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Efficacy of Indigenous entomofungal pathogens for the management of maize fall armyworm, *Spodoptera frugiperda*

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Abstract
Metarhizium (*Nomuraea*) *rileyi* was isolated from cadavers of maize fall armyworm (FAW), *Spodoptera frugiperda* noticed in epizootic form at Regional Agricultural Research Station (RARS), Anakapalli, Andhra Pradesh in the month of October, 2019. *M. rileyi* ANGRAU Anakapalli strain (AKP Nr-1) sequence was submitted to NCBI and accession number (MN 960559) was retrieved in 2020. Laboratory bioassay studies of the indigenous entomofungal strain, *M. rileyi* (ANGRAU AKP Nr1) against 2rd instar larvae of the *S. frugiperda* were conducted for three years from 2020 to 2022. Among the different concentrations tested, *M. rileyi* (ANGRAU AKP Nr1) @ 1 x10^9 spores/ml caused highest larval mortality (92.54% and 92.24%) followed by 1x10^8 spores/ml with 87.87% and 89.58% larval mortality in leaf dip method and larval treatment method respectively. *M. rileyi* (ANGRAU AKP Nr^{-1}) showed LC_{50} of (1.1 x10^8 spores/ml) and LT_{50} (1 x10^8 spores/ml) as 84.4 hours. Field efficacy studies of two indigenous entomofungal strains of *M. rileyi* (ANGRAU, Anakapalle strain and UAS, Raichur strain) against FAW conducted under ICAR-AICRP on Biological control centres located at RARS, Anakapalle (ANGRAU) and UAS, Raichur during 2020-21 to 2022-23. Native isolate, *M. rileyi* (ANGRAU AKPNr-1) at concentration 1x10^8 spores/ml @ 5 g/L as two sprays was effective with significantly low fall armyworm incidence (7.45% and 4.83%) followed by *M. rileyi* (UAS, Raichur strain) (11.46% and 4.82%) compared to high fall armyworm incidence in untreated control (28.22 % and 18.01%) in 2020 and 2021 respectively. Evaluation studies of native isolates of entomopathogens as three sprays against maize FAW conducted in 2022 showed very low FAW damage in chemical treatment, emamectin benzoate (2.05%) followed by *M. rileyi* (ANGRAU strain AKP-Nr-1) (3.88%) and *M. rileyi* (UAS, Raichur strain) (4.69%) compared to high damage in Untreated control treatment (20.85%). Percent reduction in fall armyworm damage over control was high in *M. rileyi* Anakapalle strain (65.87%) and *M. rileyi* Raichur strain (63.18%) as effective treatment after the chemical insecticide, Emamectin benzoate (87.44%). Cob yield recorded significantly high in *M. rileyi* ANGRAU Anakapalle strain (63.22 q/ha) and UAS, Raichur strain (58.22 q/ha) after chemical, emamectin benzoate (69.3 q/ha) and low in untreated control (35.04 q/ha). Yield increase over control in *M. rileyi* (Anakapalle strain) and *M. rileyi* (UAS, Raichur strain) were 66.83% and 66.15 % respectively compared to 74.37% in Chemical insecticide, Emamectin benzoate with effective against fall armyworm in maize crop.

Keywords: *Spodoptera frugiperda, Metarhizium rileyi,* Bioassay, field evaluation, maize

Introduction
Invasive pest, Fall armyworm first reported in Africa on maize plants and threatened the food security (Goergen *et al.* 2016) [9]. *S. frugiperda* outbreak caused high yield losses in maize (8.3 to 20.6 million tonnes per annum) with high crop value (US$ 2.5 to 6.2 billion) in Africa (Day *et al.* 2017) [6]. Management of fall armyworm using chemical insecticides is unsustainable due to insecticide resistance, effects natural enemies, hazards to human and environment. There is need to minimize the use of chemical insecticides with sustainable IPM technologies against *S. frugiperda* for ecofriendly safe management in India (Abraham *et al.* 2019 and Yu *et al.* 2003) [2, 18]. 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; Visalakshi *et al.*,2020) [12, 16, 14]. Native strains of entomopathogenic fungi as an alternative to chemical insecticides have to be evaluated against maize fall armyworm.

Keeping in view, laboratory bioassay and field efficacy studies for the native entomofungal pathogens against *S. frugiperda* were conducted under AICRP on Biological control (AICRP BC), RARS, Anakapalle, Andhra Pradesh.

Materials and Methods

Spodoptera frugiperda eggs and larvae obtained from insect rearing laboratory, RARS, Anakapalle were used for the bioassay and efficacy studies. Indigenous strains of *Metarhizium rileyi* (ANGRAU Anakapalle AKP Nr1 and UAS, Raichur} isolated from maize FAW larvae of Andhra Pradesh and Karnataka were used in this study. These isolates were characterized and sequences deposited in NCBI GenBank database.

Bioassay studies

Metarhizium rileyi (ANGRAU Anakapalle AKP Nr-1) isolate was multiplied on solid grain medium (broken rice) for bioassay studies. Spore suspension was prepared with one gram of 15 days old fungal culture mixed in 9 ml of distilled water and Tween 80 (0.01%). Spore suspension was diluted into six concentrations (1×10^4, 1×10^5, 1×10^6, 1×10^7, 1×10^8 & 1×10^9 spores per ml) using Neubauer haemocytometer.

Second instar fall armyworm larvae were treated with spore suspension containing different concentrations (1×10^4, 1×10^5, 1×10^6, 1×10^7, 1×10^8 & 1×10^9 spores/ml) for 15 secs with 10 larvae per treatment as three replications in larval dip method. After the treatment, FAW larvae were placed in plastic tins and fed with fresh maize leaves at room temperature (25±2 °C} and humidity (70±5%). Everyday, fresh food (maize leaves) were provided to the larvae. Larvae treated with sterile water mixed with tween 80 (0.01%) was maintained as Control treatment. Similarly maize leaves treated with spore suspension for six concentrations (1×10^4, 1×10^5, 1×10^6, 1×10^7, 1×10^8 & 1×10^9 spores/ml) and sterile water as control were kept as food for FAW larvae in leaf dip method (10 larvae/treatment, 3 replications). FAW larval mortality due to fungal infection was recorded at regular interval in larval treatment and leaf dip methods for six spore concentrations of *M. rileyi* (AKP Nr-1) and percent larval mortality was worked out (Abbott, 1925) [1].

$$\text{Corrected larval mortality \%} = \left(1 - \frac{\text{Number of larvae in Treatment}}{\text{Number of larvae in Control}}\right) \times 100$$

Freshly laid eggs of FAW were treated with fungal spore suspension of *M. rileyi* (AKP Nr-1) at1×10^8 spores/ml for 15secs and observed for larval emergence in laboratory along with untreated check. Daily data was recorded for five days on hatching of eggs and larval emergence.

Fall armyworm larval mortality studies conducted for indigenous fungal isolate, *M. rileyi* (ANGRAU Anakapalle AKP Nr-1) at six spore concentrations (1×10^4, 1×10^5, 1×10^6, $1\times10^{7,}$ 1×10^8 & 1×10^9 spores/ml). Single concentration ($1\text{x}10^8$ spores^{-1}) was used in time mortality studies. Dose to kill 50 percent of the larval population (LC_{50}) and time to kill 50 percent of the larval population (LT_{50}) were calculated (Finney, 1971) [7].

Field efficacy studies

Field efficacy studies were conducted using two native isolates of *M. rileyi* (ANGRAU AKP Nr 1 and UAS, Raichur) on maize FAW at RARS, Anakapalle during 2020, 2021 and 2022. The experiments were laid out in randomized block design sowing maize Hybrid - Advanta PAC 751 at spacing of 60cm × 30cm with plot size of 5 m × 6 m in 2020 and 2021. Three concentrations of these two fungal isolates containing 1×10^8, 1×10^{10} & 1×10^{12} spores/ml and sterile water as untreated check were sprayed two times at 20 and 30 days after sowing of maize seeds against FAW. *M. rileyi* (ANGRAU AKP Nr 1 and UAS, Raichur) talc formulations at the dose of 5 g/litre (1×10^8 Cfu/g) were applied during 20, 30 and 40 days after sowing and compared with chemical, ememectin benzoate in maize field during 2022. Pre and post treatment data on plant infestation with FAW were recorded. Plant damage was recorded based FAW infestation in 20 plants in all the treatments and reduction in plant damage due to spraying was calculated. Cob yield recorded in entomofungal pathogen treatments was compared with untreated control and chemical control. The data was analysis using statistical method.

Results and Discussions

Bioassay studies

M. rileyi (ANGRAU Anakapalle AKP Nr1) at spore concentration of 1×10^9 spores or conidia/ml caused 92.54 and 92.24% mortality followed by 1×10^8 spores/ml with 87.87 and 89.58% mortality in leaf dip and larval treatments methods respectively. The rest of the concentrations showed 68.4 to 92.54 % mortality (Table 1). *M. anisopliae* showed 78.6% FAW larval mortality at 1×10^9 conidia/ml (García *et al.*,2011). High FAW egg and early instar larval mortality ((92% and 97%) reported by Aktuse *et al.* (2019). Laboratory biassay studies conducted by Morales-Reyes *et al.* in 2013 showed that *M. anisopliae* at 10^6 concentrations showed 45% mortality on larvae of FAW. Obtained results showed that *M. rileyi* (ANGRAU AKP Nr1) was effective with 92.54% mortality of maize FAW larvae.

M. rileyi ANGRAU AKP Nr1 showed LC_{50} of 1.1 $\text{x}10^8$ spores per ml and LT_{50} of 84.4 hours (Table 2). Hundred percent mortality of FAW neonate larvae was observed with *M. anisopliae* with LC_{50} of 36 $\times10^5$ conidia^{-1} in Mexico (Cruz-Avalos *et al.*, 2019). Similarly, Romero-Arenas *et al.* reported high mortality of FAW in *M. anisopliae* (CP-MA1 isolate) at 5.3×10^5 spore concentration per ml in 2014.

Field efficacy studies

Significantly low plant damage was observed due maize FAW after two sprays with *M. rileyi* (ANGRAU AKP Nr1 isolate) at spore concentration of 1×10^{10} spores/ml (6.57%) in 2020 and at par with UAS, Raichur isolate of *M. rileyi* (11.46%) than the other spore concentrations whereas high plant damage with FAW recorded in untreated check (28.22%). Maize yield was significantly high in *M. rileyi* ANGRAU AKP Nr1 at 1×10^8 spores/ml (55.79 Q ha^{-1}) and *M. rileyi* UAS, Raichur (53.81 Q ha^{-1}) and low in untreated control (29.43 Q ha^{-1}) (Table 3).

Plant infestation by FAW was significantly low after two sprays of *M. rileyi* at 1×10^8 spores/ml concentration of ANGRAU AKP Nr1 (4.83%) and *M. rileyi* UAS, Raichur (4.82%) than the untreated control (18.01%) during 2021. Maize yield recorded high in *M. rileyi* ANGRAU AKP Nr1 (67.56Q ha^{-1}) followed by *M. rileyi* UAS, Raichur (67.17 Q ha^{-1}) and low in untreated control (35.95 Q ha^{-1}). Field experiments with *M. rileyi* against FAW in Dharwad district of Karnataka, India reported good pest reduction (Mallapur *et al*. 2018) [11].

Present results showed that *M. rileyi* ANGRAU AKP Nr1 and *M. rileyi* UAS, Raichur (1×10^8 spores/ml) showed 78.93 and 72.37 % reduction of FAW infestation, respectively and 89.5

and 87.93% increase in the cob yield, respectively during the year 2020. In 2021, *M. rileyi* ANGRAU AKP Nr1 and *M. rileyi* UAS, Raichur (1× 10^8 spores/ml) showed 42.45 and 45.89% of reduction of FAW damage and high yield increase (87.93 and 86.84% respectively). Field evaluation of talc formulation of *M. rileyi* (ANGRAU Anakapalle strain and UAS,Raichur strain) of 1× 10^8 spores/ml @ 5 g/lt as three sprays against maize FAW in 2022 showed very low fall armyworm damage in chemical treatment, emamectin benzoate (2.05%) followed by *M. rileyi* ANGRAU strain AKP-Nr-1 (3.88%) and UAS, Raichur strain(4.69%) compared to high damage in untreated control (20.85%) (Table 4). Percent reduction in fall armyworm damage over control was high in *M. rileyi* Anakapalle strain (85.26%) and *M. rileyi* Raichur strain (82.19%) next to Emamectin benzoate (92.21%). Cob yield recorded significantly high in *M. rileyi* ANGRAU Anakapalle isolate (66.3 Q ha^{-1}) and UAS, Raichur isolate (53.69 Q ha^{-1}) after chemical, emamectin benzoate (69.3 Q ha^{-1}) and low in untreated control (39.74 Q ha^{-1}). Yield increase over untreated control in *M. rileyi* (Anakapalle strain} and *M. rileyi* (UAS,Raichur strain) were 66.83% and 35.1 % respectively compared to 74.37% in Chemical insecticide treatment (Emamectin benzoate). The present results indicate the efficacy of indigenous entomofungal pathogens, *M. rileyi* ANGRAU Anakapalle strain AKP Nr1 and UAS, Raichur strain against fall armyworm in maize crop.

Table 1: Bioassay of *Metarhizium rileyi* (Anakapalle strain AKP-Nr-1) against maize fall armyworm, *Spodoptera frugiperda*

Treatment	Fall army worm Larval mortality (%) Leaf dip method				Fall army worm Larval mortality (%) Larval treatment method			
	2020	2021	2022	Mean	2020	2021	2022	Mean
T_1: *M. rileyi* (AKP-Nr-1) 1x10^4 spores / ml	66.6 (1.82)	68.75 (1.84)	69.85 (1.84)	68.4	70.8 (1.85)	70.3 (1.85)	71.12 (1.85)	70.74
T_2: *M. rileyi* (AKP-Nr-1) 1x10^5 spores / ml	70.0 (1.85}	71.23 (1.85)	70.53 (1.85)	70.58	71.6 (1.86)	72.0 (1.86)	71.25 (1.85)	71.61
T_3: *M. rileyi* (AKP-Nr-1) 1x10^6 spores / ml	73.33 (1.87)	75.0 (1.88)	76.1 (1.88)	74.81	78.2 (1.89)	77.67 (1.89)	77.86 (1.89)	77.91
T_4: *M. rileyi* (AKP-Nr-1) 1x10^7 spores / ml	80.0 (1.90)	81.25 (1.91)	80.66 (1.91)	80.63	80.42 (1.91)	81.0 (1.91)	81.48 (1.91)	80.98
T_5: *M. rileyi* (AKP-Nr-1) 1x10^8 spores / ml	86.67 (1.94)	88.75 (1.95)	88.2 (1.95)	87.87	90.23 (1.96)	89.5 (1.95)	89.01 (1.95)	89.58
T_6: *M. rileyi* (AKP-Nr-1) 1x10^9 spores / ml	91.37 (1.962)	91.75 (1.965)	90.5 (1.96)	92.54	92.33 (1.972)	92.8 (1.974)	91.61 (1.96)	92.24
CD (0.05)	0.024	0.019	0.015		0.604	0.404	0.496	
CV%	0.070	0.055	0.457		0.02	0.014	0.017	

Values in parenthesis are logarthmic transformed values

Table 2: Concentration mortality and Time mortality response of entomopathogenic fungi against maize fall armyworm, *Spodoptera frugiperda*

Isolates	LC_{50} spores/ml	95% fiducial limit	Slope±SE	X^2	P value	df
Metarhizium rileyi Anakapalle strain (Akp- Nr-1)	1.1 x10^8	5.0 x10^7-3.5 x10^8	0.671±0.11	1.04	0.78	2
Isolates	LT_{50} hours	95% fiducial limit	Slope± SE	X^2	P value	df
Metarhizium rileyi Anakapalle strain (Akp- Nr-1)	84.4 hours	76.22-109.25	5.105±1.270	1.533	0.455	2

SE- standard error, X^2- Chi square, df- degree of freedom

Table 3: Field efficacy of *Metarhizium rileyi* (Anakapalle strain) against maize FAW in kharif season

Treatment	FAW damage %						Percent reduction in FAW damage after two sprays		Cob yield Q/ha	
	2020-2021			2021-2022						
	Before first spray	After first spray	After second spray	Before first spray	After first spray	After second spray	2020-21	2021-22	2020-21	2021-22
T_1: *Metarhizium rileyi* (AKP-Nr-1) 1x10^8 spores / ml	35.36 (36.48)	20.45 (26.75)	7.45 (15.77)	10.97 (19.02)	9.0 (17.4)	4.82 (12.64)	78.93	56.06	55.79	67.56
T_2: *M. rileyi* (AKP-Nr-1) 1x10^{10} spores / ml	41.09 (39.84)	20.67 (26.99)	6.57 (14.84)	10.42 (18.84)	7.4 (15.57)	4.95 (12.26)	84.01	52.5	52.53	56.81
T_3: *M. rileyi* (AKP-Nr-1) 1x10^{12} spores / ml	40.59 (39.55)	267.78 (31.12)	12.9 (20.67)	10.57 (20.73)	5.95 (14.12)	5.27 (13.17)	68.22	50.14	50.87	51.67
T_4: *M. rileyi* (UAS, Raichur) 1x10^8 spores / ml	41.47 (40.07)	25.98 (30.54)	11.46 (19.29)	10.51 (18.89)	7.88 (16.26)	4.83 (12.23)	72.37	54.04	53.81	67.17
T_5: *M. rileyi* (UAS, Raichur) 1x10^{10} spores / ml	38.36 (38.25)	20.26 (26.64)	8.21 (16.2)	10.3 (18.64)	7.13 (15.07)	5.05 (12.75)	78.6	50.97	52.13	60.15
T_6: *M. rileyi* (UAS, Raichur) 1x10^{12} spores / ml	39.58 (38.97)	24.93 (29.92)	10.71 (18.86)	10.5 (18.82)	5.18 (12.9)	5.65 (13.27)	72.94	46.19	47.74	55.06
T_7: Untreated control	42.52 (40.7)	38.81 (38.53)	28.22 (32.01)	9.47 (17.76)	15.61 (23.23)	18.01 (24.99)			29.43	35.95
CD (0.05)	NS	4.6	7.23	NS	4.9	4.33			8.39	8.54
CV%	8.1	8.61	20.68	10.85	16.81	16.28			10.74	9.68

Values in parenthesis are logarthmic transformed values

Table 4: Evaluation of native isolates of entomopathogens against maize fall armyworm in kharif season, 2022

Treatment	FAW damage %**		FAW damage upto 60 days crop age	Percent reduction in FAW damage over control	Cob yield Q/ha	Percent increase in yield over control
	Before spray	After sprays				
T_1: *Metarhizium rileyi* (ANGRAU Anakapalle strain AKP Nr-1) 1x10^8 spores/ml @ 5 g/L	12.36 (1.069)	3.88 (0.579)	16.29 (1.191)	85.26	66.30	66.83
T_2: *Metarhizium rileyi* (UAS, Raichur strain) 1x10^8 spores/ml @ 5 g/lt	16.81 (1.221)	4.69 (0.670)	21.50 (1.33)	82.19	53.69	35.10
T_3: Emamectin benzoate 5SD @ 0.4 g/L	13.19 (1.110)	2.05 (0.310)	15.25 (1.176)	92.21	69.30	74.37
T_4: Untreated control	12.92 (1.107)	26.33 (1.413)	39.26 (1.645)	-	39.74	-
CD (0.05)	NS	0.12	0.14		16.16	
CV%	9.35	10.94	7.13		17.90	

Three sprays at 20,35,50 days after sowing

** Values in parenthesis are logarithmic transformed values

Conclusion

Based on three years field evaluation studies conducted in FAW infested maize crop in Andhra Pradesh, native entomopathogenic fungi, *M. rileyi* (ANGRAU Anakapalle strain AKP Nr1) was proved as potential entomofungal isolate against maize FAW and was recommended as suitable alternative to chemical, emamectin benzoate. The maize crop treated with the native entomopathogenic fungi, *M. rileyi* (ANGRAU Anakapalle strain AKP Nr1) showed low infestation of fall armyworm resulted in considerable yield increase compared to untreated control.

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