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RESEARCH ARTICLE

Evaluation of oil formulations of *Nomuraea rileyi* (Farlow) Samson against *Spodoptera litura* under laboratory conditions

■ **T. SHARMILA, K. MANJULA AND T. MURALI KRISHNA**

SUMMARY

Three oil formulations of entomopathogenic fungus *Nomuraea rileyi* were evaluated for their efficacy along with crude formulation against third instar *Spodoptera litura* during 2011-2012 at the Department of Entomology, S.V. Agricultural College, Tirupati. Mean larval mortalities of 96.67, 93.33, 86.67 and 76.67 per cent, respectively were recorded with groundnut, sunflower, coconut oil based formulations and crude formulation of *N. rileyi* at highest concentration *i.e.,* 1 x 10^8 spores ml^{-1} at 10 DAT. More than 70 per cent larval mortalities of *S. litura* were obtained with the concentrations above 1 x 10^6 spores per ml in case of groundnut oil and sunflower oil based formulations. The mortalities gradually reduced with concentration showing the least at 1 x 10^2 spores ml^{-1}. Incubation period, was in negative correlation with the concentration of *N. rileyi* in all the formulations. Pupal mortalities and malformed adults emergence showed positive association with concentration of *N. rileyi* against *S. litura* larvae.

Key Words : *N. rileyi*, Oil formulation, Lab evaluation, *S. litura*

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Nomuraea rileyi (Farlow) Samson is an important natural control agent of many Lepidopteran caterpillars throughout the world. It occurs in epizootic form in the humid regions and is easily multiplied at a low cost, is obviously a best candidate for exploitation at the field level. Therefore, it has great potential for development into myco-insecticide. An experiment was carried out for evaluating the oil formulations of *N. rileyi S. litura* larvae under laboratory conditions.

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MATERIAL AND METHODS

Mass production of *N. rileyi* was done in rice grain media as explained here. To 30 g of broken rice taken in 250 ml conical flask, 28 ml of 1 per cent yeast extract solution was added and soaked overnight. Next day the rice yeast mixture was autoclaved at 15 psi for 30 minutes and cooled. The cooled conical flasks were inoculated with 2ml of *N. rileyi* spore suspension (10^8 spores ml^{-1}) under aseptic conditions in laminar air flow chamber. After inoculation they were incubated at 25±2°C temperature and around 85 per cent RH for 20 days. After noticing sufficient sporulation, the spores were harvested, dried and powdered. The three oil formulations of *N. rileyi* were prepared separately by mixing @ one gram of conidial powder of *N. rileyi* obtained from broken rice culture (1 x 10^{10} spores g^{-1}) with ten ml of autoclaved and cooled oils containing tween–20 (0.05%). Ten ml of each oil (*i.e.*, sunflower oil or groundnut oil or coconut oil) formulation was mixed separately with 100 ml of distilled water along with tween-20

(0.05%) and thoroughly shaked and filtered through muslin cloth and the spore count was adjusted to 1 x 10^8 spores ml^{-1}. Crude formulation was prepared by mixing one gram of conidial powder of *N. rileyi* obtained from broken rice culture (1 x 10^{10} spores g^{-1}) with 100 ml of distilled water along with tween -20 (0.05%) and thoroughly shaked and filtered through muslin cloth and the spore count was adjusted to 1 x 10^8 spores ml^{-1}.

From this stock suspensions, serial dilutions of 1 x 10^7 to 1 x 10^2 spores ml^{-1} were prepared. All the seven concentrations of each of these three (sunflower, groundnut and coconut) oil based suspensions of *N. rileyi* and *N. rileyi* without oil (crude formulation) were used for infecting laboratory reared second generation third instar *S. litura* larvae by leaf application method with hand automizer. All the treatments were replicated thrice with 10 larvae per replication. An untreated control was also maintained. Daily observations on post treatment changes in larvae, larval mortality, pupal mortality, malformed pupae, and malformed adults were recorded by providing fresh leaves. The larval mortality was converted to percentage before subjecting to statistical analysis by using the formula.

RESULTS AND DISCUSSION

The extent of mortality of third instar larvae of *S. litura* treated with serial dilutions of three different oil formulations of *N. rileyi* and crude formulation of *N. rileyi* are presented in Table 1. The infected larvae died in a characteristic way *i.e.* with slightly raised head and anterior portion of the body by firmly adhering the posterior portion to the substrate *i.e.* food material with the prolegs and mummified due to *N. rileyi* infection. Infected larvae became sluggish and consumed less food. Shrinkage of integument and development of pinkish colouration on it particularly on ventral side was noticed. At death, the larval bodies observed were very smooth.Within few hours of death, the larval body became stiff. After 1-2 days, the dead larvae were covered by a thin coat of white mycelial mat of *N. rileyi* and covered densely in next 24 h. Soon after 2-4 days, cadavers became malachite green in colour due to sporulation of mycelia.

The larval mortalities obtained with oil formulations were significantly higher than the crude formulation of *N. rileyi*. The highest mean larval mortality of 96.67 per cent was recorded with groundnut oil formulation of *N. rileyi* at 1 × 10^8 spores ml^{-1} and thirty per cent with the lowest concentration (1×10^2 spores ml^{-1}) of it (Table 1). In sunflower oil formulation, 93.33 per cent larval mortality was recorded with 1 × 10^8 spores ml^{-1} and 26.67 per cent with 1×10^2 spores ml^{-1}. With coconut oil and crude formulations, 86.67, 76.67 per cent larval mortalities were observed with 1 × 10^8 spores ml^{-1} concentration and 20.00, 13.33 per cent with 1 × 10^2 spores ml^{-1} respectively (Table 1). More than 70 per cent larval mortalities of *S. litura* were obtained with the concentrations above 1 × 10^6 spores ml^{-1} in case of groundnut oil and sunflower oil formulations. The larval mortalities decreased gradually with reduction of concentration in all the cases *i.e.*, the larval mortalities were positively correlated with the concentration of fungus. These results are in agreement with that of Gundannavar (2001) who reported cumulative mortality of larvae of *H. armigera* increased with increase in concentration of spores of *N. rileyi* and exposure period.

The superiority of oil formulations may be due to the reason that oils may form a cover over conidial surface and prevents drying, before actual germination. Hedgecock *et al.* (1995) reported that oil based formulations allow the fungal biopesticides to be applied in a non- desiccated manner which reduces the effects of thermal stress.

The better performance of oil formulations of *N. rileyi* are in conformity with the results of several other workers. Nagaraja (2005) recorded highest cumulative larval mortality of 95 per cent of third instar *S. litura* with oil formulation compared to crude formulation. Similar results were recorded by Vimaladevi *et al.* (2002). They recorded that *N. rileyi* in

Table 1: Efficacy of *N. rileyi* applied as oil and crude formulations against 3rd instar larvae of *S. litura*

Concentration of *N. rileyi* (spores ml^{-1})	Per cent larval mortality				*Mean (% larval mortality)
	*Sunflower oil formulation	*Groundnut oil formulation	*Coconut oil formulation	*Crude formulation	
1 x 10^8	93.33 (75.03)^a	96.67 (79.53)^a	86.67 (68.56)^a	76.67 (61.09)^a	88.33 (71.05)
1 x 10^7	80.00 (64.13)^b	86.67 (68.56)^b	73.33 (58.88)^b	63.33 (52.71)^b	75.83 (61.07)
1 x 10^6	73.33 (58.88)^c	83.33 (65.88)^c	60.00 (50.74)^c	56.67 (48.55)^c	68.33 (56.01)
1 x 10^5	63.33 (52.71)^d	66.67 (54.71)^d	53.33 (46.89)^d	46.67 (43.07)^d	57.50 (49.34)
1 x 10^4	50.00 (44.98)^e	56.67 (48.81)^e	43.33 (41.15)^e	40.00 (39.21)^e	47.50 (43.54)
1 x 10^3	40.00 (39.21)^f	43.33 (41.15)^f	33.33 (35.24)^f	26.67 (31.07)^f	35.83 (36.67)
1 x 10^2	26.67 (31.07)^g	30.00 (33.19)^g	20.00 (26.55)^g	13.33 (21.39)^g	22.5 (28.05)
Untreated control	0.00 (0.00)^h	0.00 (0.00)^h	0.00 (0.00)^h	0.00 (0.00)^h	0.00 (0.00)
Mean	53.33 (45.75)	57.91 (48.98)	46.24 (41.00)	40.41 (37.14)	49.47 (44.69)

S.E.± C.D. (P=0.05) Concentration 0.20 0.57 Formulations 0.14 0.40 Concentration x formulations 0.40 1.15

Figures in the parentheses are angular transformed values. Means in a column followed by same letter (s) are not significantly different (p=0.05)

*Means of three replications

sunflower oil formulation along with triton- x-100 showed 83.9 per cent mortality of 7 to 8 days old *S. litura* larvae. Prior *et al.* (1988) reported higher mortality of grasshopper when *Metarhizium anisopliae* was applied with oil than aqueous formulation. The present results give an indication of better performance of groundnut oil over other two oils to apply *N. rileyi*. Similarly, Ramegowda (2005) stated that among oil formulations of *N. rileyi*, groundnut oil registered highest mortality of 96 per cent followed by sunflower oil and safflower oil against *S. litura* at 1×10^8 spores ml^{-1}.

Incubation period :

The incubation periods were varying with different spore concentrations of *N. rileyi.* Lowest incubation period of 4 days was recorded with 1×10^8 and 1×10^7 spores ml^{-1} concentration of groundnut oil formulation and 1×10^8 spores ml^{-1} concentration of sunflower oil (Table 2). Higher incubation period recorded was 7.5 days with 1×10^3 and 1×10^2 spores ml^{-1} concentrations of crude formulation (Table 2).

Number of conidia survived, germinated and penetrated into the integument per unit area may be high in case of oil formulations than crude suspensions. When the ramifications occurs fast inside the body, death also occurs in less time. Lower incubation periods were recorded at higher concentrations and higher at lower concentrations. Siva Sankaran *et al.* (1998) reported longer incubation periods with lower dosages of *N.rileyi* incase of *S. litura*. SivaSankaran *et al.* (1990) also reported similar results with *B. bassiana* against *Chilo infuscatellus*. Similarly, Lopez and Boucias (1994) reported that with increase in concentrations of *N. rileyi* against *S. exigua* from 5×10^2 to 5×10^4 hyphal bodies per insect, a decrease of incubation period was noticed. Babi Neeraja (2008) also recorded negative relationship between incubation period and concentration of *N. rileyi* when she treated *S. litura* with different concentration of *N. rileyi*.

Pupal mortalities :

The mortalities of pupae were higher at higher concentrations. Pupal mortalities were observed to be in reduced with reduction in concentration. Almost nil mortalities were recorded with lowest two concentrations. Pupal mortalities with different concentrations in each formulation were significantly different. Results on pupal mortalities are in agreement with Ramakrishnan *et al.* (1983) who observed enhanced mortality of *S. litura* pupae with an increase in concentration of *B. bassiana*. Similarly, higher pupal mortalities were recorded by Kamala Jayanthi and Padmavathamma (1996) when *B. bassiana* was applied to *S. litura* larvae. Lalitha (2007) reported that the pupal mortality increases with increase in concentration of *N.rileyi* when applied to *S.litura*.

Table 2 : Incubation period, pupal mortalities and malformed adults recorded when formulations of *N.rileyi* were treated to 3rd instar *S.litura*

Conc. of *N. rileyi* (spores ml^{-1})	Incubation period (days)				Pupal mortalities (%)				Malformed pupae (%)			
	SOF	GOF	COF	CF	SOF	GOF	COF	CF	SOF	GOF	COF	CF
1×10^8	4	4	5	6	66.20 (54.45)^a	100.00 (90.00)^a	54.40 (47.52)^a	58.00 (49.60)^a	33.80 (35.54)^a	0.00 (0.00)^g	45.60 (42.47)^a	42.00 (40.39)^a
1×10^7	4.5	4	5	6	51.44 (45.82)^b	53.32 (46.90)^b	19.60 (26.27)^b	27.27 (31.47)^b	13.20 (21.29)^b	46.68 (43.09)^a	32.56 (34.79)^b	21.83 (27.85)^b
1×10^6	5	5	5	6.5	32.50 (34.65)^c	33.32 (35.25)^c	16.33 (23.82)^c	18.47 (25.45)^c	12.50 (20.69)^b	34.61 (37.27)^b	26.00 (30.65)^c	20.77 (27.10)^b
1×10^5	5	5	6	6.5	15.40 (23.09)^d	21.00 (27.27)^d	12.39 (20.59)^d	7.20 (15.54)^d	8.60 (17.03)^c	33.21 (36.03)^c	23.33 (28.87)^d	7.60 (16.00)^c
1×10^4	5.5	5	6	7	8.60 (17.03)^e	18.46 (25.44)^e	8.56 (16.99)^e	2.80 (9.52)^e	3.30 (10.38)^d	21.17 (35.25)^d	16.39 (23.87)^e	0.00 (0.00)^d
1×10^3	6	6	6.5	7.5	3.33 (10.43)^f	10.58 (18.96)^f	0.00 (0.00)^f	0.00 (0.00)^f	0.00 (0.00)^e	21.00 (27.39)^e	0.00 (0.00)^f	0.00 (0.00)^d
1×10^2	6.5	6	6.5	7.5	0.00 (0.00)^g	0.00 (0.00)^g	0.00 (0.00)^f	0.00 (0.00)^f	0.00 (0.00)^e	14.28 (22.19)^f	0.00 (0.00)^f	0.00 (0.00)^d
Untreated control					0.00 (0.00)^g	0.00 (0.00)^g	0.00 (0.00)^f	0.00 (0.00)^f	0.00 (0.00)^e	0.00 (0.00)^g	0.00 (0.00)^f	0.00 (0.00)^d
S.E.±					0.54	0.37	0.42	0.56	0.55	0.36	0.34	0.33
C.D. (P=0.05)					1.67	1.16	1.29	1.72	1.70	1.12	1.06	1.03

SOF = Sunflower oil formulation GOF = Groundnut oil formulation COF = Coconut oil formulation CF= Crude formulation
All the values are means of three replications Figures in parentheses are angular transformed values.
Means in the column followed by same letter(s) are not significantly different (P = 0.05)

Malformed adults :

The higher malformed adult emergence of 46.68 per cent was recorded at 1×10^7 sporesml^{-1} concentration with groundnut oil formulation. Malformations of 33.80, 45.60 and 42.00 per cent was recorded at higher concentration *i.e.*, 1×10^8 spores ml^{-1} with sunflower oil, coconut oil and crude formulations of *N. rileyi*, respectively (Table 2). Adult malformation decreased with lowering of concentration. There was absolutely zero per cent malformed adults at concentrations below 1×10^3 spores ml^{-1} in sunflower and coconut oil formulations and below 1×10^4 spores ml^{-1} in crude formulation. Babi Neeraja (2008) recorded positive association of malformed adults of *S. litura* with concentration of *N. rileyi*. Kamala Jayanthi and Padmavathamma (1996) recorded higher malformed adult emergence and lower emergence of normal adults when *S. litura* larvae were treated with *B. bassiana*.

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