. rileyi* was applied without addition of Tween-20. After a gap of 5 minutes, the treated larvae were transferred into troughs. The experiment was replicated thrice and carried out under room temperature of 25°C ± 2°C and 80 per cent relative humidity. Untreated control was also maintained with water spray. Daily observations on symptoms of infection and larval mortalities were made.

The maximum mean larval mortality of 97.88 per cent was obtained with $1x10^8$ spores ml^{-1} concentrations of subculture-I (Table 1) which was on par with insect culture ($1x10^8$ spores ml^{-1}) with mean mortality of 96.88 per cent (Table 3), while 92.22 per cent mean mortality was obtained with subculture-II (Table 2). In all the three cultures, death of larvae was gradually reduced with the lowering of concentration of spores. More than 50 percent larval mortality were recorded with the concentrations $1x10^4$ and above. Significant differences in mortality was observed in all the instars tested with various concentrations.

Table 1. Mortality of *Achaea janata* larvae due to subculture–I of *Nomuraea rileyi*

Concentration of *N. rileyi* (spores ml^{-1})	Per cent larval mortality			
	*Instar II	*Instar III	*Instar IV	Mean
1 x 10^8	100.00[a] (90.00)	100.00[a] (90.00)	93.66[a] (75.56)	97.88 (85.18)
1 x 10^7	91.00[b] (72.55)	94.33[b] (76.37)	85.00[b] (67.38)	90.11 (72.10)
1 x 10^6	85.33[c] (67.73)	82.66[c] (65.45)	81.33[c] (64.87)	83.11 (66.02)
1 x 10^5	75.66[d] (60.49)	75.66[d] (60.45)	58.66[d] (50.000	70.00 (56.98)
1 x 10^4	66.33[e] (54.54)	63.33[e] (52.74)	49.66[e] (44.809)	59.77 (50.699)
1 x 10^3	54.00[f] (47.29)	46.66[f] (43.08)	34.66[f] (36.05)	45.11 (42.14)
1 x 10^2	52.33[f] (46.33)	36.66[g] (37.26)	18.67[g] (25.542)	35.88 (36.38)
Untreated control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Mean	65.58 (54.87)	62.42 (53.17)	52.70 (45.52)	60.23 (51.19)

* Mean of three replications.

	SEm ±	CD (*P*=0.05)
Instars	1.53	1.52
Concentrations	0.87	2.48
Instars x concentrations	1.51	4.30

Figures in the parentheses are angular transformed values. Figures with same alphabet(s) in superscript are statistically not significant.

Table 2. Mortality of *Achaea janata* larvae due to subculture–II of *Nomuraea rileyi*

Concentration of *N. rileyi* (Spores ml^{-1})	Per cent larval mortality			
	*Instar II	*Instar III	*Instar IV	Mean
1 x 10^8	93.66a (75.99)	93.66a (75.56)	89.33a (71.50)	92.22 (74.35)
1 x 10^7	85.33b (67.62)	81.66b (64.67)	79.33b (63.02)	82.11 (65.10)
1 x 10^6	77.33 c (62.03)	71.66 c (57.85)	67.66c (55.36)	72.22 (58.41)
1 x 10^5	73.33d (58.97)	66.66 d (54.75)	56.33 d (48.69)	65.44 (54.14)
1 x 10^4	67.00e (54.94)	52.66 e (46.53)	46.33 e (42.88)	55.33 (48.12)
1 x 10^3	51.33f (45.76)	42.00 f (40.36)	33.66 f (35.45)	42.33 (40.52)
1 x 10^2	40.66g (39.62)	27.00 g (31.02)	16.00 g (23.12)	27.88 (31.25)
Untreated control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Mean	61.08 (50.62)	54.41 (46.34)	48.58 (42.50)	54.69 (46.49)

* Mean of three replications.

	SEm ±	CD (*P*=0.05)
Instars	0.77	2.20
Concentrations	1.26	3.60
Instars x concentrations	2.19	NS

Figures in the parentheses are angular transformed values. Figures with same alphabet(s) in superscript are statistically not significant.

There were significant differences among the first, second sub cultures and insect culture of *N. rileyi* in showing the pathogenicity to *A. janata*. The earlier report by Morrow *et al*. (1989) indicated that serial sub culturing of *N. rileyi* alters both growth and development on *in vitro* and *in vivo* substrates. According to Morrow *et al.* (1989), six conidial transfers on SMAY plates, resulted in reduced virulence against *Anticarsia gemmatalis* (Hubner) larvae and after 16th conidial transfer, progeny conidia became avirulent. Similarly, reduced virulence on repeated conidial subculturing of *Metahizium anisopliae* against *H. armigera* was reported by Pallavi *et al*. (2008).

Influence of larval age on *Nomuraea rileyi* infection

The maximum (100 per cent) infection was recorded in second and third instars of *A. janata* with higher concentration (1x10^8 spores ml^{-1}) of subculture I of *N. rileyi*. Fourth instar *A. janata* was also susceptible up to 93.66 per cent with the above concentration. More than 50 per cent second instar larvae were dead even with 1x10^2 spores ml^{-1}. Whereas, it was 35.00 and 18.00 per cent with respect to third and fourth instars respectively. Similarly, second and third instar larvae were observed to be more susceptible than fourth instar to second subculture also. The observed mortality with 1x10^2 to 1x10^7 spores ml^{-1} of subculture II were 40-85 per cent for II instar; 27 to 82 per cent for III instar and 16-79 per cent for IV instar of *A. janata*.

When insect culture was applied to second and third instar larvae of *A. janata,* 100 per cent and 99 per cent mortality was obtained respectively at higher concentration i.e., 1x10^8 spores ml^{-1}. Larval mortality of 91.66 per cent recorded in case of IV instar larvae. The concentrations 1x10^4 to 1x10^7 spores ml^{-1} recorded 60 to 91 per cent,

Table 3. Mortality of *Achaea janata* larvae due to *Nomuraea rileyi*

Concentration of *N. rileyi* (Spores ml^{-1})	Per cent larval mortality			
	*Instar II	*Instar III	*Instar IV	Mean
1 x 10^8	100.00a (90.00)	99.00a (85.37)	91.66a (73.31)	96.88 (82.89)
1 x 10^7	91.33b (72.98)	82.00b (65.17)	87.33b (69.26)	86.88 (69.14)
1 x 10^6	83.33c (65.93)	74.33c (59.67)	87.33c (69.21)	81.66 (64.94)
1 x 10^5	72.66d (58.49)	54.33d (47.49)	73.66d (59.16)	66.88 (55.04)
1 x 10^4	60.00e (50.77)	41.66e (40.20)	57.66e (49.42)	53.11 (46.79)
1 x 10^3	42.66f (40.77)	33.66f (35.43)	40.66f (39.60)	39.00 (38.60)
1 x 10^2	30.33g (33.28)	27.66g (31.705)	29.00g (32.56)	29.00 (32.52)
Untreated control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Mean	60.04 (51.53)	51.58 (45.63)	58.41 (49.06)	56.681 (48.74)

* Mean of three replications.

	SEm ±	CD (*P*=0.05)
Instars	0.53	1.53
Concentrations	0.88	2.50
Instars x concentrations	1.52	4.33

Figures in the parentheses are angular transformed values. Figures with same alphabet(s) in superscript are statistically not significant

40-82 per cent and 57 to 87 per cent in second, third and fourth instars respectively. The lowest concentration i.e., 1x10^2 spores ml^{-1} also recorded nearly 30 per cent larval mortality in all the three instars.

In all the three types of cultures tested, As the age of the larvae advanced there was decrease in mortality. The fungal spores need to germinate and penetrate through the integument for infection to occur, the increased toughness of the cuticle in grown up larvae prohibits fungal development further.

Our observations are in accordance with the findings of Habib and Patel (1990) who reported that third instar larva of *Spodoptera frugiperda* was susceptible than fourth instar when infected with to *N. rileyi* with concentrations of 1.03x10^7 and 1.2x10^7 conidial spores ml^{-1}. Susceptibility decreased with the increasing age of *S. littoralis* to *N. rileyi* (Fargues and Rodriguez, 1980). Boman (1981) reported that chemical constituents vary with increasing larval age and this results in hardening of the cuticle and increased hormonal defense mechanisms to the microbial infections, leading to lesser susceptibility of later instars. Khan and Rajak (1986) also reported that first two instars of *H. armigera* were highly susceptible to *B. bassiana.*

The present results showed considerable pathogenicity of *N. rileyi* to the castor semilooper, *A. janata* and corroborate with the findings of several workers on effects of entomopathogenic fungi on lepidopteran larvae (Kulkarni and Lingappa, 2002). Maximum mortality of all noctuids was observed at concentration of 1.2x10^8 spores ml^{-1} . They also stated that *S. litura* and *A. janata* were relatively susceptible species than others. Rao and

Phadke (1977) found that a dense aqueous spore suspension of the fungus, *N. rileyi* caused 100.00 per cent mortality of *S. litura* larvae. Lezama *et al.,* (1993) reported 100 per cent mortality of II, III, IV and V instars of *Spodoptera frugiperda* (Smith) at $1x10^8$ spores ml^{-1} concentration. Vimaladevi (1994) recorded cumulative mortality of 88-97 per cent in *S. litura* with *N. rileyi* at $2x10^{11}$ spores ml^{-1}. Gopalakrishnan and Narayanan (1989) observed 100 per cent mortality of III instar larvae of *H. armigera*. Goh *et al.,* (1992) stated that application of *N. rileyi* at $1x10^7$ spores ml^{-1} concentration caused 50-76% mortality in first to fourth instar larvae of *S. litura*, whereas fifth instar was less susceptible. Faria *et al.,* (1993) reported the 100% infection of *N. rileyi* in a population of *Anticarsia gemmatalis* (Hubner) in soybean crop in Brazil.

The findings of the present study has established that *N. rileyi* is pathogenic to castor semilooper*, Achoea janata* also. The later instars of *Achaea janata* resist the fungus. Subculturing leads to reduced virulence of the fungus and virulence is regained when it is passage through any host insect.

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