Development and evaluationofdifferentformulationsof*Nomuraearileyi*(Farlow)Samsonagainst*Spodopteralitura*(Fabricius)

Abstract: The 26 different formulations of *Nomuraearileyi*(Farlow) Samson were developed and tested against third instar larvae of *Spodopteralitura*(Fabricius). The experiment was conducted at Bio control laboratory, Department of Entomology, Kittur Rani Chanamma College of Horticulture (KRCCH), Arabhavi. Among all formulations tested, thegroundnutoilandcombinationofgroundnutoil+ricegrainformulationsrecordedcent per cent mortality of *S. litura*at seven days of treatment. The per cent mean mortality was 83.33, 80.00, 73.33 and 73.33 per cent in groundnut oil, combination of groundnut oil + rice grain, mustard oil +ricegrainandgroundnut oil +wheat grain, respectively.

Keywords:*Nomuraearileyi*,*Spodopteralitura*,Formulations, Spore viability

1. Introduction

The tobacco leaf eating caterpillar, *Spodopteralitura*(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 andcastor.Biopesticidesusedforpestmanagementareenvironmentallysafe,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 (Gokak *et al*., 2017)

The pathogenicity of fungi towards insects has been mainly attributed to various hydrolytic enzymes, such as chitinases, proteases and lipases. Among the entomopathogenic fungi, *Nomuraearileyi*(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 (Ignoffo, 1981). The formulation of fungi still awaits a serious effort in formulation technology. Efforts with entomopathogenic fungi tend to be concentrated on conidial formulation (Pereira and Roberts, 1990).

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Recently, many efforts have been made to formulate the *N. rileyi*commerciallyforthefarmersusehowever,maintainingthesporeviabilityfor 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. MaterialandMethods

Rearing of *Spodopteralitura*

The field population of the test insect, the cabbage leaf eating caterpiller, *Spodopteralitura* 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 (Sowmya *et al.,* 2017)

Maintenance of pure culture of *Nomuraearileyi*

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-7was 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 (Gokak *et al.,* 2017 and Sowmya *et al.,* 2017).

Mass multiplication of *Nomuraearileyi*

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 ± 1˚C) for15 days. After complete growth of *N. rileyi* on broth medium, it was used for the development of oil-based formulations of the *N. rileyi*.

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Development of different formulation

Preparationofoilformulationsof*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 oilswere sterilized at 121°C for 15 minutes. They were later cooled under room temperature and utilizedforthepreparationofoilformulationof*N.rileyi*.Sixoilformulationsof*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).

Preparationofpowderformulationsof*N. rileyi*

Fivepowderformulationsof*N.rileyi*weredevelopedbymixingthesporepowderof*N.rileyi*obtainedfromdifferentgrains(rice,bajra,wheat,sorghumandragi)withtalc powder in the ratio of 1:1. Later combination of both spore and talc powder were grinded for30 seconds to make it as fine dust. This method was followed as per the previously reported procedure of Sharmila *et al.* (2015) (Table 1).

Preparationofcombinationofoilandpowderformulationsof*N. rileyi*

About 15 combinations of oil and spore powder of *N. rileyi*were prepared by mixing differentoils,sporedustandsporesuspensioninaproportionof10:10:20withtween20 (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 thereported procedure by Patil and Jadhav (2016) (Table 1).

Evaluationofdevelopedformulationsof*N. rileyi*against*S.litura*underlaboratorycondition

Atotalof26differentformulationsof*N.rileyi*weredevelopedwhichincludedsix oils,fivepowderand15combinationformulations(Table1).Allthedevelopedformulations of *N. rileyi*and control (distilled water treatment) were evaluated against *S. litura*under laboratory conditions for their efficacy.

Bioassaystudy

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 layerof one per cent agar-agar media. Petri dishes were covered with the muslin cloth and maintained at room temperature of 27.0 ± 1.0°C

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andtherelativehumidityof70.0±5.0percent.Anothertenthirdinstarlarvaeof*S. litura*were released on castor leaves as control and treated with double distilled sterilized water. Each treatment was replicatedtwice with ten larvae per replication. This procedure followedas previouslyreported bioassay study by Al-Keridis (2016) and Sowmya*et al.* (2017).

Observationsrecorded

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

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

Statistical analysis

The data on cumulative mortality rate was transformed by arcsine and analysed by ANOVA of a completely randomizes design. The data on the larval mortality was subjected to arcsin transformation and means were compared at p ≤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. Resultsanddiscussion

Evaluation of different formulations of *N. rileyi*against *S.litura*

The significant highest (83.33 %) mean mortality of *S. litura*was recorded in groundnutoilformulation.Thecombinationofgroundnutoil+ricegrainformulation recorded 80.00 per cent of mean mortality of *S. litura*followed by combination of groundnutoil + wheat grain, mustard oil + rice grain (73.33 %), combination of mustard oil + ragi grain, mustardoil+wheatgrain(70.00%)meanmortalityof*S.litura*.Alltheformulations exhibited significantly higher mean mortality of *S. litura*than the untreated control (6.66 %) (Table 2, Fig.1).

Thehigherefficacyofoilformulationswasmainlydue topreventionofthe desiccationoftheconidiawhichhelpedinlongersurvivalperiodandbetterpenetrationof peg into the integument as reported by Burges (1998). This was also true with the present finding. All the developed formulations (26) of *N. rileyi*tested under laboratory conditiongiventhegoodresults,sincecontrolledclimaticconditionwithlowtemperatureandhighhumidity,inadditiontothis,localisolate(NR-7)usedforthedevelopmentofformulationsof*N.*

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*rileyi*favored for their higher efficacy. Sharmila *et al.* (2015) recorded *S. litura*larval mortalities of 96.67, 93.33, 86.67 and 76.67 per cent in groundnut oil, sunflower oil, coconutoilandcrudeformulationsof*N.rileyi*(1Ẋ10^8cfu/ml),respectivelyat10daysafter 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 followedby 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 (Banu, 2013). Patil and Padhye (2015) 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.

4. Conclusion

The developed efficient formulationof *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 temperatureto 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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Table 1: Formulations of *Nomuraearileyi* evaluated against *Spodopteralitura*underlaboratory conditions

Tr. No.	Formulations	Tr. No.	Formulations
T_1	Ground nut oil formulation (GNO)	T_{14}	Combination of GNO + BJG
T_2	Sunflower oil formulation (SFO)	T_{15}	Combination of GNO + WHG

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T_3	Coconut oil formulation (CNO)	T_{16}	Combination of GNO + SRG
T_4	Soybeanoil 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 + BJG
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 + BJG
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.)

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Table 2:Efficiency of different formulations of *Nomuraearileyi*(2 X 10^8cfu/ml) against *Spodopteralitura*

Treatments	Cumulative larval mortality (%) of *Spodopteralitura*			
	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}
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)

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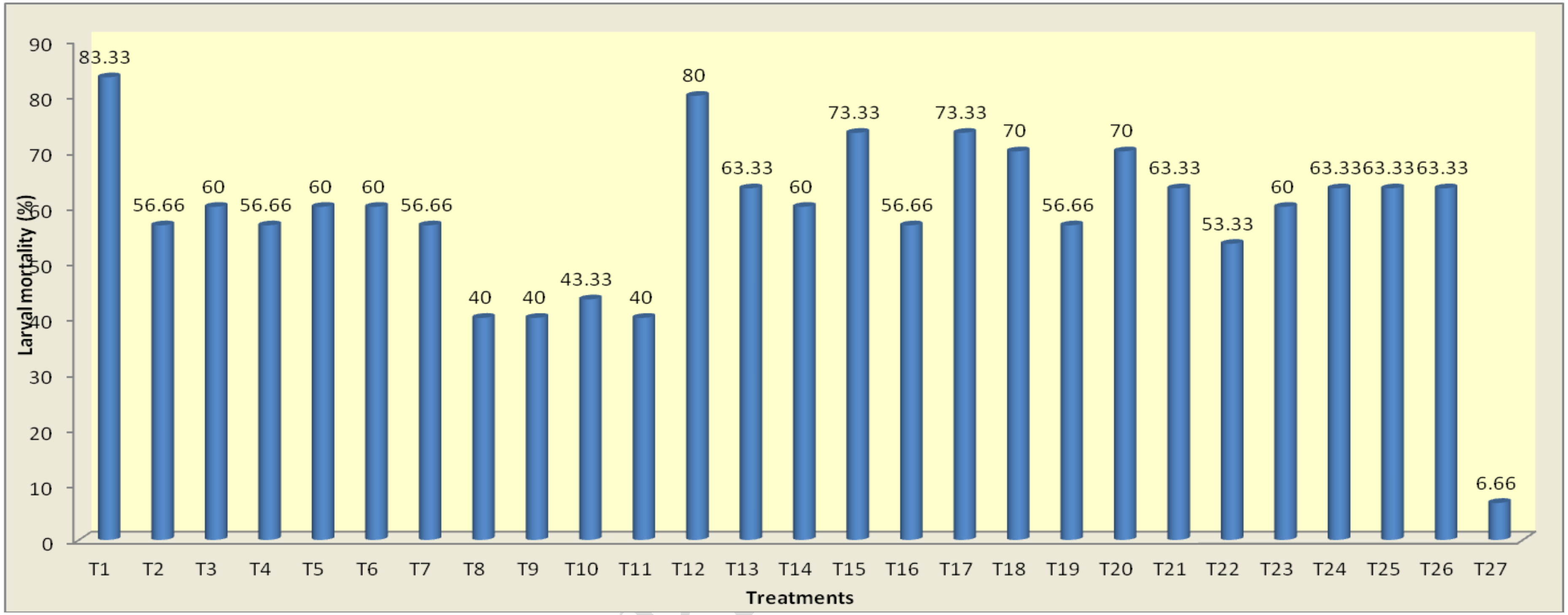

Fig. 1: Efficacy of different formulations of *Nomuraearileyi*against *Spodopteralitura*

T_1-Groundnut oil formulation (GNO)
T_2 -Sun flower oil formulation (SFO)
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T_{16} -Combination of GNO+SRG
T_{17}- Combination of MSO+RCG
T_{18} -Combination of MSO+RGG
T_{19} -Combination of MSO+BJG
T_{20} -Combination of MSO+WHG
T_{21} -Combination of MSO+SRG
T_{22} -Combination of SEO +RCG
T_{23} -Combination of SEO +RGG
T_{24} -Combination of SEO +BJG
T_{25} -Combination of SEO +WHG
T_{26} -Combination of SEO +SRG
T_{27} -Distilled water as control