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Efficacy of Oil Based Formulations of *Nomuraea rileyi* (Farlow) Samson against Pupae of *Spodoptera litura In vitro*

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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 pupae of S. litura was calculated.

Among the 16 oil based formulations of N. rileyi, rice bran oil with 0.2g spores and 0.1ml triton-X 100 oil formulation recorded highest pupal mortality of 60-74 per cent followed by 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 formulation which recorded 56-70 and 55-69 per cent respectively. The remaining formulations recorded 19-68 per cent pupal mortality.

Key words: *Nomuraea rileyi, Oil formulations, Vegetable oils, Mineral oils, Triton-X 100, Pupal mortality.*

INTRODUCTION

An indiscriminate use of chemical pesticides is posing threat to 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 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.

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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[6].

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.

The main objective of this study is to evaluate the oil based formulations of *N. riley* against pupae of *Spodoptera litura.*

MATERIAL AND METHODS

The standard medium used for isolation and mass production of *N. rileyi* was SDAY medium (Saboraud's Dextrose Agar enriched with Yeast extract medium).

Composition of SDAY medium

Ingredients	Weight/Volume
Agar	20 g
Peptone	10 g
Dextrose	40 g
Yeast	5 g
Distilled water	1000 ml
Chloramphenicol	80 mg l^{-1}

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.

Likewise a total of 16 treatments and an untreated control were maintained. These prepared oil formulations were stored in incubator at 22°C.

Preparation of spray suspensions from oil based formulations for laboratory studies:

At the time of treatments for laboratory studies, from each oil formulation (*i.e.*, olive oil, rice bran oil, liquid paraffin oil, and heavy grade mineral oil) 0.5 ml quantity was taken with the help of micro pipette and mixed with 100 ml of water taken in a beaker and shaken thoroughly.

Culturing of *Spodoptera litura* in the Laboratory:

Culture of *S. litura* was maintained in the laboratory during the entire experimental period on natural feed *i.e.* castor leaves. The fresh castor leaves were collected from the field and washed thoroughly with tap water. Field collected eggs were kept in rearing

troughs for hatching on a moist filter paper. Freshly hatched larvae were provided with fresh castor leaves in transparent plastic rearing containers and covered with muslin cloth. Castor leaves were changed daily till pupation and rearing troughs were cleaned daily. After pupation, pupae were kept for adult emergence in cages (35 × 25 × 45 cm). The emerged adults were provided with absorbent cotton swabs dipped in diluted honey (10%) as adult food. The Castor leaves dipped in water present in a conical flask were placed inside the cage for egg laying. After egg laying the egg masses were collected into plastic containers every day and were reared on castor leaves under aseptic conditions to get disease free larvae. The hatched larvae were provided with fresh castor leaves every day. The third instar larvae were selected for laboratory studies.

BIOASSAY METHOD

For testing the virulence, lab reared third instar larvae of *S. litura* was used to study larval mortality at monthly intervals up to 150 days. All the 16 treatments were replicated thrice. An untreated control was also maintained. In some larvae the infection was carried to the pupal stage and death of pupae recorded. Pupal malformations also were recorded (Plate 1.).

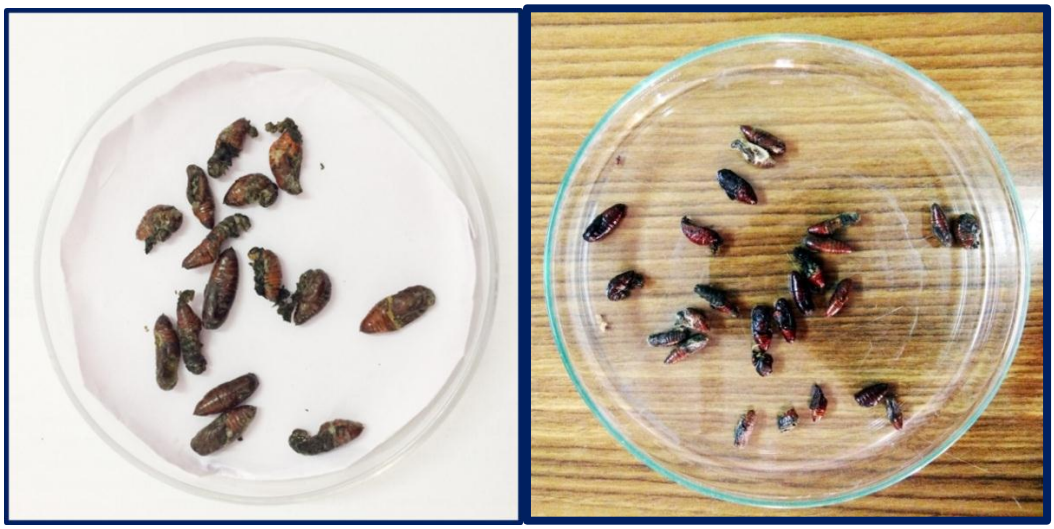

Plate 1. Malformed and dead pupae of *S. litura* due to carry over infection of *N. rileyi* from the treated larvae

Analysis of the Data

The pupal mortality due to oil based formulations of *N. rileyi* was expressed as per cent mortality before subjecting to statistical analysis by using the formula[5].

$$\text{Per cent pupal mortality} = \frac{\text{No. of pupae dead/malformed due to infection}}{\text{Total number of pupa}} \times 100$$

RESULTS AND DISCUSSION

The present results indicate that the virulence of *N. rileyi* conidia reduces with increase in storage period.

The results showed that all the treatments were significantly different with each other in recording pupal infection at 30, 60, 120 and 150 days of preparation (Table 1. and Fig 1.). Rice bran oil with 0.2g spores and 0.1ml triton-X 100 oil formulation recorded 67.34 and 60.34 per cent pupal mortality/malformed pupae at 60 and 150 DAP. Liquid paraffin with 0.2g spores and 0.1ml triton-X 100 oil formulation recorded 66.34 and 56.34 per cent pupal mortality/malformed pupae at 60 and 150 DAP. Lowest reduction (14%) in pupal mortality/malformed pupae from the day of preparation to 150 days of preparation was shown by rice bran oil with 0.2g spores and 0.1ml triton-X 100 as well as liquid paraffin

with 0.2g spores and 0.1ml triton-X 100 oil formulations respectively. The next best treatment was heavy grade mineral oil with 0.2g spores and 0.1ml triton-X 100 oil formulation, which recorded 62.34 and 55.00 per cent pupal mortality/malformed pupae at 60 and 150 DAP and showed relatively lowest reduction of 14.33 per cent of virulence of *N. rileyi* conidia.

Rice bran oil with 0.2g spores and 0.05ml of triton-X 100, liquid paraffin with 0.2g spores and 0.05 ml of triton-X 100, heavy grade mineral oil with 0.2g spores and 0.05ml of triton-X 100, rice bran oil with 0.1g spores and 0.1ml of triton -X 100 and olive oil with 0.2g spores and 0.1 ml of triton-X100 recorded 15-16 per cent of reduction in the virulence of conidia of *N. rileyi* from the day of preparation to 150 days of preparation.

The remaining oil formulations *i.e.,* rice bran oil with 0.1g spores and 0.05ml triton-X 100, heavy grade mineral oil with 0.1g spores and 0.1ml of triton -X 100, liquid paraffin with 0.1g spores and 0.1 ml of triton-X 100, heavy grade mineral oil with 0.1g spores and 0.05ml of triton-X 100, liquid paraffin with 0.1g spores and 0.05 ml of triton-X 100 and olive oil with 0.2g spores and 0.05 ml of triton-X 100 recorded 17-22 per cent reduction in virulence of *N. rileyi* conidia.

Table 1: The pupal mortalities/malformed pupae of *S. litura* recorded when third instar larvae treated with different oil based formulations of *N. rileyi*

Treatments	Mean per cent Pupal mortality/Malformed pupae					
	1 DAP	30 DAP	60 DAP	90 DAP	120 DAP	150 DAP
T_1	51.34^{a} (45.75)	50.34^{e} (45.17)	47.00^{e} (43.26)	46.34^{d} (42.88)	38.34^{e} (38.24)	30.34^{e} (33.41)
T_2	58.34^{g} (49.78)	54.34^{g} (47.47)	50.00^{g} (44.98)	49.00^{e} (44.41)	43.34^{g} (41.15)	39.34^{g} (38.82)
T_3	67.34^{kl} (55.12)	63.34^{m} (52.72)	59.34^{m} (50.36)	57.34^{j} (49.19)	53.34^{m} (46.89)	52.34^{m} (46.32)
T_4	70.34^{n} (56.98)	68.34^{p} (55.74)	66.34^{p} (54.51)	64.34^{m} (53.31)	59.34^{p} (50.36)	56.34^{p} (48.62)
T_5	43.00^{b} (40.96)	42.00^{b} (40.38)	41.34^{b} (39.99)	39.34^{b} (38.82)	25.34^{b} (30.21)	19.00^{b} (25.83)
T_6	45.00^{c} (42.11)	43.34^{c} (41.15)	42.00^{c} (40.38)	40.00^{b} (39.22)	32.34^{c} (34.64)	22.00^{c} (27.96)
T_7	48.34^{d} (44.02)	46.34^{d} (42.88)	43.34^{d} (41.15)	41.34^{c} (39.99)	35.00^{d} (36.26)	26.34^{d} (30.86)
T_8	63.34^{i} (52.71)	58.34^{j} (49.78)	56.34^{j} (48.62)	55.00^{h} (47.85)	50.00^{j} (44.98)	47.00^{j} (43.26)
T_9	62.00^{h} (51.92)	57.00^{i} (49.00)	55.00^{i} (47.85)	53.34^{g} (46.89)	47.00^{i} (43.26)	45.00^{i} (42.11)
T_{10}	65.34^{j} (53.91)	60.00^{k} (50.75)	57.34^{k} (49.19)	55.34^{h} (48.04)	51.34^{k} (45.75)	49.34^{k} (44.60)
T_{11}	68.34^{lm} (55.73)	64.34^{n} (53.31)	60.34^{n} (50.94)	58.34^{k} (49.78)	55.34^{n} (48.04)	53.34^{n} (46.89)
T_{12}	74.34^{o} (59.54)	70.34^{q} (56.98)	67.34^{q} (55.12)	65.34^{n} (53.91)	62.34^{q} (52.12)	60.34^{q} (50.94)
T_{13}	55.00^{f} (47.85)	52.34^{f} (46.32)	49.00^{f} (44.41)	47.00^{d} (43.26)	41.34^{f} (39.99)	35.00^{f} (36.26)
T_{14}	61.34^{h} (51.53)	55.34^{h} (48.04)	52.34^{h} (46.32)	50.34^{f} (45.17)	45.34^{h} (42.31)	43.34^{h} (41.15)
T_{15}	66.34^{jk} (54.52)	62.00^{l} (51.92)	58.34^{l} (49.78)	56.34^{i} (48.62)	52.34^{l} (46.32)	50.34^{l} (45.17)
T_{16}	69.34^{mn} (56.35)	65.00^{o} (53.71)	62.34^{o} (52.12)	60.34^{f} (50.94)	57.00^{o} (49.00)	55.00^{o} (47.85)
T_{17}	0.00^{a} (0.00)	0.00^{a} (0.00)	0.00^{a} (0.00)	0.00^{a} (0.00)	0.00^{a} (0.00)	0.00^{a} (0.00)
General mean	**56.99**	**53.69**	**51.03**	**49.35**	**44.06**	**40.25**
SE(m) ±	**0.44**	**0.27**	**0.27**	**0.28**	**0.28**	**0.26**
C.D.(p = 0.05)	**1.28**	**0.77**	**0.77**	**0.81**	**0.81**	**0.74**

Figures in parenthesis indicate angular transformed values.

DAP = Days after Preparation

Means in the column followed by same letter(s) are not significantly different by DMRT

Data are the means of three replications

T_1: Liquid paraffin with 0.1g spores and 0.05 ml of Triton-X100, T_2: Liquid paraffin with 0.1g spores and 0.1 ml of Triton-X100, T_3: Liquid paraffin with 0.2g spores and 0.05 ml of Triton-X100, T_4: Liquid paraffin with 0.2g spores and 0.1 ml of Triton-X100, T_5: Olive oil with 0.1g spores and 0.05 ml of Triton-X100, T_6: Olive oil with 0.1g spores and 0.1 ml of Triton-X100, T_7: Olive oil with 0.2g spores and 0.05 ml of Triton-X100, T_8: Olive

oil with 0.2g spores and 0.1 ml of Triton-X100, T_9: Rice bran oil with 0.1g spores and 0.05ml of Triton-X100, T_{10}: Rice bran oil with 0.1g spores and 0.1ml of Triton-X100, T_{11}: Rice bran oil with 0.2g spores and 0.05ml of Triton-X100, T_{12}: Rice bran oil with 0.2g spores and 0.1ml of Triton-X100, T_{13}: Heavy grade mineral oil with 0.1g spores and 0.05ml of Triton-X100,T_{14}: Heavy grade mineral oil with 0.1g spores and 0.1ml of Triton-X100, T_{15}: Heavy grade mineral oil with 0.2g spores and 0.05ml of Triton-X100, T_{16}: Heavy grade mineral oil with 0.2g spores and 0.1ml of Triton-X100, T_{17}: Untreated check. The highest per cent reduction in virulence of *N. rileyi* conidia was recorded in olive oil with 0.1g spores and 0.05 ml of triton-X 100 (24%), followed by olive oil with 0.1g spores and 0.1 ml of triton-X 100 (23%) respectively.

Rice bran oil with 0.2g spores and 0.1ml triton-X 100 oil formulation was found to be superior by recording highest per cent pupal mortality/malformed pupae from the day of preparation to 150 days of storage followed by liquid paraffin with 0.2g spores and 0.1ml triton-X 100, heavy grade mineral oil with 0.2g spores and 0.1ml triton-X 100.

Rice bran oil is extracted from the hard outer brown layer of rice grains after chaffing. It has mild flavor and has high smoke point of 232^{o}C (stabile at high temperatures). Rice bran oil consists of higher food energy of 880 k Cal per 100 gms. It is rich in antioxidants. This oil contains 38% monosaturated, 37% polyunsaturated d and 25% saturated fatty acids. The above properties of rice bran oil may be suitable for *N. rileyi* conidia for being more viable and virulent.

Daud *et al.*[1], reported that rice bran oil is rich in natural antioxidants such as tocopherols, tocotrienols, oryzanol and phenolic compound. The total phenol content ranges from 190-450 mg/kg.

Mineral oils are liquid by-products of refining crude oil to make gasoline and other petroleum products. They are transparent, colorless oil composed mainly of alkanes from a mineral source and cycloalkanes, related to petroleum jelly.

The rice bran oil with 0.2g spores and 0.05ml of triton-X 100, liquid paraffin with 0.2g spores and 0.05 ml of triton-X 100, heavy grade mineral oil with 0.2g spores and 0.05ml of triton-X 100, rice bran oil with 0.1g spores and 0.1ml of triton -X 100, olive oil with 0.2g spores and 0.1 ml of triton-X 100 and rice bran oil with 0.1g spores and 0.05ml triton-X 100 oil formulations were considered as the next best oil formulations. The olive oil with 0.1g spores and 0.05 ml of triton-X 100 was found to be inferior in recording least pupal mortality/malformed pupae.

Olive oil is a liquid fat, produced by pressing whole olives. It mainly consists of oleic acid, with smaller amount of other fatty acids like linoleic acid and palmitic acid. It has favorable flavor. It has a smoke point of 190-210^{o}C. This oil contains 13.33g of saturated and 66.6g of monosaturated fattyacids, with 800 k Cal energy per 100g. When compared to rice bran oil, olive oil has relatively lower smoke point and less saturated fatty acids. These properties of olive oil may be comparatively less favorable for the conidia of *N. rileyi* to maintain viability and virulence.

Muco *et al.*[3], reported that olive oil contains palmitic, palmitoleic, stearic, oleic, linoleic, linolenic, and arachidic fatty acids. The total phenol content ranged from 117-304 mg/kg. These physico-chemical properties of olive oil might be highly unfavorable for *N. rileyi* spores for retaining the viability and pathogenicity

The superiority of these formulations is due to high spore concentration coupled with higher concentration of triton-X 100 and high spore germination. The decreased virulence of *N. rileyi* with the time is due to reduction in germination per cent of conidia.

Lalitha[2], also recorded 61.11 per cent *S. litura* pupal mortality when *N. rileyi* was applied. She reported that the pupal mortality increased with increase in concentration.

Ramakrishnan *et al.*[4], observed increase in pupal mortality of *S.litura* with an increase in concentration of *B. bassiana*.

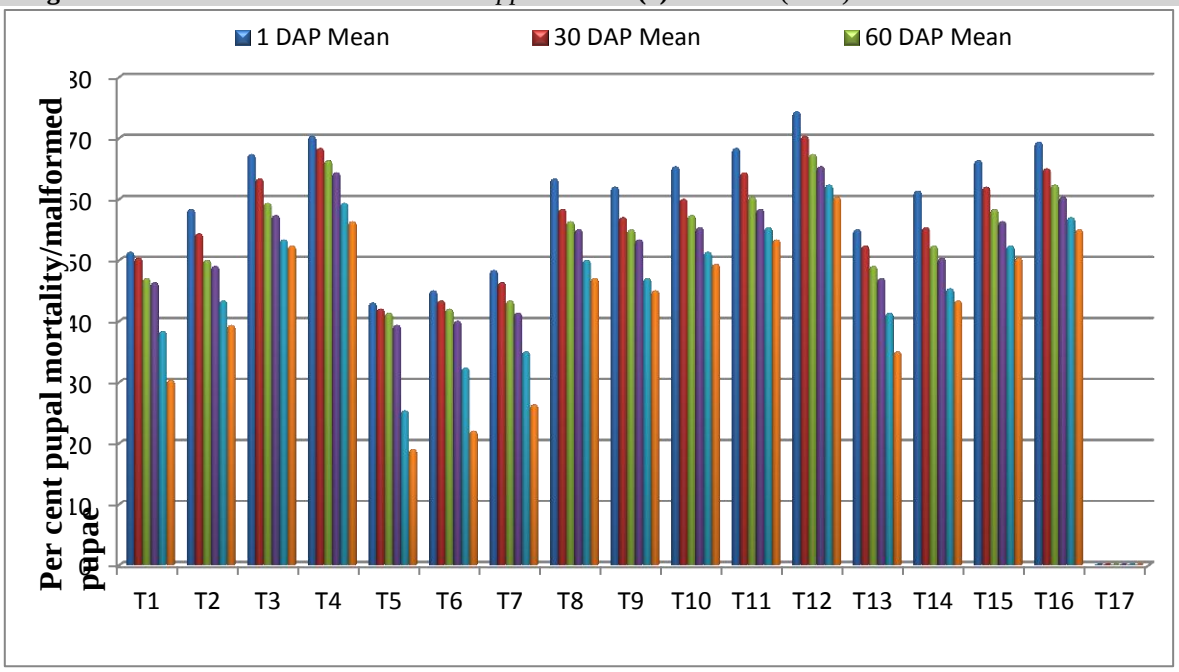

Fig. 1: Pupal mortality/ malformed pupae of *S. litura* with different oil based formulations of *N. rileyi* treated to third instar

T_1: Liquid paraffin with 0.1g spores and 0.05 ml of Triton-X100, T_2: Liquid paraffin with 0.1g spores and 0.1 ml of Triton-X100, T_3: Liquid paraffin with 0.2g spores and 0.05 ml of Triton-X100, T_4: Liquid paraffin with 0.2g spores and 0.1 ml of Triton-X100, T_5: Olive oil with 0.1g spores and 0.05 ml of Triton-X100, T_6: Olive oil with 0.1g spores and 0.1 ml of Triton-X100, T_7: Olive oil with 0.2g spores and 0.05 ml of Triton-X100, T_8: Olive oil with 0.2g spores and 0.1 ml of Triton-X100, T_9: Rice bran oil with 0.1g spores and 0.05ml of Triton-X100, T_{10}: Rice bran oil with 0.1g spores and 0.1ml of Triton-X100, T_{11}: Rice bran oil with 0.2g spores and 0.05ml of Triton-X100, T_{12}: Rice bran oil with 0.2g spores and 0.1ml of Triton-X100, T_{13}: Heavy grade mineral oil with 0.1g spores and 0.05ml of Triton-X100,T_{14}: Heavy grade mineral oil with 0.1g spores and 0.1ml of Triton-X100, T_{15}: Heavy grade mineral oil with 0.2g spores and 0.05ml of Triton-X100, T_{16}: Heavy grade mineral oil with 0.2g spores and 0.1ml of Triton-X100, T_{17}: Untreated check.

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