



Development and Evaluation of Different Formulations of *Nomuraea rileyi* (Farlow) Samson against *Spodoptera litura* (Fabricius)

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Authors' contributions

This work was carried out in collaboration among all authors. Authors Rashmi S. H. and Ramanagouda S. H. were major contributor in research work and writing the manuscript. Author Rashmi S. H. did all experiments. All authors read and approved the final manuscript.

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ABSTRACT

The 26 different formulations of *Nomuraea rileyi* (Farlow) Samson were developed and tested against third instar larvae of *Spodoptera litura* (Fabricius). The experiment was conducted at Bio control laboratory, Department of Entomology, Kittur Rani Chanamma College of Horticulture (KRCCH), Arabhavi. Among all formulations tested, the groundnut oil and combination of groundnut oil + rice grain formulations recorded cent per cent mortality of *S. litura* at seven days of treatment. The percent mean mortality was 83.33, 80.00, 73.33 and 73.33 per cent in groundnut oil,

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combination of groundnut oil + rice grain, mustard oil + rice grain and groundnut oil + wheat grain, respectively.

Keywords: *Nomuraea rileyi*; *Spodoptera litura*; formulations; spore viability.

1. INTRODUCTION

The tobacco leaf eating caterpillar, *Spodoptera litura* (Fabricius), (Lepidoptera: Noctuidae) is a polyphagous sporadic pest with high mobility and reproductive capacity. *S. litura* devastates a large host range of more than 120 host plants. The major ones include tobacco, cotton, groundnut, jute, maize, rice, cauliflower, cabbage, capsicum and castor. Biopesticides used for pest management are environmentally safe, selective, specific in their action and easily biodegradable. They can be used in combination with other control measures in integrated pest management programs. As the days passed, slowly the chemical pesticides have become less attractive to the producers due to the increased input cost, development of insecticide resistance, concerns about human health and deleterious effect on nontarget organisms [1].

The pathogenicity of fungi towards insects has been mainly attributed to various hydrolytic enzymes, such as chitinases, proteases and lipases. Among the entomopathogenic fungi, *Nomuraea rileyi* (Farlow) Samson seems to be promising because of its widespread occurrence and relative abundance due to its wide host range which includes all the major caterpillar pests [2]. The formulation of fungi still awaits a serious effort in formulation technology. Efforts with entomopathogenic fungi tend to be concentrated on conidial formulation [3].

Recently, many efforts have been made to formulate the *N. rileyi* commercially for the farmers use however, maintaining the spore viability for longer period of time is a challenging issue. Therefore, the present work was carried out on development and evaluation of formulations of *N. rileyi* for the management of *S. litura*.

2. MATERIALS AND METHODS

2.1 Rearing of *Spodoptera litura*

The field population of the test insect, the cabbage leaf eating caterpillar, *Spodoptera litura* was collected from vegetable fields of KRC College of Horticulture, Arabhavi and nearby

vegetable fields of farmers. The culture of the test insect was maintained on castor, *Ricinus communis* leaves under laboratory conditions in plastic tubs of size 45 cm diameter. The larvae of third instar were taken from the culture, as and when required [4].

2.2 Maintenance of Pure Culture of *Nomuraea rileyi*

The 15 different strains of *N. rileyi* were isolated from soil samples collected from different regions of Belagavi district under DST Project and most virulent strain (NR-7) was identified. Further, the NR-7 was used for present study. The pure fungus culture of NR-7 was maintained by sub culturing on Sauboured maltose agar medium and the virulence of *N. rileyi* was retained by inoculating on its natural host (*S. litura*) and further isolated from the infected *S. litura* by series of treatment viz., washing the infected *S. litura* with sodium hypochlorite solution (2 %) followed by alcohol (70 %) and distilled water. Later the cadavers of *S. litura* were placed in the Petri plates (10 cm) containing Sabouraud maltose agar medium and incubated for 15 days at room temperature for fungal growth. The pure culture was isolated and maintained under refrigerated conditions to carry out various experiments [1 and 4].

2.3 Mass Multiplication of *Nomuraea rileyi*

The multiplication of *N. rileyi* was undertaken in Sauboured Maltose Broth (SMB). The sterilized SMB media was inoculated with pure culture of *N. rileyi* under laminar air flow chamber (LFC) and incubated at room temperature ($28 \pm 1^\circ\text{C}$) for 15 days. After complete growth of *N. rileyi* on broth medium, it was used for the development of oil-based formulations of the *N. rileyi*.

2.4 Development of Different Formulation

2.4.1 Preparation of oil formulations of *N. rileyi*

The oil formulations were prepared by following the method of previously reported procedure with slight modifications (Bhanu Prakash et al., 2015). The different edible oils were sterilized at 121°C

for 15 minutes. They were later cooled under room temperature and utilized for the preparation of oil formulation of *N. rileyi*. Six oil formulations of *N. rileyi* were prepared separately by mixing 20 ml spore suspension of *N. rileyi* with 10 ml of autoclaved cooled oils with tween 20 (0.05 %) in one liter of sterilized distilled water and filtered through muslin cloth (Table 1).

2.4.2 Preparation of powder formulations of *N. rileyi*

Five powder formulations of *N. rileyi* were developed by mixing the spore powder of *N. rileyi* obtained from different grains (rice, bajra, wheat, sorghum and ragi) with talc powder in the ratio of 1:1. Later combination of both spore and talc powder were grinded for 30 seconds to make it as fine dust. This method was followed as per the previously reported procedure of Sharmila et al. [5] (Table 1).

2.4.3 Preparation of combination of oil and powder formulations of *N. rileyi*

About 15 combinations of oil and spore powder of *N. rileyi* were prepared by mixing different oils, spore dust and spore suspension in a proportion of 10:10:20 with tween 20 (0.05 %) in 1 liter of sterilized distilled water and finally filtered through muslin cloth. The method for the

preparing combination formulations of *N. rileyi* was followed as per the reported procedure by Patil and Jadhav [6] (Table 1).

2.4.4 Evaluation of developed formulations of *N. rileyi* against *S. litura* under laboratory condition

A total of 26 different formulations of *N. rileyi* were developed which included six oils, five powder and 15 combination formulations (Table 1). All the developed formulations of *N. rileyi* and control (distilled water treatment) were evaluated against *S. litura* under laboratory conditions for their efficacy.

2.5 Bio Assay Study

The third instar larvae of *S. litura* were treated with formulations of *N. rileyi* by larval dip (2 seconds) method and released on castor leaf placed on petriplate containing a thin layer of one per cent agar-agar media. Petri dishes were covered with the muslin cloth and maintained at room temperature of $27.0 \pm 1.0^\circ\text{C}$ and the relative humidity of 70.0 ± 5.0 per cent. Another ten third instar larvae of *S. litura* were released on castor leaves as control and treated with double distilled sterilized water. Each treatment was replicated twice with ten larvae per replication. This procedure followed as previously reported bioassay study by Al-Keridis [7] and Sowmya et al.[4].

Table 1. Formulations of *Nomuraea rileyi* evaluated against *Spodoptera litura* under laboratory conditions

Tr. No.	Formulations	Tr. No.	Formulations
T ₁	Ground nut oil formulation (GNO)	T ₁₄	Combination of GNO + BJJ
T ₂	Sunflower oil formulation (SFO)	T ₁₅	Combination of GNO + WHG
T ₃	Coconut oil formulation (CNO)	T ₁₆	Combination of GNO + SRG
T ₄	Soybean oil formulation (SYO)	T ₁₇	Combination of MSO + RCG
T ₅	Mustard oil formulation (MSO)	T ₁₈	Combination of MSO + RGG
T ₆	Sesamum oil formulation (SEO)	T ₁₉	Combination of MSO + BJJ
T ₇	Rice grain formulation (RCG)	T ₂₀	Combination of MSO + WHG
T ₈	Ragi grain formulation (RGG)	T ₂₁	Combination of MSO + SRG
T ₉	Bajra grain formulation (BJG)	T ₂₂	Combination of SEO + RCG
T ₁₀	Wheat grain formulation (WHG)	T ₂₃	Combination of SEO + RGG
T ₁₁	Sorghum grain formulation (SRG)	T ₂₄	Combination of SEO + BJJ
T ₁₂	Combination of GNO + RCG	T ₂₅	Combination of SEO + WHG
T ₁₃	Combination of GNO + RGG	T ₂₆	Combination of SEO + SRG
T ₂₇ : Control			

(The abbreviations for different oils and grains were indicated by three letters instead of two letters to avoid the confusion among the different formulations. Ex. Rice grain and ragi grain, Sunflower oil and sesumum oil)

2.6 Observations Recorded

The mortality of *S. litura* was recorded after three, five and seven days after treatment (DAT). The moribund larvae were counted as dead and percent larval mortality was worked out by using following formula.

$$\text{Larval mortality (\%)} = \frac{\text{Number of moribund and dead larvae}}{\text{Total number of larvae}} \times 100$$

2.7 Statistical Analysis

The data on cumulative mortality rate was transformed by arcsine and analysed by ANOVA of a completely randomized design. The data on the larval mortality was subjected to arc sin transformation and means were compared at $p \leq 0.01$ CD using Duncan's multiple range test (DMRT). Web Based Agricultural Statistics Software Package (WASP v. 2.0) was used for all the statistical analysis.

3. RESULTS AND DISCUSSION

3.1 Evaluation of Different Formulations of *N. rileyi* against *S. litura*

The significant highest (83.33 %) mean mortality of *S. litura* was recorded in groundnut oil formulation. The combination of groundnut oil + rice grain formulation recorded 80.00 per cent of mean mortality of *S. litura* followed by combination of groundnut oil + wheat grain, mustard oil + rice grain (73.33 %), combination of mustard oil + ragi grain, mustard oil + wheat grain (70.00 %) mean mortality of *S. litura*. All the formulations exhibited significantly higher mean mortality of *S. litura* than the untreated

control (6.66 %) (Table 2, Fig. 1).

The higher efficacy of oil formulations was mainly due to prevention of the desiccation of the conidia which helped in longer survival period and better penetration of peg into the integument as reported by Burges [8]. This was also true with the present finding. All the developed formulations (26) of *N. rileyi* tested under laboratory condition given the good results, since controlled climatic condition with low temperature and high humidity, in addition to this, local isolate (NR-7) used for the development of formulations of *N. rileyi* favored for their higher efficacy. Sharmila et al. [5] recorded *S. litura* larval mortalities of 96.67, 93.33, 86.67 and 76.67 per cent in groundnut oil, sunflower oil, coconut oil and crude formulations of *N. rileyi* (1×10^8 cfu/ml), respectively at 10 days after treatment.

In present findings, among the different grain formulations of *N. rileyi* tested, the formulation of rice grain recorded significantly highest mortality of *S. litura* larvae followed by wheat grain. The similar findings were also obtained by earlier workers in other insects wherein, different grains tested, the spores multiplied on rice and sorghum grains recorded maximum mortality of mealy bug (96.00 %) after nine days of treatment [9]. Patil and Padhye [10] have revealed that, the adjuvants viz., sunflower oil, glycerol and honey were effective in reducing the adverse effect of UVC rays on *N. rileyi* compared to other adjuvants tested.

Table 2. Efficiency of different formulations of *Nomuraea rileyi* (2×10^8 cfu/ml) against *Spodoptera litura*

Treatments	Cumulative larval mortality (%) of <i>Spodoptera litura</i>			
	3 DAT	5 DAT	7 DAT	Mean
T ₁ -Ground nut oil formulation (GNO)	60.00 (50.77) ^a	90.00 (71.57) ^a	100.00 (89.71) ^a	83.33 (65.70) ^a
T ₂ -Sun flower oil formulation (SFO)	40.00 (39.23) ^c	60.00 (50.77) ^d	70.00 (56.79) ^d	56.66 (49.02) ^g
T ₃ -Coconut oil formulation (CNO)	40.00 (39.23) ^c	70.00 (56.79) ^c	70.00 (56.79) ^d	60.00 (50.77) ^f
T ₄ - Soybean oil formulation (SYO)	50.00 (45.00) ^b	60.00 (50.77) ^d	60.00 (50.77) ^e	56.66 (49.02) ^g
T ₅ - Mustard oil formulation (MSO)	40.00 (39.23) ^c	60.00 (50.77) ^d	80.00 (63.44) ^c	60.00 (50.77) ^f
T ₆ - Sesamum oil formulation (SEO)	40.00 (39.23) ^c	70.00 (56.79) ^c	70.00 (56.79) ^d	60.00 (50.77) ^f

Treatments	Cumulative larval mortality (%) of <i>Spodoptera litura</i>			
	3 DAT	5 DAT	7 DAT	Mean
T ₇ -Rice grain formulation (RCG)	30.00 (33.21) ^d	70.00 (56.79) ^c	70.00 (56.79) ^d	56.66 (49.02) ^g
T ₈ -Ragi grain formulation (RGG)	20.00 (26.57) ^e	50.00 (45.00) ^e	50.00 (45.00) ^f	40.00 (39.23) ^j
T ₉ -Bajra grain formulation (BJG)	20.00 (26.57) ^e	50.00 (45.00) ^e	50.00 (45.00) ^f	40.00 (39.23) ^j
T ₁₀ -Wheat grain formulation (WHG)	10.00 (18.44) ^f	60.00 (50.77) ^d	60.00 (50.77) ^e	43.33 (40.98) ^h
T ₁₁ - Sorghum grain formulation (SRG)	10.00 (18.44) ^f	50.00 (45.00) ^e	60.00 (50.77) ^e	40.00 (39.23) ^j
T ₁₂ - Combination of GNO+RCG	60.00 (50.77) ^a	80.00 (63.44) ^b	100.00 (89.71) ^a	80.00 (63.44) ^b
T ₁₃ -Combination of GNO+RGG	50.00 (45.00) ^b	70.00 (56.79) ^c	70.00 (56.79) ^d	63.33 (52.54) ^e
T ₁₄ - Combination of GNO+BJG	40.00 (39.23) ^c	70.00 (56.79) ^c	70.00 (56.79) ^d	60.00 (50.77) ^f
T ₁₅ -Combination of GNO+WHG	50.00 (45.00) ^b	80.00 (63.44) ^b	90.00 (71.57) ^b	73.33 (58.69) ^c
T ₁₆ -Combination of GNO+SRG	40.00 (39.23) ^c	60.00 (50.77) ^d	70.00 (56.79) ^d	56.66 (49.02) ^g
T ₁₇ - Combination of MSO+RCG	50.00 (45.00) ^b	80.00 (63.44) ^b	90.00 (71.57) ^b	73.33 (58.69) ^c
T ₁₈ -Combination of MSO+RGG	50.00 (45.00) ^b	70.00 (56.79) ^c	90.00 (71.57) ^b	70.00 (56.79) ^d
T ₁₉ -Combination of MSO+BJG	40.00 (39.23) ^c	60.00 (50.77) ^d	70.00 (56.79) ^d	56.66 (49.02) ^g
T ₂₀ - Combination of MSO+WHG	50.00 (45.00) ^b	70.00 (56.79) ^c	90.00 (71.57) ^b	70.00 (56.79) ^d
T ₂₁ -Combination of MSO+SRG	50.00 (45.00) ^b	60.00 (50.77) ^d	80.00 (63.44) ^c	63.33 (52.54) ^e
T ₂₂ -Combination of SEO +RCG	30.00 (33.21) ^d	60.00 (50.77) ^d	70.00 (56.79) ^d	53.33 (46.72) ⁱ
T ₂₃ -Combination of SEO +RGG	30.00 (33.21) ^d	70.00 (56.79) ^c	80.00 (63.44) ^c	60.00 (50.77) ^f
T ₂₄ -Combination of SEO +BJG	40.00 (39.23) ^c	70.00 (56.79) ^c	80.00 (63.44) ^c	63.33 (52.54) ^e
T ₂₅ -Combination of SEO +WHG	30.00 (33.21) ^c	80.00 (63.44) ^b	80.00 (63.44) ^c	63.33 (52.54) ^e
T ₂₆ -Combination of SEO +SRG	40.00 (39.10) ^c	70.00 (56.79) ^c	80.00 (63.44) ^c	63.33 (52.54) ^e
T ₂₇ - Control	0.00 (0.29) ^g	10.00 (17.85) ^f	10.00 (17.85) ^g	6.66 (15.34) ^k
S. Em ±	1.13	0.95	0.95	0.31
C. D. at 1%	4.45	3.72	3.72	0.96
CV (%)	4.36	2.50	2.24	0.89

DAT-Days after treatment; Figures in the parenthesis are Arc sin transformed values In a column, means followed by same alphabet (s) do not differ significantly by DMRT (P=0.01)

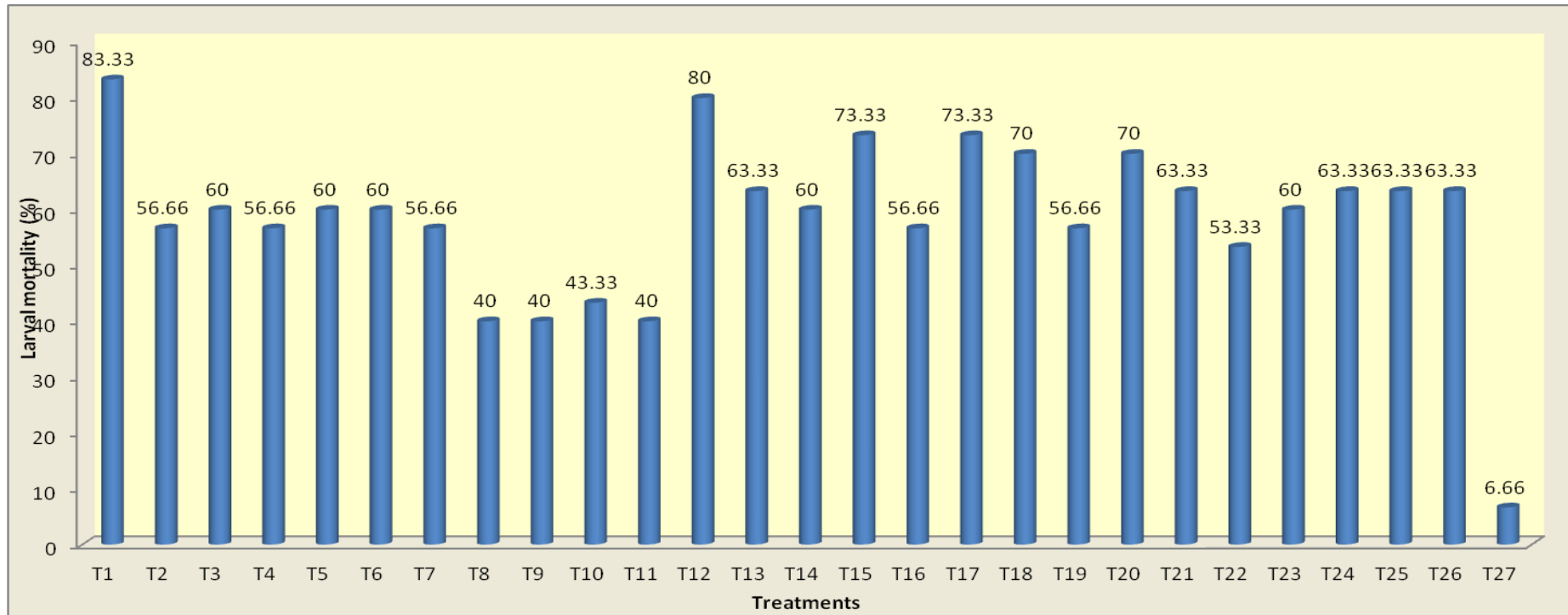


Fig. 1. Efficacy of different formulations of *Nomuraea rileyi* against *Spodoptera litura*

T₁-Groundnut oil formulation (GNO)
 T₂-Sun flower oil formulation (SFO)
 T₃-Coconut oil formulation (CNO)
 T₄- Soybean oil formulation (SYO)
 T₅- Mustard oil formulation (MSO)
 T₆- Sesamum oil formulation (SEO)
 T₇-Rice grain formulation (RCG)

T₈-Ragi grain formulation (RGG)
 T₉-Bajra grain formulation (BJG)
 T₁₀-Wheat grain formulation (WHG)
 T₁₁- Sorghum grain formulation (SRG)
 T₁₂- Combination of GNO+RCG
 T₁₃-Combination of GNO+RGG
 T₁₄- Combination of GNO+BJG

T₁₅-Combination of GNO+WHG
 T₁₆-Combination of GNO+SRG
 T₁₇- Combination of MSO+RCG
 T₁₈-Combination of MSO+RGG
 T₁₉-Combination of MSO+BJG
 T₂₀- Combination of MSO+WHG
 T₂₁-Combination of MSO+SRG

T₂₂-Combination of SEO +RCG
 T₂₃-Combination of SEO +RGG
 T₂₄-Combination of SEO +BJG
 T₂₅-Combination of SEO +WHG
 T₂₆-Combination of SEO +SRG
 T₂₇-Distilled water as control

4. CONCLUSION

The developed efficient formulation of *N. releyi* using vegetable oils and grain media showed positive response on growth, development of *N. releyi* and increased mortality of *S. litura*. Incorporation of different edible oils as adjuvant, improves the efficacy of formulations and the virulence of the fungi. These formulations are best stored under lower temperature to increase the shelf life of developed formulations and these formulations should be used well before three months of their mass production. The developed efficient formulation (groundnut oil) can be used for the management of *S. litura* after testing under field conditions. Finally, these formulations could be included in the Integrated Pest Management (IPM) programmes for managing *S. litura* to reduce the insecticide residue and cost of cultivation.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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