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Field Evaluation of Oil Based Formulations of *N. rileyi* Against *Spodoptera litura* in Rabi Groundnut

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ABSTRACT

The liquid formulations of Nomuraea rileyi, an important entomopathogenic fungus were prepared by using two vegetable oils and two mineral oils viz., olive oil, rice bran oil, liquid paraffin oil, heavy grade mineral oil. N. rileyi spore mass was harvested from culture plates and mixed to autoclaved test oils in the proportions of 0.1g (0.5 x 10^8 spores/0.1 g) and 0.2g (0.1 x 10^9 spores/0.2 g) per 100ml. Triton-X 100, a wetting agent was also used in two different concentrations i.e., 0.05% and 0.1% for all four test oils. The pathogenicity of N. rileyi conidia was studied at monthly intervals up to 5 months and mortality percentages of third instar larva of S. litura was calculated.

Among the 16 oil based formulations of N. rileyi tested in the laboratory, based on the better performance, eight were selected for field evaluation against S. litura in Rabi groundnut. In the field also, rice bran oil with 0.2g spores and 0.1ml triton-X 100 oil formulation recorded highest per cent larval reduction of 82 per cent at 20 days after treatment. Liquid paraffin with 0.2g spores and 0.1ml triton-X 100 and heavy grade mineral oil with 0.2g spores and 0.1ml triton-X 100 oil formulations also stood as next best treatments. Least performance was observed by olive oil with 0.1g spores and 0.05 ml of triton-X 100 oil formulation (49 per cent larval reduction). The remaining formulations recorded 58-71 per cent larval reduction.

Key words: *Nomuraea rileyi, Oil formulations, Spodoptera litura, Rabi, Groundnut, Larval reduction.*

INTRODUCTION

The cultivated groundnut (*Arachis hypogaea* L.) is an important oilseed crop of tropical and subtropical areas of the world. It is considered as 13th most important food crop, 4th important source of edible oil and 3rd substantial source of vegetable protein around the world[1]. The seeds are rich source of edible oil (43-45%) and protein (25-28%) and also a valuable source of vitamins namely B, E and K. Groundnut cake, after the oil extraction is a high protein animal feed and haulm provides quality fodder. The cake is used as cattle and poultry feed and also serves as organic manure with high nitrogen content. Among several pests attacking groundnut, *Spodoptera litura* (F.) is the major defoliator causing considerable yield loss.

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In India, *S. litura* has become a major pest and created a serious threat to agricultural industry due to the development of resistance towards commonly used insecticides. An indiscriminate use of chemical pesticides is posing threat to the environment and human health. Many species of insect pests have significantly developed resistance to different group of chemical insecticides. So, works on alternate ecofriendly strategies have been initiated, that reduces the negative influence of chemical pesticides.

One line of such strategies is the use of microbial agents/microbial pesticides such as bacteria, virus, fungi, nematodes, protozoa *etc.*

Usage of entomopathogenic fungi against insect pests gained importance from the last few decades. More than 750 species of fungi, mostly deuteromycetes and entomophthorales, are pathogenic to insects.

Species that have been most intensively investigated as mycoinsecticides in the crop pest control include *Beauveria bassiana, Lecanicillium lecanii, Metarhizium anisopliae, Nomuraea rileyi, Paecilomyces fumosoroseus, P. farinosus, Entomophthora* sp., *Fusarium* sp. and *Aspergillus* sp. They are specific to insects and do not infect host plants. These fungi are cosmopolitan in their distribution and diversity.

Due to their eco-friendly and bio-persistence behavior and easily preference to kill pest species at different developmental stages, their utilization increases day-by-day[10].

Nomuraea rileyi (Farlow) Samson is a deuteromycetous fungus of cosmopolitan nature. *N. rileyi* is an important mortality factor for many lepidopteran insects throughout the world. It has the potential to cause spectacular epizootics under favorable environmental conditions. In India, epizootics of *N. rileyi* were recorded on lepidopteran insect pests in field crops and forest trees. In Andhra Pradesh also regular occurrence of *N. rileyi* is being observed on *Helicoverpa armigera, Spodoptera litura, Plusia sps etc.,* in crops like groundnut, cotton under favorable ecosystem.

MATERIAL AND METHODS

Preparation of oil based formulations of *N. rileyi*

The test oils used for the preparation of *N. rileyi* formulations are commonly and commercially available vegetable and mineral oils *viz.,* Olive oil, rice bran oil, liquid paraffin oil, heavy grade mineral oil. The selected oils manufactured by standard companies were purchased. The oils were poured into sterilized conical flasks/blue cap bottles of 250 ml and autoclaved at 15 psi pressure at 121°C for 15 min. Each oil was considered as a treatment and three replications were maintained (100ml/replication). The harvested spores of *N. rileyi* were mixed to the test oils in the proportions of 0.1g and 0.2g per 100 ml of test oil. Triton-X 100, a wetting agent was also used in two different concentrations *i.e.,* 0.05% and 0.1% for all four test oils for uniform mixing of spores under aseptic conditions.

Details of the Field Experiment

The oil based formulations of *N. rileyi* that recorded considerably higher larval and pupal mortalities under laboratory conditions were used for evaluating against *S. litura*, in *Rabi* groundnut crop, at dry land farm, S.V. Agricultural College, Tirupati. A Randomized Block Design (RBD) was laid with three replications of 5m x 4m plot size. Seeds of the Narayani variety of Groundnut treated with mancozeb @ 3 g kg^{-1} seeds were sown in rows at 22.5 cm apart and 10 cm spacing was maintained between plants. All the recommended package of practices was followed to raise successful crop except plant protection measures. When considerable damage of *S. litura* was noticed reaching above ETL, then the sprayings of *N. rileyi* was done (at 40 DAS). The experiment includes eight treatments and an untreated control with three replications each.

Preparation of spray suspension from oil formulations for field spraying:

For field spraying, from each oil based formulation, 5ml was taken with the help of measuring cylinder and dissolved in a litre of water and mixed thoroughly. A quantity of 3.5 lit of this spray fluid was used to sufficiently wet the groundnut foliage (20 m^2 area) with the help of foot sprayer.

OBSERVATIONS

Assessment of infected larvae of *S. litura*

In each replication, in all the treatments, five plants were selected for taking observations. Observations on total number of larvae per five plants of *S. litura* were recorded in each replication, in all the treatments at 5, 10 and 20 days after spray. The cadavers found in the field infected with *N. rileyi* were collected and confirmed the disease in the laboratory. A pre count was also taken to calculate mean per cent larval reduction over pre-treatment with the following formula[9].

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

Statistical Analysis

The data obtained on larval population was subjected to statistical analysis (ANOVA). Per cent values were transformed to arc-sin values before subjecting to statistical analysis. Means were separated by Duncan's Multiple Range Test (DMRT). The statistical analysis carried out through SPSS.20.00.

RESULTS AND DISCUSSION

A day before the application of treatments the larval population was above ETL (9-11 larvae/ five plants) and uniform. The per cent reduction of *S. litura* larval population at 5, 10 and 20 days after treatment (Plate 1.) is presented in Table 1. and Fig 1.

The results indicate that rice bran oil with 0.2g spores and 0.1ml triton-X 100 oil formulation treated plot recorded highest larval reduction of 31.21, 59.39 and 81.51 per cent with respect to *S. litura* larva at 5, 10 and 20 DAT. The treatments liquid paraffin oil with 0.2 g spores and 0.1 ml of triton-X 100, heavy grade mineral oil with 0.2 g spores and 0.1 ml of triton-X 100 recorded 23-76 per cent larval reduction up to 20 DAT.

Plate 1: ***S. litura* cadavers in groundnut crop treated with oil formulations of *N. rileyi***

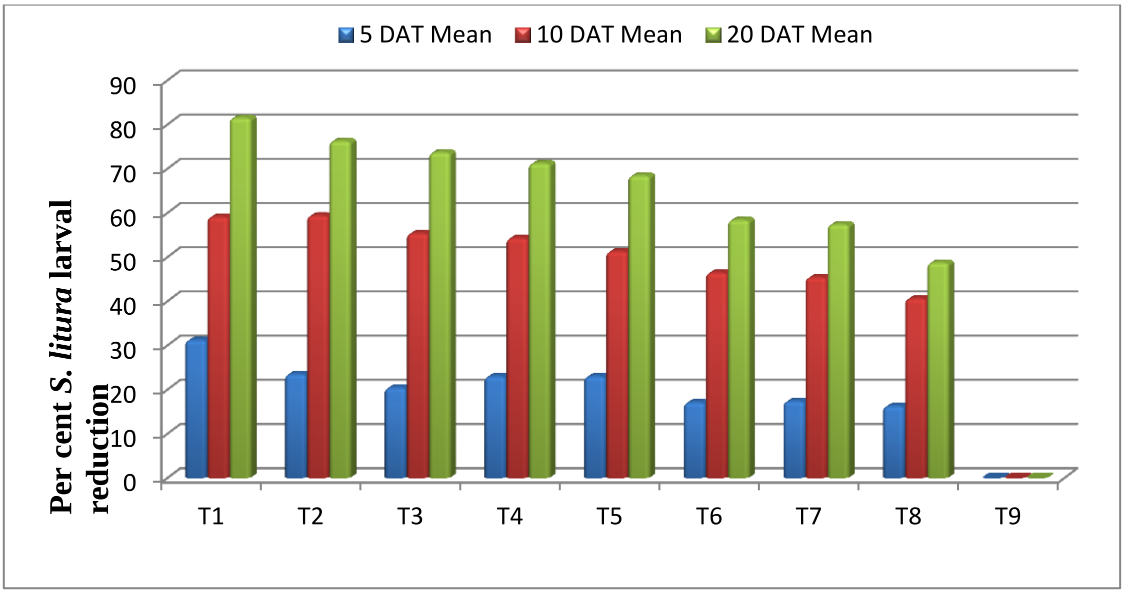
Table 1: ***S. litura*** **larval population reduction in *Rabi* groundnut due to application of different oil based formulations of *N. rileyi***

Treatments	Pre-treatment population per 5 plants	Mean per cent larval population reduction		
		5 DAT	10 DAT	20 DAT
T_1	10.66	31.21^{c} (33.91)	59.39^{d} (50.40)	81.51^{e} (64.91)
T_2	11.33	23.48^{bc} (28.89)	59.08^{d} (50.25)	76.25^{de} (60.95)
T_3	11.33	22.97^{bc} (28.51)	55.35^{cd} (48.07)	73.68^{de} (59.26)
T_4	11.66	22.97^{bc} (28.51)	54.29^{cd} (47.44)	71.20^{de} (57.82)
T_5	11.66	20.41^{b} (26.82)	51.26^{cd} (45.71)	68.43^{cd} (55.85)
T_6	11.33	17.34^{b} (24.57)	46.43^{bc} (42.92)	58.38^{bc} (49.83)
T_7	9.66	17.08^{b} (24.11)	45.38^{bc} (42.32)	57.39^{bc} (49.23)
T_8	12.33	16.23^{b} (23.27)	40.59^{b} (39.55)	48.71^{b} (44.24)
T_9	11.66	0.00^{a} (0.00)	0.00^{a} (0.00)	0.00^{a} (0.00)
SE(m) ±		**2.80**	**3.16**	**3.69**
C.D.(p = 0.05)		**8.48**	**9.58**	**11.16**

T_1: Rice bran oil with 0.2g spores and 0.1ml triton-X 100, T_2: Liquid paraffin with 0.2g spores and 0.1ml triton-X 100, T_3: Heavy grade mineral oil with 0.2g spores and 0.1ml triton-X 100, T_4: Rice bran oil with 0.2g spores and 0.05ml triton-X 100, T_5: Liquid paraffin with 0.2g spores and 0.05ml triton-X 100, T_6: Heavy grade mineral oil with 0.2g spores and 0.05ml triton-X 100 , T_7: Rice bran oil with 0.1g spores and 0.1ml triton-X 100, T_8: Olive oil with 0.2g spores and 0.1 ml triton-X 100, T_9: Untreated control

Figures in parenthesis indicate angular transformed values.
Means in the column followed by same letter(s) are not significantly different by DMRT
DAT = Days after Treatment
Data are the means of three replications

Fig 1: The reduction of *S. litura* larval population with different oil based formulations of *N. rileyi* in *Rabi* groundnut



T_1: Rice bran oil with 0.2g spores and 0.1ml triton-X 100, T_2: Liquid paraffin with 0.2g spores and 0.1ml triton-X 100, T_3: Heavy grade mineral oil with 0.2g spores and 0.1ml triton-X 100, T_4: Rice bran oil with 0.2g spores and 0.05ml triton-X 100, T_5: Liquid paraffin with 0.2g spores and 0.05ml triton-X 100, T_6: Heavy grade mineral oil with 0.2g spores and 0.05ml triton-X 100 , T_7: Rice bran oil with 0.1g spores and 0.1ml triton-X 100, T_8: Olive oil with 0.2g spores and 0.1 ml triton-X 100, T_9: Untreated control

Except olive oil with 0.2g spores and 0.1ml triton-X 100, which recorded 16, .23, 40.59 and 48.71 per cent larval reduction at 5, 10 and 20 DAT, all the other oil formulations of *N. rileyi* recorded the larval reduction above 50 per cent at 20 days after treatment.

It has been reported that oil formulations of *N. rileyi* with triton-X-100 has recorded 65.9 per cent mortality in post-rainy season of groundnut and 62.8 per cent mortality in rainy season castor against *S. litura*[12].

The effectiveness of oil based formulations (Diesel: Sunflower oil 7:3) of conidia of fungal isolate *N. rileyi* N812 against *H. armigera* has been evaluated. The conidia were found to be most effective in controlling *H. armigera*. The per cent efficacy was 61 per cent. Pod damage was 15.48 and yield was 12.62 per ha in *N. rileyi* treated plots[6].

Field performance of *N. rileyi* applied at 2 x 10^8 conidia/ l against *S. litura*, *H armigera* and *Thysanoplusia orichalcea* was compared with sequential sprays of monocrotophos @ 1.0 ml/l and λ-cyhalothrin @ 0.6 ml/l on large scale. Larval population was reduced to the extent of the 28 and 62 per cent in 10 days after first and second application of *N. rileyi*, respectively as against 42 and 65 per cent after first (monocrotophos) and second (λ-cyhalothrin) spray, respectively[3].

It has been 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[7].

The two formulations of *B. bassiana i.e.* oil palm kernel cake based formulations of conidia (OPKC) and conidial powder (CP) against adult banana weevils has been evaluated. The same level of mortality of 75.5 per cent was recorded compared with only one per cent mortality in the untreated control[2].

The oil formulations of *M. anisopliae* (M 34412), *B. bassiana* (B 3301) and *N. rileyi* (N 3.12) under field conditions in pigeonpea against *H. armigera* has been evaluated. After two sprays, per cent efficacy from various treatments ranged from 55.51 to 70.93. The treatment with *M. anisopliae* was found to be effective with maximum efficacy of 70.93 per cent, *N. rileyi* with 62.95 per cent. The per cent pod damage in *M. anisopliae*, *N. rileyi* was 8.76 and 10.24, respectively[5].

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 *H. armigera* larvae in laboratory bioassays and field trials on sunflower[11].

Three entomopathogenic fungi *B. bassiana*, *P. fumosoroseus, V. lecaniii* has been evaluated on groundnut pests *Aphis craccivora, Aproarema modicella, Mylabris pustulata* and *Spodoptera litura* in field conditions. The infestation of *S. litura* and *A. modicella* were greatly reduced after the treatment of *B. bassiana*. *P. fumosoroseus* and *V. lecanii* were less effective than *B.* bassiana[8].

The entomopathogenic fungus, *N. rileyi* on *S. litura* larvae infesting groundnut was observed during 32-37th Standard Meteorological Week (SMW) of 2014 in Eastern Region of India on second and third instar larvae of *S.litura*. Maximum larval mortality (93.67%) was observed on 37th SMW and high larval mortality positively relates to rainfall and relative humidity. Pathogenicity of *N. rileyi* (3.4 x 10^{-9}cfu) on second and third larval instars revealed high mortality within 3-4 days and visible fungal growth were observed on fifth and sixth day of treatment. *N. rileyi* can be easily isolated and could be incorporated in biological management modules for wide area management of lepidopteran insect-pests especially against the second and third instars larvae of *S.* litura[4].

CONCLUSION

Rice bran oil with 0.2g spores and 0.1ml triton-X 100 formulation treated plot recorded highest larval reduction of 31.21, 59.39 and 81.51 per cent with respect to *S. litura* larva at 5, 10 and 20 DAT. Except olive oil with 0.2g spores and 0.1ml triton-X 100, all the other oil formulations of *N. rileyi* recorded the larval reduction above 50 per cent at 20 days after treatment.

The present results proved that storage of *N. rileyi* in best suited vegetable oils such as rice bran oil and mineral oils such as liquid paraffin would be more advantages for maintenance of satisfactory viability and virulence up to 5 months.

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