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Biocontrol potential of native *Metarhizium* isolates against fall army worm *(Spodoptera frugiperda)*

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ABSTRACT

Entomopathogenic fungi infected *Spodoptera frugiperda* cadavers were collected from maize fields of Krishna Delta regions of Andhra Pradesh. Two isolates of *Metarhizium rileyi,* one isolate each of *Metarhizium anisopliae* and *Beauveria bassiana* were isolated using standard protocols and were identified through morphological characters and molecular techniques at Regional Agricultural Research Station, Lam, Guntur during the year 2023-24. Bioassay studies with native *Metarhizium* isolates against *Spodoptera frugiperda* revealed significant mortality rates with the isolates, *M. anisopliae* Gu1 (61.67%), followed by *M. rileyi* Gu1 (53.3%), *M. rileyi* Gu2 (46.67%) and *M. rileyi* Ch (40.0%). The present studies revealed more than 50 per cent mortality and supported the integration of *Metarhizium* based bio pesticides in integrated pest management strategies for sustainable maize crop production.

Key words: Bioassay, entomopathogenic fungi *Metarhizium rileyi*, *Metarhizium anisopliae*, *Spodoptera frugiperda*

Entomopathogenic fungi (EPF) are natural regulators of insect populations and have gained attention as myco-insecticidal agents due to their effectiveness in controlling various agricultural pests. They infect insects at all life stages and offer an eco-friendly alternative to synthetic pesticides, playing a key role in integrated pest management (IPM). EPFs like *Beauveria*, *Metarhizium*, and *Isaria* are increasingly used for sustainable pest control due to their environmental safety and cost-effectiveness. Native EPF strains, isolated from local habitats, (Sayed *et al*., 2018) show promise in controlling indigenous pests, with fungi invading insect hosts through the cuticle rather than ingestion.

Spodoptera frugiperda is an invasive polyphagous pest was first reported in Karnataka in 2018 and caused significant economic loss in maize production (Sharanabasappa *et al.,* 2018). Larvae damage the foliage, leaf whorls, tassels, and cobs depending on the stage of infection (Goergen *et al.,* 2016). FAW attack on maize might result in yield losses ranging from 8.3 to 20.6 million tonnes annually. This is a nightmare for farmers and poses a major threat to food security and agricultural trade. Insecticides are quick tools to eliminate pests in the field, however controlling *S. frugiperda* is challenging due to its feeding behaviour and protective inner leaves (Paredes *et al*., 2021). To combat this pest, farmers rely on use of insecticides.

Entomopathogenic fungi are useful biocontrol agents as they are detrimental to target pest, being safe for humans, non-target species, and the environment. . When ideal conditions prevail, the fungal spores germinate and breach the insect cuticle through enzymatic degradation. The EPFs have fast multiplication after invading the insect tissues, and emerge from the dead insect to produce more fungal spores. A few studies have been performed on the effectiveness of native EPF for the management of FAW in India. Keeping this in view in the present study on native EPF strains was investigated against fall army worm *Spodoptera frugiperda* to offer a potential alternative to chemical insecticides for sustainable agricultural management.

MATERIALS AND METHODS

Roving survey was conducted in Krishna Delta Region of Andhra Pradesh situated in Bapatla and Guntur districts during 2023-24 for collection of the Entomopathogenic Fungi (EPF) focusing on maize ecosystems. Live larvae and EPF-infected larval cadavars were collected during the survey were

brought to the laboratory, surface sterilised and placed on moistened filter paper to encourage fungal growth. EPFs were isolated using Sabouraud's Maltose Yeast Extract Agar (SMAY). Entomopathogenic fungal structures were examined using a compound microscope (NIKON Eclipse E 200) at 10x and 40x power, with appropriate magnification and fungal spore morphology, size, colour and length/width ratio and measurements were recorded digitally using V-image-2013 software. The molecular identity of the potent EPF isolate was established through homology analysis of the internal transcribed spacer (ITS) region of ribosomal DNA (rDNA).

After the isolation and identification of the entomopathogenic fungi, four *Metarhizium* isolates were studied for their biocontrol potential against the fall army worm, *Spodoptera frugiperda*. Bio assay study was executed with 5 treatments viz., T1-*M.rileyi* $Gu_{1,}$ T_2-*M.rileyi* $Gu_{2,}$ T_3-*M.rileyi* Ch, T_4-*M. anisopliae* Gu_1 and T_5-Untreated control which were replicated six times in a Completely Randomized Design (CRD). Topical application of conidial suspension (2 × 10x spores/ml) of *Metarhizium* isolates were done on third instar larvae (10 per treatment) fed with artificial diet (Shashikala and Saicharan, 2022) in culture vials. In untreated control, larvae were sprayed with sterile distilled water. Larval infection and mortality were recorded for every 24 hours until pupation (Dutta *et al.* 2014).

Mortality (%) = (No. of dead insects / total no. of insects released) X 100 Corrected mortality = (Test mortality (%) - Control Mortality (%)) / (100 - Control mortality (%)). Data were subjected to a one-way Analysis of Variance. Homogeneity of variances in mean data of percent mortality between treatments was estimated using Tukey's post hoc test. Statistical analyses were done in R studio (2019) software.

RESULTS AND DISCUSSION

In the Krishna delta region, 12 major Maize growing villages were surveyed for collection of entomo-pathogenic fungi infected cadavers. Entomo-pathogenic fungi infected fall armyworm larval cadavers were found in three villages out of 12 villages surveyed (Plate 1). The cadavers collected from Lam village of Tadikonda mandal of Guntur district identified as *Metarhizium rileyi* which was coded as M.r-Gu1. The insect cadaver collected from Nidumukkala and Chebrolu villages of Guntur district were identified as *Metarhizium rileyi* and *Metarhizium anisopliae*, which were coded as M.r-Gu2 and M.a Gu1, respectively. Insect cadavers collected from Vaddamanu village of Guntur district was identified as *Beauveria bassiana* and coded as Bb-Gu1. One *Metarhizium rileyi* isolate was taken from the Plant Pathology lab, RARS, Lam which was collected from Chintapalli was coded as Mr-Ch (Table 1).

Morphological, Molecular identification and phylogeny of EPF isolates

Metarhizium rileyi colonies were initially white for up to ten days and then changed to olive green during sporulation. The mycelium was white, septate, and flocculent, becoming green as sporulation progressed. Its conidiophores were erect with short, blocky branches, while conidia were aseptate, spherical to ovoid and arranged in short divergent chains, varying from pale to dark green (Vimaladevi and Prasad 1997 and Visalakshi *et al*. 2020). The colonies of *Metarhizium anisopliae* started white and turned dark green during sporulation. Its conidiophores were found in compact patches, heavily entangled, with rounded to conical apices grouped in a dense hymenium. The conidia were cylindrical, aseptate, and formed prismatic or parallel strands (Tangthirasunun *et al*., 2010). *Beauveria bassiana* exhibited white mould colonies that produced dry, powdery conidia and characteristic white spore balls. Its colonies were round, with conidiospores densely clustered in whorls, appearing hyaline and short. The conidia were smooth, globose to sub-globose, and white in color. (Plates 2 and Plate 3).

The molecular identity of *Metarhizium rileyi*, *Metarhizium anisopliae*, and *Beauveria bassiana* was confirmed through Polymerase Chain Reaction (PCR) by amplifying the Internal Transcribed Spacer (ITS 1 and ITS 4) region of ribosomal DNA. The partial sequence data base of ITS were processed and Sequence analysis through NCBI BLAST revealed that *M. rileyi* isolates (M.r-Gu_1, M.r-Gu_2, and M.r -Ch) had 100%, 100%, and 99.6% similarity with NCBI-accessioned isolates from Delhi, Telangana, and Punjab (NCBI accession numbers PP068366, OR727483and MK697304, respectively. whereas M.a-Gu1 was recorded 99.80 per cent similarity with *Metarhizium anisopliae* and Bb-Gu1 was closely related to *Bauveria bassiana* with 99.2 per cent similarity with NCBI accession numbers PP776609 (*M. anisopliae*, Coimbatore

A B

Plate 1 (A and B). Fungal infected cadavers collected in fields during survey

Table 1. Details of samples collected from maize fields of Bapatla and Guntur districts during survey

S. No.	Village	Latitude	Longitude	Mandal	District	Collected sample (Soil or insect	Isolated organism (EPF)	Code
1	Lam	16.3641°N	80.428° E	Tadikonda	Guntur	Insect cadaver	*Metarhizium rileyi*	M.r- Gu_1
2	Nidumukkala	16.4429 °N	80.4118° E	Tadikonda	Guntur	Insect cadaver	*Metarhizium rileyi*	M.r-Gu_2
3	Chebrolu	16.200°N	80.533° E	Chebrolu	Guntur	Insect cadaver	*Metarhizium*	M.a-Gu_1
4	Gollamudi Palem	16.3416°N	80.4728° E	Peddakakani	Guntur	Soil	-	-
5	Munnangi	16.3308° N	80.7218° E	Kollipara	Guntur	Soil	-	-
6	Athota	16.2910° N	80.6805° E	Kollipara	Guntur	Soil	-	-
7	Vallabhapuram	16.3321° N	80.6273° E	Kollipara	Guntur	Soil	-	-
8	Dharanikota	16.5323° N	80.3731° E	Amaravathi	Guntur	Soil	-	-
9	Dondapadu	16.5878° N	80.4442° E	Thullur	Guntur	Soil	-	-
10	Bapatla	15.8966° N	80.4604° E	Bapatla	Bapatla	Soil	-	-
11	Chirala	15.8120° N	80.3553° E	Chirala	Bapatla	Soil	-	-
12	Ponnuru	16.0685° N	80.5481° E	Ponnuru	Bapatla	Soil	-	-
13	Vaddamanu	16.523° N	80.460° E	Thullur	Guntur	Insect cadaver	*Beauveria bassiana*	Bb-Gu_1
14	Chintapalli			Chintapalli	Alluri Sitharama Raju	Insect cadaver	*Metarhizium rileyi*	M.r.Ch.

Plate 2. Mycelial growth in petriplate and Microscopic view of the *Metarhizium anisopliae isolate*

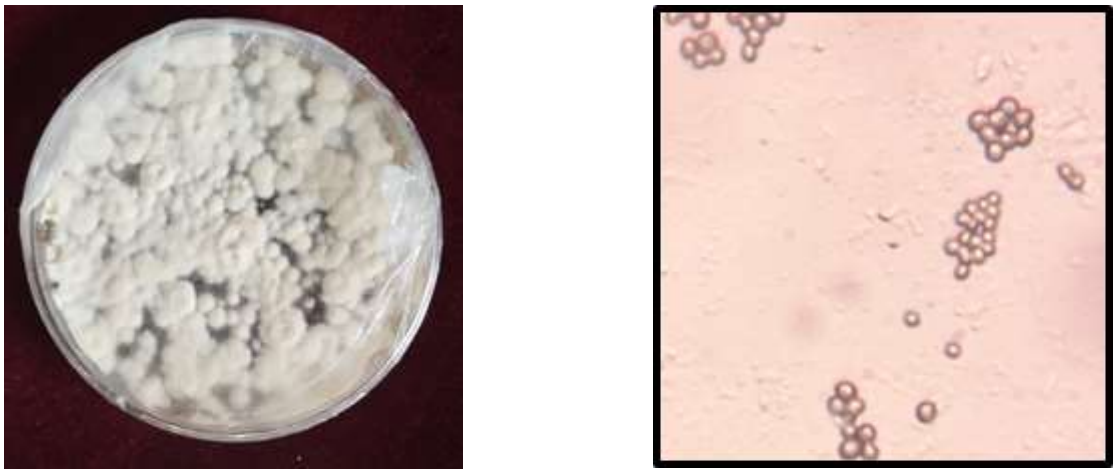

Plate 3. Mycelial growth in Petri plate and microscopic view of the *Beauveria bassiana isolate*

isolate) and PP703149 (*B. bassiana*, Raichur isolate).

Two major clades were formed when phylogenetic tree was constructed for the five entomopathogenic fungal isolates using the Neighbor-Joining method in MEGA XI software. *B. b-Gu1* was part of Clade II, clustering with the *B. bassiana* Coimbatore isolate (OR436904) and the Raichur isolate (PP703149). *M. a-Gu1* clustered with the *M. anisopliae* Gazipur isolate (OQ581920) and the Coimbatore isolate (PP776609). All the *M. rileyi* isolates formed Clade I, where the Guntur isolates (*M. r-Gu1* and *M. r*-Gu2) closely clustered with the *M. rileyi* Karnataka isolate (MN602591) and *M.r. Ch* isolate clustered with *M. rileyi* Philippines isolate (OR826619)(Fig 1).

Biocontrol Potential of Native *Metarhizium* isolates

Third instar fall army worm larvae treated with *Metarhizium* isolates revealed significant differences in mortality rates upto 10 days of post treatment. No mortality was observed at one and three days after treatment in any of the isolates, including the untreated control. High corrected mortality of 22.0, 45.7 and 58.7 was recorded with M.a Gu1 isolate of Metarhizium at five, seven and ten days after the treatment, respectively. Among the *M. rileyi* isolates, *M. rileyi* Gu1 recorded high mortality of 20.3, 41.8 and 50.2, respectively at five, seven and ten days of post-treatment followed by *M. rileyi* Gu2 (16.9, 33.2 and 42.8 per cent) (Table 4.13). Low mortality of 13.6, 27.8 and 35.6 percent at five, seven and ten days after the treatment was recorded in *M. rileyi* Ch isolate (Table 2 and Fig 2)). These results were similar to the findings of Parjane *et al*. (2023), who studied the pathogenicity of *Metarhizium anisopliae* against second and third instar larvae of *Spodoptera frugiperda*, finding that second instar larvae exhibited mortality of 0.0 to 72.77% at different time intervals against third instar larvae when treated with different doses of *Metarhizium anisopliae* 1.15% WP. Similar results were obtained by Herlinda *et al.* (2020) who reported that 14 *Metarhizium* spp. isolates proved highly lethal to *S.frugiperda* larvae causing mortality rates ranging from 70.67% to 78.67%. The most virulent isolate suppressed adult emergence by up to 81.2%. Shahzad *et al*. (2021) evaluated the efficacy of *M. anisopliae* and *B. bassiana* against *S. frugiperda* 2nd instar larvae using spore concentrations ranging from 1×10^4 to 1×10^8 spores/ml. They found that the LC50 values for *M. anisopliae* and *B. bassiana* were $1.3x10^7$ and $1.8x10^7$ spores/ml, respectively. The LT50 values recorded were 84.01 hours for *M. anisopliae* and 80.99 hours for *B. bassiana*, indicating the time required to achieve 50% mortality. Shylesha (2020) evaluated ten indigenous strains of *Beauveria bassiana*, *Metarhizium anisopliae,* and *M. rileyi* against second instar larvae of *Spodoptera frugiperda* found *M. anisopliae* ICAR-NBAIR Ma-35 caused 67.8% mortality, followed by *B.bassiana* ICAR-NBAIR Bb-45 at 64.3%, and ICAR-NBAIR Bb-11 at 57.1%. Other strains exhibited mortality rates between 10.7 and 28.6 per cent.

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Fig 1. Phylogenetic tree based on the nucleotide sequences of ITS -1 & 4 of an isolated fungus with relevant sequences of *Metarhizium* spp. from NCBI database

Table 2. Corrected larval mortality of *S. frugiperda* with *Metarhizium* isolates under laboratory con ditions

Tr. No	*Metarhizium* isolate	Larval Mortality (%)				
		1 DAS	3 DAS	5 DAS	7 DAS	10 DAS
T_1	*M.rileyi* – M.r Gu_1	0	0	21.67^{a}	45.00^{a}	53.33^{ab}
T_2	*M.rileyi* - M.r Gu_2	0	0	18.33^{ab}	36.67^{b}	46.67^{bc}
T_3	*M.rileyi* Ch	0	0	15.0^{b}	31.67^{b}	40.00^{c}
T_4	*M. anisopliae* M.aGu_1	0	0	23.33^{a}	48.3^{a}	61.67^{a}
T_5	Untreated Control	0	0	3.33^{c}	5.00^{c}	6.67^{d}
	CD (P=0.05)			0.49	0.59	0.4
	CV(%)			12.3	16.9	11.5

Grouping is based on DMRT test. Treatments with same letters are not significantly different at 1% level.

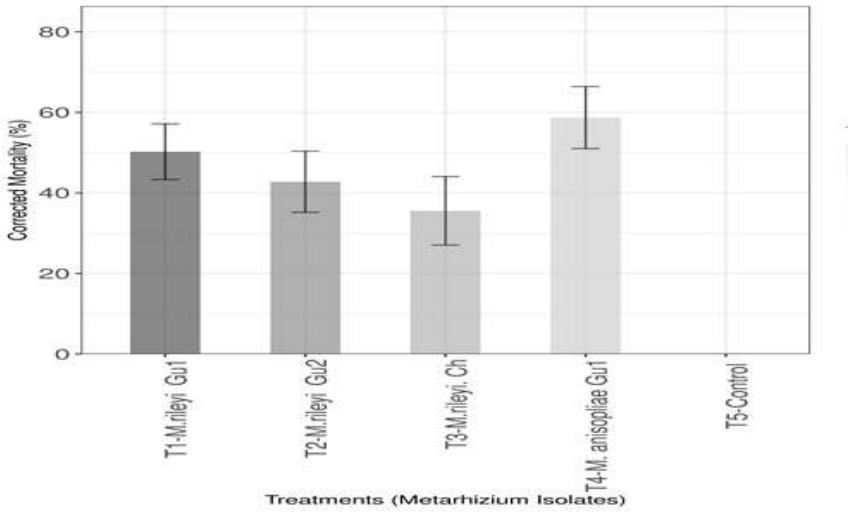

Fig 2. Bioassay studies of *Metarhizium* isolates against fall armyworm

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