Current Biotica 7(1&2): 32-39, 2013 ISSN 0973-4031

Pathogenicity of *Nomuraea rileyi* (Farlow) Samson against castor semilooper, *Paralellia algira* Linnaeus

E. Hemasree*, K. Manjula* and *T. Muralikrishna

Department of Entomology, S.V. Agril. College, Tirupati – 517 502, Acharya N.G.Ranga Agricultural University, Andhra Pradesh, India

*E-mail:hemprasad34@yahoo.com

ABSTRACT

The larvae of *Paralellia algira* L. (II, III and IV instars) were treated with subculture I, II and insect cultures of *Nomuraea rileyi* to evaluate the pathogenicity. Results showed larval mortalities at all the concentrations of the three cultures. First subculture and insect culture were almost equally efficacious in causing the disease. Slightly lowered mortalities were recorded with subculture II. Reduction in larval mortalities was noticed with advancement of the age in *P. algira* larvae. Almost 100 per cent mortality of II instar larvae was observed with $1x10^8$ spores ml^{-1} concentration whereas it was reduced by 5 – 10 per cent against III and IV instars.

KEY WORDS : Larval mortality, *Nomuraea rileyi*, *Paralellia algira*, pathogenicity

INTRODUCTION

The castor semilooper, *Paralellia algira* Linnaeus, one of the important pests of castor infests along with *Achoea janata* causing severe defoliation in several areas of Andhra Pradesh. The management by synthetic chemical insecticides result in environmental hazards and hence biological control agents, microbial pathogens, parasitoids, predators etc., are encouraged and integrated in the management of the pests. The microbes *viz.*, Nuclear polyhedrosis virus, Granulosis virus, *Bacillus thuringiensis, Beauveria bassiana* and *Nomuraea rileyi* are widely used in the suppression of lepidopteran pests targeting larval stages.

Nomuraea rileyi (Farlow) Samson 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, Achoea janata* etc.

Studies on the pathogenicity and field evaluation of *N. rileyi* with respect to *S. litura* and *H. armigera* were carried out by several scientists, however, the documented literature is scanty against castor semiloopers in Andhra Pradesh. Hence, a study was conducted to record the pathogenicity of *N. rileyi* on *P. algira.*

MATERIAL AND METHODS

The study was carried out in the Department of Entomology, S. V. Agricultural College, Tirupati, A. P., India during 2009-10.

Culturing of the test insect and pathogen:

Cultures of *P. algira* was maintained in the laboratory by using sterilized rearing containers. The eggs of *P. algira* were collected from castor plants from dry land farm, wet land farm at S.V. Agricultural College, Tirupati. After hatching, larvae were reared on castor leaves in the laboratory. Fresh food material was 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 culture of *N. rileyi* available in the department was passed through the insect larvae *(Spodoptera litura)* 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.

Application of *N. rileyi:*

Stock suspensions of $1x10^8$ spores ml^{-1} were prepared in distilled water, measuring the density of spores with Neubaur haemocytometer and a compound microscope. Then, serial dilutions were prepared. 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. *Nomuraea rileyi* spore suspensions were sprayed with the help of hand automizer on the larvae in petriplates separately for each treatments. The treated larvae were transferred into troughs after 5 minutes. The experiment was replicated thrice and carried out under room temperature of 25°C ± 2°C and 80 per cent relative humidity. Check was also maintained with water spray on larvae. Daily observations on symptoms of infection and larval mortalities were recorded.

RESULTS AND DISCUSSION

The maximum mean larval mortality of 96.55 and 95 per cent were obtained with $1x10^8$ spores ml^{-1} concentrations of subculture-I and insect culture (Table 1& 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 was recorded with the concentrations $1x10^4$ and above. A significant difference in mortality was observed in all the instars tested with various concentrations.

From the findings, the subculture II of *N. rileyi* recorded least efficacy towards larval mortality when compared to the insect and subculture I. Hence, significant reduced virulence were resulted in two conidial transfers of *N.rileyi* against to *P. algira.* This findings confirms the previous reports by Morrow *et al*. (1989) who indicated that serial sub culturing of *N.rileyi* alters both growth and development on *in vitro* and *in vivo* substrates. According to him, six conidial transfers on SMAY plates, resulted in reduced virulence against *Anticarsia gemmatalis* larvae and after 16^{th} 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 N.rileyi infection*:**

The maximum (100 per cent) infection was recorded in second and third instars of *P.algira* with higher concentration ($1x10^8$ spores ml^{-1}) of subculture I of *N.rileyi*. Fourth instar *P. algira* was also susceptible upto 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 34.00 and 19.00 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 mortalities with $1x10^2$ to $1x10^7$ spores ml^{-1} of subculture II were 34-90 per cent for II instar; 27 to 82 per cent for III instar and 16-79 per cent for IV instar of *P. algira*.

When insect culture of *N. riley* was applied to second and third instar larvae of *P. algira,* 100 per cent and 95 per cent mortalities were obtained at higher concentration i.e., $1x10^8$ spores ml^{-1}. Larval mortality of 90.00 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, 40-82 per cent and 51 to 84 per cent in second, third and fourth instars respectively. The lowest concentration i.e., $1x10^2$ spores ml^{-1} also recorded nearly 27 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 rate. 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.

The above observations are in accordance with the findings of Habib and Patel (1990) who reported that third instar larvae of *S.frugiperda* was susceptible than fourth instar when infected with *N.rileyi* with concentrations of $1.03x10^7$ and $1.2x10^7$ conidial spores ml^{-1} applied topically to maize leaves on which larvae were fed. Susceptibility decreased with increasing the age of *S.littoralis* to *N.rileyi* (Fargues and Rodriguez, 1980). Boman (1981) reported chemical constituents vary with increasing the larval age results in hardening of the cuticle and increased hormonal defense mechanisms to the microbial infections, leads to lesser susceptibility of later instars. Khan and Rajak (1986) reported that, first

two instars of *H.armigera* were highly susceptible to *B.bassiana.*

The present results showed significant pathogenicity of *N. rileyi* to the semilooper, *P. algira* and corroborate with the findings of several workers on effects of entomopathogenic fungi on lepidopteran larvae. Kulkarni and Lingappa (2002), applied *N.rileyi* at different concentrations viz., $1.2x10^8$ to $1.2x10^4$ conidia L^{-1} on the larvae of different noctuids viz., *Spodoptera litura, Achoea janata, Cydia ptychora* Meyrick, *Mythimna separata* Walker, *Earias vitella F.* and *Galleria mellonella* L. highest concentration of $1.2x10^8$ spores ml^{-1} resulted in maximum mortalities of all the noctuids. They also stated that *S.litura* and *A.janata* were relatively susceptible species than others tested. 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*, 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), sprayed *N. rileyi* spores on III instar larvae of *H. armigera* and observed 100 per cent mortality. 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 susceptible upto 36 per cent only. Faria *et al.* (1993), reported the hundred per cent infection of *N.rileyi* in a population of *Anticarsia gemmatalis* (Hubner) in soybean crop in Federal district, Brazil during 1990-91.

CONCLUSION

The findings of the present study had concluded that *N. rileyi* is pathogenic to castor semilooper, *Paralellia algira* and repeated conidial transfers leads to reduced virulence of the fungus and virulence is regained when it is passed through any host insect.

Table 1 : Mortality of *P. algira* larvae due to subculture-I of *N. rileyi*

Concentration of *N.rileyi* (Spores ml^{-1})	Per cent larval mortality			Mean (% larval mortality)
	*Instar II	*Instar III	*Instar IV	
1 x 10^8	100.00^a (90.00)	97.33^a (84.52)	92.33^a (73.97)	96.55 (82.83)
1 x 10^7	92.66^b (74.39)	94.66^b (76.73)	86.66^b (70.06)	91.33 (73.73)
1 x 10^6	85.33^c (68.05)	79.66^c (63.20)	74.66^c (59.83)	79.88 (63.69)
1 x 10^5	76.66^d (61.14)	74.66^d (59.81)	59.33^d (50.38)	70.22 (57.11)
1 x 10^4	64.33^e (53.35)	56.66^e (48.83)	48.33^e (44.04)	56.44 (48.74)
1 x 10^3	54.33^f (47.49)	41.33^f (40.00)	31.00^f (33.83)	42.22 (40.44)
1 x 10^2	51.33^g (45.76)	32.66^g (34.85)	19.33^g (26.08)	34.44 (35.56)
Untreated control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Mean	65.58 (55.02)	59.62 (50.99)	51.45 (44.77)	58.88 (50.26)

	SEm ±	CD (P=5%)
Instars	0.72	2.05
Concentrations	1.18	3.35
Instar x concentrations	2.04	5.81

Figures in the parentheses are angular transformed values.

*Mean of three replications.

Figures indicated with same alphabet(s) are statistically insignificant

Table 2 : Mortality of *P. algira* larvae due to Subculture-II of *N. rileyi*

Concentration of *N.rileyi* (Spores ml^{-1})	Per cent larval mortality			Mean (% larval mortality)
	*Instar II	*Instar III	*Instar IV	
1 x 10^8	96.33^a (79.90)	92.33^a (74.19)	89.33^a (70.96)	92.66 (75.02)
1 x 10^7	89.33^b (71.37)	83.33^b (65.92)	82.00^b (64.91)	84.88 (67.40)
1 x 10^6	76.33^c (61.108)	73.33^c (58.93)	63.66^c (52.96)	71.11 (57.66)
1 x 10^5	75.66^c (60.48)	66.33^d (54.56)	55.33^d (48.07)	65.77 (54.37)
1 x 10^4	64.33^d (53.34)	51.66^e (45.39)	42.00^e (40.39)	52.66 (46.56)
1 x 10^3	52.66^e (46.53)	40.00^f (39.22)	37.00^f (37.45)	43.22 (41.06)
1 x 10^2	47.00^f (43.27)	15.00^g (22.59)	13.00^g (21.18)	25.00 (28.96)
Untreated control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Mean	62.70 (52.00)	52.75 (45.17)	47.79 (41.99)	54.41 (46.38)

	SEm ±	CD (P=5%)
Instars	0.58	1.65
Concentrations	0.95	2.70
Instars x concentrations	1.64	4.68

Figures in the parentheses are angular transformed values.

* Mean of three replications.

Figures indicated with same alphabet(s) are statistically insignificant

Table 3 : Mortality of *P. algira* larvae due to *N. rileyi*

Concentration of *N.rileyi* (Spores ml^{-1})	Per cent larval mortality			Mean (% larval mortality)
	*Instar II	*Instar III	*Instar IV	
1 x 10^8	100.00^a (90.00)	95.00^a (79.45)	90.00^a (71.57)	95.00 (80.34)
1 x 10^7	91.33^b (72.88)	78.66^b (62.54)	84.00^b (66.46)	84.66 (67.29)
1 x 10^6	84.66^c (67.01)	79.00^b (62.83)	82.33^b (65.69)	82.00 (65.17)
1 x 10^5	75.66^d (60.74)	54.66^c (47.68)	74.33^c (59.59)	68.22 (56.00)
1 x 10^4	58.33^e (49.82)	42.00^d (40.39)	55.33^d (48.07)	51.88 (46.09)
1 x 10^3	45.33^f (42.31)	38.33^e (38.25)	36.33^e (36.96)	40.00 (39.17)
1 x 10^2	34.33^g (35.84)	26.33^f (30.85)	19.66^f (26.31)	26.77 (31.00)
Untreated control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Mean	61.20 (52.33)	51.75 (45.25)	55.25 (46.83)	56.06 (48.13)

	SEm±	CD (P=5%)
Instars	0.71	2.03
Concentrations	1.17	3.32
Instars x concentrations	2.02	5.76

Figures in the parentheses are angular transformed values.

* Mean of three replications.

Figures indicated with same alphabet(s) are statistically insignificant

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[MS received 28 June 2013;
MS accepted 24 August 2013]