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Viability of *Nomuraea rileyi* Conidia in Vegetable and Mineral Oil Based Formulations

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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 viability of N. rileyi conidia was studied at monthly intervals up to 5 months and germination percentages of N. rileyi conidia 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 conidial germination. It was ranged from 68-93 per cent up to 150 days. 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 recorded 64-90 per cent and 62-89 per cent conidial germination respectively. The relatively lowest conidial germination of 35-71 per cent was observed in olive oil with 0.1g spores and 0.05 ml of triton-X 100 oil formulation. The remaining formulations recorded 35-87 per cent conidial germination.

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

INTRODUCTION

Biological control is one of the components of integrated management of many economically important insect pests. The biocontrol agents include the parasitoids, predators and entomopathogenic microorganisms. 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.

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Nomuraea rileyi (Farlow) Samson is a deuteromycetous fungus of cosmopolitan nature. Nomuraea 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.

Formulation of biological control agent is an important criterion for sustainable agriculture. Formulation can improve the product stability and viability of biological control agents. Many of the microbial agents have been formulated with dried milk, powdered casein, gelatin, saponins, oils, soaps, *etc.* It is a common phenomenon that the efficiency of entomopathogenic fungi enhances when they are prepared as oil formulations. The formulations of *N. rileyi* were prepared by using two vegetable and two mineral oils.

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.

Testing the viability of *N. rileyi* in different oil formulations

The germination of conidia of *N. rileyi* was studied at monthly intervals up to 150 days. At each time, two to three drops of spore suspension was placed in cavity slide. The cavity slide was placed in the humidity chamber which was prepared by arranging moistened cotton in petriplates and it was incubated at 22^{o} C. After 24 hours, the spore suspension was observed under the microscope for counting of total number of spores and germinated spores in the microscopic field. The germination percentage of *N. rileyi* conidia was calculated.

RESULTS AND DISCUSSION

The data on viability of *N. rileyi* spores at monthly intervals up to 5 months indicated that majority of the treatments are significantly different from each other. There was 25-36 per cent reduction of conidial germination from the day of preparation to 150 days after preparation (Table 1).

The results showed that rice bran oil with 0.2g spores and 0.1ml triton-X 100 oil formulation recorded highest germination at monthly intervals. At 60 and 150 DAP, it recorded 82.34 and 68.00 per cent conidial germination. The next best is liquid paraffin with 0.2g spores and 0.1ml triton-X 100 which recorded 80.34 and 64.34 per cent conidial germination at 60 and 150 DAP respectively. Heavy grade mineral oil with 0.2g spores and 0.1ml triton-X 100 also good with 77.34 and

62.00 per cent conidial germination at 60 and 150 DAP respectively. In the above three formulations, the reduction in conidial germination was comparatively low *i.e.* 25-27 per cent (Table 1).

Rice bran oil with 0.2g spores and 0.1ml triton-X 100 oil formulations is superior among all. It 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.

It is rich in natural antioxidants such as tocopherols, tocotrienols, oryzanol and phenolic compounds. The total phenol content ranges from 190-450mg/kg [4].

Gopalakrishnan and Mohan[5] reported that rice was the most suitable substrate for quicker and better mass multiplication of *N. rileyi*. Krishnaveni[7] reported that maize and rice grains stands in first and second places with 96 and 80 per cent of germination of conidia of *N. rileyi*. According to Preez *et al.*[9], rice contains higher proportion of starch and amylase. Hydrolysis of starch in rice resulted in the release of glucose and maltose depending on clarification.

When two spore loads *i.e.* 0.1g and 0.2g per 100ml oils were compared, the higher per cent viable spores were recorded in the latter, whereas the two concentrations of wetting agent, triton-X 100 *i.e.* 0.05% and 0.1%, the higher concentration of wetting agent found better suited for *N. rileyi* spores to retain the viability. In none of the formulation the viability was declined less than 30 per cent. Alves *et al.*[1], reported the viability of conidia of *Metarhizium anisopliae* mixed with eight emulsifiable adjuvant oils (EAO), seven wetter/spreaders, three vegetable oils and four mineral oils after 24h and 48h spreading over the SDA medium surface. The oil formulations did not cause any negative effect on conidial germination.

Table 1: Viability of *N. rileyi* conidia in different oil based formulations up to 5 months

Treatments	Mean per cent germination of *N. rileyi* spores					
	0 DAP	**30 DAP**	**60 DAP**	**90 DAP**	**120 DAP**	**150 DAP**
T_1	78.00^{bc} (62.02)	72.34^{b} (58.25	64.00^{c} (53.11)	58.34^{c} (49.78)	53.67^{cde} (47.08)	46.00^{e} (42.69)
T_2	79.34^{cde} (62.94)	77.34^{c} (61.56)	68.67^{d} (55.94)	59.67^{c} (50.55)	60.00^{f} (50.75)	49.00^{g} (44.41)
T_3	87.00^{fg} (68.88)	80.67^{de} (63.89)	76.34^{f} (60.87)	71.00^{fg} (57.39)	65.34^{g} (53.92)	58.34^{l} (49.78)
T_4	89.66^{g} (71.25)	84.67^{f} (67.06)	80.34^{g} (63.66)	72.00^{fg} (58.04)	68.00^{g} (55.53)	64.34^{o} (53.31)
T_5	70.66^{a} (57.18)	66.00^{a} (54.31)	56.34^{a} (48.62)	52.00^{a} (46.13)	43.34^{a} (41.15)	35.34^{a} (36.46)
T_6	75.66^{b} (60.44)	69.67^{b} (56.56)	60.34^{b} (50.94)	55.00^{b} (47.85)	45.67^{a} (42.49)	41.00^{b} (39.78)
T_7	76.66^{bc} (61.10)	70.00^{b} (56.77)	61.00^{bc} (51.34)	55.34^{b} (48.04)	49.00^{b} (44.40)	41.67^{c} (40.19)
T_8	80.66^{e} (63.93)	78.00^{cd} (62.02)	74.34^{ef} (59.54)	67.34^{e} (55.12)	56.00^{e} (48.43)	51.00^{i} (45.56)
T_9	80.00^{cde} (63.43)	78.34^{cd} (62.24)	71.67^{e} (57.82)	62.67^{d} (52.32)	54.34^{de} (47.47)	50.00^{h} (44.98)
T_{10}	81.66^{e} (64.64)	78.67^{cd} (62.48)	75.34^{f} (60.20)	69.67^{ef} (56.56)	60.67^{f} (51.14)	53.00^{j} (46.70)
T_{11}	87.33^{fg} (69.16)	81.00^{de} (64.14)	76.67^{f} (61.10)	71.66^{fg} (57.83)	65.67^{g} (54.12)	59.67^{m} (50.55)
T_{12}	93.00^{h} (74.860	86.00^{f} (68.03)	82.34^{g} (65.15)	73.67^{g} (59.12)	76.34^{h} (60.87)	68.00^{p} (55.53)

T_{13}	76.66^{bc} (61.10)	71.00^{b} (57.39)	62.00^{bc} (51.93)	58.34^{c} (49.78)	50.67^{bc} (45.36)	44.00^{d} (41.54)
T_{14}	78.33^{bcde} (62.24)	76.00^{c} (60.65)	67.34^{d} (55.13)	60.00^{cd} (50.75)	52.67^{cd} (46.51)	47.34^{f} (43.45)
T_{15}	85.67^{f} (67.73)	78.67^{cd} (62.48)	75.34^{f} (60.20)	70.34^{f} (56.98)	65.00^{g} (53.71)	56.67^{k} (48.81)
T_{16}	88.66^{fg} (70.32)	83.34^{ef} (65.89)	77.34^{f} (61.55)	71.67^{fg} (57.82)	67.67^{g} (53.33)	62.00^{n} (51.92)
General mean	**81.82**	**76.98**	**70.58**	**64.29**	**58.38**	**51.70**
SE(m) ±	**1.14**	**1.09**	**0.97**	**0.99**	**1.21**	**0.22**
C.D.(p = 0.05)	**3.29**	**3.15**	**2.82**	**2.88**	**3.51**	**0.64**

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

Ignoffo *et al.*[6], reported that the infectivity of conidia *(N. rileyi)* layered on the soil was lost to the extent of 10 % in 10 days. Balardin and Loch [2] reported that *N. rileyi* culture stored at 3°C in sterilized mineral oil had no changes in viability or pathogenicity even after 6 months. Batta[3] observed the viability of *M. anisopliae* in water emulsion (water in oil) formulation with coconut or soybean oil and recorded 50% reduction (half-life) in conidial viability after 4 - 6 months at 20 ± 1°C. Pallavi Nahar et *al.*[8] , tested viability of *N. rileyi* in different formulations such as diesel + sunflower oil, diesel + ground nut oil, diesel + safflower oil and their combinations in diesel in the ratio of 7:3 and Tween 80 (0.1 %). Conidial germination of *N. rileyi* (N812) was more than 80% in the presence of sunflower oil, diesel: sunflower oil mixture, diesel: groundnut oil mixture and Tween - 80 (0.1%) after 36 hours whereas less germination occurred in safflower oil, ground nut oil and their combination with diesel.

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