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Effect of entomopathogenic fungi against invasive pest *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) in maize

Ramanujam B.*, Poornesha B. and Shylesha A. N.

Abstract

Ten indigenous entomofungal strains of *Beauveria bassiana*, *Metarhizium anisopliae*, and *M. rileyi* were evaluated against 2nd instar larvae of the maize fall armyworm (FAW) *Spodptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) in a laboratory bioassay. Among the ten strains tested, *M. anisopliae* ICAR-NBAIR Ma-35 caused 67.8% mortality, followed by *B. bassiana* ICAR-NBAIR Bb-45 with 64.3%, and ICAR-NBAIR Bb-11 with 57.1% mortality. Rest of the isolates showed 10.7–28.6% mortality. ICAR-NBAIR Ma-35 showed LC_{50} of 1.1×10^7 spores/ml and LT_{50} at 1×10^8 spores/ml is 86.04 h and ICAR-NBAIR Bb-45 showed LC_{50} of 1.9×10^7 spores/ml and LT_{50} at 1×10^8 spores/ml is 88.30 h. Field evaluation with these two promising strains were conducted against maize fall armyworm for 2 years (2018 and 2019) at ICAR-National Bureau of Agricultural Insect Resources experimental farm, Bengaluru, Karnataka, India. Field trial results indicated 68 and 69% reduction of FAW infestation and 55 and 62% increase in yield in the plots treated with *M. anisopliae* ICAR-NBAIR Ma-35/*B. bassiana* ICAR-NBAIR Bb-45, respectively, during 2018. In 2019, 70 and 76% reduction of FAW infestation and 44 and 55% increase in yield were observed in the plots treated with these two entomofungal pathogens, respectively.

Keywords: *Spodoptera frugiperda*, *Beauveria bassiana*, *Metarhizium anisopliae*, *Metarhizium rileyi*, Maize

Background

The maize fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) was reported for the first time in India in 2018. It was widely distributed in Karnataka regions like Chikkaballapur, Hassan, Shivamogga, Davanagere, Bangalore, and Chitradurga during July–August 2018 (Shylesha et al. 2018). The FAW is native of the tropical and sub-tropical regions of North, Central, and South America (Chittenden 1901; Luginbill 1928). In Africa, first report of fall armyworm on maize plants was made in 2016 (Goergen et al. 2016). It has become invasive and threatened the food security in Africa causing more than 200 million people whose staple crop is maize. In the absence of proper control methods, *S. frugiperda* has the potential to cause maize yield losses of 8.3 to 20.6 million tonnes per annum in Africa. The value of crop losses is estimated between US$ 2.5 and 6.2 billion (Day et al. 2017). In addition, the pest is known to be highly polyphagous, spreading to other crops from the invaded regions (Goergen et al. 2016). The application of chemical insecticides is unsustainable because it develops insecticide resistance, cost-effective, effects natural enemies, and environmental hazards as well as health risks. Therefore, it is important to minimize the use of insecticides and need to develop sustainable IPM technologies against *S. frugiperda* for safe, ecofriendly management practices in India (Abrahams et al. 2017 and Yu et al. 2003). FAW larvae are susceptible to entomopathogenic microorganisms, such as bacteria, fungi, nematodes, viruses, and protozoa (Molina-Ochoa et al. 2003; Ríos-Velasco et al. 2010). Native strains of entomopathogenic fungi as an alternative

* Correspondence: bonamramanujam58@gmail.com
Division of Germplasm, Conservation and Utilisation, ICAR-National Bureau of Agricultural Insect Resources, P.O. Box. 2491, H. A. Farm post, Bellary Road, Hebbal, Bangalore, Karnataka 560024, India

Table 1 Screening of entomopathogenic fungal isolates against fall armyworm

Isolates	% mortality*	Source of the isolate	NCBI Genbank accession number	Location
Beauveria bassiana ICAR-NBAIR Bb-5a	28.6 ± 0.88[b]	*Hypothenemus hampei* (coffee berry borer)	JF837134	Karnataka
Beauveria bassiana ICAR-NBAIR Bb-11	57.1 ± 0.10[a]	*Tetranychus urticae* (red spider mite)	JF837121	Karnataka
Beauveria bassiana ICAR-NBAIR Bb-45	64.3 ± 1.67[a]	Rhizosphere soil of carrot	JF837094	Tamil Nadu
Metarhizium anisopliae ICAR-NBAIR Ma-4	21.4 ± 0.88[bc]	*Plocaederus ferrugineus* (cashew stem and root borer)	JF837157	Karnataka
Metarhizium anisopliae ICAR-NBAIR Ma-11	10.7 ± 0.88[bc]	*Agrotis ipsilon* (black cutworm)	JF837146	Assam
Metarhizium anisopliae ICAR-NBAIR Ma-14a	21.4 ± 0.88[bc]	Rhizosphere soil of chili	JF837110	Assam
Metarhizium anisopliae ICAR-NBAIR Ma-15	17.8 ± 0.88[bc]	Soil from termite mound	JF837154	Assam
Metarhizium anisopliae ICAR-NBAIR Ma-35	67.8 ± 0.58[a]	Soil (fallow lands)	JQ518481	Gujarat
Metarhizium rileyi ICAR-NBAIR Nr-1	14.3 ± 0.00[bc]	*Spodoptera litura* (tobacco cutworm)	MN907775	Andhra Pradesh
Metarhizium anisopliae ICAR-NBAIR Nr Sf-1	21.4 ± 0.33[bc]	*Spodoptera frugiperda* (fall armyworm)	MN602591	Karnataka
Control	6.67 ± 0.88[c]	–	–	
CD@1%	0.590	–	–	

*Values in columns followed by the different letters are significantly ($P < 0.01$) different with each other

to chemical insecticides have to be evaluated against maize fall armyworm.

The present study was undertaken up to evaluate the entomofungal pathogens against *S. frugiperda* under laboratory and field-level conditions.

Materials and methods

Insect culture

The second instar larvae and egg patches of *Spodoptera frugiperda* was obtained from mass production unit of ICAR-NBAIR, Bangalore, Karnataka, India, were used for the bioassay.

Source of fungal cultures

Three strains of *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycetes: Hypocreales: Cordycipitaceae) (ICAR-NBAIR Bb-5a, Bb-11 Bb-45), five strains of *Metarhizium anisopliae* (Metchnikoff) Sorokin (Ascomycetes: Hypocreales: Clavicipitaceae) (ICAR-NBAIR Ma-4, Ma-35, Ma-11, Ma-14a, Ma-15), and two strains of *M. rileyi* (Farlow) Kepler S.A. Rehner & Humber (Ascomycetes: Hypocreales: Clavicipitaceae) (ICAR-NBAIR Nr-1 and NrSf-1) derived from insect and soil samples of different geographical locations of India were maintained in the culture repository at the National Bureau of Agricultural Insect Resources (NBAIR), Bangalore, India, were used in this study. All these isolates were molecular characterized and sequenced using ITS region and were deposited in NCBI GenBank database (Table 1).

Laboratory bioassay

Fungal spore suspension

Each fungal isolate was grown on broken rice for laboratory bioassay. Spore suspension was prepared by taking 1 g of 15 days old conidiated rice in 9 ml of sterile distilled water containing 0.01% Tween 80. The suspension was filtrated through three layers of muslin cloth to get hyphal-free spore suspension, and the spore concentration was adjusted to various concentrations like (1×10^4, 1×10^5, 1×10^6, 1×10^7, and 1×10^8 spores/ml) using Neubauer' s improved hemocytometer.

In vitro screening

The 2nd instar larvae (10 larvae/replication-3 replications) of *S. frugiperda* were dipped in the suspension containing 1×10^8 spores/ml for 15 s. The treated larvae were transferred into plastic containers and supplied with fresh detached maize leaves. Then the larvae were maintained under controlled conditions of 25 ± 2 °C temperature and 70 ± 5% relative humidity for 10 days. Fresh maize leaves were provided as a food to the larvae in 24 h interval. Controls were treated with sterile water containing 0.01% Tween 80. The observation on mortality due to fungal infection was carefully noted and recorded. The percent mortality of the larvae for each

Table 2 Concentration mortality response of entomopathogenic fungi against fall armyworm

Isolates	LC_{50} spores/ml	95% fiducial limit	Slope ± SE	χ^2	*P* value	df
Metarhizium anisopliae ICAR-NBAIR Ma-35	1.1×10^7	5.0×10^6–3.5×10^7	0.661 ± 0.106	1.035	0.793	2
Beauveria bassiana ICAR-NBAIR Bb-45	1.9×10^7	7.4×10^6–8.1×10^7	0.589 ± 0.102	1.700	0.637	2

SE standard error, χ^2 chi square, and *df* degree of freedom

Table 3 Time mortality response of entomopathogenic fungi against fall armyworm

Isolates	LT_{50} hours	95% fiducial limit	Slope ± SE	χ^2	*P* value	df
Metarhizium anisopliae ICAR-NBAIR Ma-35	86.04 h	74.55–113.02	5.505 ± 1.390	1.433	0.488	2
Beauveria bassiana ICAR-NBAIR Bb-45	88.30 h	79.10–101.31	6.611 ± 1.255	1.945	0.584	2

SE standard error, χ^2 chi square, and *df* degree of freedom

isolate was calculated using Abbott's formula (Abbott, 1925). The data were subjected to one-way analysis of variance (ANOVA) using statistical software SPSS windows version 20.0. Further studies were carried out using promising isolates.

$$\text{Corrected\%mortality} = \left(1 - \frac{\text{n in T after treatment}}{\text{n in Co after treatment}}\right) * 100$$

where n = insect population, T = treated, Co = control

The egg patches of *S. frugiperda* laid on maize leaves were used for bioassay studies of two promising strains (30 eggs/replication-5 replications). Each egg patch containing 30 eggs were dipped in the spore suspension containing 1×10^8 spores/ml for 15 s and the treated eggs were maintained on maize leaves under controlled conditions of 25 ± 2 °C temperature and 70 ± 5% relative humidity until they hatched. Similarly, the eggs in the untreated control were treated using sterile distilled water and maintained on maize leaves. Observations on egg hatching were recorded carefully at 24 intervals for a period of 5 days.

Concentration and time mortality studies

Two promising isolates were subjected to dose and time mortality studies. Five spore concentrations (1×10^4, 1×10^5, 1×10^6, 1×10^7, and 1×10^8 spores^{-1}) were used to study LC_{50} and bioassays were carried out as described above. In case of time mortality studies, single concentration (1×10^8 spores^{-1}) was used to determine LT_{50}. The dose and time to kill 50% of the population (LC_{50} and LT_{50}) were determined by probit analysis (Finney 1971). Statistical analysis was done using SPSS windows version 20.0.

Field evaluation

The field trials were carried out using 2 promising strains of *B. bassiana* ICAR-NBAIRBb-45 and *M. anisopliae* ICAR-NBAIRMa-35 on maize FAW at ICAR-NBAIR Attur Farm, Bengaluru, Karnataka, India, during 2018 (November to February) and 2019 (June to September).

The field trials were laid out in completely randomized block design with 3 treatments and eight replications using maize hybrid variety (BRMH-1) obtained from Karnataka State Seeds Corporation, India, for this study. The maize seeds were sown manually in the experimental plot with a plot size of 5 m × 6 m for each treatment and spacing of 60 cm × 30 cm were maintained and irrigated regularly. All the agronomic practices with recommended doses of fertilizers were followed to maintain good plant health till harvest of crop as per package of practice by UAS, Bengaluru, Karnataka, India, and harvesting was done manually. The talc formulations of these isolates were applied at the dose of 5 g/l (1×10^8 Cfu/g) during 20, 30, and 40 days after sowing (10 days interval) of maize seeds in the experimental field during both the seasons as soon as pest incidence was observed. Pre- and post-observations on number of plants infested with FAW were recorded after 5 days of treatment. The plant infestation with FAW was recorded based on the number of plants infested by FAW out of 120 plants (15 plants per replication in each treatment, total 120 plants). The percent reduction in plants infestation by FAW was calculated. Yield was recorded at the time of crop harvest for each treatment. The data was subjected to statistical analysis for drawing inferences using SPSS windows version 20.0. Significant level was set at 0.05 and the means were separated by Turkey test.

Table 4 Field evaluation of entomofungal pathogens against fall armyworm in maize during 2018

Treatments	Pre-treatment Fall armyworm incidence before first spray		Post-treatment* Fall armyworm incidence after three sprays		Cob yield* Q/ha
	Infested plants by FAW	Plant infestation (%)	Infested plants by FAW	Plant infestation (%)	
T1: *Metarhizium anisopliae* ICAR-NBAIR Ma-35	82	68.33	24.33^a	20.28^a	27.08^a
T2: *Beauveria bassiana* ICAR-NBAIR Bb-45	79	65.83	25.67^a	21.39^a	22.68^a
Control	77	64.17	80.33^b	66.94^b	10.18^b
CD@5%	NS	NS	0.220	0.568	0.588
CV	–	–	9.21	9.21	12.611

*Values in columns followed by the different letters are significantly ($P < 0.01$) different with each other
NS non-significant

Table 5 Field evaluation of entomofungal pathogens against fall armyworm in maize during 2019

Treatments	Pre treatment Fall armyworm incidence before first spray		Post treatment* Fall armyworm incidence after three sprays		Cob yield* Q/hectare
	Infested plants by FAW	Plant infestation (%)	Infested plants by FAW	Plant infestation (%)	
T1: *Metarhizium anisopliae* ICAR-NBAIR Ma-35	47	39.17	14.0^{a}	11.67^{a}	30.52^{a}
T2: *Beauveria bassiana* ICAR-NBAIR Bb-45	51	42.5	17.33^{a}	14.44^{a}	24.33^{b}
Control	50	41.67	58.67^{b}	48.89^{b}	13.52^{c}
CD@5%	NS	NS	0.235	0.607	0.321
CV	–	–	12.048	12.038	6.364

*Values in columns followed by the different letters are significantly ($P < 0.01$) different with each other
NS non-significant

Results and discussion

Laboratory bioassay

Among the ten strains tested, *M. anisopliae* ICAR-NBAIR Ma-35 caused 67.8% mortality followed by *B. bassiana* ICAR-NBAIR Bb-45 with 64.3% and Bb-11 with 57.1% mortality. The rest of the strains showed 10.7–28.6% mortality (Table 1). No mortality was observed on FAW eggs when treated with these promising isolates. García et al. (2011) reported 96.6 and 78.6% mortality with *B. bassiana* and *M. anisopliae* strains, respectively on 2nd instar larvae of the FAW at a concentration of 1×10^9 conidia/ml. Aktuse et al. (2019) reported that, *M. anisopliae* isolates showed 92 and 97% mortality on FAW eggs and neonate larvae, respectively, and were less susceptible to 2nd instar larvae of FAW, whereas only *B. bassiana* showed 30% mortality on 2nd instar larvae of FAW in Kenya. Cruz-Avalos et al. (2019) reported that, *M. anisopliae* and *B. bassiana* isolates showed 19 and 100% mortality on FAW eggs and neonate larvae, respectively. Morales-Reyes et al. (2013) reported that *M. anisopliae* and *B. bassiana* at 10^6, 10^7 concentrations showed in the range of 45 to 65% mortality, respectively, on the 2nd instar larvae of FAW in laboratory bioassay. Obtained results showed that *B. bassiana* and *M. anisopliae* were effective against 2nd instar larvae of FAW with 10–67% mortality where no mortality was observed on eggs. Fang et al. (2005) reported that entomopathogenic fungal enzymes play an important role on virulence of insect pests, which varies with different strains.

Concentration and time mortality studies

ICAR-NBAIR Ma-35 showed LC_{50} of 1.1×10^7 spores ml^{-1} and LT_{50} of 86.04 h and ICAR-NBAIR Bb-45 showed LC_{50} of 1.9×10^7 spores ml^{-1} and LT_{50} of 88.30 h respectively (Tables 2 and 3). Cruz-Avalos et al. (2019) found that 19 and 100% mortality was observed with *B. bassiana* and *M. anisopliae* strains, respectively, and LC_{50} in the range of 7.4×10^4 to 36×10^5 conidia^{-1} on FAW eggs and neonate larvae, respectively, in Mexico. Also, *M. anisopliae* isolate CP-MA1 at the dose of 5.3×10^5 conidia/ml showed 72.5% mortality of 3rd instar larvae of FAW (Romero-Arenas et al. 2014).

Field evaluation

In 2018, significantly low percentages of plant infestation by FAW were observed after 3 sprays of *M. anisopliae* ICAR-NBAIR Ma-35 (20.28%) and *B. bassiana* ICAR-NBAIR Bb-45 (21.39%) compared to the untreated control (66.94%). Cob yield was significantly high in *M. anisopliae* ICAR-NBAIR Ma-35 (27.08 Q ha^{-1}) and *B. bassiana* ICAR-NBAIR Bb-45 (22.68%) compared to the untreated control (10.18 Q ha^{-1}) (Table 4). In 2019, significantly lower percentage of plant infestation by FAW was observed after 3 sprays of *M. anisopliae* ICAR-NBAIR Ma-35 (11.67%) and *B. bassiana* ICAR-NBAIR Bb-45 (14.44%) than the untreated control (48.89%). Cob yield was significantly high in *M. anisopliae* ICAR-NBAIR Ma-35 (30.52 Q ha^{-1}) followed by *B. bassiana* ICAR-NBAIR Bb-45 (24.33%) compared to the untreated control (13.52 Q ha^{-1}) (Table 5). No other pests and diseases were observed in the treated and untreated experimental plot. The results indicate the efficacy of *M. anisopliae* ICAR-NBAIR Ma-35 and *B. bassiana* ICAR-NBAIR-Bb-45 against maize FAW. Field trial with *M. rileyi* (*Nomuraea rileyi*) against FAW in Dharwad district of Karnataka, India, showed 58–62% of pest reduction after 15 days of spraying (Mallapur et al. 2018). Obtained results showed that *M. anisopliae* ICAR-NBAIR Ma-35 and *B. bassiana* ICAR-NBAIR Bb-45 showed 68 and 69% of reduction, respectively, of FAW infestation and 55 and 62% increase in the yield in 2018, respectively. In 2019, they showed 70 and 76% of reduction of FAW infestation and 44 and 55% increase in the yield.

Conclusion

Based on 2-year field trials conducted in FAW infested plots in Karnataka state of India, *M. anisopliae* ICAR-NBAIR Ma-35 and *B. bassiana* ICAR-NBAIR-Bb-45 were recommended as potential isolates for management of FAW in maize crop, as the plots treated with this biocontrol agent showed minimum infestation levels of FAW and considerable increases in the yield than the untreated control.

Abbreviations
ICAR: Indian Council of Agricultural Research; NBAIR: National Bureau of Agricultural Insect Resources; NCBI: National Center for Biotechnology Information; UAS: University of Agricultural Sciences; FAW: Fall armyworm; IPM: Integrated pest management; Bb: *Beauveria bassiana*; Ma: *Metarhizium anisopliae*; Nr: *Nomuraea rileyi* (*Metarhizium rileyi*); Cfu: Colony forming units; LC_{50}: Lethal concentration; LT_{50}: Lethal time

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Authors' contributions
The concept and design of the experiments were prepared by all authors. BP conducted the experiments in laboratory as well as in field and analyzed. All the authors read and approved the manuscript.

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Availability of data and materials
All data are available in the article and the materials used in this work are of high quality and grade.

Ethics approval and consent to participate
All experimental works were approved by ICAR-National Bureau of Agricultural Insect Resources, Bengaluru, India.

Consent for publication
I agree to publish this scientific paper in the EJBPC.

Competing interests
The authors declare that they have no competing interests.

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