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

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A B S T R A C T

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*, rice bran oil with 0.2g spores and 0.1ml triton-X 100 oil formulation recorded highest larval mortality of 78-91 per cent followed by liquid paraffin with 0.2g spores and 0.1ml triton-X 100and heavy grade mineral oil with 0.2g spores and 0.1ml triton-X 100 oil formulation which recorded 74-89 and 69-86 per cent respectively. The remaining formulations recorded 28-85 per centlarval 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. 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 (Sultana *et al.,* 2017). *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 third instar larvae of *Spodoptera litura.*

Materials 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).

Maintenance of entomopathogenic fungus *Nomuraea rileyi*

The medium was plated into petriplates under aseptic conditions @ 20 ml per plate. The media plated petriplates were allowed to solidify in the laminar air flow chamber for about 15-20 min. The *N. rileyi* culture plates were taken and cut into discs with flame sterilized cork borer and the cut discs were inoculated on to the media plated plates with the help of cork borer and inoculation needle under aseptic conditions. The inoculated plates were sealed with paraffin tape, stored in incubator at 25^{o} C and observed daily for the development of white distinct colonies at initial stage (Plate 1) and later for pale green to malachite green distinct colonies (Plate 2).

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^{o}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. For this fresh castor leaves were collected from the field and washed with tap water and allowed to dry. The prepared *N. rileyi* spray suspensions of all the 16 treatments were applied to castor leaves separately on both sides with the help of atomizer or hair brush for infecting the larva. The treated leaves were kept on newspapers and allowed to air dry for about 20 min. The treated castor leaves were placed in sterilized plastic troughs (20 cm diameter). To these plastic troughs 10 uniform sized freshly moulted third instar *S. litura* larvae were placed, allowed to crawl and feed. From the next day, larvae are provided with fresh castor leaves.

Daily observations on post treatment changes in larvae and larval mortality were recorded. The virulence test was done at monthly intervals up to 150 days. An untreated control was also maintained. All the treatments were replicated thrice.

Analysis of the Data

The larval mortality due to oil based formulations of *N. rileyi* was expressed as per cent mortality before subjecting to statistical analysis by using the formula (Sharmila *et al.*, 2015).

$$\text{Per cent larval mortality} = \frac{\text{No. of larvae dead due to infection}}{\text{Total number of larva treated}} \times 100$$

Results and Discussion

External changes / symptoms observed in *N. rileyi* treated larvae

Symptoms before death

The infected larvae became sluggish and consumed less amount of food. Shrinked integument and pinkish discoloration was noticed on the ventral side of the body. Most of the larvae died in a characteristic way *i.e.* with slightly raised head and anterior portion of the body by firmly adhering the posterior portion to the substrate *i.e.,* food material with the prolegs. At death, the larval bodies were very smooth.

Changes after death

Within few hours of death, the larval body became stiff. The cadavers were placed in glass containers with good RH. After one to two days, the dead larvae were covered by a thin coat of white mycelia mat of *N. rileyi* (Plate 3.) and covered densely in next 24 h. Soon after two to four days, cadavers became malachite green in colour due to sporulation of mycelia (Plate 4.).

The results indicate that rice bran oil with 0.2g spores and 0.1ml triton-X 100 formulation recorded highest larval mortality at monthly intervals. At 60 and 150 DAP rice bran oil with 0.2g spores and 0.1ml triton-X 100 oil formulation recorded 86.33 and 78.00 per cent larval mortality and showed lesser reduction of 12.67 per cent larval mortality from the day of preparation to 150 days after preparation (Table 1 and Fig. 1).

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.,* (2015) 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.

Gopalakrishnan and Mohan (2000) reported that rice was the most suitable substrate for quicker and better mass multiplication of *N. rileyi.* Krishnaveni (2014) 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.,* (1985) rice contains higher proportion of starch and amylase. Hydrolysis of starch in rice resulted in the release of glucose and maltose depending on clarification.

The next best treatments are 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 which recorded 82.00 and 79.33 per cent larval mortality at 60 DAP and 74.33 and 69.00 per cent larval mortality at 150 DAP. They showed relatively lesser reduction of 14.34 and 16.67 per cent respectively.

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.

When cost of oils also concerned, rice bran oil is found to be cheaper followed by liquid paraffin. The olive oil with 0.1g spores and 0.05 ml of triton-X 100 formulation recorded 44.00 and 28.00 per cent larval mortality at 60 and 150 DAP and has shown relatively highest reduction of 22.00 per cent of virulence of conidia of *N. rileyi*.

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 fatty acids, 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.,* (2015) 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 remaining treatments, 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, 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, olive oil with 0.2g spores and 0.05 ml of triton-X 100 and olive oil with 0.1g spores and 0.1 ml of triton-X 100 oil formulations has shown 18-21 per cent reduction in virulence of conidia of *N. rileyi* from the day of preparation to 150 days of preparation.

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.

Plate.1 Vegetative growth of *N. rileyi* on SDAY medium (white mycelium)

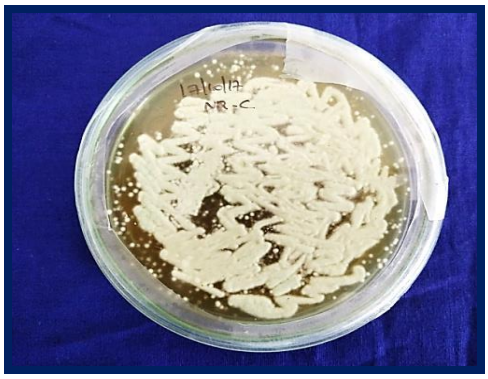


Plate.2 Sporulated *N. rileyi* on SDAY medium (malachite green)

Plate.3 Third in star *S. litura* cadavers

Plate.4 Sporulation of *N. rileyi* on cadavers (White cover is the mycelium)

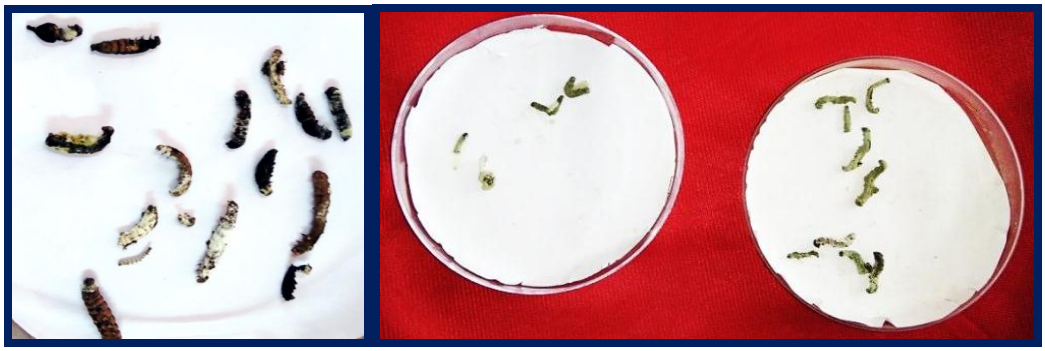
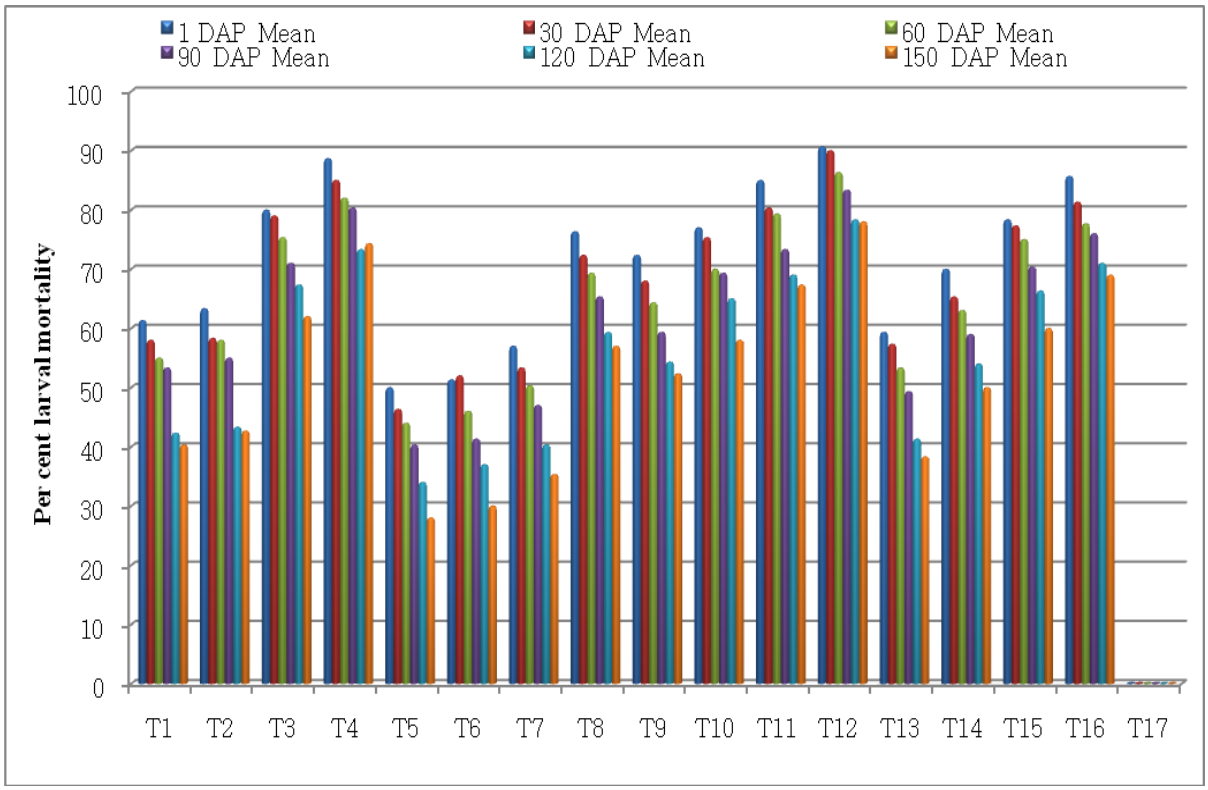


Table.1 *Spodoptera litura* larval mortalities recorded when third instar treated with different oil based formulations of *N. rileyi*

Treatments	Mean per cent larval mortality					
	1 DAP	**30 DAP**	**60 DAP**	**90 DAP**	**120 DAP**	**150 DAP**
T_1	61.33^{e} (51.53)	58.00^{d} (49.58)	55.00^{f} (47.85)	53.33^{f} (46.89)	42.33^{f} (40.57)	40.33^{f} (39.41)
T_2	63.33^{f} (52.71)	58.33^{d} (49.77)	58.00^{g} (49.58)	55.00^{g} (47.850	43.33g (41.15)	42.67^{g} (40.77)
T_3	80.00^{k} (63.40)	79.00^{j} (62.72)	75.33^{k} (60.19)	71.00^{k} (57.39)	67.33^{l} (55.12)	62.00^{l} (51.92)
T_4	88.67^{m} (70.30)	85.00^{l} (67.19)	82.00^{n} (64.87)	80.33^{n} (63.65)	73.33^{o} (58.89)	74.33^{o} (59.54)
T_5	50.00^{b} (44.98)	46.33^{b} (42.89)	44.00^{b} (41.53)	40.33^{b} (39.41)	34.00^{b} (35.65)	28.00^{b} (31.94)\
T_6	51.33^{b} (45.75)	52.00^{c} (46.13)	46.00^{c} (42.69)	41.33^{c} (39.99)	37.00^{c} (37.45)	30.00^{c} (33.19)
T_7	57.00^{c} (49.00)	53.33^{c} (46.89)	50.33^{d} (45.17)	47.00^{d} (43.26)	40.33^{d} (39.41)	35.33^{d} (36.45)
T_8	76.33^{i} (60.87)	72.33^{g} (58.24)	69.33^{j} (56.35)	65.33^{i} (53.90)	59.33^{i} (50.36)	57.00^{j} (49.00)
T_9	72.33^{h} (58.24)	68.00^{f} (55.53)	64.33^{i} (53.31)	59.33^{h} (50.36)	54.33^{h} (47.47)	52.33^{i} (46.32)
T_{10}	77.00^{ij} (61.32)	75.33^{h} (60.19)	70.00^{j} (56.76)	69.33^{j} (56.36)	65.00^{j} (53.71)	58.00^{j} (49.58)
T_{11}	85.00^{l} (61.19)	80.33^{jk} (63.65)	77.67^{l} (61.77)	73.33^{l} (58.89)	69.00^{m} (56.14)	67.33^{m} (55.12)
T_{12}	90.67^{n} (72.34)	90.00^{m} (71.55)	86.33^{o} (68.28)	83.33^{o} (65.88)	78.33^{p} (62.24)	78.00^{p} (62.00)
T_{13}	59.34^{d} (50.39)	57.33^{d} (49.19)	53.33^{c} (46.89)	49.33^{e} (44.60)	41.33^{e} (39.99)	38.33^{e} (38.24)
T_{14}	70.00^{g} (56.76)	65.33^{e} (53.90)	63.00^{h} (52.50)	59.00^{h} (50.16)	54.00^{h} (47.27)	50.00^{h} (44.98)
T_{15}	78.33^{jk} (62.23)	77.33^{i} (61.55)	75.00^{k} (59.98)	70.33^{k} (56.98)	66.33^{k} (54.51)	60.00^{k} (50.75)
T_{16}	85.67^{l} (67.73)	81.33^{k} (64.38)	79.33^{m} (62.94)	76.00^{m} (60.64)	71.00^{n} (57.39)	69.00^{n} (56.14)
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	**67.43**	**64.67**	**61.71**	**58.45**	**52.73**	**49.57**
SE(m) ±	**0.62**	**0.49**	**0.30**	**0.39**	**0.35**	**0.35**
C.D.(p = 0.05)	**1.78**	**1.42**	**0.87**	**1.12**	**1.01**	**1.02**

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.

Fig.1 Efficacy of oil based formulations of *N. rileyi* treated to third instar *S. litura* larvae under laboratory conditions



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.

Triton-X 100 is a non-ionic detergent used in various protein methods. It is accurate with precise 10% detergent solution in ultrapure water. It is easy to use and simple to dilute and dispense. It is exceptionally pure with less than 1.0 µeq/ml peroxides and carbonyls. It is stable, packed under inert nitrogen gas in glass.

The similar results were reported by Vimaladevi *et al.,* (2002) who reported that the entomopathogenic fungus *N. rileyi* in sunflower oil formulation along with Triton-X 100 showed 83.9 per cent mortality of 7 to 8 days old *S. litura* larvae, whereas, the same in sunflower oil + Tween-80 (0.02%) has recorded only 65.7 per cent mortality under laboratory conditions

Sharma and Sharma (2017) studied intrinsic toxicity of *N. rileyi* against different larval instars of *S. litura* (Fabricius) by larval and leaf-dip method. *N. rileyi* when applied at concentrations between 10^2 and 10^9 conidia/ml resulted in 20–83.34, 26.67–100, 20–80, 20–96.67 and 16.67–83.34 per

cent mortality of first, second, third, fourth and fifth instar larvae respectively. LC_{50} values 5.7×10^3, 4.4×10^4, 1.2×10^3, 1.3×10^5 and 8.3×10^4 and LC_{90} values 1.1×10^7, 4.6×10^7, 7.7×10^7, 2.7×10^8 and 1.5×10^8 conidia/ml were for different instars. Fungus at 10^7 conidia/ml took 6.9, 6.2, 7.1, 6.9 and 7.6 days to kill 50 per cent and 10.2, 8.9, 10.2, 9.5 and 12.6 days to kill 90 per cent of first, second, third, fourth and fifth instar larvae respectively.

Padanad (2009) evaluated the Pathogenicity of ten *N. rileyi* isolates against *S. litura*by exposing third instars to topical application of a spore concentration of 10^8 conidia/ml. All ten isolates of *N rileyi* were active against third instars of *S. litura*, resulting in 85 to 97% mortality.LT_{50} values of *N. rileyi* isolates against third instars of *S. litura* ranged from 5.5 to 6.6 days.

Vegetable oils at 0.5 per cent concentrations *(v/v)* were emulsified in 0.02 per cent Tween 80 solution containing 2 x 10^{10} conidia / 100 ml of spray solution. These preparations were applied after one hour to *S. litura* larvae on castor leaves and allowed to feed for 48 hr. Cumulative larval mortality nine days after treatment due to fungus indicated the similarity between treatments with vegetable oils (Safflower, groundnut, sunflower, rapeseed, mustard, sesame, cotton seed and coconut oil) indicating the safety of these vegetable oils to the fungus (Vimaladevi and Prasad, 1996).

Lezama *et al.,* (1993) reported cent per cent mortality in I, II, III and IV instar larvae of *Spodoptera frugiperda* and 97.5 and 77.5 in V and VI instar larvae when treated with spore suspension of *N. rileyi* at 2.8 X 10^7 spores per ml.

Habib and Patel (1990) carried out a laboratory experiment to study the pathogenicity of *N. rileyi* to larvae of *S. frugiperda,* at 27 ±2°C temperature and 70 percent RH. They reported that third instar larvae were more susceptible than fourth instar larvae.

Lopez and Boucias (1994) evaluated the effect of *N. rileyi* against *Spodoptera exigua* and recorded 50 per cent mortality at five days, 100 per cent morality at three to four with 5 x 10^2, 5 x 10^3 spores ml $^{-1}$.

Vimaladevi *et al.,* (2003) conducted laboratory bioassays at a concentration of 2×10^8 conidia ml^{-1} of *N. rileyi* and recorded 77-85 per cent mortalities in *S. litura* and *H. armigera.*

Nagaraja (2005) reported that the oil formulation of *N. rileyi*@ 2×10^8 spores per ml exerted considerable pathogenicity (26.2 per cent mortality) to the third instar larvae of *H. armigera*at 3 DAT. The cumulative mortality steadily increased to reach 93.20 per cent on the 10^{th} day after treatment. The laboratory evaluation of *N. rileyi* formulations *viz.,* oil formulation (sunflower oil + Tween-80 (0.02%), wettable powder (talc) and crude formulation at 2×10^8 conidia per ml concentration against third instar larvae of *S. litura* also was carried out. At the end of 10^{th} day, the highest cumulative mortality of 95 per cent was obtained in oil formulations whereas 83.10 and 77 per cent mortalities were recorded with WP and crude *N. rileyi* respectively.

Sharmila *et al.,* (2015) evaluated oil formulations of *N. rileyi* against *S. litura* under laboratory conditions and reported more than 70 % larval mortalities of *S. litura* in case of groundnut and sunflower oil based formulations with concentrations above 1x 10^6 spores per ml and mortality reduced with concentrations showing least with 1x 10^2 spores per ml.

Prior *et al.,* (1988) who reported that laboratory studies primarily with grasshopper have shown that oil formulation of aerial conidia of *M. anisopliae* are more efficacious than aqueous formulation under various temperature and moisture conditions.

Prithiva *et al.,* (2017) reported that among the different formulations tested *viz.,* crude, talc and oil formulations, *B. bassiana* (Bb 112) oil formulation was most effective against whitefly on tomato with 45.86 % reduction in population over control followed by talc (29.62 %) and crude formulations (21.63 %).

Shweta and Sobita (2017) reported that, per cent mortality was quiet high in earlier instars as compared to later instars. The highest dose of 5% 2.3×10^6 conidia/ml brought 91.66, 90.00, 88.33, 78.77, 66.11 and 49.99 percent mortality in 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 5^{th} and 6^{th} instar respectively as compared to only 11.66, 07.21, 01.10, 00.00, 01.11 and 00.00 per cent mortality in control of respective stages. They also reported that higher the dose of *B. bassiana* higher will be the mortality of tobacco caterpillar.

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