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Evaluation of *Metarhizium (Nomuraea) rileyi* Rice Bran Oil Formulations against 3rd instar *Spodoptera litura* under Laboratory

Y. Peeru Saheb[1*], K. Manjula[2], K. Devaki[3], R. Sarada Jayalakshmi Devi[4], B. Ravindra Reddy[5] and Archana Anokhe[6]

[1]Assistant Professor, Department of Entomology, SBVR Agricultural College, Badvel, Kadapa, (Andhra Pradesh), India.
[2]Professor and HOD, Department of Entomology, S.V. Agricultural College, Tirupati, Chittoor, (Andhra Pradesh), India.
[3]Scientist, Department of Entomology, RARS, Tirupati, Chittoor, (Andhra Pradesh), India.
[4]Scientist, Department of Plant Pathology, RARS, Tirupati, Chittoor, (Andhra Pradesh), India.
[5]Associate Professor, Department of Statistics and Computer Applications, S.V. Agricultural College, Tirupati, Chittoor, (Andhra Pradesh), India.
[6]Scientist, IARI, Department of Entomology (New Delhi), India.

(Corresponding author: Y. Peeru Saheb)*
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ABSTRACT: Formulation of biological control agent is an important criterion for sustainable agriculture. Formulation can improve the product stability and viability may result in consistency of field performance of many potential biological control agents. Formulation of biocontrol products has been used against insect pests (bioinsecticides), diseases (biofungicides) and weeds (bioherbicides). Many of the biocontrol agents have been formulated with dried milk, powdered casein, gelatin, saponins, oils, soaps, *etc*. So far as microbial insecticides are concerned, it is essential that the compound used should not inhibit the successful establishment of the pathogens. Oil based formulation of *M. rileyi* reduced the pest populations distinctly than other formulations. Ten types of rice bran oil formulations of *Metarhizium* (*Nomuraea*) *rileyi* were prepared by using 5 wetting agents at two different concentrations each. The ten formulations along with an untreated control were evaluated against third instar *Spodoptera litura* at monthly intervals upto one year under laboratory conditions. The results pertaining to the shelf life of *M. rileyi* rice bran oil formulations showed that T_1 (rice bran oil @ 100 ml + 5 g *M. rileyi* + Triton X-100 (0.1%) and T_7 (Rice bran oil @ 100 ml + 5 g *M. rileyi* + Tween-40 (0.1%) recorded higher per cent conidial viabilities from after preparation of formulation to 12 months after storage in T_1 as 89.28-29.27 per cent, T_7 (85.29-24.97%) and lower were noted in the treatments T_6 (60.82-20.54%) to T_{10} (63.87-20.63%) whereas higher per cent larval mortalities in laboratory varied as T_1 (93.33-46.67%), T_7 (86.67-40%) and T_3 (80-33.33%) while lower were observed in T6 (56.67-20.00%), T_{10} (63.33-20.00%). Among the five wetting agents (surfactants) used in preparation of oil formulations of *M. rileyi*, Triton X-100 @ 0.1% concentration was proved superior in maintaining the viability and virulence of spores.

Keywords: *Metarhizium (Nomuraea) rileyi*, rice bran oil formulations, third instar, *Spodoptera litura*, laboratory.

INTRODUCTION

Concerns about the negative effects of chemical insecticides have led to emphasis on alternative strategies for pest control. Pest management involving biocontrol agents is assuming prominence and have been considered as an important and safe strategy in insect population reduction. Among the several micro-organisms viz. bacteria, fungi, virus, protozoans and nematode, entomopathogenic fungi fills an extremely important niche for control of insect pests.

The fungus *Metarhizium rileyi* (Farlow) Kepler, S.A.Rehner and Humber, formerly known as *Nomuraea rileyi* (Kepler *et al.*, 2014). *Spodoptera litura* (Fab.) (*Lepidoptera*: *Noctuidae*), commonly known as tobacco caterpillar, is a polyphagous pest, which feeds on different species of plants and is widely distributed in various parts of the world. Crops like soybean, oilseeds, pulses, cotton and vegetables are seriously affected by this pest, which causes great yield losses (Srivastava *et al.*, 2018).

Formulation of biological control agent is an important criterion for sustainable agriculture (Sharma, 2004). Formulation will improve the product stability and viability which will result in consistency of field performance of many potential biological control agents. Formulation of biocontrol products has been used against insect pests (bioinsecticides), diseases (biofungicides) and weeds (bioherbicides) (Gopalakrishnan and Mohan, 2000). Biopesticides have a specific activity only towards target pests and result in lower exposure and rapid decomposition without leaving any residues behind (Namasivayam and Arvind 2015). Many of the biocontrol agents have been formulated with dried milk, powdered casein, gelatin, saponins, oils, soaps, *etc*. So far as microbial

insecticides are concerned, it is essential that the compound used should not inhibit the successful establishment of the pathogens (Tincilley *et al*., 2000). Oil based formulation of *M. rileyi* reduced the pest populations distinctly than other formulations (Devi, 2000). Among the several existing entomogenous fungi, *Nomuraea rileyi* is a cosmopolitan species infecting many noctuids such as *Helicoverpa armigera, Spodoptera litura, Tricoplusia ni, Anticarsia gemmatalis, Pseudoplusia* and has potential for development into mycoinsecticide (Shanthakumar *et al*., 2010). Though work had been done on different formulations of *Metarhizium (Nomuraea) rileyi*, much work had not been done with different wetting agents at different concentrations.

MATERIALS AND METHODS:

Production of Rice Grain Based *M. rileyi* for Preparation of Rice Bran Oil Formulations

Broken rice 60 g, distilled water of 60 ml, yeast extract powder (1%) 600 mg were taken into each 500 ml conical flasks and mixed well with glass rod. The flasks were plugged with non absorbent cotton plugs and soaked for 4-6 hours. Then the rice yeast mixture was sterilized under autoclave at 121°C and 15 Psi for 15 minutes and was left for cooling. After cooling, the media in conical flasks was loosened with sterilized glass rod or spatula under aseptic conditions in laminar airflow chamber. Then the discs of *M. rileyi* culture (NNR5 isolate) were placed into the media. The flasks were incubated at 25 ± 2°C temperature. After noticing sufficient sporulation, *i.e* after 10 days, the rice grain spore mass was harvested into glass Petri plates (13.5 cm diameter). Allowed to dry in laminar airflow chamber. The dried material was made into powder with the help of mixer grinder. The assessment of spore load g^{-1} of powder formulation preparation was done using Neubaeur Haemocytometer. This powder was used for preparation of rice bran oil formulation.

The number of conidia per gram were determined with a Neubaeur's haemocytometer under compound microscope and calculated by using the following formula.

No. of spores per unit = N × 400 × 1000 × 10 × D

where,

D: Dilution factor

N: Mean number of spores per square of the haemocytometer.

Preparation of Rice Bran Oil Formulations of *M. rileyi*

Conical flasks of 500 ml capacity with caps were sterilized and used. For T_1, 100 ml of rice bran oil was taken and added Triton-X 100 @ 0.1% (*i.e.* 100 microlitre) with the help of pipette. It was stirred well. To this solution, 5 g of *M. rileyi* powder was added and stirred well again. All the formulations with five wetting agents and at two concentrations were prepared. The formulations were maintained in the laboratory at 25 ± 2°C. The following were the prepared formulations:

T_1 : Rice bran oil + 5 g *M. rileyi* + Triton X-100 (0.1%)

T_2 : Rice bran oil + 5 g *M. rileyi* + Triton X-100 (0.2%)

T_3 : Rice bran oil + 5 g *M. rileyi* + Teepol (0.1%)

T_4 : Rice bran oil + 5 g *M. rileyi* + Teepol (0.2%)

T_5 : Rice bran oil + 5 g *M. rileyi* + Tween-20 (0.1%)

T_6 : Rice bran oil + 5 g *M. rileyi* + Tween-20 (0.2%)

T_7 : Rice bran oil + 5 g *M. rileyi* + Tween-40 (0.1%)

T_8 : Rice bran oil + 5 g *M. rileyi* + Tween-40 (0.2%)

T_9 : Rice bran oil + 5 g *M. rileyi* + Tween-80 (0.1%)

T_{10} : Rice bran oil + 5 g *M. riley i*+ Tween-80 (0.2%)

Evaluation of *M. rileyi* Rice Bran Oil Formulations under Laboratory

M. rileyi rice bran oil formulation of 0.25 ml from each treatment was poured into 50 ml of distilled water into conical flasks. Sterilized Petri plates were taken, into which the groundnut leaves were placed.

M. rileyi spore suspensions were applied on the groundnut leaves with an automizer and 10 uniform sized freshly moulted third instar *Spodoptera litura* larvae were allowed feed. Totally eleven treatments were maintained including untreated control. All the treatments were replicated thrice. The experiments were conducted at 25 ± 2°C. Daily observations on post treatment changes in larvae, larval mortality, were recorded. The prepared formulations were evaluated at monthly intervals. Viability tests of *M. rileyi* spores in the formulations were carried out at monthly intervals upto one year.

Analysis of the Data: The larval mortality and conidia viability were converted to percentage values before subjecting to statistical analysis through SPSS. Means were separated by DMRT. The larval mortality was expressed as per cent larval mortality by using the formula.

Percent larval mortality =

$$\frac{\text{No. of larvae dead due to infection}}{\text{Total no. of treated larvae}} \times 100$$

Percent conidia viability =

$$\frac{\text{No. of germinated conidia}}{\text{Total conidia}} \times 100$$

RESULTS AND DISCUSSION

Efficacy of Rice Bran Oil Formulations against Third Instar *Spodoptera litura* under Laboratory Conditions (2018-2019)

It was observed that soon after preparation of *M. rileyi* rice bran oil formulations, the treatment T_1 (rice bran oil @ 100 ml + 5 g *M. rileyi* + Triton X-100 (0.1%)) recorded highest mean per cent conidia viability of 89.28. The next best treatments were T_7 (85.29%), T_3 (81.20%), T_9 (76.46%), T_5 (72.39%), T_2 (69.99%), T_6 (60.82%) and T_8 (67.49%). Most of the treatments have shown significant differences among them except the treatment T_4 (65.49%) and T_{10} (63.87%) which were on par with each other in recording the germination (Table 1). The maximum mean per cent larval mortality of 93.33 against third instar *Spodoptera litura* larvae was noted in T_1 treatment which was found at par with T_7 (86.67%) and significantly different from all other treatments. These were followed by the treatment T_3

with 80.00 mean per cent larval mortality which was on par with T_7 (86.67%), T_9 (76.67%). The treatments T_6 (56.67%) T_4 and T_{10} (63.33%) were at par with each other. There was no larval mortality in untreated control (Table 2). Wiwat, (2004) evaluated 12 different oil based formulations of *N. rileyi* for conidial germination on the day of formulation and two weeks after formulation at two different temperatures of 40°C and 30°C. Most of the oil formulations resulted in >62 per cent germination after two weeks of storage at 40°C. Nagaraja *et al*., (2006) reported that *N. rileyi* with sunflower oil (2%) and Tween 80 (0.02%) resulted in maximum cumulative mortality of third instar *Spodoptera litura* (95.00%) followed by talc based wettable powder (83.10%) and unformulated crude formulation (77.00%) under laboratory conditions.

In the investigations after one month of storage of *M. rileyi* rice bran oil formulations in the laboratory, the treatment T_1 was found superior with 86.25 mean per cent conidia viability. The treatments that followed this were T_7 (81.07%), T_3 (76.92%), T_9 (72.39%), T_5 (67.49%), T_2 (62.90%), T_{10} (62.29%), T_4 (61.28%), T_8 (54.32%) and T_6 (52.11%) recorded least viabilities (Table 1). The observations of mean per cent larval mortality of 86.67was found in T_1 that was on par with T_7 (80.00%) and T_3 (76.67%). Treatments T_{10}, T_6 and T_4 were found on par with mean per cent larval mortality of 56.67. In untreated control there was no larval mortality (Table 2).

After 2 months of storage of *M. rileyi* rice bran oil formulations T_1 was observed with maximum of 86.04 mean per cent conidia viability. The next better treatments were T_7 (79.06%), T_3 (73.51%), T_9 (69.53%). Treatments T_{10} (59.96%) and T_4 (58.81%) were at a par with each other. These were followed by T_8 (53.80%) and T_6 (48.54%) (Table 1). Highest mean per cent larval mortality of 83.33 per cent was observed in the T_1 which was found on par with T_7 (76.67%) and significantly different from all other treatments. These were followed by T_3 (70.00%), T_9 (66.67%) and T_5 (63.33%) which were at a par with each other. The treatments followed by this are T_8, T_2 (56.67%), T_{10}, T_4 (53.33%) were found at par. Treatment T_6 showed 43.33 mean per cent larval mortality. There was no larval mortality in untreated control, T_{11} (Table 2).

After three months of storage of various *M. rileyi* rice bran oil formulations, T_1 recorded 83.98 mean per cent conidia viability and significantly different from other treatments. The next superior treatment followed was T_7 (74.99%). Treatments T_3 (63.61%) and T_9 (62.46%) were found on par with each other. T_8 (51.81%) was on par with T_{10} (49.95%). Treatment T_6 recorded lowest viability of 44.42 per cent which is significantly different from other treatments (Table 1). Highest mean larval per cent mortality of 80.00 against third instar larvae of *Spodoptera litura* was observed in T_1 which was found at a par with T_7 (70.00%) but significantly different from all other treatments. Treatments T_4, T_8 and T_{10} showed on par with each other with mean per cent larval mortalities ranging from 43.33 to 53.33. Treatment T_6 recorded 36.67 mean per cent larval mortality (Table 2). Krishnaveni *et al*., (2016) evaluated wettable powder formulations of *N. rileyi* against 3rd instar of *Spodoptera litura* under laboratory conditions at different storage intervals and temperatures which were prepared by using six inert materials *i.e.*, talc, starch, rice flour, jowar flour, wheat flour and ragi flour. Among these, talc was found superior by recording 60 per cent of larval mortality at 30 days after storage, 50 per cent at 60 days after storage and 47 per cent larval mortality at the end of 90 days of storage with higher concentration of 1×10^8 spores ml^{-1}.

The data after 4 months of storage of *M. rileyi* rice bran oil formulations revealed that among all the treatments highest mean per cent conidia viability observed was 82.59 per cent in T_1 which was at par with T_7 (69.56%) and T_3 (62.14%). The next better treatments T_9 (61.50%) and T_5 (59.22%) were at par with each other. In T_6, T_{10}, T_4 and T_2 42.08-52.91 per cent germination of conidia was recorded. The least mean per cent conidia viability was noted in T_8 (37.68%) (Table 1). The highest mean per cent larval mortality against third instar *Spodoptera litura* larva observed was 76.67 per cent in T_1 which was on par with T_7 (66.67%) and statistically differs from all other treatments. The next better treatment was T_3 (63.33%) which was on par with T_9 and T_5 (56.67%). The treatments T_6, T_2, T_4, T_8 and T_{10} were on par with each other with mean per cent larval mortalities varying from 40.00 to 43.33 (Table 2).

The mean per cent viability of conidia after storage for 5 months in the laboratory revealed that maximum of 73.32 was observed in the treatment T_1 which was significantly different from all treatments. This was followed by T_7 (68.88%) and T_9 (61.50%) which are significantly different from others. Treatments T_3 (58.80%) and T_5 (57.65%) were on par with each other. Treatment T_{10} (48.10%) was on par with T_8 (46.38%). The treatments T_6 and T_4 recorded 40.51 and 43.38 mean per cent conidia viability respectively (Table 1). The results of mean per cent larval mortality after 5 months of storage of *M. rileyi* rice bran oil formulations in the laboratory, indicated that 73.33 per cent was observed in T_1 which was on par with T_7 (66.67%) and significantly different from other treatments. The next followed treatments T_3 (60.00%) and T_9 (53.33%) were also on par with each other. T_5 (50.00%) was on par with T_9 (53.33%). Treatments T_6, T_4, T_{10}, T_2 and T_8 were recorded on par with each other with mean larval per cent mortalities ranging from 33.33 to 40.00 (Table 2). Bhargavi *et al.*, (2018) who reported pathogenicity of *N. rileyi* conidia against third instar larva of *Spodoptera litura* at monthly intervals up to five months. They prepared liquid formulations of *N. rileyi*, 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 of 0.1g (0.5×10^8 spores/0.1 g) and 0.2 g (0.1×10^9 spores/0.2 g) per

100 ml of test oils and Triton-X 100 was also used in two different concentrations *i.e.,* 0.05 per cent and 0.1 per cent for all four test oils. Likewise a total of 16 treatments and an untreated control were maintained. Among the 16 treatments of oil based formulations of *N. rileyi*, 100 ml rice bran oil with 0.2 g *N. rileyi* spores and 0.1 ml triton-X 100 oil formulation recorded highest per cent larval mortality of 86.33 and 78.00 at 60 and 150 days after preparation respectively.

The results after 6 months of storage of treatments revealed the highest mean per cent conidia viability of 60.45 per cent in T_1 which was followed by T_7 (65.10%) and these two were significantly different from other treatments. The next better treatments T_3 (51.58%), T_9 (52.11%) and T_5 (49.94%) which were statistically indifferent. Treatment T_2 recorded (46.84%) which is significantly different from others. In T_4, T_{10} and T_8 39.20-42.25 per cent viability was recorded. The least mean per cent conidia viability was noted in T_6 (37.11%) (Table 1). The maximum of 70.00 mean per cent larval mortality was observed in T_1 which was on par with T_7 (63.33%). Treatments T_3 (56.67%), T_9 (50.00%) and T_5 (46.67%) were on par with each other in the mean per cent larval mortality. The treatments T_6, T_4, T_{10}, T_2, and T_8 were also on par with each other with per cent mortalities of 30.00 to 36.67 (Table 2).

The data regarding mean per cent viability of conidia, after 7 months of storage of *M. rileyi* rice bran oil formulations indicated that among all the treatments highest conidia viability of 58.96 per cent was observed in T_1 which was significantly different from all the treatments. This was followed by T_9 (49.94%), T_7 (48.82%), T_3 (48.23%), T_5 (47.76%) which were at par with one another. These were followed by T_{10} (37.43%) and T_4 (37.24%). The least mean per cent conidia viability was noted in T_6 (32.31%) which was significantly different from other treatments (Table 1). T_1 in which highest of 66.67 mean per cent larval mortality was noted which was also on par with T_7 (56.67%) and significantly differs from other treatments. The next effective treatments were T_5, T_9 and T_3 were on par with each other with mean per cent larval mortality ranging from 43.33 to 50.00. The treatments T_6, T_4, T_{10}, T_2 and T_8 were also found on par with one another with mean per cent mortalities varying from 26.67 to 33.33 (Table 2).

The results after storage of *M. rileyi* rice bran oil formulations for 8 months of the per cent viability clearly shows that among all the treatments, highest mean per cent conidia viability observed was 47.60 per cent in T_7 was followed by T_1 (47.48%) which were on par with each other. These were followed by T_3 (46.63%), T_5, T_9 (45.38%) which were found on par with each other. T_2 (43.22%) was at par with T_5, T_9 and T_3. Treatments T_8 (39.23%), T_6 (36.32%) and T_4 (35.98%) were found at a par. The least mean per cent conidia viability was noted in T_{10} (30.70) (Table 1). Highest mean per cent larval mortality of 63.33 in T_1 that was found at a par with T_7 (53.33%). Treatments T_3, T_9 and T_5 were also found to be at par with one another with mean per cent larval mortality ranging from 36.67 to 46.67. The treatments T_6, T_4, T_{10}, T_2, and T_8 were recorded on par with one another with mean per cent larval mortalities ranging from 23.33 to 30.00 (Table 2).

The data after 9 months of storage on observation of mean per cent conidia viability among all thetreatments inferred that the highest mean per cent conidia viability of 42.77 was observed in T_5. This was followed by T_2 (42.08%), T_3 (41.90%) found on par with each other. Treatments T_7 (39.00%) and T_1 (39.00%) were on par with each other. The next treatments were T_6 (34.33%), T_8 (36.98%), T_9 (38.00%) and T_4 (33.95%). The least mean per cent conidia viability was noted in T10 (27.92%) which was significantly different from other treatments (Table 1). At 9 months of storage, the highest mortality of 60.00 per cent was obtained in T_1 which was on par with T7 (50.00%) and differs significantly from other treatments. Treatments T_3 (46.67%), T9 (40.00%) and T_5 (36.67%) were on par with one another. T_8, T_2 (30.00%), T_{10}, T_4 (26.67%) and T_6 (23.33%) were also found on par with one another (Table 2).

It was observed that data after 10 months of storage of *M. rileyi* rice bran oil formulations among all the treatments, highest mean per cent conidia viability observed was 38.20 per cent in T_2. It was followed by T_5 (35.93%), T_7 (34.86%) which were on par with each other. Treatment T_1 (33.30%), T_3 (33.29%) and T_9 (33.26%) were found on par with one another. It was followed by T_6 (27.23%) and T_8 (26.85%) which were at par with one another. The least mean per cent conidia viability was noted in T_{10} (25.58%) (Table 1). Highest mean per cent larval mortality of 56.67 against third instar *Spodoptera litura* was recorded in T_1 which was significantly different from others. This was followed by the treatments T_7 (46.67%) and T_3 (40.00%) which were on par with each other. The next treatments that followed were T_9 (36.67%) and T_5 (33.33%) that had no significant statistical difference. The treatments T_6, T_4, T_{10}, T_2 and T_8 were observed with mean per cent mortalities of 20.00 to 26.67 which were comparatively low and had no statistical significant difference (Table 2).

The observations after 11 months of storage of *M. rileyi* rice bran oil formulations among all the treatments revealed highest mean per cent conidia viability observed was 33.31 per cent in T_7. This was followed by T_5, T_9 (31.72%), T_3, T_1 (31.21%) which were on par with one other. The treatments T_4 (27.76%) and T_2 (27.47%) were at par with each another. Treatment T_8 recorded (24.94%). The least mean percentage conidia germination were noted in T_6 (21.16%) and T_{10} (23.00%) and were found on par with each other (Table 1).

Table 1: Viability of conidia of *M. rileyi* in rice bran oil formulations under laboratory conditions (2018-2019).

Sr. No.	Treatment	Mean Percent Conidia germination												
		At preparation of formulation	1st MAS	2nd MAS	3rd MAS	4th MAS	5th MAS	6th MAS	7th MAS	8th MAS	9th MAS	10th MAS	11th MAS	12th MAS
1	T_1	89.28^{a} (70.89)	86.25^{a} (68.23)	86.04^{a} (68.06)	83.98^{a} (66.41)	82.59^{a} (65.34)	73.32^{a} (58.90)	60.45^{b} (51.03)	58.96^{a} (50.16)	47.48^{a} (43.55)	39.00^{abc} (38.64)	33.30^{bc} (35.24)	30.21^{ab} (33.34)	29.97^{a} (33.19)
2	T_2	69.99^{f} (56.78)	62.90^{f} (52.47)	62.48^{e} (52.23)	57.55^{e} (49.34)	52.91^{cd} (46.67)	49.97^{e} (44.98)	46.84^{d} (43.19)	44.42^{c} (41.79)	43.22^{b} (41.10)	42.08^{ab} (40.44)	38.20^{a} (38.17)	27.47^{bc} (31.60)	22.82^{cde} (28.51)
3	T_3	81.20^{c} (64.31)	76.92^{c} (61.29)	73.51^{c} (59.03)	63.61^{c} (52.90)	62.14^{ab} (52.03)	58.80^{d} (50.07)	51.58^{c} (45.91)	48.23^{b} (43.99)	46.63^{ab} (43.07)	41.90^{ab} (40.33)	33.29^{bc} (35.23)	31.21^{ab} (33.95)	26.43^{abcd} (30.92)
4	T_4	65.49^{h} (54.03)	61.28^{g} (51.52)	58.81^{g} (50.08)	53.83^{f} (47.20)	49.99^{cd} (44.99)	43.38^{g} (41.20)	39.20^{ef} (38.76)	37.24^{d} (37.60)	35.98^{c} (36.86)	33.95^{e} (35.63)	29.61^{cd} (32.97)	27.76^{bc} (31.79)	23.62^{bcde} (29.07)
5	T_5	72.39^{e} (58.30)	67.49^{e} (55.24)	66.63^{e} (54.72)	59.96^{d} (50.75)	59.22^{bc} (50.32)	57.65^{d} (49.40)	49.94^{c} (44.97)	47.76^{b} (43.72)	45.38^{ab} (42.35)	42.77^{a} (40.84)	35.93^{ab} (36.82)	31.72^{ab} (34.26)	26.85^{abc} (31.19)
6	T_6	60.82^{i} (51.25)	52.11^{i} (46.21)	48.54^{i} (44.17)	44.42^{h} (41.79)	42.08^{d} (40.44)	40.51^{h} (39.53)	37.11^{f} (37.53)	32.31^{e} (34.64)	36.32^{c} (37.06)	34.33d^{e} (35.86)	27.23^{de} (31.44)	21.16^{d} (27.37)	20.54^{e} (26.93)
7	T_7	85.29^{b} (67.44)	81.07^{b} (64.21)	79.06^{b} (62.77)	74.99^{b} (60.00)	69.56^{ab} (56.51)	68.88^{b} (56.09)	65.10^{a} (53.79)	48.82^{b} (44.32)	47.60^{a} (43.62)	39.00^{abc} (38.64)	34.86^{ab} (36.18)	33.31^{a} (35.25)	24.97^{bcd} (29.97)
8	T_8	67.49^{g} (55.24)	54.32^{fg} (47.48)	53.80^{h} (47.18)	51.81^{fg} (46.04)	37.68^{cd} (37.44)	46.38^{f} (42.92)	42.25^{e} (40.54)	41.33^{c} (40.01)	39.23^{c} (38.78)	36.98^{cde} (37.45)	26.85^{de} (31.19)	24.94^{cd} (29.94)	22.15^{de} (28.05)
9	T_9	76.46^{d} (60.98)	72.39^{d} (58.30)	69.53^{d} (56.50)	62.46^{c} (52.22)	61.50^{bc} (51.65)	61.50^{c} (51.65)	52.11^{c} (46.21)	49.94^{b} (44.97)	45.38^{ab} (42.35)	38.00^{bcd} (38.05)	33.26^{bc} (35.21)	31.72^{ab} (34.26)	27.92^{ab} (31.88)
10	T_{10}	63.87^{h} (53.05)	62.29^{h} (52.11)	59.96^{g} (50.75)	49.95^{g} (44.97)	46.38^{cd} (42.92)	48.10^{ef} (43.91)	39.94^{ef} (39.19)	37.43^{d} (37.71)	30.70^{d} (33.63)	27.92^{f} (31.88)	25.58^{e} (30.36)	23.00^{d} (28.63)	20.63^{e} (26.98)
	F	**Sig.**	**Sig.**	**Sig.**	**Sig.**	**Sig.**	**Sig.**	**Sig.**	**Sig.**	**Sig.**	**Sig.**	**Sig.**	**Sig.**	**Sig.**
	Sig.	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**

MAS: Months after storage, Values are the means of three replications, Values in parenthesis are arc sine transformed values, Values followed by same letter are not significantly different as per DMRT, Means are significantly different at P (0.01)

T1 = Rice bran oil @100 ml + 5 g *M. rileyi* + Triton X-100 (0.1%), T2 = Rice bran oil @100 ml + 5 g *M. rileyi* + Triton X-100 (0.2%); T3 = Rice bran oil @100 ml + 5 g *M. rileyi* + Teepol (0.1%); T4 = Rice bran oil @100 ml + 5 g *M. rileyi* + Teepol (0.2%); T5 = Rice bran oil @ 100 ml + 5 g *M. rileyi* + Tween-20 (0.1%); T6 = Rice bran oil @ 100 ml + 5 g *M. rileyi* + Tween-20 (0.2%); T7 = Rice bran oil @ 100 ml + 5 g *M. rileyi* + Tween-40 (0.1%); T8 = Rice bran oil @100 ml + 5 g *M. rileyi* + Tween-40 (0.2%); T9 = Rice bran oil @ 100 ml + 5 g *M. rileyi* + Tween-80 (0.1%); T10 = Rice bran oil @100 ml + 5 g *M. rileyi* + Tween-80 (0.2%)

T_1 showed highest of 50.00 mean per cent larval mortality which was on par with T_7 (43.33%) and significantly different from other treatments. Treatments T_3 (36.67%) and T_9 (33.33%) were the next best which were found on par with each other. Then the treatments followed were T_5 (26.67%), T_{10}, T_8, T_2 (23.33%) and T_6, T_4 (20.00%). These were also found to be on par with one another (Table 2).

The results after 12 months of storage of different *M. rileyi* rice bran oil formulations shows that the highest mean per cent conidia viability of 29.97 was observed in T_1 which was followed by T_9 (27.92%), T_5 (26.85%) and T_3 (26.43%). The lowest of 20.54, 20.63 percentages viabilities were noticed in T_6 and T_{10} respectively which were on par with each other (Table 1). The maximum of 46.67 per cent larval mortality was recorded in T_1 which issignificantly different from other treatments. Treatment T_7 recorded 40.00 mean per cent mortality which is also significantly different from other treatments. T_3 (33.33%) and T_9 (30.00%) were on par with each other. Treatments T_5, T_2, (23.33%) and T_{10}, T_8, T_6, T_4 (20.00%) were also observed on par with one another (Table 2). The present results are in accordance with the report of Ramegowda (2005) who tested nine vegetable oils and seven wettable powder formulations of *N. rileyi*. He recorded that the viability of conidia after one year of storage was 22.21 per cent in refrigerated condition, while it was only 15.64 per cent at ambient room temperature. He also recorded that rice flour, talc and sorghum flour emerged as the best among carrier materials evaluated, while skimmed milk powder and gram flour appeared to be non-suitable. Grijalba *et al.*, (2018) reported that a Colombian isolate of *M. rileyi* was produced in bulk and conidia were formulated as an emulsifiable concentrate (EC) and viability of formulated conidia was studied. Conidial viability was maintained at >85 per cent for 12 months under refrigeration (8°C) and for more than three months at 18°C. The efficacy of the EC to control *S. frugiperda* was correlated with the storage time by using different mathematical models and conservative values of 6 and 12 months at 8°C and 18°C respectively.

The higher mean per cent conidia germination were observed in treatments T_1, T_7 and T_3 from at preparation of formulation to 12 months after storage varied in T_1 as 89.28 to 29.27 per cent, T_7 (85.29 to 24.97%) and T_3 (81.20 to 26.43%) where as lower were noted in the treatments T_6 (60.82 to 20.54%) to T_{10} (63.87 to 20.63%). The higher mean per cent larval mortalities were observed in treatments T_1, T_7 and T_3 when treated against third instar *Spodoptera litura* at monthly intervals and the per cent larval mortalities ranged as T_1 as 93.33 to 46.67, T_7 (86.67 to 40%) and T_3 (80 to 33.33%) while lower were noted in the treatments T_6 (56.67 to 20.00%), T_{10} (63.33 to 20.00%) and in T_{11} untreated control 0.00 per cent was observed (Tables 1 and 2).

Pathogenicity levels also were recorded according to conidia viabilities. Gradual decrease in viability and virulence was recorded as the period of storage advances upto 12 months of observations.

In the present study, rice bran oil was proved as effective in maintaining the viability and virulence of conidia of *M. rileyi.* Rice bran oil is the oil extracted from the hard outer brown layer of rice called chaff. It is known for its high smoke point of 232°C and mild flavor and can retain its properties evenexposed to high temperatures. Its shelf life is 1-2 years. It is considered by some to be the "world's healthiest edible oil" containing many vitamins, antioxidants and nutrients. By considering the above properties, rice bran oil is used as a main storage medium for conidia of *N. rielyi.*

Table 2: Efficacy of rice bran oil formulations of *M. rileyi* against third instar *Spodoptera litura* under laboratory conditions (2018-2019).

Sr. No.	Treatment	Mean Per cent Larval Mortality of third instar *Spodoptera litura* larva												
		At preparation of formulation	1st MAS	2nd MAS	3rd MAS	4th MAS	5th MAS	6th MAS	7th MAS	8th MAS	9th MAS	10th MAS	11th MAS	12th MAS
1.	T_1	93.33^{a} (77.71)	86.67^{a} (68.86)	83.33^{a} (66.14)	80.00^{a} (63.43)	76.67^{a} (61.22)	73.33^{a} (59.00)	70.00^{a} (56.79)	66.67^{a} (54.78)	63.33^{a} (52.78)	60.00^{a} (50.77)	56.67^{a} (48.85)	50.00^{a} (45.00)	46.67^{a} (43.08)
2.	T_2	66.67^{cde} (54.78)	63.33^{cd} (52.78)	56.67^{de} (48.85)	56.67^{bcd} (48.85)	43.33^{cd} (41.15)	40.00^{e} (39.23)	36.67^{de} (37.22)	33.33^{cd} (35.22)	30.00^{def} (33.21)	30.00^{def} (33.21)	26.67^{de} (31.00)	23.33^{e} (28.78)	23.33^{d} (28.78)
3.	T_3	80.00^{bc} (63.43)	76.67^{abc} (61.22)	70.00^{bc} (56.79)	66.67^{bc} (54.78)	63.33^{b} (52.78)	60.00^{bc} (50.77)	56.67^{bc} (48.85)	50.00^{b} (45.00)	46.67^{bc} (43.08)	46.67^{bc} (43.08)	40.00^{bc} (39.23)	36.67^{bc} (37.22)	33.33^{c} (35.22)
4.	T_4	63.33^{de} (52.78)	56.67^{d} (48.85)	53.33^{ef} (46.92)	43.33^{de} (41.15)	43.33^{cd} (41.15)	36.67^{e} (37.22)	33.33^{de} (35.22)	30.00^{d} (33.21)	26.67^{ef} (31.00)	26.67^{ef} (31.00)	23.33^{e} (28.78)	20.00^{e} (26.57)	20.00^{d} (26.57)
5.	T_5	70.00^{cde} (56.79)	66.67^{cd} (54.78)	63.33^{cde} (52.78)	56.67^{bcd} (48.85)	56.67^{bc} (48.85)	50.00^{d} (45.00)	46.67^{cd} (43.08)	43.33^{bc} (41.15)	36.67^{cde} (37.22)	36.67^{cde} (37.22)	33.33^{cd} (35.22)	26.67^{de} (31.00)	23.33^{d} (28.78)
6	T_6	56.67^{e} (48.85)	56.67^{d} (48.85)	43.33^{f} (41.15)	36.67^{e} (37.22)	40.00^{d} (39.23)	33.33^{e} (35.22)	30.00^{e} (33.21)	26.67^{d} (31.00)	23.33^{f} (28.78)	23.33^{f} (28.78)	20.00^{e} (26.57)	20.00^{e} (26.57)	20.00^{d} (26.57)
7.	T_7	86.67^{ab} (68.86)	80.00^{ab} (63.43)	76.67^{ab} (61.22)	70.00^{ab} (56.79)	66.67^{ab} (54.78)	66.67^{ab} (54.78)	63.33^{ab} (52.78)	56.67^{ab} (48.85)	53.33^{ab} (46.92)	50.00^{ab} (45.00)	46.67^{b} (43.08)	43.33^{ab} (41.15)	40.00^{b} (39.23)
8.	T_8	66.67^{cde} (54.78)	63.33^{cd} (52.78)	56.67^{de} (48.85)	53.33^{cd} (46.92)	43.33^{cd} (41.15)	40.00^{e} (39.23)	36.67^{de} (37.22)	33.33^{cd} (35.22)	30.00^{def} (33.21)	30.00^{def} (33.21)	26.67^{de} (31.00)	23.33^{e} (28.78)	20.00^{d} (26.57)
9.	T_9	76.67^{bcd} (61.22)	73.33^{bc} (59.00)	66.67^{cd} (54.78)	63.33^{bc} (52.78)	56.67^{bc} (48.85)	53.33^{cd} (46.92)	50.00^{c} (45.00)	46.67^{b} (43.08)	40.00^{cd} (39.23)	40.00^{bcd} (39.23)	36.67^{cd} (37.22)	33.33^{cd} (35.22)	30.00^{c} (33.21)
10.	T_{10}	63.33^{de} (52.78)	56.67^{d} (48.85)	53.33^{ef} (46.92)	43.33^{de} (41.15)	43.33^{cd} (41.15)	36.67^{e} (37.22)	33.33^{de} (35.22)	30.00^{d} (33.21)	26.67^{ef} (31.00)	26.67^{ef} (31.00)	23.33^{e} (28.78)	23.33^{e} (28.78)	20.00^{d} (26.57)
11.	T_{11}	0.00^{f} (0.00)	0.00^{e} (0.00)	0.00^{g} (0.00)	0.00^{f} (0.00)	0.00^{e} (0.00)	0.00^{f} (0.00)	0.00^{f} (0.00)	0.00^{e} (0.00)	0.00^{g} (0.00)	0.00^{g} (0.00)	0.00^{f} (0.00)	0.00^{f} (0.00)	0.00^{e} (0.00)
	F	**Sig.**	**Sig.**	**Sig.**	**Sig.**	**Sig.**	**Sig.**	**Sig.**	**Sig.**	**Sig.**	**Sig.**	**Sig.**	**Sig.**	**Sig.**
	Sig.	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**

MAS: Months after storage, Values are the means of three replications, Values in parenthesis are arc sine transformed values Values followed by same letter are not significantly different as per DMRT, Means are significantly different at P (0.01)

T_1 = Rice bran oil @100 ml + 5 g *M. rileyi* + Triton X-100 (0.1%), T_2 = Rice bran oil @100 ml + 5 g *M. rileyi* + Triton X-100 (0.2%); T_3 = Rice bran oil @ 100 ml + 5 g *M. rileyi* + Teepol (0.1%); T_4 = Rice bran oil @100 ml + 5 g *M. rileyi* + Teepol (0.2%); T_5 = Rice bran oil @ 100 ml + 5 g *M. rileyi* + Tween-20 (0.1%); T_6 = Rice bran oil @ 100 ml + 5 g *M. rileyi* + Tween-20 (0.2%);T_7 = Rice bran oil @ 100 ml + 5 g *M. rileyi* + Tween-40 (0.1%); T_8 = Rice bran oil @100 ml + 5 g *M. rileyi* + Tween-40 (0.2%); T_9 = Rice bran oil @ 100 ml + 5 g *M. rileyi* + Tween-80 (0.1%); T_{10} = Rice bran oil @100 ml + 5 g *M. rileyi* + Tween-80 (0.2%); T_{11} = Untreated control

Wetting agents are surfactants that reduce the surface tension of liquid medium, coat the surface of suspension particles and thereby facilitate the wetting of each particle.

In the present study, among the five wetting agents (surfactants) used in preparation of oil formulations of *M. rileyi*, Triton X-100 was proved superior in maintaining the viability and virulence of spores. Next suited surfactants were Tween-40, Teepol, Tween-80 and Tween-20 were somewhat lower in performances. So, the ingredients of Tween-40 than Teepol, Tween-80 and Tween-20 may be suitable. When the two concentrations were considered (0.1 and 0.2%) at 0.1% higher viabilities and larval mortalities were recorded. Triton X-100 is one of the most widely used non-ionic surfactants for lysing cells to extract protein and cellular organelles or to permeabilize the living cell membrane for transfection. It has a hydrophilic polyoxylene oxide chain and an aromatic hydrocarbon lipophilic or hydrophobic group. It is a good emulsifier, mild detergent and non-denaturing agent. Teepol is a natural liquid detergent, equally effective in hard, soft and salt water. Teepol is well suited to laboratory usage. Tween 40 is used as emulsifier, solubulizer, stabilizer, diffusant and fiber lubricant etc. Bukhari *et al*., (2011) tested fungal spores of both *Metarhizium anisopliae* and *Beauveria bassiana* against malaria mosquito larvae (*Anopheline* larvae) in the laboratory. The fungal spores, were formulated with wheat flour, white pepper, WaterSavr (WaterSavr™, Sodium bicarbonate version, Flexible Solutions International Ltd., Victoria BC, Canada), 0.1% Tween 80 aqueous solution, Ondina oil 917 (Shell Ondina® Oil 917, Shell, The Netherlands) and ShellSol T (Shellsol T®, Shell, The Netherlands) and were tested for their potential as carrier of fungal spores. Among these, ShellSol T was easy to mix and apply to the water surface and it was more effective against *Anopheline* larvae than 0.1% Tween 80. ShellSol T also improved the persistence of fungal spores after application to the water. Aquino *et al*., (2010) reported that among the five oil formulations *i.e*., mineral oil, canola oil, sunflower oil, olive oil and peanut oil of *N. rileyi* tested against larvae of *S. exigua, S. frugiperda, Heliothis zea* and *Heliothis virescens* the mineral oil formulation was proved more effective.

FUTURE SCOPE

Development of dry and oil formulations of *M. rileyi* isolates, combination of different microbial agents and field evaluation against pest complex in crops like groundnut, blackgram and maize. Evaluation of nanomaterial based formulations of *M. rileyi* against insects. To conduct compatibility studies with novel pesticides under fieldconditions. To study the efficacy of *M. rileyi* against insect pests under storage. Development of safety tests data of *M. rileyi* and documentation, submission for registration as biopesticide.

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Conflict of Interest. None.

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