Research Article

Entomopathogenic Fungi: As an Eco-Friendly Approach for the Management of Thrips *Megalurothrips distalis* Karny (Thysanpotera: Thripidae) and their Impact on the Yield in Mungbean (*Vigna radiata* (L.) Wilczek)

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Abstract | The use of living organisms, Predators, parasitoids and microorganisms likewise fungi, bacteria and viruses has proven to be a viable and sustainable pest management technique. Entomopathogenic fungi (EPF) are currently used as biocontrol agents and are alternatives of synthetic insecticides in sustainable agriculture. The bio-efficacy of entomopathogenic fungi (EPF); *Metarhizium anisopliae* (PacerMA), *Verticillium lecanii* (Zimm) (Mealikil-VL), *Isaria fumosorosea* (Wise) and *Beauveria bassiana* (Bals) (Racer-BB) were investigated against the mung bean thrips, *Megalurothrips distalis* Karny (Thysanoptera: Thripidae). Evaluations were based on thrips population, percentage reduction in number of thrips per flower, and percentage damage of the mung bean pods. On an accumulative basis, *B. bassiana* at 7.5 % concentration resulted in the reduction of thrips population per flower (59.42 %) and it was observed more superior than other tested EPFs. Application of *B. bassiana* resulted highest number of flowers (185.40) with the maximum number of pods/plant (56) followed by *M. anisopliae* which produced 180.8 flowers and 51.27 pods per plant at the same concentration. Moreover, a *B. bassiana* caused a maximum (36.31%) flower shedding reduction. However, flower shedding, total number of flowers, yield deformed pods and total pods was influenced by the applications of different concentrations of EPFs. Overall, *B. bassiana* at 7.5 % concentration significantly increased the yield to 1018.9 kg per hectare than the other tested EPFs. *B. bassiana* was a potential candidate for thrips management in mung bean and had a significant impact on the total return. Consequently, the EPF, *B. bassiana*, may potentially be incorporated into the Mung bean thrips IPM program.

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Introduction

The Mung bean, *Vigna radiata* (L.) Wilczek, is an important pulse crop cultivated in Pakistan (Mansoor *et al*., 2017; Rani *et al*., 2018). It is broadly distributed all over the world throughout the tropical as well as subtropical regions with the primary growing regions in South and Southeast Asia (Pratap *et al*., 2021; Chadha, 2010; Bairwa and Singh, 2017). Because of the mung bean short life cycle, it can be adapted easily into different cropping systems. It is usually cultivated and used by the farmers because of its maximum protein contents (Tang *et al*., 2014; Ratnasekera and Subhashi, 2015; Hou *et al*., 2019; Pratap *et al*., 2021).

Mung bean crop is susceptible to numerous insect pests including mung bean thrips, *Megalurothrips distalis* Karny (Thysanoptera: Thripidae) (Sani and Umar, 2017; Rani *et al*., 2018; Gehlot and Prajapat, 2021; Sequeros *et al*., 2021). Damage from this insect pest includes a drop in flowers, distorted pods, inferior quality grains, and low yield (Kooner *et al*., 1983; Rani *et al*., 2018; Gehlot and Prajapat, 2021; Sequeros *et al*., 2021). Thrips is considered a sucking feeder, extensively utilizing proteins, carbohydrates, lipids, vitamins, water and inorganic salts from the host, and it affects badly to the nutritional value and resistance trait of the host (Haile and Higley, 2003; Bayu and Prayogo, 2018; Gehlot and Prajapat, 2021).

In addition to killing off the beneficial insects that would otherwise keep the mung bean flower thrips population in check, the use of chemical insecticides for thrips management also raises concerns about phytotoxicity and goes against the "3Rs" (resistance, resurgence and residue) (Siegwart *et al*., 2015). In recent years, entomopathogens have increasingly gained a high importance for managing numerous insect pests (Arthus *et al*., 2013; Shiberu *et al*., 2013; Ain *et al*., 2021; Gulzar *et al*., 2021). Biological control and the use of EPFs in particular, have received a lot of interest as a promising method of management (Camara *et al*., 2022). Microbial pesticides, including entomopathogenic; fungi, viruses, and bacteria have been proven to play a significant role in sustainable crop production (Ekesi *et al*., 2000; Niassy *et al*., 2012; Arthus *et al*., 2013; Shiberu *et al*., 2013; Ain *et al*., 2021; Gulzar *et al*., 2021). These microbial controls could provide a long lasting pest management and generally safer for the environment and non-target organisms than the chemical control (Khetan, 2000). Using virulent isolates of entomopathogenic fungi as part of biointensive integrated pest control could be a good way to get rid of thrips (Savariya and Jethva, 2023). The world's interest in the use of entomopathogenic fungi (EPFs), as biological control in different pest management programs, has been rising in recent years (Yang *et al*., 2020).

EPFs as management tools for pests of mung bean have not been studied extensively. Present investigations were carried out to determine the impact of different EPFs against mung bean flower thrips under field conditions and to evaluate the efficacy of EPFs on mungbean yield parameters (flower shedding, total number of pods, total number of flowers and damaged pods and finally on yield) with suitable concentrations.

Materials and Methods

Plant source

The mung bean variety NM-2011 was cultivated. The experimental plot size was (5 × 2.4 m^2). Mung bean was cultivated, using a hand drill method, at a recommended seed rate of 8-10 kg per acre. All treatments were completely randomized and replicated thrice. All cultural practices, including hoeing and removal of weeds were performed in all treatments manually.

Entomopathogenic fungi

Four different EPFs including *Isaria fumosorosea*, (Paecilomite®), *Verticillium lecanii* (Zimm) (Mealikil-®), *Beauveria bassiana* (Bals) (Racer®) and *Metarhizium anisopliae* (Pacer®) were obtained from ALM (Agri life Meda Hyderabad), Andhra Pradesh, India. The virulence of above cited EPFs *Verticillium lecanii* (Zimm) (Mealikil-VL) (1.3 x 10^9 CFU/g), *Metarhizium anisopliae* (Pacer-MA) (1.2 x 10^9 CFU/g), *Beauveria bassiana* (Bals) (Racer-BB) (1.4 x 10^9 CFU/g), and *Isaria fumosorosea* (Wise) (1x 10^9 CFu/g), was assessed at 2.5, 5.0, and 7.5 % concentration levels, in field trials.

Field pathogenicity bioassays

The pathogenicity of the different EPFs, at different concentrations, was assessed under field conditions. The EPF's were applied at the occurrence of thrips in the experimental plots. Different concentrations of the treatments were repeated after a resurgence of thrips populations in the experimental plot, except the control treatments. Before the application of EPF's treatment, the population of thrips was estimated from five randomly selected plants per treatment. From each plant, three opened flowers were gently shaken over a white cardboard paper. The dislodged thrips feeding inside flowers were recorded, using a magnifying lens. After EPFs' applications, data were recorded after 3, 7 and 15 days post-treatments. Pathogenicity of different EPF concentrations was evaluated based on the reduction of thrips numbers, flower shedding, and deformed pods per plant by using the following equations (Mumutaj, 2014).

$$\text{\% Reduction of thrips} = \frac{\text{No.of thrips in control} - \text{No.of thrips in treatments}}{\text{No.of thrips in control}} \times 100$$

$$\text{\% Flower shedding by thrips} = \frac{\text{No.of flower shedding}}{\text{Total no.of flowers}} \times 100$$

$$\text{Flower shedding reduction \% over control} = \frac{FSC-FST}{FSC} \times 100$$

Where; FSC = Flower shedding in the control, FST = Flower shedding in the treatment.

$$\text{\% Increase of flowers over control} = \frac{TFT-TFC}{TFC} \times 100$$

TFT= Total flowers in treatment; TFC= Total flowers in control).

$$\text{Pods increasing \%} = \frac{TPT-TPC}{TPC} \times 100$$

TPT= Total pods in treatment; TPC= Total pods in control).

After harvesting, the plants were sun-dried and threshed manually. Collected seeds were weighed, and the total yield per hectare was estimated manually.

Statistical analysis

All the data were analyzed using Statistix 8.1, USA. The data regarding the differences in the mean population at different time intervals and potential yield were determined, using analysis of variance (ANOVA). The means were compared, using the LSD test at a 5 % level of significance (Gomez and Gomez, 1984).

Results and Discussion

Impact of EPFs on thrips population and percent reduction of thrips population

Pathogenicity of the different EPFs against thrips in the mung bean was assessed at three different concentration levels. The results revealed that on an accumulative basis, all concentrations of EPF's showed a highly significant differences among the tested treatments (Table 1). Aplication of *B. bassiana* at 7.5 % concentration exhibited significant results and showed a minimum thrips population (1.76) per flower, and it was statistically different than the other treatments, followed by *M. anisopliae* along with 2.06 thrips/flower (Table 1).

The percentage reduction of thrips population on an accumulative basis at the three different concentrations levels had also showed significant differences among the treatments (Table 2). Application of *B. bassian* at the 7.5 % concentration exhibited maximum 59.42 % reduction in thrips population followed by *M. anisopliae*, *V. lecanii*, and *I. fumosorosea*, with 52.64, 43.69, and 38.38 % reductions on comparison to control (Table 2).

Table 1: *Assessment of entomopathogenic fungi against thrips population.*

Treatments	2.5% concentration			Mean	5.0% concentrations			Mean	7.5% concentration			Mean
	3 DAS	7 DAS	15 DAS		3 DAS	7 DAS	15 DAS		3 DAS	7 DAS	15 DAS	
V. lecanii	3.66 bc	2.48 f	3.11 de	3.09 c	3.51 b	2.22 fg	2.69 de	2.81 c	3.20 b	1.81 gh	2.34 de	2.45 c
M. anisopliae	3.12 de	2.43 f	2.87 e	2.81 d	2.68 de	1.98 gh	2.34 ef	2.33 d	2.49 cd	1.69 gh	1.99 fg	2.06 d
B. bassiana	3.34 cd	2.01 g	2.31 fg	2.56 e	2.92 cd	1.61 i	1.86 hi	2.13 d	2.30 def	1.35 i	1.63 hi	1.76 e
I. fumosoroseus	3.75 b	3.07 de	3.56 bc	3.46 b	3.64 b	2.57 def	3.12 c	3.11 b	3.20 b	2.14 ef	2.69 c	2.68 b
Control	4.46 a	4.15 a	4.38 a	4.33 a	4.46 a	4.15 a	4.38 a	4.33 a	4.46 a	4.15 a	4.38 a	4.33 a
LSD value at 5%	0.35			0.20	0.36			0.21	0.31			0.18
Mean	3.67 a	2.83 c	3.25 b		3.44 a	2.51 c	2.88 b		3.13 a	2.23 c	2.61 b	
LSD value at 5%	0.16				0.16				0.14			

Means sharing similar letters are not significantly different by LSD test at P = 0.05

Table 2: *Assessment of entomopathogenic fungi against percent reduction of thrips population.*

Treatments	2.5% Concentration			5.0% Concentrations			7.5% Concentration			Mean
	3 DAS	7 DAS	15 DAS	3 DAS	7 DAS	15 DAS	3 DAS	7 DAS	15 DAS	
V. lecanii	17.86 g	40.29 cd	28.77 ef	21.43 g	46.51 cd	38.53 e	28.42 f	56.34 c	46.30 d	43.69 c
M. anisopliae	30.09 ef	41.42 bc	34.42 de	39.91 de	52.36 bc	46.38 cd	44.14 de	59.23 bc	54.55 c	52.64 b
B. bassiana	25.13 f	51.60 a	47.27 ab	34.46 ef	61.22 a	57.67 ab	48.15 d	67.28 a	62.82 ab	59.42 a
I. fumosoroseus	15.97 g	25.97 f	18.58 g	18.59 g	38.20 e	28.64 f	28.26 f	48.29 d	38.60 e	38.38 d
Control	0.00 h	0.00 h	0.00 h	0.00 h	0.00 h	0.00 h	0.00 g	0.00 g	0.00g	0.00 e
LSD Value at 5%	5.99			6.85			6.06			3.5

Means sharing similar letters are not significantly different by LSD Test at P = 0.05

Table 3: *Assessment of different entompathogenic fungi at different concentrations against flowers and pods formation of mungbean.*

Treatments	% increase of flowers over control	Flower shedding per plant	% Flower shedding	% Reduction of flower shedding over control	% Increase of pods number over control	Deformed Pods per plant
V. lecanii (zimm) (Mealikil-VL) 2.5%	7.59 (136.53) efg	101.47 abc	74.50 bc	6.16 cde	15.50 (38.13) fg	9.27 b
V. lecanii (zimm) (Mealikil-VL) 5.0%	15.79 (146.93) cde	100.60 bc	68.62 de	6.96 cd	22.93 (40.67) def	6.40 cd
V. lecanii (zimm) (Mealikil-VL) 7.5%	27.32 (161.53) b	95.20 c	58.88 f	11.95 c	35.69 (44.87) cd	5.93 cdef
M. anisopliae (Pacer-MA) 2.5%	16.99 (148.40) cd	82.60 d	55.66 f	23.61 b	29.58 (42.80) cde	5.27 efg
M. anisopliae (Pacer-MA) 5.0%	28.58 (163.13) b	77.47 d	47.49 gh	28.36 b	37.36 (45.33) c	5.00 fg
M. anisopliae (Pacer-MA) 7.5%	42.48 (180.80) a	77.07 d	42.63 h	28.73 b	55.23 (51.27) b	4.93 fg
B. bassiana (Bals) (Racer-BB)2.5%	22.06 (154.87) bc	76.40 d	49.38 g	29.34 b	30.82 (43.20) cde	5.33 defg
B. bassiana (Bals) (Racer-BB) 5.0%	37.97 (175.07) a	75.93 de	43.34 h	29.79 ab	50.78 (49.80) b	5.00 fg
B. bassiana (Bals) (Racer-BB)7.5%	46.14 (185.40) a	68.87 e	37.14 i	36.31 a	69.33 (56.00) a	4.27 g
I. fumosoroseus 2.5%	6.50 (135.13) fg	105.13 ab	77.85 b	2.78 de	9.05 (36.07) gh	9.87 b
I. fumosoroseus 5.0%	13.13 (143.47) def	101.33 abc	70.61 cd	6.28 cde	19.75 (39.53) efg	6.67 c
I. fumosoroseus 7.5%	22.85 (155.87) bc	100.93 abc	64.90 e	6.66 cde	32.46 (43.73) cde	6.13 cde
Control	126.87 g	108.13 a	85.24 a	-	(33.07) h	10.53 a
LSD (0.05)	10.52	7.48	4.98	6.93	4.27	1.1
CV (%)	4.07	4.93	4.95	24.63	5.83	10.07

Values in parenthesis represent mean No. of total flowers and total pods per plant in their respective columns. Means sharing similar letters are not significantly different by LSD Test at P = 0.05

Impact of entompathogenic fungi on flowers and pods formation of mungbean

Results revealed that application of *B. bassiana* at maximum concentration 7.5% expressed significant results and increased 46.14% flowers followed by *M. anisopliae* (42.48) and *V. lecanii* (27.32), respectively. Similarly, application of *B. bassiana* at 7.5% concentration exhibited minimum flower shedding 68.87 per plant followed by *M. anisopliae* (77.07) and *V. lecanii* (95.20) respectively. Moreover, in case of % flower shedding and % reduction of flower shedding application of *B. bassiana* at 7.5% concentration showed minimum 37.14% flower shedding and 36.31% reduction in flower shedding followed by the *M. anisopliae* and *V. lecanii*, respectively at the same concentration (Table 3).

Impact of entomopathogenic fungi on % increase of pods number and deformed pods per plant in Mungbean

The results in response to different EPFs concentrations application against total pods and deformed pods formation significantly affected deformed pods and the total numbers of pods formation/plant (Table 3). Application of *B. bassiana* at the concentrations of 7.5 % produced the maximum 56.0 pods/plant than the control (Table 3). Similarly, *M. anisopliae* with 7.5 % and *B. bassiana* at 5.0 % showed a significant increase in pods formation, which was 51.27 and 49.80 pods per plant than the control, respectively (Table 3). The lowest number of 36.07 pods per plant was observed in *I. fumosorosea* at 2.5 % concentration on comparison to control (Table 3).

Impact of entomopathogenic fungi on seed yield of mungbean

All EPFs at different concentrations exhibited significantly higher yields than the control (Figure 1). The plot treated with *B. bassiana* 7.5 % produced the highest yield potential of 1018.9 kg/ha, followed by *M. anisopliae* (7.5%) and *B. bassiana* (5.0 %), with 941.1 kg ha^{-1} and 841.1 kg/ha^{-1} yields, respectively. The minimum yield of 551.7kg ha^{-1} was obtained in untreated plants.

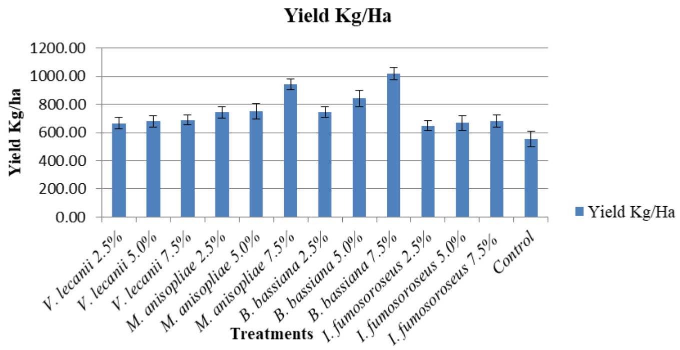

Figure 1: *Effect of different entomopathogenic fungi at different concentrations on seed yield of mungbean.*

The EPFs had a significant role against thrips in mung bean (Shiberu *et al.*, 2013; Ain *et al.*, 2021; Gulzar *et al.*, 2021). The application of EPFs, has gained a lot of attention as a viable way of thrips management (Camara *et al.*, 2022). Results of the present study are in accordance with the outcomes of other studies (Ekesi *et al.*, 2000; Niassy *et al.*, 2012; Arthus *et al.*, 2013; Shiberu *et al.*, 2013; Ain *et al.*, 2021; Gulzar *et al.*, 2021) who reported that different entomopathogenic fungi are used to control thrips. The findings of the contemporary study expressed that EPF treated plots are more superior to non-treated plots for the reduction of thrips population (Ekesi *et al.*, 2000; Arthus *et al.*, 2013; Shiberu *et al.*, 2013; Mfuti *et al.*, 2016; Hemalatha *et al.*, 2017; Singh *et al.*, 2018; Ain *et al.*, 2021; Gulzar *et al.*, 2021). Comparable results were obtained by (Singh *et al.*, 2013, 2018; Ain *et al.*, 2021; Gulzar *et al.*, 2021), who reported that bio-pesticides were helpful in reducing thrips populations. Savariya and Jethva (2023) also reported that entomopathogenic fungi could be used as an effective method of thrips control.

The results of the present study showed that on a cumulative basis, application of *B. bassiana* (7.5%) had an effective capacity of killing 48.15 and 67.28%, after a 3rd and 7th day of applications. After 15 days of application, the efficacy decreased to 62.82% on comparison to the control, which mostly differed significantly than other treatments. Present results are in line with Vestergaard *et al.* (1995) who described that *B. bassiana* efficacy was 46.18, 54.31, and 60.67%, on the third, fifth and seventh day, respectively. Weekly application of the EPFs could provide a significant control against mung bean thrips in mung bean crops. Similar results were reported by Singh *et al.* (2018) and Maniania *et al.* (2003a, b). The present findings are comparable with Gill *et al.* (1998) who reported that Botanigard, a commercial formulation of *B. bassiana*, on weekly intervals expressed the full control of western flower thrips (*Frankliniella occidentalis*). Similarly, Singh *et al.* (2018) also showed that the *B. bassiana* showed a significant decline in thrips population in mung bean. Findings revealed that *B. bassiana* was superior against thrips inhibition than other tested EPFs likewise *V. lecanii*, which is in agreement with (Singh *et al.*, 2013, 2018; Hemalatha *et al.*, 2017; Ain *et al.*, 2021; Gulzar *et al.*, 2021), who described that *B. bassiana* showed significant results in inhibiting thrips populations.

Present findings showed that the highest yield potential 1018.9 kg/ha was reported by *B. bassiana* (7.5%), followed by the application of *M. anisopliae* (7.5%) and *B. bassiana* (5.0%) treated plots, with 941.1 and 841.1 kg ha-1 seed yield, respectively. These findings are in line the results of Bayu and Prayogo (2018), Singh *et al.* (2013, 2018), who reported that the application of *B. bassiana* can inhibit pest population in mung bean and results in higher seed weight (659.7g/plot). These results are also in partial conformity with the findings of Maniania *et al.* (2003a). Application of *B. bassiana* at the concentration of 7.5 % significantly controlled thrips in mung bean and significantly increased flowers (46.14 %) and pods (69.334 %) over the control. Moreover, *B. bassiana* application at the rate of 7.5 % plays a pivotal role in controlling flower shedding and deformed pods and it significantly influences the crop yield potential.

Conclusions and Recommendations

Present study revealed that applications of *B. bassiana* could significantly reduce thrips population and their damage in mung bean. Application of *B. bassiana* influenced the potential yield than the other tested EPFs. Application of *B. bassiana* can result in a significant yield increase of mung bean through a reduction in thrips and is recommended as a biological control in an IPM component on mung bean. However, further research work is required to understand the efficacy of *B. bassiana* in combination with botanical extracts and other biological agents (EPFs) for thrips control in an environmentally safe manner.

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Novelty Statement

Entomopathogenic fungi (EPF) are currently used as biocontrol agents and are alternatives of synthetic insecticides in sustainable agriculture. *Beauveria bassiana* is ecofriendly approach which could significantly reduce the Megalurothrips distalis population in mungbean. Moreover, *B. bassiana* can provide highest number of flowers and pods which ultimately increases the yield of mungbean.

Author's Contribution

Muhammad Nadeem: Conducted research trial collected data.
Jamshaid Iqbal: Project administration.
Tariq Mustafa and Gul Rehman: Project administration.
Muhammad Faisal: Conceived the idea.
Muhammad Younas: Corrected the paper.
Aftab Ahmad Khan: Review of literature.
Ameer Hamza: Helped in data collection.
Abdul Ghaffar: Analyzed the data.
Munir Abbas: Compiled the data.

List of abbreviations
EPF: entomopathogenic fungi; DAS: days after spray

Conflict of interest
The authors have declared no conflict of interest.

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