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Management of Fall Armyworm (*Spodoptera frugiperda*) in Maize with Oil Based Formulations of *Metarhizium rileyi*

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Maize, a vital cereal crop globally and in India, faces productivity challenges exacerbated by invasive pests like the fall armyworm. In response, eco-friendly strategies like microbial-based pesticides, including *Metarhizium rileyi*, is emerging as promising solution. This study addresses the challenge for effective fall armyworm management by utilizing *M. rileyi* oil based formulations. Results demonstrate significant larval reduction, with sunflower oil-based formulations achieving the highest reduction of 49.12% compared to control. These findings underscore the potential of *M. rileyi* formulations, particularly in sunflower oil, as valuable tools in integrated pest management strategies for maize crops.

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1. INTRODUCTION

Maize (*Zea mays* L.) holds a significant position as the "Queen of cereals" due to its remarkable yield potential. It stands out as a versatile cereal crop that thrives in diverse seasons and environments, playing a pivotal role in the global agricultural economy. The global production of maize exceeds 1,147.7 million tonnes across 170 countries, cultivated on approximately 193.7 million hectares, with an average productivity of 5.75 tonnes per hectare [1]. In India, maize stands as a crucial cereal crop, following rice and wheat, with an extensive cultivation area of 9.89 million hectares and an annual production of 33.62 million tonnes [2].

Despite maize's growing significance as an industrial crop in India, its productivity lags behind the global average, with only 3.2 tonnes per hectare. This disparity can be attributed to various factors, including the lack of elite hybrids and varieties capable of withstanding biotic and abiotic stresses. Socioeconomic challenges, such as the adoption of new hybrids and inadequate support for maize growers, further contribute to the issue. Biotic factors pose an additional challenge, with 250 insect pests associated with maize in both field and storage conditions. Notably, pests like maize stalk borer, pink stem borer, shoot bug, armyworm, shoot fly, aphids, cob borer, and termites create significant bottlenecks [3].

In recent years, a particularly destructive invasive pest, the fall armyworm (*Spodoptera frugiperda*), has emerged as a major threat to maize production. This pest, native to tropical and subtropical regions of the Americas, remained confined to its region of origin until 2016. Subsequently, it rapidly spread across Sub-Saharan African countries, causing substantial losses in maize production [4]. The first report of fall armyworm in India was documented in Shivamogga, Karnataka, in 2018 [5]. Fall armyworm is a polyphagous pest known to attack 353 plant species across 76 plant families, with a preference for grasses, including maize.

The economic impact of fall armyworm is substantial, with estimated annual yield losses reaching approximately US $300 million, rising to over US $500 million during major outbreaks in the USA [6]. In Brazil, the cost of controlling fall armyworm reached US $600 million in 2009, equivalent to roughly US $40 per hectare [7]. Extrapolated estimates for 12 African countries suggest potential losses of US $2.5–6.3 billion in 2017 [8]. Furthermore, Abrahams et al. [9] predict annual economic losses of up to US $13 billion in Sub-Saharan Africa across crops like maize, rice, sorghum, and sugarcane. In India, crop losses due to fall armyworm are estimated at around US $2,481 million, affecting an area of 1.4 lakh hectares [10].

Efforts to manage these economic losses include various control approaches such as cultural methods, chemical pesticides, and natural plant products. The Indian government has provided ad hoc recommendations for the use of chemical pesticides or biopesticides [11]. However, excessive use of conventional insecticides can lead to issues like insecticide resistance, pest resurgence, environmental pollution, and food residue. Additionally, insecticides have adverse effects on non-target organisms, disrupting natural ecosystems and posing long-term challenges for ecosystem stability.

In response to these challenges, biocontrol and biopesticide strategies have emerged as sustainable and eco-friendly alternatives to chemical insecticides. Among these, microbial-based pesticides have proven effective and are a promising solution for managing invasive pests [12]. Microbial pesticides, particularly entomopathogenic fungi (EPF), have gained attention in integrated pest management. EPFs are abundant in terrestrial environments and play a vital role in regulating insect and arachnid populations. Among EPFs, species within the *Metarhizium*, *Beauveria*, and *Isaria* genera have been effectively used in biological pest control, targeting various insect pests.

Metarhizium rileyi, formerly known as *Nomureae rileyi*, is one such entomopathogenic fungus known for its efficacy against lepidopteran insects. It exhibits a high rate of natural incidence under field conditions and is capable of causing epizootics. *M. rileyi* has been recognized for its eco-friendly nature and host specificity, making it a suitable candidate for pest management.

However, lack of suitable formulations further complicates the management of fall armyworm infestations. Previous laboratory studies evaluating oil-based formulations of *M. rileyi* against fall armyworm found that sunflower and sesame oil-based formulations performed well. Consequently, this study aims to assess the efficacy of these formulations at the field level, potentially providing a more effective solution for fall armyworm management.

2. MATERIALS AND METHODS

2.1 Culture Maintenance of *M. rileyi*

Rice grains were soaked overnight in water at a quantity of 50 g in 50 ml of one per cent yeast extract solution per conical flask of 250 ml capacity. After thorough mixing, the flasks were plugged with non-absorbent cotton and autoclaved at 15 PSI pressure at 121 °C for 30 min. After thorough mixing, the flasks were plugged with non-absorbent cotton and autoclaved at 15 PSI pressure at 121 °C for 30 min. The disc of fully grown *M. rileyi* (UASRBC Mr19) culture plate was inoculated into respective substrates and incubated at 22-26 °C at 75- 85 per cent RH in BOD. The inoculated grains were completely covered by the malachite green coloured spores in 12 days after inoculation. The spores were harvested with the help of sterilized sieve after drying the grains along with the spores for 2-3 days under shade condition and were used to prepare powder formulation and oil-based formulations.

2.2 Oil based Formulations of *M. rileyi*

The test oils used for the preparation of *M. rileyi* formulations are sunflower oil, groundnut oil, coconut oil, mineral oil, sesamum oil, pongamia oil and neem oil. The oils were poured into sterilized borosil bottles of 250 ml and autoclaved at 15 PSI pressure at 121°C for 15 min. Harvested spores of *M. rileyi* were mixed to the test oils in the proportion of 0.2 g per 100 ml of test oil. Tween – 80 as a wetting agent was also used in concentration of 0.1 per cent for test oils for uniform mixing of spores under aseptic conditions. The prepared oil based formulations were stored in HDPE bottles for further use in laboratory and field evaluation trials.

2.3 Powder Formulation of *M. rileyi*

Powder formulation was prepared by mixing of dry spore suspension in sterilized talcum powder in the ratio of 1:2 (500g: 1 kg talc) in sterilized tray under laminar air flow and dried to 8% moisture. The talc formulation of *M. rileyi* (UASRBC-*Mr*19) contains 1.0 × 10^8 cfu/g. The formulation was stored in polypropylene pouches for further use in field evaluation trials.

2.4 Pathogenicity of Different Oil Based Formulations of *M. rileyi* against fall Armyworm *S. frugiperda* under Laboratory Condition

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

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

$$\text{Per cent pupal morality} = \frac{\text{No.of pupae dead due to infection}}{\text{Total number of pupae treated}} \times 100$$

Per cent mortality was analyzed after transforming into angular values.

2.5 Field Studies

a) Details of experiment:

Two oil based formulations of *M. rileyi* (UASRBC-Mr19) were evaluated against the *S. frugiperda* in maize crop, during rabi season of 2021-22 at Biocontrol block. A Randomised Block Design (RBD) was laid out with three replications of 4 m × 4 m plot size. Sowing was taken up with a spacing of 60 cm × 30 cm. All the recommended package of practices were followed to raise the crop except plant protection measures. When considerable damage of *S. frugiperda* were noticed reaching ETL, the following treatments were imposed at 30 days after sowing (Table 1).

b) Assessment of infected larvae of *S. frugiperda* in field

In each replication, five plants were selected and tagged for taking observations. The tagged plants were observed for healthy *S. frugiperda* larvae at pre count and healthy and infected (mummified) larvae after treatment. The infected larvae were counted after 5, 10 and 15 days of treatment. Pre counts were used to calculate the mean per cent larval reduction over pre-treatment with the following formula.

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

c) Statistical analysis:

The data obtained on the larval population were subjected to statistical analysis. Percent values were transferred to arc-sine values before subjecting to statistical analysis. Means were separated by DMRT [13].

Table 1. Different treatments of *M. rileyi* against fall armyworm *S. frugiperda*

Tr. No.	Oil formulation	Concentration (CFU/ ml)	Dosage (ml or g/lit)
T_1	Sesame oil formulation	$1×10^8$	4
T_2	Sesame oil formulation	$1×10^8$	6
T_3	Sesame oil formulation	$1×10^8$	8
T_4	Sunflower oil formulation	$1×10^8$	4
T_5	Sunflower oil formulation	$1×10^8$	6
T_6	Sunflower oil formulation	$1×10^8$	8
T_7	Talc based formulation	$1×10^8$	5
T_8	Untreated control	-	-

3. RESULTS AND DISCUSSION

a) One day before spray

One day before spray the larval population ranged from 10.08 to 11.32 larvae per five plants and it was at statistically non-significant.

b) First spray

Five days after first spray, highest reduction of 16.25 per cent was noticed in the highest dosage of sunflower oil based formulation $1×10^8$ @ 8 ml/L and it was at par with its next lowest dosage $1×10^8$ @ 6 ml/L which recorded 14.54 per cent. The highest dosage of sesame oil based formulation recorded 13.25 per cent reduction and it was at par with its next lowest dosage $1×10^8$ @ 6 ml/L which recorded 12.75 per cent. Similar trend was noticed at seven and ten days after first spray with increased per cent reduction in larval population over control (Table 2).

c) Second spray

Per cent reduction of *S. frugiperda:* Highest reduction of 49.12 per cent larvae over control was noticed in the highest dosage of sunflower oil based formulation and it was at par with its next lowest dosage which recorded 47.86 per cent. The highest dosage of sesame oil based formulation $1×10^8$ @ 8 ml/L recorded 44.68 per cent and it was at par with its next dosage $1×10^8$ @ 6 ml/L which recorded 41.32 per cent and similar trend was noticed at seven and ten days after second spray (Table 3).

Table 2. Efficacy of oil based formulations of *M. rileyi* against *S. frugiperda* during first spray in maize ecosystem

Tr. No.	Oil formulation	Concentration (CFU/ ml)	Dosage (ml or g/lit)	Reduction in larval population over control (%)			
				First Spray			
				1 DBS	5 DAS	7 DAS	10 DAS
T_1	Sesame oil formulation	$1×10^8$	4	11.18 (19.53)	9.25 (17.71)	11.75 (20.05)	24.73 (29.82)
T_2	Sesame oil formulation	$1×10^8$	6	10.46 (18.87)	12.75 (20.92)	16.58 (24.03)	31.84 (34.35)
T_3	Sesame oil formulation	$1×10^8$	8	10.22 (18.64)	13.25 (21.35)	19.82 (26.44)	37.18 (37.57)
T_4	Sunflower oil formulation	$1×10^8$	4	11.32 (19.66)	8.54 (16.95)	10.16 (18.59)	21.56 (27.67)
T_5	Sunflower oil formulation	$1×10^8$	6	10.76 (19.15)	14.54 (22.38)	18.84 (25.72)	35.52 (36.58)
T_6	Sunflower oil formulation	$1×10^8$	8	10.08 (18.51)	16.25 (23.77)	21.25 (27.45)	36.28 (37.04)
T_7	Talc based formulation	$1×10^8$	5	10.74 (19.13)	14.25 (22.18)	19.15 (25.95)	35.55 (36.60)
T_8	Untreated control	-	-	10.88 (19.26)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
S Em±				0.18	0.56	0.48	0.78
CD @ P=0.05				NS	1.68	1.44	2.33

CFU= Colony Forming Unit, DBS = Days Before Spraying, DAS = Days After Spraying
Figures in the parenthesis are arcsine values

Table 3. Efficacy of oil based formulations of *M. rileyi* against *S. frugiperda* during second spray in maize ecosystem

Tr. No.	Oil formulation	Concentration (CFU/ ml)	Dosage (ml or g/lit)	Reduction in larval population over control (%)		
				Second Spray		
				5 DAS	7 DAS	10 DAS
T_1	Sesame oil formulation	1×10^8	4	33.84 (35.57)	42.58 (40.73)	48.55 (44.17)
T_2	Sesame oil formulation	1×10^8	6	41.32 (40.00)	59.36 (50.39)	61.55 (51.68)
T_3	Sesame oil formulation	1×10^8	8	44.68 (41.95)	61.82 (51.84)	63.85 (53.04)
T_4	Sunflower oil formulation	1×10^8	4	29.78 (33.07)	38.62 (38.42)	41.82 (40.29)
T_5	Sunflower oil formulation	1×10^8	6	47.86 (43.77)	62.88 (52.46)	71.24 (57.57)
T_6	Sunflower oil formulation	1×10^8	8	49.12 (44.50)	65.14 (53.81)	73.45 (58.98)
T_7	Talc based formulation	1×10^8	5	48.16 (43.95)	62.65 (52.33)	71.58 (57.78)
T_8	Untreated control	-	-	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
S Em±				0.51	0.47	0.61
CD @ P=0.05				1.52	1.41	1.83

CFU= Colony Forming Unit, DBS = Days Before Spraying, DAS = Days After Spraying
Figures in the parenthesis are arcsine values

Overall, the sunflower oil based formulation was found to be superior in reduction of *S. frugiperda* and next best treatment was talc based formulation followed by sesame oil based formulation (Fig. 1). The present findings with respect to the superiority of oil based formulations was in line with Prior et al. [14] who reported that conidial suspension of *B. bassiana* in oil was effective for field application due to its non-drying properties and exhibited the additional advantage of prolonged survival ability of spores.

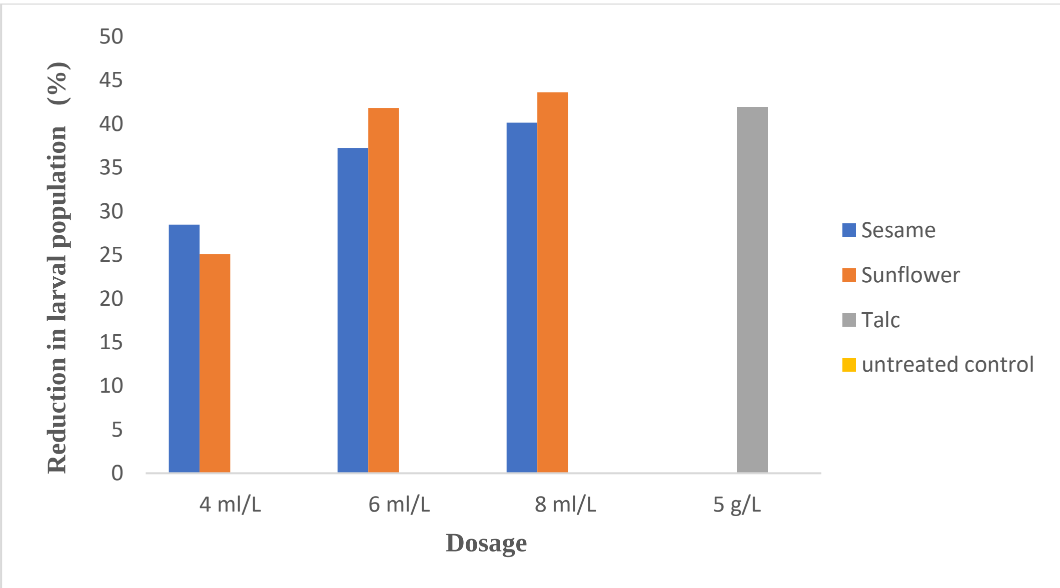

Fig. 1. Mean larval reduction of fall armyworm

Similarly, Naik [15] also reported the oil based formulation of *M*. *anisopliae*, *B*. *bassiana* and *V*. *lecanii* resulted in highest mortality of leaf hoppers in rice. The efficacy of the oil based formulation was in line with Nahar et al. [16] who reported that oil formulations of *M. anisopliae* (M 34412), *B. bassiana* (B 3301) and *N. rileyi* (N 3.12) recorded highest mortality of pod borer, *H. armigera* in pigeon pea.

The superiority of sunflower oil f"rmul'tion was also supported by Vega et al. [17] where in oil based formulations viz., mineral, canola, sunflower, olive and peanut oils of two fungal strains of *N. rileyi* and *Isaria tenuipes* against larvae of *S. exigua*, *S. frugiperda* recorded highest activity of *N. rileyi* in suppressing the pest population [18-20].

4. CONCLUSION

The present study evaluated the efficacy of two oil-based formulations along with the talc based formulation in managing the larval population of *S. frugiperda*. Results indicated that both sunflower oil and sesame oil-based formulations demonstrated significant reductions in larval population compared to the control group. Particularly, the sunflower oil-based formulation exhibited superior efficacy, achieving the highest reduction percentages across different dosages and application intervals. It was observed that the non-drying properties of oil-based carriers contribute to prolonged spore survival, enhancing the efficacy of fungal agents against target pests. These findings contribute to the development of sustainable and environmental friendly pest management strategies, emphasizing the importance of exploring natural compounds for enhancing the efficacy of biocontrol agents in agricultural practices.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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