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Field evaluation of oil formulations of *Nomuraea rileyi* (Farlow) Samson against *Spodoptera litura* and *Helicoverpa armigera* in groundnut

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ABSTRACT

Nomuraea rileyi (Farlow) Samson is a potential entomopathogenic fungus against lepidopteran pests. In groundnut crop ecosystem, along with three oil formulations (sunflower, coconut and groundnut), two Wettable powder (corn flour and talc) and one crude formulation were evaluated against *Spodoptera litura* and *Helicoverpa armigera* during 2011-2012 at dry land farm, S.V. Agricultural College, Tirupati. The results indicated that groundnut oil based formulation of *N. rileyi* recorded significantly higher reduction of both *S. litura* (82.46%) and *H. armigera* (74.58%) larval population. The groundnut oil based formulation also recorded significantly lower per cent leaf damage by both *S. litura* (14.27%) and *H. armigera* (13.27%). However sunflower oil and coconut oil formulations also proved better than WP and crude formulations. Crude formulation was also found to be effective when compared to WP formulations in reducing the larval population, in recording lower per cent leaves damaged at 15 days after treatment against both pests.

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INTRODUCTION

The cultivated groundnut (*Arachis hypogaea* L.) is an important oilseed crop of tropical and subtropical areas of the world. In India, the crop occupies an area of 8.61 lakh ha with a production of 15.76 lakh tonnes and productivity of 1960 kg ha^{-1} during *Rabi* (Indiastat.com, 2009-2010) season. Andhra Pradesh ranks first among major groundnut producing states of India, occupying an area of 2.9 lakh hectare, with total production of 6.17 lakh tonnes and productivity of 2317 kg ha^{-1} (Indiastat.com, 2009-2010). Insect pests and diseases are the major constraints for groundnut production. Among the insects, defoliators like *Spodoptera litura* Fabricius, *Helicoverpa armigera* Hubner, *Aproaerema modicella* Devanter and the sucking pests *viz.*, thrips, leaf hoppers, aphids are equally important yield reducing pests. *S. litura* and *H. armigera* have become major pests and creating serious threat to agricultural industry due to the development of resistance towards several commonly used insecticides (Ramakrishnan *et al.*, 1983; Ramegowda, 1999). As indiscriminate use of chemical pesticides has witnessed the alarming situation in present agro-ecosystem creating health hazards to the extent of human genetic level and this resulted in increased interest towards biological control in recent years. Among the competent pathogen genera *Nomuraea rileyi* (Farlow) Samson (Moniliales: Deuteromycetes) is one candidate fungus which was to cause spectacular epizootics under favourable environmental conditions on lepidopteran larvae. In Andhra Pradesh, regular occurrence of *Nomuraea rileyi* (Farlow) is being recorded from Guntur district on *H. armigera* and *S. litura* particularly in Groundnut and Cotton crops. This fungus failed to form epizootics under low relative humidity with higher temperature conditions. Application of *N. rileyi* as oil formulation may be a possible effort for suppression of caterpillars in the areas of occasional occurrence of epizootics. Suitable formulation help in uniform distribution of conidia on the foliage as well as on insect body.

MATERIAL AND METHODS

An experiment was conducted to evaluate oil formulations of *N. rileyi* against *S. litura*, *H. armigera* (Hubner) in groundnut crop along with WP and crude formulations, during *Rabi* season of 2011-2012 at dry land farm, S.V. Agricultural College, Tirupati. A Randomized Block Design was laid with three replications of 3 m × 4 m plot size. Seeds of Narayani variety of groundnut treated with mancozeb @ 3 g kg^{-1} seed were sown in rows at 22.5 cm apart and 10 cm spacing was maintained between plants. All the recommended package of practices were followed to raise successful crop except plant protection measures. When considerable damage of *S. litura* and *H. armigera* was noticed reaching above ETL then the sprayings of *N. rileyi* was done (at 40 DAS). The experiment includes seven treatments with three replications each.

Preparation of formulations for field spraying :

Oil formulations :

The oil formulations @ 10 ml (1 × 10^{9} spores ml^{-1}) were dissolved in a lt of water along with Tween-20 to get 1 × 10^{7} spores ml^{-1} concentration and the mix is filtered through muslin cloth. A quantity of 2.5 lt of this spray fluid was used to sufficiently wet the groundnut foliage (12 m^{2} area) with the help of knap sack sprayer.

Wettable powder formulations :

For preparation of WP formulaions @ one gram of dried conidial powder of *N. rileyi* harvested from broken rice grains was mixed with 5 g each of two carrier materials *i.e.*, corn flour and talc. Before mixing, these carrier materials were sieved through 0.5 mm mesh to maintain uniformity in particle size of conidial powder and the carrier material. These carrier materials were sterilized in an autoclave at 121^{0}C and 15 psi for 30 min and mixed with conidial powder and two to three drops of Tween-20 (0.05%) was added to the mixture as a wetting agent for uniform mixing of spores with carriers. The prepared formulations of *N. rileyi* were transferred to sterilized polythene bags. 2g of these WP formulations were mixed with one lt water for spraying under field conditions by knap sack sprayer.

Crude formulation :

One gram (1 × 10^{10} spores g^{-1}) of spores of *N. rileyi* harvested from rice grain were mixed with a lt of water along with Tween-20 (0.05 %) to get 1 × 10^{7} spores ml^{-1} concentration and thoroughly shaked and filtered through muslin cloth. A quantity of 2.5 lt of this spray fluid was used to sufficiently wet the groundnut foliage (12 m^{2} area) with the help of knap sack sprayer. The observations on per cent larval reduction at 3, 7 and 15 days after treatment (DAT) was worked out by following formula :

$$\text{Per cent larvel reduction} = \frac{\text{Post treatment population}}{\text{Pre treatment population}} \times 100$$

The observations on leaflet damage was also made after spraying by counting number of damaged leaves and total number of leaves at 3, 7 and 15 days after treatment by the following formula :

$$\text{Per cent leaves damaged} = \frac{\text{Total number of damaged leaves}}{\text{Total number of healthy leaves}} \times 100$$

The pod yield obtained in individual treatments was recorded for assessing the impact of different *N. rileyi* formulation on yield and economics.The data obtained on larval population and leaf damage were subjected to statistical analysis (ANOVA). Percentage values were transformed to arc-sine values before subjecting to statistical analysis. Means were separated by DMRT.

RESULTS AND DISCUSSION

The findings of the present study as well as relevant discussion have been presented under the following heads :

Leaf damage reduction :

Per cent leaf damage reduction increased with an increase in duration after spraying under field condition. Among different formulations of *N. rileyi*, the least leaf damage of 14.27 per cent due to *S. litura* was noticed with groundnut oil based formulation followed by sunflower oil (16.91%) and coconut oil formulation (19.48%) which differ significantly from one another. Higher leaf damage of 30.37 per cent was recorded with control treatment which differ significantly from all the treatments. Leaf damage recorded in plots treated with crude (20.24%), corn flour (21.27%) and talc based (21.06%) formulations were found at par with each other (Table 1). In case of *H. armigera* the least leaf damage of 13.27 per cent was noticed with groundnut oil formulation, followed by sunflower oil (15.14%) and coconut oil formulation (18.20%). Higher leaf damage of 28.38 per cent was recorded with control treatment which differ significantly from all the treatments. Results of crude (18.39%), corn flour (20.63%) and talc based

(20.81%) formulations were found at par with one another (Table 2).

Per cent larval reduction :

Groundnut oil formulation recorded *S. litura* population reduction upto 82.46 per cent followed by sunflower oil formulation (73.81%). With coconut oil formulation, 68.75 per cent reduction was obtained which was found at par with crude formulation (66.59%) and talc based formulation (65.35%). Corn flour formulation recorded 62.07 per cent larval reduction (Table 3).

With groundnut oil formulation, *H. armigera* population was reduced upto 74.58 per cent followed by sunflower oil formulation (61.98%). Per cent population reduction due to coconut oil formulation was 51.38 per cent. Results of sunflower oil and coconut oil formulations was found at par with crude formulation (60.27%). Results of talc based formulation (31.66%) and corn flour formulation (30.33%) were found at par with each other (Table 4).

Grain yield :

Pod yield obtained in different treatments are presented in Table 5. Yield was significantly influenced by formulations of *N. rileyi*. Among all formulations evaluated, higher yield was obtained in treatment with groundnut oil based formulation (16.83 q ha^{-1}). Yield obtained in sunflower oil formulation (14.69 q ha^{-1}) and coconut oil formulation (13.72 q ha^{-1}) were statistically at par with each other. Yield obtained with crude formulation (13.05 q ha^{-1}) was at par with talc formulation (12.47 q ha^{-1}). The least yield was obtained in untreated control (10.08 q ha^{-1}).

Net returns :

The plot which received groundnut oil formulation of *N. rileyi* recorded the highest net returns (Rs. 55792 ha^{-1}) followed by the plots with sunflower oil formulation with *N. rileyi* (Rs. 45562 ha^{-1}) and coconut oil formulation (Rs. 38140 ha^{-1}). The lowest net return (Rs. 23884 ha^{-1}) was recorded in control (Table 5).

Benefit : Cost ratios (BCR) :

B:C ratios of treatments were in the range of 0.97 to 2.23. The groundnut oil formulation of *N. rileyi* emerged as the best treatment with B:C ratio of 2.23, sunflower oil formulation with a B:C ratio of 1.82 found to be next best treatment. Whereas, the lowest B:C ratio of 0.97 was observed in control plot (Table 5).

The present results showed superiority of oil

Table 1 : Effect of different *N. rileyi* formulations on per cent leaf damage due to *S. litura* in groundnut

Treatments	Per cent leaf damage			
	Pre- treatment	3 DAT	7 DAT	15 DAT
	Mean	Mean	Mean	Mean
T_1 : *N. rileyi* with 1% groundnut oil @1×10^{7} spores ml^{-1}	31.06	22.84 28.55)^{a}	17.73 (24.90)^{a}	14.27 (23.08)^{a}
T_2 : *N. rileyi* with 1% sunflower oil @1×10^{7} spores ml^{-1}	33.01	23.84 29.22)^{ab}	20.32 (26.79)^{b}	16.91 (24.28)^{b}
T_3 : *N. rileyi* with 1% coconut oil @1×10^{7} spores ml^{-1}	33.20	24.02 (29.34)^{ab}	21.68 (27.75)^{c}	19.48 (26.19)^{c}
T_4 : *N. rileyi* without oil @1×10^{7} spores ml^{-1}	28.94	24.98 (29.98)^{b}	22.57 (28.37)^{cd}	20.24 (26.73)^{cd}
T_5 : Talc based formulation of *N. rileyi* @ 2 g lt^{-1}	28.21	26.66 (31.08)^{c}	23.23 (28.82)^{d}	21.06 (27.31)^{cd}
T_6 : Corn flour based formulation of *N. rileyi* @ 2 g lt^{-1}	30.18	26.95 (31.27)^{c}	23.96 (28.37)^{d}	21.27 (26.73)^{d}
T_7 : Untreated control	31.39	29.11 (32.65)^{d}	31.38 (34.06)^{e}	30.37 (33.44)^{e}
S.E. ±		0.31	0.29	0.33
C.D. (P = 0.05)		0.98	0.90	1.03

Table 2 : Effect of different *N. rileyi* formulations on per cent leaf damage due to *H. armigera* in groundnut

Treatments	% leaf damage			
	Pre- treatment	3 DAT	7 DAT	15 DAT
	Mean	Mean	Mean	Mean
T_1 : *N. rileyi* with 1% groundnut oil @1×10^{7} spores ml^{-1}	28.00	23.35 (28.89)^{a}	17.21 (24.51)^{a}	13.27 (21.36)^{a}
T_2 : *N. rileyi* with 1% sunflower oil @1×10^{7} spores ml^{-1}	27.81	24.32 (29.54)^{a}	19.03 (25.86)^{b}	15.14 (22.89)^{ab}
T_3 : *N. rileyi* with 1% coconut oil @1×10^{7} spores ml^{-1}	27.97	25.38 (30.24)^{b}	21.34 (27.51)^{c}	18.20 (25.25)^{bc}
T_4 : *N. rileyi* without oil @1×10^{7} spores ml^{-1}	28.36	26.89 (31.23)^{bc}	21.47 (27.60)^{c}	18.39 (25.38)^{c}
T_5 : Talc based formulation of *N. rileyi* @ 2 g lt^{-1}	28.10	27.17 (31.41)^{c}	22.32 (28.19)^{c}	20.81 (27.07)^{c}
T_6 : Corn flour based formulation of *N. rileyi* @ 2 g lt^{-1}	27.89	26.92 (31.25)^{bc}	23.69 (29.13)^{d}	20.63 (27.00)^{c}
T_7 : Untreated control	27.56	27.28 (31.48)^{c}	28.71 (32.39)^{e}	28.38 (32.19)^{d}
S.E. ±		0.34	0.25	0.72
C.D. (P = 0.05)		1.06	0.77	2.22

formulations over the crude and wettable powder formulations in reducing the *S. litura* and *H. armigera* larval population and the leaf damage caused by them.

These results are in accordance with Burges (1998) who reported higher efficacy of oil based formulation of *N. rileyi*, against lepidopteran pests which might be due to the following reasons: oil wets the dry, dusty type of conidia allowing conidia to suspend easily in oil and spread rapidly over the surface of leaves, which helps better contact of conidia with insect cuticle carrying many conidia to protected spaces between the infolded intersegmental membrane which helps easy penetration of the fungus into body for further multiplication resulted in higher mycosis. Whereas, in wettable powder and crude formulation, faster dessication of conidia fails to penetrate the target site resulting in lesser efficacy.

The present investigations were in accordance with Vimaladevi *et al.* (2002) who reported that oil formulation of *N. rileyi* with triton-x-100 @ 2×10^{11} conidia per lit significantly reduced the *S. litura* population to 44.3 per cent in post-rainy season of groundnut and 62.7 per cent in rainy season castor. Similarly the results were in support of Nagaraja (2005) who indicated that significantly higher per cent mycosis (26.56%) was recorded in oil formulation of *N. rileyi* @ 2×10^{11} conidia per ha which reflected on least pod damage (26.43%) in chickpea ecosystem.

In the same way Prior *et al.* (1988) reported that conidial suspension of *B. bassiana* in oil was effective for field application because of its non-drying properties. The oil formulation of *B. bassiana* exhibited the additional advantage of prolonged conidial survival. The application of oil based formulation of *M. anisopliae*, *B. bassiana* and *V. lecanii* resulted in minimization of leaf hoppers and maximization of yield of okra (Harischandra Naik and Shekharappa, 2009).

Similarly, Jenkins and Thomas, 1996 also reported significantly shorter mean survival times of *S. gregaria* than aerial conidia in oil or submerged conidia in water plus adjuvant or water alone on application of *M. anisopliae* var. acridum submerged conidia against *Schistocerca gregaria* in field.

Vimaladevi and Prashanth (2009) showed that the performance of ITCC 4513 and HaBb DOR the two isolates of *B. bassiana* when formulated in oils was superior over unformulated conidia as reflected by the higher mortality of

Table 3 : Effect of different *N. rileyi* formulations on *S. litura* larval population reduction in groundnut

Treatments	Pre –treatment population m^{-2}	% of population reduction		
		3 DAT	7 DAT	15 DAT
		Mean	Mean	Mean
T_1 : *N. rileyi* with 1% groundnut oil @1×10^7 spores ml^{-1}	23.56	9.51 (16.97)^a	60.84 (51.26)^a	82.46 (65.26)^a
T_2 : *N. rileyi* with 1% sunflower oil @1×10^7 spores ml^{-1}	23.00	7.10 (15.01)^a	58.10 (49.68)^a	73.81 (59.29)^{ab}
T_3 : *N. rileyi* with 1% coconut oil @1×10^7 spores ml^{-1}	21.33	4.60 (10.05)^a	48.34 (44.04)^b	68.75 (56.11)^{bc}
T_4 : *N. rileyi* without oil @1×10^7 spores ml^{-1}	21.33	6.16 (14.24)^a	48.33 (44.04)^b	66.59 (54.88)^{bc}
T_5 : Talc based formulation of *N. rileyi* @ 2 g lt^{-1}	24.41	8.24 (16.37)^a	47.34 (43.45)^b	65.35 (53.95)^{bc}
T_6 : Corn flour based formulation of *N. rileyi* @ 2 g lt^{-1}	22.72	4.55 (12.31)^a	42.41 (40.63)^b	62.07 (51.99)^c
T_7 : Untreated control	23.33	0.00 (0.00)^a	0.00 (0.00)^c	0.00 (0.00)^d
S.E. ±		2.84	2.16	2.14
C.D. (P=0.05)		8.77	6.66	6.62

Table 4 : Effect of different *N. rileyi* formulations on *H. armigera* larval population reduction in groundnut

Treatments	Pre –treatment population/ 5 plants	% of population reduction		
		3 DAT	7 DAT	15 DAT
		Mean	Mean	Mean
T_1 : *N. rileyi* with 1% groundnut oil @1×10^7 spores ml^{-1}	4.30	11.66 (19.94)^a	54.16 (47.41)^a	74.58 (59.83)^a
T_2 : *N. rileyi* with 1% sunflower oil @1×10^7 spores ml^{-1}	6.00	5.48 (13.38)^b	38.57 (38.30)^{ab}	61.98 (51.96)^{ab}
T_3 : *N. rileyi* with 1% coconut oil @1×10^7 spores ml^{-1}	4.65	4.72 (12.54)^b	33.33 (34.48)^b	51.38 (45.83)^b
T_4 : *N. rileyi* without oil @1×10^7 spores ml^{-1}	5.30	3.88 (11.04)^b	34.72 (36.06)^b	60.27 (51.02)^b
T_5 : Talc based formulation of *N. rileyi* @ 2 g lt^{-1}	6.00	3.19 (10.06)^b	23.17 (27.80)^b	31.66 (33.85)^c
T_6 : Corn flour based formulation of *N. rileyi* @ 2 g lt^{-1}	5.30	3.11 (10.06)^b	20.88 (26.96)^b	30.33 (33.29)^c
T_7 : Untreated control	5.65	0.00 (0.00)^c	0.00 (0.00)^c	0.00 (0.00)^d
S.E. ±		1.31	4.00	3.06
C.D. (P=0.05)		4.06	12.35	10.76

Figures in paranthesis indicates angular transformed values; Means in the column followed by same letter(s) are not significantly different (P = 0.05); DAT = Days After Treatment

H. armigera larvae in laboratory bioassays and field trials on sunflower.

Paulina *et al.* (2010) evaluated oil formulations (mineral, canola, sunflower, olive and peanut oils) of two fungal strains of *N. rileyi* and *Isaria tenuipes* against larvae of *S. exigua, S. frugiperda*, *H. zea* and *H. virescens*. They recorded highest activity of *N. rileyi* in mineral oil. The present results were in disagreement with Silva and Silva (2001) who recorded the better efficacy of aqueous suspension of *B. bassiana* than mineral oil suspension when used against boll weevil in cotton.

The results on effect of WP formulations are in accordance with Nankinga and Moore (2000) who reported that application of *B. bassiana* maize flour formulated wettable powder at the rate of 2×10^6 conidia per ha proved most effective in reducing the weevil population by 65.72 per cent within 8 weeks after a single application. Ramarethinam *et al.* (2000) evaluated commercial wettable powder formulation of "Priority" of *Paecilomyces fumosoresus* (Wize) Brown and Smith against red spider mite, *Oligonychus coffeae* in tea under laboratory conditions. The mortality of *O. coffeae* ranged from 58.2 to 64.83 per cent on 10th day after spraying and 75.68 to 95.68 on the 15th day after spraying.

Bhagat *et al.* (2003) also reported that the field efficacy of talc based formulations of *M. anisopliae* against white grubs in potato @ 5×10^{13} conidia per ha along with chlorpyriphos 20 EC at 200 g *a.i.* per ha was found effective with maximum reduction in plant damage (75-80%) and tuber damage (63.7%). Of the eight WP formulations prepared, crude WP had registered lowest LC_{50} value of 80.09×10^3 conidia per ml followed by talc based WP and rice flour. Among the WP formulations, crude formulation recorded 82 per cent mortality followed by talc and rice flour (Ramegowda, 2005).

The present results are in Tang and Hou (2002) who sprayed soil surface with *N. rileyi* spore suspension (10^7 conidia per ml) against *H. armigera* in corn and recorded nearly 95 per cent mortality with 20 per cent water content in soil.

The present results are supported by findings of Naik and Shekharappa (2009) who recorded that the yield of okra was significantly higher in oil based formulation of *M. anisopliae* (38.80 q ha^{-1}) and *V. lecanii* (38.50 q ha^{-1}) with monitory returns of Rs. 14720 and Rs. 14480 ha^{-1}, respectively followed by *B. bassiana*.

Similarly, Saxena and Ahmad (1997) tested *Beauveria bassiana* under field conditions to control *H. armigera* infesting chickpea for two crop seasons and found it very effective. At a spore concentration of 2.68×10^7 spores per ml, the average pod damage was 6.8 per cent and yield of 2377 kg ha^{-1}. Where untreated control recorded 16.3 per cent pod damage with an yield of 1344 kg ha^{-1}. Nahar *et al.* (2004) also reported 62.95 per cent mortality of *H. armigera* with *N. rileyi* in pigeonpea.

Mallikarjuna *et al.* (2006) also recorded that sunflower oil formulation of *N. rileyi* emerged as the best treatment with B:C ratio of 17.63. Quinalphos 0.05 per cent with a B:C ratio of 15.98 found to be next best treatment. Whereas, the lowest B:C ratio of 4.73 was observed in plots which received bentonite + sucrose (7:1) formulation.

Nahar *et al.* (2003) evaluated oil based formulations of conidia of fungal isolate *N. rileyi* N812 to control *H. armigera* (Diesel : Sunflower oil 7:3) and recorded yield of 12.62 ha^{-1} in *N. rileyi* treated plots. Demerits of chemicals *i.e.*, high cost with several undesirable consequence such as development of resistant in insect pest, threat to natural bio-agent, contamination of food with residues and environment pollution. Management through entomopathogen like *N. rileyi* which is highly cost effective and safe towards non-target organism will form the best mycoinsecticide for the management of lepidopteran pests. Similar work related to the present investigation was also carried out by Jagdish Babu and Mallikarjun (2012); Gandhi *et al.* (2013); Rathod *et al.* (2014); Karkar *et al.* (2014) and Prasanna and Manjula (2014).

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Table 5 : Yield and economics of different formulations of *N. rileyi* evaluated against *S. litura* and *H. armigera* in groundnut crop

Treatments	*Yield (q ha^{-1})	*Gross returns (Rs. ha^{-1})	*Cost of cultivation (Rs. ha^{-1})	*Net returns (Rs. ha^{-1})	*B : C ratio
T_1 : *N. rileyi* with 1% groundnut oil @1×10^7 spores ml^{-1}	16.83^a	80784	24992	55792	2.23
T_2 : *N. rileyi* with 1% sunflower oil @1×10^7 spores ml^{-1}	14.69^b	70512	24950	45562	1.82
T_3 : *N. rileyi* with 1% coconut oil @1×10^7 spores ml^{-1}	13.72^{bc}	65856	25310	40546	1.6
T_4 : *N. rileyi* without oil @ 1×10^7 spores ml^{-1}	13.05^{cd}	62640	24500	38140	1.55
T_5 : Talc based formulation of *N. rileyi* @ 2 g lt^{-1}	11.86^d	56928	24614	32314	1.31
T_6 : Corn flour based formulation of *N. rileyi* @ 2 g lt^{-1}	12.47^{cd}	59856	24620	35236	1.43
T_7 : Untreated control	10.08^e	48384	24500	23884	0.97

Means in the column followed by same letter (s) are not significantly different (P = 0.05); *Mean of three replications : Cost of groundnut = Rs. 4800 q^{-1}; Groundnut oil = Rs. 82 kg^{-1}; Sunflower oil = Rs. 75 kg^{-1}; Coconut oil = Rs. 135 kg^{-1}; Corn = Rs. 19 kg^{-1}; Talc = Rs. 20 kg^{-1}; N. rileyi (conidia) = Rs. 80 100g^{-1}

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