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Bioefficacy of entomopathogenic fungi against the leafhopper, *Amrasca biguttula biguttula* (ishida) infesting Okra

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Abstract
The present study evaluated the effectiveness of different entomopathogenic fungi against leafhopper under laboratory and field conditions during *rabi* and *kharif* 2023. Seasonal incidence, influence of weather parameters, laboratory bioassay and field evaluation of fungal formulations were studied. Leafhopper populations reached a peak during May (14.02 insects/3 leaves) in *rabi* and August (13.23 insects/3 leaves) in *kharif*. Weather factors significantly influenced pest incidence. Laboratory evaluation revealed that thiamethoxam 25 WG @ 0.4 g L^{-1} recorded the highest mortality (93.33–100%), while among the biological treatments, the combined formulation of *Beauveria bassiana, Metarhizium anisopliae, and Verticillium lecanii* @ 15 g L^{-1} recorded the highest mortality (73.33%) at 72 Hat. Field studies also confirmed that the combined fungal formulation effectively reduced leafhopper populations, recording 68.66% and 71.10% reduction over untreated control during *rabi* and *kharif*, respectively. The study indicates that the combined entomopathogenic fungal formulation can be incorporated into integrated pest management programmes as an eco-friendly alternative to chemical insecticides.

Keywords: *Amrasca biguttula biguttula*, *Beauveria bassiana*, biological control, *Metarhizium anisopliae*, okra, *Verticillium lecanii*

Introduction

Okra (*Abelmoschus esculentus* L. Moench) is an important vegetable crop cultivated throughout tropical and subtropical regions. Among the insect pests attacking okra, the leafhopper, *Amrasca biguttula biguttula*, is one of the most destructive sucking pests. Both nymphs and adults feed on plant sap, resulting in leaf curling, yellowing, hopper burn symptoms, reduced plant vigour, and considerable yield loss (Gosh *et al*., 2016). Farmers mainly depend on chemical insecticides for its management, but excessive use has resulted in insecticide resistance, environmental contamination, and harmful effects on beneficial organisms. Therefore, environmentally safe alternatives are required for sustainable pest management. Entomopathogenic fungi such as *Beauveria bassiana, Metarhizium anisopliae, and Verticillium lecanii* have shown promising potential as biological control agents against sucking pests. Hence, the present study was undertaken to evaluate their efficacy against *A. biguttula biguttula* under laboratory and field conditions. The yield loss due to leafhopper desapping in okra extents 54 to 66 per cent (Sharma and Sharma, 2001; Dhandapani *et al*., 2003 [5, 17]; Satpathy *et al*., 2004). The pest is extremely difficult to control owing to its robust reproductive potential, ability to travel quickly from one plant to another, and living environment (Fouly *et al*., 2011) [6]. To tackle the pest development, a number of chemical insecticides are liberally sprayed on this vegetable crop which leads to several problems like toxic residues, elimination of natural enemies, environmental disharmony and development of resistance. Okra being a fresh vegetable that is harvested at regular intervals, it is critical to evaluate safer alternatives like botanicals and mycopathogens which possess no residual toxicity, is best suited for vegetables like okra, where we use fresh vegetables for consumption (Palthiya and Nakat, 2017) [11]. Biological control of insect pests using different entomopathogenic microorganisms is gaining importance due to their target specificity, self-perpetuity, and obvious safety to the environment. The pest control prospects chiefly of entomopathogenic fungi (EPF) *viz*., *Beauveria bassiana* (Balsamo), *Metarhizium anisopliae* (Metchnikoff), and *Verticillium lecanii* (Zimmerman), have been proved beyond doubt over the decades without any resistance development (Halder *et al*., 2017) [9].

Materials and Methods

The study was conducted during the *rabi* (March–May 2023) and *kharif* (June–September 2023) seasons at the Eastern Farm of Pandit Jawaharlal Nehru College of Agriculture and Research Institute (PAJANCOA & RI), Karaikal. The experiment was conducted in an area of 40 x 10 square meter with a spacing of 45 x 30 cm using okra hybrid COBh 4 under Randomized Block Design (RBD) with three replications. Different entomopathogenic fungi, namely *Beauveria bassiana*, *Metarhizium anisopliae*, and *Verticillium lecanii*, were evaluated individually and in combinations, along with thiamethoxam 25 WG as the chemical check and an untreated control.

Seasonal population dynamics of the Okra leafhoppers, *Amrasca biguttula biguttula* and its relationship with weather parameters

The incidence of *A. biguttula biguttula* was recorded in situ on 50 randomly selected plants and the observations were made at weekly intervals from March 2023 to September

2023 and it was correlated with the weather parameters *viz*., maximum and minimum temperature, relative humidity and rainfall. The recommended dose of fertilizer of 100: 100: 100 kg/ha NPK and FYM @ 40 t/ ha as basal, N @ 100 Kg/ha as top dressing at 30 days after sowing (Bebitha, 2009 & Kumari *et al*., 2022) [2, 14] (Plate 1).

Plate 1: Field experiment

In-*vitro* evaluation of entomopathogens against the leafhoppers *Amrasca biguttula biguttula* in okra

A supervised laboratory experiment was conducted in the Department of Agricultural Entomology, PAJANCOA and RI, Karaikal. The field collected leafhoppers were starved one hour before the treatment and released in petriplates containing leaf discs of okra treated with entomopathogens and other treaments. The experiment was laid down in a Completely Randomized Design (CRD) with 9 treatments and 3 replications (Naik and Shekharappa, 2009) [10] (Plate 2 & 3).

Plate 2 : In-*vitro* evaluation of leafhoppers

Plate 3: leafhopper adult

Field evaluation of entomopathogens against the leafhoppers, *Amrasca biguttula biguttula* (Ishida)

Field efficacy of the treatments against leafhoppers was evaluated through four foliar spray applications under field conditions. The observation on the population of leafhoppers and aphids were made on three leaves per plant one each from top, middle and bottom region from randomly selected 10 plants per plot leaving the border rows. The observations were taken a day before spraying and at 1, 5, 9, 12, 14 DAT. (Prem Kumar and Ashwani Kumar, 2017) [13] Leafhopper populations were recorded in both Field Experiment I and Field Experiment II before each spray (pre-treatment count) and at regular intervals up to 14 days after each application to assess treatment effectiveness. The observations obtained from successive sprays were subjected to statistical analysis using Analysis of Variance (ANOVA). Treatment means were compared at the 5% level of significance to determine significant differences among the treatments.

Results and Discussion

Seasonal incidence of leafhoppers *Amrasca biguttula biguttula* (Ishida) in okra during *rabi* and *kharif* 2023

The seasonal incidence of leafhopper, *Amrasca biguttula biguttula*, on okra during *rabi* and *Kharif* 2023 were presented in (Table 1) Fig 1. During *rabi* 2023, the leafhopper population ranged from 5.77 to 14.02 insects per 3 leaves, with the lowest population recorded in March (5.77 insects/3 leaves) and the highest in May (14.02 insects/3 leaves), indicating a gradual increase throughout the season. During *kharif* 2023, the population ranged from 4.26 to 13.23 insects per 3 leaves, with the lowest population observed in June (4.26 insects/3 leaves) and the highest in August (13.23 insects/3 leaves). Anurag and Chandrakar (2018) [1] reported that the major activity period of jassid *A. biguttula biguttula* were observed from August to October with one distinct peak during 39th SMW (Standard Mean week) (10.36 jassid/plant), 40th SMW (39.24 aphids/ plant).

Table 1: Seasonal incidence of leafhoppers *Amrasca biguttula biguttula*

Sl.No.	Month and Year	Incidence of Leafhoppers *Amrasca biguttula biguttula* (number)
		Rabi 2023
1.	March 2023	5.77
2.	April 2023	9.89
3.	May 2023	14.02
		Kharif 2023
4.	June 2023	4.26
5.	July 2023	7.55
6.	August 2023	13.23
7.	September 2023	7.25

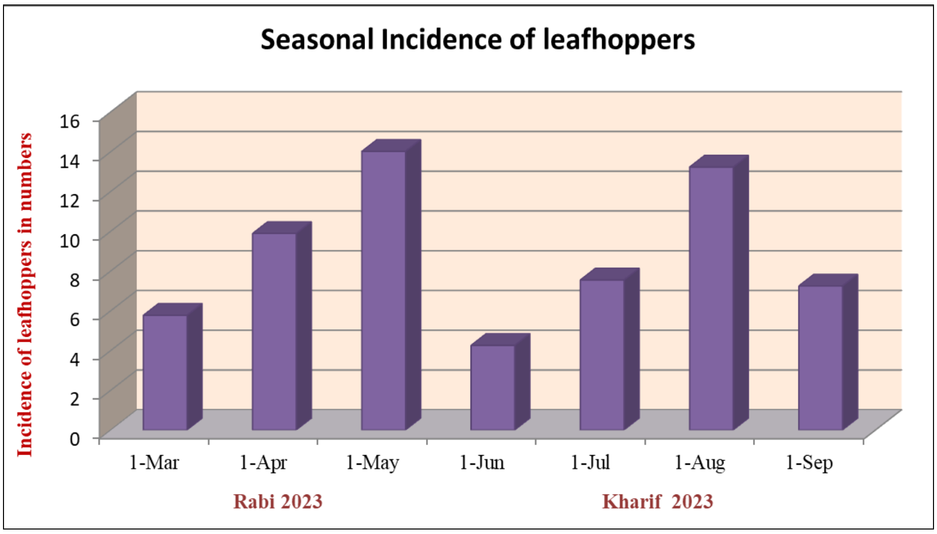


Fig 1: Seasonal incidence of leafhoppers *Amrasca biguttula biguttula* (Ishida) in okra during *rabi* and *kharif* 2023

Influence of weather parameters on the seasonal incidence of leafhoppers *Amrasca biguttula biguttula* in okra during *rabi* and *kharif* 2023

Regression analysis revealed a statistically significant relationship between leafhopper incidence and weather parameters in both seasons. During *rabi* 2023, the regression model showed a high coefficient of determination ($R^2 = 0.833$), with leafhopper incidence positively correlated with maximum temperature, minimum temperature, evening relative humidity, and rainfall, while a negative correlation was observed with morning relative humidity. Similarly, during *kharif* 2023, the regression model also showed a significant fit ($R^2 = 0.796$). Leafhopper incidence exhibited a positive correlation with morning relative humidity and rainfall, whereas maximum temperature, minimum temperature, and evening relative humidity showed negative correlations (Table 2). The results on the seasonal incidence of insect pests of okra revealed that the activity of leafhopper *A. biguttula biguttula*, was more in kharif season than in rabi season (Deevaraja *et al*., 2020) [4].

Table 2: Multiple regression on coefficient of weather parameters against the incidence of, leafhoppers *Amrasca biguttula biguttula*

Sl. No.	Source	Regresssion equation	R2
1.	Weekly count - *rabi* 2023	Y=-6.990+1.092X1+0.243X2-0.390X3 +0.123X4+0.045X5	0.833*
2.	Weekly count - *kharif* 2023	Y= 62.015-0.667X1-1.226X2+0.242X3-0.390X4 +0.006X5	0.796*

In vitro* evaluation of entomopathogenic fungi against the leafhoppers *Amrasca biguttula biguttula

Under laboratory conditions, adults of leafhoppers were released in okra leaf discs treated with entomopathogenic fungi. The adult mortality of leafhoppers ranged from 0.00 to 93.33, 0.00 to 100.00, 11.11 to 100.00 per cent at 24, 48, 72 hours after treatment (HAT). The highest mortality was recorded in the treatment with thiamethoxam 25WG @ 0.4 g/l (93.33 to 100.00%) followed by *B. bassiana* + *M. anisopliae* + *V. lecanii* @ 15 g/l (0.00 to 73.33%) while a lower mortality was observed in the treatment with *B. bassiana* @ 5 g/l (0.00 to 13.33 %) compared to the untreated check (0.00 to 11.11%) (Table 3) Fig 2.

Naik and Shekharappa (2009) [10] reported that, among the entomopathogenic fungal formulations against the sucking pests of okra, *Beauveria bassiana* oil, *Metarhizium anisopliae* oil and their WP formulations @ 1 X 10^8 cfu/ml recorded 96.67 per cent mortality of leafhoppers followed by *Verticillum lecanii* oil and *B. bassiana* WP formulations @ 1 X 10^8 cfu/ml (93.33%). Baladaniya *et al*. (2010) revealed that *V. lecanii* @ 7 g/ l gave higher mortality of jassids *A. biguttula biguttula* in okra under laboratory condition. Rekha *et al.* (2017) reported that the thiamethoxam 25 WDG @ 0.2 g/l and flonicamid 50 WG @ 0.3 g/l showed the highest mortality against the okra leafhoppers.

Table 3: In *vitro* evaluation of entomopathogenic fungi against leafhoppers *Amrasca biguttula biguttula*

Sl. No	Treatments	Dosage g/l	Mortality of leafhoppers (%)		
			24 Hat	48 Hat	72 Hat
1.	*B. bassiana*	5	0.00 (0.55) b	0.00 (0.55) b	13.33 (3.07) g
2.	*M. anisopliae*	5	0.00 (0.55) b	6.66 (2.28) b	20.00 (5.73) ef
3.	*V. lecanii*	5	0.00 (0.55) b	0.00 (0.55) b	26.66 (6.53) de
4.	*V. lecanii + M. anisopliae*	10	0.00 (0.55) b	13.33 (3.07) b	46.66 (10.49) ab
5.	*B. bassiana + M. anisopliae*	10	0.00 (0.55) b	6.66 (2.28) b	40.00 (9.26) bc
6.	*V. lecanii + B. bassiana*	10	0.00 (0.55) b	0.00 (0.55) b	33.33 (8.13) cd
7.	*B. bassiana + M. anisopliae + V. lecanii*	15	0.00 (0.55) b	13.33 (3.07) b	73.33 (11.53) ab
8.	Thiamethoxam 25 WG	0.4	93.33 (12.46) a	100.00 (12.92) a	100.00 (12.92) a
9.	Untreated check	-	0.00 (0.55) b	0.00 (0.55) b	11.11 (2.28) g
	CD (P=0.05)		0.457**	4.288**	3.369**

** - Significant at P = 0.01
In a column mean followed by a common letter are significantly different by DMRT (P=0.05)
Values in Parentheses are arc sine transformed values
HAT – Hours after treatment

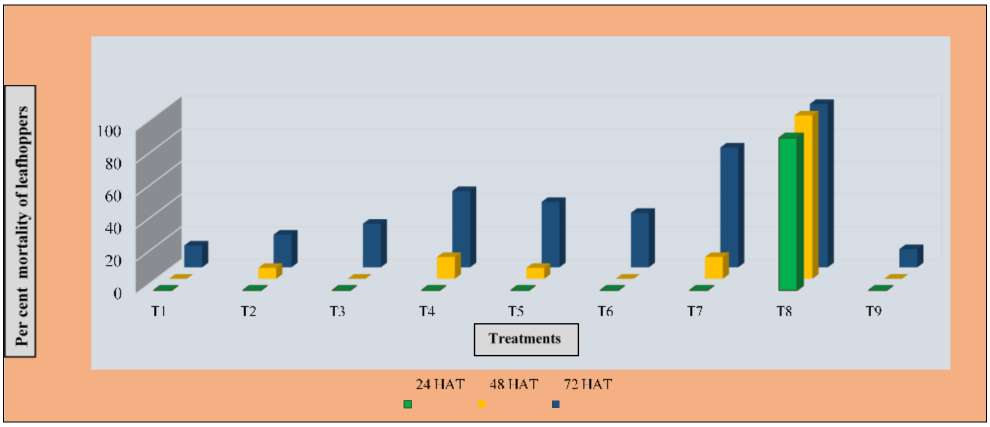


Fig 2: In-*vitro* evaluation of entomopathogenic fungi against the leafhoppers *Amrasca biguttula biguttula*

T1 - *B. bassiana* @ 5 g/l, T2 - *M. anisopliae* @ 5 g/l, T3 - *V. lecanii* @ 5 g/l, T4 - *V. lecanii* + *M. anisopliae* @ 10 g/l, T5 - *B. bassiana* + *M. anisopliae* @ 10 g/l, T6 - *V. lecanii* + *B. bassiana* @ 10 g/l, T7 - *B. bassiana* + *M. anisopliae* + *V. lecanii* @ 15 g/l, T8 - Thiamethoxam 25 WG @ 0.4 g/l, T9 - Untreated check

Field evaluation of entomopathogens against the leafhoppers, *Amrasca biguttula biguttula* (Ishida) during *rabi* and *kharif* 2023

Field evaluation conducted during the *rabi* and kharif seasons demonstrated significant differences among the treatments in reducing leafhopper populations. In both seasons, thiamethoxam 25 WG @ 0.4 g L^{-1} was the most effective treatment, recording the lowest pooled mean leafhopper population of 6.40 and 6.08 insects per three leaves, with 72.65% and 75.11% reduction over the untreated control during *rabi* and *kharif*, respectively. Among the biological treatments, the combined application of *Beauveria bassiana* + *Metarhizium anisopliae* + *Verticillium lecanii* @ 15 g L^{-1} consistently recorded the highest efficacy, with pooled mean populations of 7.33 (*rabi*) and 7.06 insects per three leaves (*kharif*), corresponding to 68.66% and 71.10% reduction over the untreated control. The two-fungus combinations provided moderate control and were more effective than individual fungal applications. The untreated control plots recorded the highest leafhopper populations of 23.38 and 24.43 insects per three leaves during *rabi* and *kharif* respectively (Table 4 and Table 5) Fig 3. Overall, the results demonstrated that the combined fungal formulation effectively suppressed *Amrasca biguttula biguttula* under field conditions and could serve as a promising eco-friendly alternative to chemical insecticides for sustainable leafhopper management in okra.

The results of the various experiments indicated that the thiamethoxam 25 WG @ 20 g a.i./ha on okra (Sinha and Sharma, 2007) [18], 0.0125 per cent in cotton (Bharpoda *et al.*, 2014) [3], 25 g a.i/ha on okra (Ghosh *et al.*, 2016) [7], 100 g/ha in cotton (Pritam Kumari *et al.*, 2022) [14], 0.5 g/l on okra (Saini *et al.*, 2023) was found to be effective against the leafhoppers A. biguttula biguttula. Pawan kumar *et al.* (2021) [12] reported that the spray of neem oil @ 3 per cent recorded the highest reduction of leafhoppers (82.84%) followed by *L. lecanii* 2 x 10^8 spores/ml @ 5 ml/l (73.91%) in okra. Shankar *et al.* (2021) [16] concluded that, the spraying of entomopathogenic fungi *Lecanicillium lecanii* @ 5 g/ l three times at an interval of 10 days from 25 days after sowing (DAS) significantly reduced the incidence of *A. biguttulla biguttula* in Bt cotton. The present findings are inagreement with the above findings.

Table 4 A: Effect of entomopathogens against the leafhoppers, *Amrasca biguttula biguttula* in okra (*Rabi* 2023)

	Treatments	Dosage (g/l)	I Foliar application						Overall Mean	% reduction over control	II Foliar application					overall mean	% reduction over control
			Pre Count	1 DAT	5 DAT	9 DAT	12 DAT	14 DAT			1 DAT	5 DAT	9 DAT	12 DAT	14 DAT		
1.	*B. bassiana*	5	11.59 (2.54)	11.21 (3.34) g	10.00 (3.16) g	10.81 (3.28) h	11.08 (3.31) h	11.69 (3.44) h	11.06	27.23	10.73 (3.27) f	9.70 (3.11) g	9.65 (3.10) g	10.42 (3.22) g	10.87 (3.29) e	10.27	45.13
2.	*M. anisopliae*	5	12.21 (3.54)	10.89 (3.30) fg	9.21 (3.03) f	10.22 (3.19) g	10.56 (3.24) g	11.07 (3.38) g	10.69	29.67	10.56 (3.25) ef	9.46 (3.07) fg	9.37 (3.06) fg	10.02 (3.16) fg	10.63 (3.26) e	10.00	46.58
3.	*V. lecanii*	5	11.07 (3.45)	9.06 (3.01) d	7.22 (2.68) d	8.46 (2.90) d	9.47 (3.07) d	9.90 (3.15) d	9.19	39.53	9.63 (3.10) d	8.46 (2.90) d	8.49 (2.91) d	8.96 (2.99) cd	9.75 (3.12) cd	9.05	51.65
4.	*V. lecanii + M. anisopliae*	10	10.37 (3.33)	8.16 (2.85) c	6.58 (2.56) c	7.22 (2.68) c	8.60 (2.93) c	9.11 (3.00) c	8.34	45.13	9.15 (3.02) c	7.55 (2.74) c	7.82 (2.79) c	8.59 (2.93) c	9.43 (3.07) c	8.50	54.59
5.	*B. bassiana + M. anisopliae*	10	11.07 (3.37)	10.56 (3.25) f	8.89 (2.98) f	9.81 (3.13) f	10.45 (3.23) f	10.93 (3.31) f	10.28	32.36	10.33 (3.21) ef	9.26 (3.04) ef	9.09 (3.01) ef	9.68 (3.11) ef	10.47 (3.23) e	9.76	47.86
6.	*V. lecanii + B. bassiana*	10	10.66 (3.36)	9.77 (3.12) e	8.00 (2.82) e	9.11 (3.01) e	9.88 (3.14) e	10.34 (3.24) e	9.62	36.71	10.17 (3.19) e	9.03 (3.00) e	8.85 (2.97) de	9.36 (3.05) de	9.96 (3.15) d	9.47	49.41
7.	*B. bassiana + M. anisopliae + V. lecanii*	15	9.71 (3.23)	7.68 (2.77) b	5.71 (2.38) b	6.35 (2.52) b	7.49 (2.73) b	8.45 (2.89) b	7.56	50.26	8.41 (2.90) b	6.79 (2.60) b	6.90 (2.62) b	7.64 (2.76) b	8.57 (2.92) b	7.66	59.08
8.	Thiamethoxam 25 WG	0.4	10.79 (3.32)	6.23 (2.49) a	4.34 (2.08) a	5.56 (2.35) a	6.68 (2.58) a	7.25 (2.69) a	6.80	55.26	7.12 (2.66) a	5.62 (2.37) a	5.98 (2.44) a	6.82 (2.61) a	7.71 (2.77) a	6.65	64.47
9.	Untreated check	-	14.02 (3.64)	14.31 (3.78) h	14.93 (3.86) h	15.79 (3.93) i	15.86 (3.98) i	16.34 (4.04) i	15.20	-	16.97 (4.11) g	18.06 (4.25) h	19.01 (4.36) h	19.54 (4.42) h	20.03 (4.47) f	18.72	-
	CD (P=0.05)	-	NS	0.059**	0.065**	0.058**	0.053**	0.043**	-	-	0.070**	0.069**	0.070**	0.090**	0.061**	-	-

NS – Not In a column mean followed by a common letter are significantly different by DMRT (P=0.05)
** - Significant at P = 0. Values in Parentheses are $\sqrt{X} + 0.5$ transformed values
# Mean of 10 DAT – Days after treatment Mean of 3 replications

Table 4 B: Effect of entomopathogens against the leafhoppers, *Amrasca biguttula biguttula* in okra (*Rabi* 2023)

Sl. No.	Treatments	Dosage (g/l)	III Foliar application						Overall Mean	% reduction over control	IV Foliar application					overall mean	% reduction over control
			Pre Count	1 DAT	5 DAT	9 DAT	12 DAT	14 DAT			1 DAT	5 DAT	9 DAT	12 DAT	14 DAT		
1.	*B. bassiana*	5	10.87 (3.29) e	10.41 (3.22) f	9.54 (3.08) f	9.62 (3.10) g	10.20 (3.19) g	10.84 (3.29) g	10.24	52.76	9.75 (3.12) f	9.01 (3.00) g	9.77 (3.12) g	10.35 (3.21) f	10.81 (3.28) g	10.03	57.10
2.	*M. anisopliae*	5	10.63 (3.26) e	10.26 (3.20) f	9.35 (3.05) f	9.44 (3.07) fg	9.90 (3.16) fg	10.75 (3.28) fg	10.05	53.64	9.42 (3.06) ef	8.78 (2.96) fg	9.55 (3.09) g	10.18 (3.19) ef	10.63 (3.26) g	9.83	57.97
3.	*V. lecanii*	5	9.75 (3.12) cd	8.85 (2.97) d	7.53 (2.75) d	8.42 (2.90) cd	9.07 (3.01) d	9.81 (3.13) d	8.90	58.94	8.77 (2.96) d	7.36 (2.71) d	8.08 (2.84) d	9.15 (3.02) d	9.33 (3.05) d	8.64	63.06
4.	*V. lecanii + M. anisopliae*	10	9.43 (3.07) c	8.36 (2.89) c	6.44 (2.53) c	8.16 (2.85) bc	8.60 (2.93) c	9.55 (3.09) c	8.42	61.16	8.15 (2.86) c	6.44 (2.53) c	7.43 (2.72) c	8.46 (2.91) c	9.12 (3.02) c	8.07	65.48
5.	*B. bassiana + M. anisopliae*	10	10.47 (3.23) e	9.83 (3.09) e	9.10 (3.01) f	9.02 (3.00) ef	9.71 (3.11) ef	10.57 (3.25) f	9.78	54.88	9.26 (3.04) def	8.45 (2.90) ef	9.07 (3.01) f	9.93 (3.15) e	10.22 (3.19) f	9.52	59.30
6.	*V. lecanii + B. bassiana*	10	9.96 (3.15) d	9.43 (3.07) e	8.33 (2.88) e	8.86 (2.97) de	9.37 (3.06) de	10.30 (3.21) e	9.37	56.78	9.03 (3.00) de	8.23 (2.87) e	8.55 (2.92) e	9.52 (3.08) d	9.75 (3.12) e	9.14	60.92
7.	*B. bassiana + M. anisopliae + V.*	15	8.57 (2.92) b	7.57 (2.75) b	5.62 (2.37) b	7.68 (2.77) b	8.12 (2.84) b	9.28 (3.04) b	7.80	64.02	7.51 (2.74) b	5.35 (2.31) b	6.39 (2.52) b	7.39 (2.72) b	8.35 (2.89) b	7.33	68.66

	lecanii															
8.	Thiamethoxam 25 WG	0.4	7.71 (2.77) a	6.71 (2.59) a	4.53 (2.13) a	6.70 (2.58) a	7.55 (2.74) a	8.31 (2.88) a	6.91	68.12	6.42 (2.53) a	4.37 (2.09) a	5.37 (2.31) a	6.63 (2.57) a	7.36 (2.71) a	6.40	72.65
9.	Untreated check	-	20.03 (4.47) f	20.44 (4.52) g	21.41 (4.62) g	22.35 (4.72) h	22.80 (4.77) h	23.09 (4.80) h	21.68	-	23.63 (4.86) g	24.33 (4.93) h	24.95 (4.99) h	25.26 (5.02) g	25.52 (5.05) h	23.38	-
CD(P=0.05)			0.061**	0.075**	0.081**	0.084**	0.066**	0.029**	-	-	0.085**	0.093**	0.043**	0.061**	0.034**	-	-

** - Significant at P = 0. In a column mean followed by a common letter are significantly different by DMRT (P=0.05)
# Mean of 10 Values in Parentheses are $\sqrt{X}$ + 0.5 transformed values
Mean of 3 DAT – Days after treatment

Table 5 A: Effect of entomopathogens against the leafhoppers, *Amrasca biguttula biguttula* in okra (*Kharif* 2023)

Sl. No.	**Treatment**	**Dosage (g/L)**	**I Foliar application**						**Overall Mean**	**% reduction over control**	**II Foliar application**					**Overall Mean**	**% reduction over control**
			Pre coun t	**1 DAT**	**5 DAT**	**9 DAT**	**12 DAT**	**14 DAT**			**1 DAT**	**5 DAT**	**9 DAT**	**12 DAT**	**14 DAT**		
1	*B. bassiana*	5	13.21 (3.63)	11.68 (3.41) f	10.31 (3.36) h	10.98 (3.31) g	11.65 (3.41) f	12.24 (3.50) g	11.67	27.06	10.98 (3.31) h	9.98 (3.16) h	10.06 (3.17) g	11.25 (3.35) f	11.90 (3.45) f	10.83	42.60
2	*M. anisopliae*	5	13.78 (3.71)	11.01 (3.32) e	9.56 (3.09) g	10.24 (3.20) f	11.36 (3.31) e	11.79 (3.43) fg	11.29	29.43	10.60 (3.26) g	9.59 (3.10) g	9.40 (3.07) f	10.98 (3.31) ef	11.45 (3.38) ef	10.40	44.88
3	*V. lecanii*	5	12.76 (3.57)	9.78 (3.13) d	7.67 (2.77) d	8.90 (2.98) d	9.95 (3.15) d	10.23 (3.20) d	9.88	38.25	8.52 (2.92) d	6.80 (2.61) d	8.05 (2.83) d	9.13 (3.02) c	10.57 (3.25) d	8.61	54.37
4	*V. lecanii + M. anisopliae*	10	12.57 (3.54)	8.57 (2.93) c	6.90 (2.63) c	7.85 (2.80) c	8.35 (2.89) c	9.56 (3.09) c	8.96	44.00	7.64 (2.76) c	5.48 (2.34) c	7.54 (2.74) c	8.32 (2.88) b	9.98 (3.16) c	7.79	58.71
5	*B. bassiana + M. anisopliae*	10	13.45 (3.66)	10.88 (3.30) e	9.05 (3.01) f	10.11 (3.18) ef	10.98 (3.31) e	11.34 (3.37) ef	10.96	31.50	10.04 (3.17) f	9.00 (3.00) f	9.10 (3.01) f	10.58 (3.25) e	11.00 (3.32) de	9.94	47.32
6	*V. lecanii + B. bassiana*	10	12.88 (3.58)	9.91 (3.14) d	8.67 (2.94) e	9.76 (3.12) e	10.03 (3.17) d	10.90 (3.30) e	10.35	35.31	9.65 (3.11) e	7.61 (2.76) e	8.67 (2.94) e	9.80 (3.13) d	10.90 (3.30) d	9.32	50.60
7	*B. bassiana + M. anisopliae + V. lecanii*	15	11.47 (3.38)	7.31 (2.70) b	5.79 (2.41) b	6.87 (2.62) b	7.45 (2.73) b	8.92 (2.99) b	7.96	50.25	6.77 (2.60) b	4.67 (2.16) b	6.93 (2.63) b	7.98 (2.82) b	8.89 (2.98) b	7.04	62.69
8	Thiamethoxam 25 WG	0.4	10.56 (3.25)	6.78 (2.60) a	4.74 (2.18) a	5.86 (2.42) a	6.52 (2.55) a	7.80 (2.79) a	7.04	56.00	5.45 (2.33) a	3.86 (1.96) a	5.69 (2.38) a	6.90 (2.63) a	7.86 (2.80) a	6.00	68.20
9	Untreated check	–	14.23 (3.86)	15.21 (3.92) g	15.89 (3.99) i	16.45 (4.06) h	16.99 (4.12) g	17.20 (4.15) h	16.00	-	17.58 (4.19) i	18.20 (4.27) i	19.07 (4.36) h	19.56 (4.342) g	19.98 (4.47) g	18.87	-
CD (P=0.05)		-	NS	0.060**	0.061**	0.066**	0.074**	0.068**	-	-	0.052**	0.056*	0.067**	0.078**	0.080**	-	-

** - Significant at P = 0. In a column mean followed by a common letter are significantly different by DMRT (P=0.05)
# Mean of 10 Values in Parentheses are $\sqrt{X}$ + 0.5 transformed values
Mean of 3 DAT – Days after treatment

Table 5 B: Effect of entomopathogens against the leafhoppers, *Amrasca biguttula biguttula* in okra (*Kharif* 2023)

Sl. No.	**Treatment**	**Dosage (g/L)**	**III Foliar application**						**Overall mean**	**% reduction over control**	**IV Foliar application**					**Overall mean**	**% reduction over control**
			Pre-count	**1 DAT**	**5 DAT**	**9 DAT**	**12 DAT**	**14 DAT**			**1 DAT**	**5 DAT**	**9 DAT**	**12 DAT**	**14 DAT**		
1	*B. bassiana*	5	11.90 (3.45) f	10.99 (3.31) f	9.30 (3.05) h	10.57 (3.25) h	11.00 (3.31) g	11.88 (3.45) g	10.94	48.61	10.96 (3.31) g	9.42 (3.07) g	10.05 (3.17) f	10.89 (3.30) g	11.20 (3.35) f	10.50	57.02
2	*M. anisopliae*	5	11.45	10.50	8.91	9.99	10.55	11.43	10.47	50.82	10.53	8.89	9.80	10.48	10.65	10.07	58.78

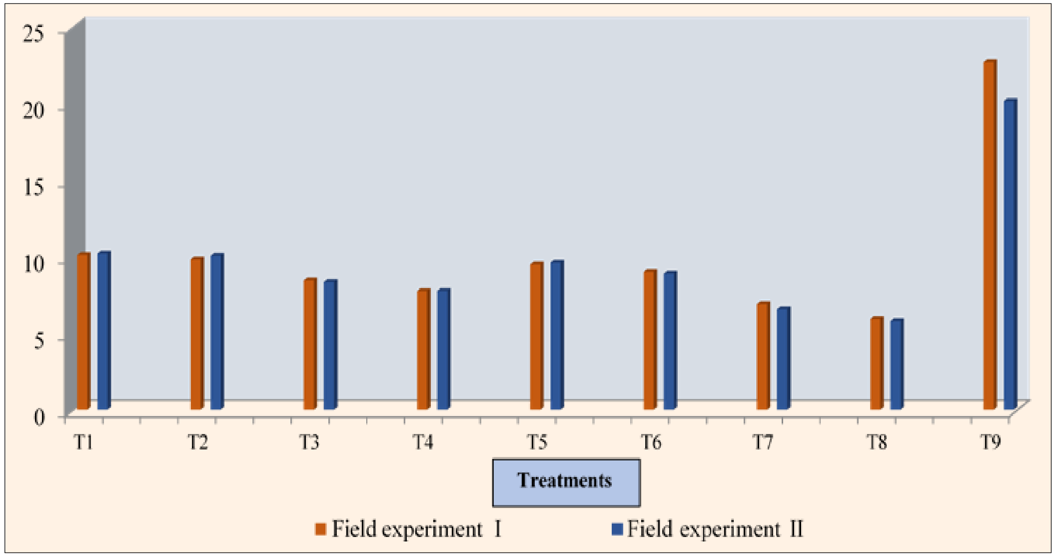
			(3.38) ef	(3.24) f	(2.98) g	(3.16) g	(3.25) fg	(3.38) g			(3.24) f	(2.98) f	(3.13) f	(3.24) fg	(3.26) e		
3	*V. lecanii*	5	10.57 (3.25) d	8.90 (2.98) d	7.06 (2.66) d	7.92 (2.81) d	8.87 (2.98) d	9.56 (3.09) d	8.81	58.61	9.00 (3.00) d	7.58 (2.75) d	8.61 (2.93) d	9.43 (3.07) d	9.90 (3.15) d	8.90	63.56
4	*V. lecanii + M. anisopliae*	10	9.98 (3.16) c	8.00 (2.83) c	6.50 (2.55) c	7.06 (2.66) c	8.01 (2.83) c	8.87 (2.98) c	8.07	62.18	8.43 (2.90) c	6.82 (2.61) c	7.90 (2.81) c	8.55 (2.92) c	9.20 (3.03) c	8.18	66.51
5	*B. bassiana + M. anisopliae*	10	11.00 (3.32) de	9.93 (3.15) e	8.10 (2.85) f	9.54 (3.09) f	9.98 (3.16) ef	10.90 (3.30) f	9.90	53.49	9.80 (3.13) e	8.54 (2.92) f	9.31 (3.05) e	10.10 (3.18) ef	10.68 (3.27) ef	9.68	60.37
6	*V. lecanii + B. bassiana*	10	10.90 (3.30) d	9.21 (3.03) d	7.60 (2.76) e	8.66 (2.94) e	9.57 (3.09) e	10.02 (3.16) e	9.32	56.22	9.63 (3.10) e	8.00 (2.83) e	8.99 (3.00) de	9.80 (3.13) de	10.18 (3.19) de	9.32	61.85
7	*B. bassiana + M. anisopliae + V. lecanii*	15	8.89 (2.98) b	7.21 (2.68) b	5.44 (2.32) b	6.04 (2.46) b	7.11 (2.67) b	8.06 (2.83) b	7.12	61.87	7.41 (2.72) b	5.45 (2.33) b	6.66 (2.58) b	7.20 (2.68) b	8.61 (2.93) b	7.06	71.10
8	Thiamethoxam 25 WG	0.4	7.86 (2.80) a	6.13 (2.47) a	4.07 (2.02) a	5.10 (2.26) a	6.23 (2.50) a	7.00 (2.65) a	6.06	66.50	6.80 (2.61) a	4.56 (2.13) a	5.73 (2.39) a	6.21 (2.49) a	7.11 (2.67) a	6.08	75.11
9	Untreated check	–	19.98 (4.47) g	20.33 (4.51) g	21.00 (4.58) i	21.76 (4.66) i	22.06 (4.70) h	22.64 (4.76) h	21.29	-	23.00 (4.80) h	23.89 (4.89) h	24.78 (4.98) g	25.06 (5.01) h	25.46 (5.05) g	24.43	–
CD(P=0.05)		-	0.080**	0.081**	0.041**	0.050**	0.094**	0.073**	-	-	0.060**	0.071**	0.072*	0.069**	0.083**	-	-

** - Significant at P = 0.01 In a column mean followed by a common letter are significantly different by DMRT (P=0.05)

Mean of 10 plants Values in Parentheses are $\sqrt{X} + 0.5$ transformed values

Mean of 3 replications DAT – Days after treatment

Fig 3: Effect of entomopathogens against the leafhoppers, *Amrasca biguttula biguttula* during *rabi* 2023 (Field experiment I) and *kharif* 2023 (Field experiment II) in okra



T1 - *B. bassiana* @ 5 g/l, T2 - *M. anisopliae* @ 5 g/l, T3 - *V. lecanii* @ 5 g/l, T4 - *V. lecanii* + *M. anisopliae* @ 10 g/l, T5 - *B. bassiana* + *M. anisopliae* @ 10 g/l, T6 - *V. lecanii* + *B. bassiana* @ 10 g/l, T7 - *B. bassiana* + *M. anisopliae* + *V. lecanii* @ 15 g/l, T8 - Thiamethoxam 25 WG @ 0.4 g/l, T9 - Untreated check

Conclusion

The present study demonstrated that the combined application of the entomopathogenic fungi *Beauveria bassiana, Metarhizium anisopliae, and Verticillium lecanii* effectively suppressed the leafhopper, *Amrasca biguttula biguttula,* under both laboratory and field conditions. The fungal consortium consistently reduced leafhopper populations and exhibited sustained efficacy across successive field applications. Although the chemical insecticide thiamethoxam recorded the highest level of leafhopper suppression, the fungal combination provided appreciable control while offering significant ecological advantages, including reduced environmental contamination and lower risks to non-target organisms. The findings indicate that the combined use of entomopathogenic fungi represents a promising eco-friendly alternative to conventional insecticides for the management of *A. biguttula biguttula* in okra. The integration of this fungal consortium into integrated pest management (IPM) programmes has the potential to reduce reliance on synthetic insecticides, delay the development of insecticide resistance, and promote sustainable crop protection. Therefore, the use of *B. bassiana, M. anisopliae, and V. lecanii* in combination can be recommended as an effective biological management strategy for leafhopper control, contributing to environmentally sustainable and economically viable okra production.

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