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Field appraisal of entomopathogenic fungi horizontal transmission device for entomo-vectoring of *Beauveria bassiana* and *Metarhizium anisopliae* in bitter gourd field against *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae)

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Abstract

Background *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae) infestation poses a serious risk to bitter gourd cultivation. Traditionally, *B. cucurbitae* has been controlled using synthetic pesticides, which have drawbacks such as non-target toxicity and pest resistance. Entomopathogenic fungi (EPF) provide concentrated ecological alternatives, which support ongoing pest reduction and sustainable agriculture by adhering to Integrated Pest Management principles. Therefore, EPF provides a viable alternative for chemical control of *B. cucurbitae*, addressing its shortcomings and promoting environmentally friendly pest control technology. This study evaluated the effectiveness of entomo-vectored horizontal transmission devices (EV-HTD) against *B. cucurbitae* in bitter gourd fields, focusing on GF-120 and Butanone acetate. Assessment parameters include converting fruit infestation data into yield loss per plant, marketable fruit yield per plant, marketable yield per hectare, and yield loss per hectare.

Results The highest mean percentage of entomo-vectored *B. cucurbitae* (70.50%) was found in plots treated with Butanone acetate + *B. bassiana*-based EV-HTD. This was followed by GF-120 + *B. bassiana*-based EV-HTD (66.18%), Butanone acetate + *M. anisopliae*-based EV-HTD (58.95%), and GF-120 + *M. anisopliae*-based EV-HTD (54.78%). The Butanone acetate + *B. bassiana*-based EV-HTD produced the highest mean number of spores per *B. cucurbitae* (7.80 spores/cm^2), while the other treatments produced low spore counts. Plots treated with Butanone acetate + *B. bassiana*-based EV-HTD had the highest percentage mortality of *B. cucurbitae* (81.20%). The percentage of fruit infestation varied between 9.00 and 34.00%, with the least amount of infestation seen in plots treated with *B. bassiana* + Butanone acetate. There were minimal yield losses in Butanone acetate. The Butanone acetate + *B. bassiana*-based EV-HTD showed the lowest yield losses (66.66 g/plant), while the other treatments showed high losses. Plots treated with Butanone acetate + *B. bassiana*-based EV-HTD had the highest marketable yield per plant (673.87 g/plant), while yields in control treatments were low. Plots treated with Butanone acetate + *B. bassiana*-based EV-HTD

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had the highest marketable yield (2217.85 kg/ha). Lastly, plots treated with Butanone acetate + *B. bassiana*-based EV-HTD (219.40 kg/ha) showed the lowest yield losses per hectare.

Conclusions According to the study's findings, Butanone acetate-based EV-HTD was more successful than GF-120. Furthermore, *B. bassiana* was more effective at controlling *B. cucurbitae* than *M. anisopliae*. With a maximum cost–benefit ratio of 14.99, the treatment Butanone acetate + *B. bassiana* was shown to be the most advantageous economically, suggesting its potential for use in practical pest management techniques.

Keywords *Bactrocera cucurbitae*, *Beauveria bassiana*, *Metarhizium anisopliae*, Entomo-vectored horizontal transmission devices, Bitter gourd, Pest management

Background

In many tropical and subtropical regions, bitter gourd (*Momordica charantia* L.) is an important crop appreciated for its culinary and medicinal properties (Jat et al. 2023). It belongs to the Cucurbitaceae family, along with other important commercial crops such as cucumbers, pumpkins and melons (Chomicki et al. 2020). However, bitter gourd cultivation faces numerous challenges, with insect pests being one of the most important threats to productivity and profitability (Hajong et al. 2020).

The cucurbit fruit fly, *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae), is a feared pest that attacks a variety of cucurbit crops, including bitter gourd (Gayathry and John 2022). This species is found in parts of Australia, the Pacific Islands, Asia and Africa. Larval infestations are caused by female cucurbit worms laying eggs under the surface of the host fruit (Tian et al. 2023). In addition to direct yield losses, *B. cucurbitae* infestations can have a negative economic impact on marketing and increase production expenses associated with pest control (Tian et al. 2023).

Historically, chemical pesticides have been the method of choice for controlling *B. cucurbitae* populations in bitter melon fields (Bhat et al. 2022). However, the widespread use of chemical pesticides has raised concerns about the development of resistance in pest populations, negative impacts on non-target organisms, and environmental contamination (Rather et al. 2022). Furthermore, pesticide residues in food can lead to trade restrictions in export markets and harm people's health (Leskovac et al. 2023). Therefore, there is a growing demand for alternative pest management (IPM) technologies that are environmentally sound, sustainable and comply with IPM standards (Deguine et al. 2021).

Entomopathogenic fungi (EPF) are a class of potential biocontrol agents that can be used to control a variety of pests, such as *B. cucurbitae* (Sharma et al. 2020; Paschapur et al. 2021; Irsad et al. 2023). EPF is a natural fungus that infects and kills insects, providing a safe and effective alternative to chemical pesticides (Ahmed et al. 2022; Mishra 2023). Both EPF, *Beauveria bassiana* and *Metarhizium anisopliae* have shown particular promise against fenugreek bugs in laboratory and field tests (Hintènou et al. 2023). Upon contact, these fungi penetrate the insect's cuticle, multiply within the host, and ultimately kill the insect (Mannino et al. 2019). Fundamentally, EPF can be used in IPM programs because they have little effect on non-target organisms (Skinner et al. 2014).

In addition to direct infection, EPF can also be spread horizontally within pest populations through the entomo-vectoring process (Menzler-Hokkanen and Hokkanen 2017). Infected insects can enhance the impact of biocontrol agents through horizontal transmission by acting as vectors and transmitting fungal spores to healthy humans (Fujiwara-Tsujii and Yasui 2021). Better distribution of EPF, higher infection rates within pest populations, and reduced reliance on external application techniques are just some of the benefits of this form of transmission (Gálvez et al. 2023). A variety of mechanisms, including attractants, behavior-modifying agents, and specialized delivery systems, can achieve horizontal transmission (Gálvez et al. 2023).

A notable achievement in biocontrol technology is the creation of new delivery mechanisms, such as EPF horizontal delivery devices (Opisa et al. 2019). The purpose of these devices is to attract target pests (such as *B. cucurbitae*) and facilitate the spread of EPF at the field level (Gálvez et al. 2023). These devices can effectively attract and infect insect populations by adding attractants such as the synthetic attractant Butanone acetate and the protein bait GF-120. This provides a targeted and long-lasting pest control solution (Hummadi et al. 2022).

Although EPF and horizontal transmission devices have the ability to manage *B. cucurbitae* in bitter gourd fields, their effectiveness in real field environments is unknown. Most previous studies have focused on field trials or laboratory evaluations in other farming systems, emphasizing the need for comprehensive field evaluations for bitter gourd production (Iqbal et al. 2021). Understanding the dynamics of EPF infection, entomovectoring efficiency, spore dispersal and its impact on *B. cucurbitae* populations and bitter gourd yield parameters is crucial to optimize biocontrol strategies and promote their adoption by producers. Furthermore, evaluating the practical feasibility and scalability of EPF-based pest control treatments in commercial bitter gourd production requires an economic analysis, such as a cost–benefit analysis. These assessments quantify the economic benefits of reducing dependence on chemical inputs and improving quality and yield protection, providing valuable information for practitioners, policymakers and stakeholders in sustainable agriculture.

The present research aimed to investigate the efficacy of the EV-HTD in bitter gourd fields, focusing on various parameters over time intervals. This included evaluating the impact of treatments involving *M. anisopliae* and *B. bassiana* combined with GF-120 and Butanone acetate on the percentage of entomo-vectored *B. cucurbitae*, analyzing spore count dynamics (spores/cm^2) of the EPF in *B. cucurbitae*, and assessing the mortality percentage of the pest. Additionally, the study aimed to understand the impact of entomo-vectoring EPF, along with GF-120 and Butanone acetate, on fruit infestation percentage, yield loss per plant (g), marketable fruit yield per plant (g), marketable yield per hectare (kg), and yield loss per hectare (kg) by *B. cucurbitae* over time intervals. Furthermore, a cost–benefit analysis was conducted to evaluate the effectiveness of these treatments against *B. cucurbitae* over specified time intervals.

Methods

Present research was carried out for 2 years (2021–2022) under field condition to appraise the efficiency of EV-HTD for entomo-vectoring of *B. bassiana* and *M. anisopliae* in melon fruit fly, *B. cucurbitae* in bitter gourd field and assess ultimate effect of its field implementation on infestation and yield of bitter gourd fruits.

Source and culturing of entomopathogenic fungal strains

Formulation of two EPF strains viz: *B. bassiana* (MBC 076) and *M. anisopliae* (F52) were imported from The National Center for Agriculture Research Service US department for Agriculture. These two EPF strains were cultured following the procedure described by Iqbal et al. (2020). A volume of one liter of distilled water was taken in conical flask and a 16.25 g SDAY (Sabouraud Dextrose Agar Yeast), 11.25 g agar and 1.25 g yeast was added to it. The materials of conical flask were homogenized in an electric homogenizer and then were autoclaved at 20 psi and 121 °C for 20 min. This autoclaved SDAY medium was transferred into Petri plates and was let to cool at room temperature inside the biosafety cabinet. One gram powder of each of *B. bassiana* and *M. anisopliae* formulation was weighed with electric balance and added to vortex tubes each of 15 ml volume. Then a volume of 1 ml of distilled water was added separately in each vortex tube. These vertex tubes were vortexed on shaker for 1 min to prepared homogeneous conidial suspension in water. Then these vertex tubes were covered with aluminum foil. A volume of 1 ml of conidial suspension of each EPF strain was pipetted and inoculated onto respective SDAY media plates. The inoculation procedure was completed inside the biosafety cabinet to avoid any contamination. These inoculated plates were wrapped around with parafilm and incubated at 28 ± 2 °C for 20–30 d inside the incubator till the culture was ready. This culturing of both EPF strains was done to confirm the viability of the imported formulations EPF strains. Both the formulations of *B. bassiana* (MBC 076) and *M. anisopliae* (F52) exhibited more than 90% germination and very luxurious growth of hyphae and conidia in the Petri plates.

Entomo-vectoring horizontal transmission device (EV-HTD)

In this experiment, Entomo-Vectoring Horizontal Transmission devices (EV-HTD) were developed as infection-station (Fig. 1). The EV-HTD consisted of a cylindrical barrel shaped plastic container and a wooden bar. One end of the wooden bar was used to fix deep in soil while its other end was inserted into the container. The end of the bar inserted in the container was wrapped with absorbent material (sponge). The container of EV-HTD was 20.0 cm high × 10 cm in diameter and its surfaces were engraved with evenly distributed holes each of 2.5 mm diameter for the release of smell of the sex-pheromone

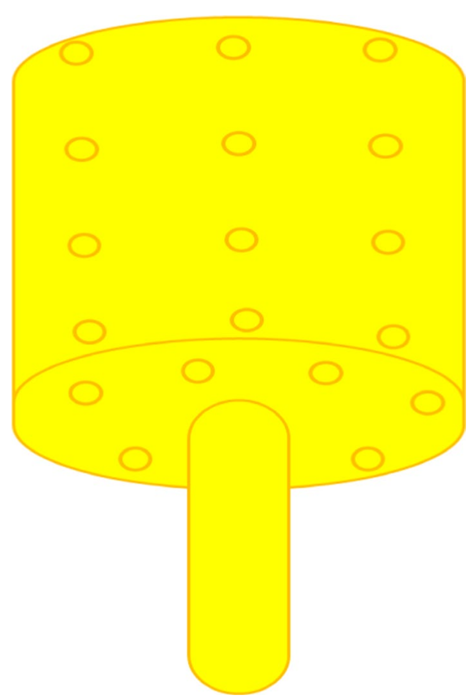

Fig. 1 Entomo-Vectoring Horizontal Transmission Device (EV-HTD)

(Butanone acetate) or proteinaceous food source (GF-120). The container as well as bar was wrapped up with yellow colored fluffy/furry/valvet tulle cloth to hold the conidia of EPF and ensure slow release of Butanone acetate or GF-120.

These EV-HTD were modified somewhat to attract and infect fruit flies with an EPF. These attractive and infective EV-HTD were set up in the cucurbit field according to the elaborated layout @ 8 EV-HTD per acre. Half of these EV-HTD were baited with Butanone acetate (cue lure pheromones) for attraction and entomo-vectoring of male fruit flies while half were baited with GF-120 (Protein Hydrolysate) attraction and entomo-vectoring of female fruit flies. The end of the bar inserted in the container and wrapped with absorbent material (sponge) of each EV-HTD was saturated soaked with either Butanone acetate or with GF-120 solution. Each of the Butanone acetate and GF-120 baited EV-HTD was implemented in the field alternatively according to the given layout. After implementation, dry powdery formulation of each of the *B. bassiana* and *M. anisopliae* were densely dusted on the yellow colored fluffy/furry/valvet tulle cloth of the EV-HTD, separately. Overall, half of the Butanone acetate baited and half of the GF-120 baited EV-HTD were dusted with *B. bassiana*, while the rest of the Butanone acetate baited and half of the GF-120 baited EV-HTD were dusted with *M. anisopliae*.

Experimental layout

Bitter gourd cultivar, Green Long, was cultivated on an acre area with a plant-to-plant distance of 45 cm on 5 March, 2021 and 28 February, 2022. The dimension of each bed was (6×2 m) with bed-to-bed distance of 1 m. All the recommended agronomic measures were practiced uniformly and no plant protection measure against fruit fly, except implementation of EV-HTD, was adopted. Experiment was repeated thrice in three different fields at least two Km away from each other including; experimental area of entomology department, vegetable area of horticulture department (both at main campus), and farmer field at Chak No. 204 RB Faisalabad. Whole of the experiment was layout according to Randomized Complete Block Design (RCBD). All around the bitter gourd field, four rows of maize crop were cultivated as border crop on 5th February of both years (2021 and 2022). The maize crop was used as fruit fly resting vegetation as well as for the installation of EV-HTD. Overall, 8 EV-HTD per acre (2 EV-HTD baited with Butanone acetate and dusted with *B. bassiana*; 2 EV-HTD baited with Butanone acetate and dusted with *M. anisopliae*; 2 EV-HTD baited with GF-120 and dusted with *B. bassiana*; 2 EV-HTD baited with GF-120 and dusted with *M. anisopliae*) were installed in the rows of border maize crop (Fig. 2).

Data collection

Net sweepings were operated in cucurbit field as well as maize at 5-days interval. The fruit fly captured in net were observed for presence of spores under microscope and taken in laboratory to assess their mortality after five days intervals up to 2 months of fruiting period. At fruiting stage, twenty fruits from each replicate were taken randomly from each lot harvesting at fortnightly interval up to 2 months of fruiting period. Totally, five pickings were done, at each locality. After each picking, fruits were separated into marketable (un-infested) and unmarketable (infested) lots and weighed, with a weighing balance, in the field. The infested fruits were counted, and the % fruit infestation was calculated. Yield data, after each picking, were also recorded. At the end of five pickings, the yield data were pooled and the % fruit infestation, number of marketable fruits/plant, yield loss/plant (yield of infested fruits/plant) and marketable yield/plant were calculated. At the end, Cost Benefit Ratio (CBR) was also be calculated.

Data analysis

The data collected on percentage fruit fly entomo-vectored, number of spores per fruit fly and percentage mortality of fruit flies, percentage fruit infestation, yield loss per plant (g), marketable fruit yield/plant (g), marketable yield/ha (kg) and yield loss/ha (kg) were analyzed, by using the following formula, using the General Linear Model (GLM) through analysis of variance (ANOVA) technique at 5% probability level with STATISTICA-10 software to compute various ANOVA parameters and means for various independent variables (treatments). Tukey's honestly significant difference test was performed to compare the mean values of significant treatments (Danho et al. 2002).

$$\%\mathrm{EVFF\ after}\ x\ \mathrm{day} = \frac{\mathrm{N_{EVFF}}}{\mathrm{N_{FF}}} \times 100$$

where EVFF = Entomo-vectored fruit flies; x = Specific time duration; N_{EVFF} = Total number of entomo-vectored fruit flies; N_{FF} = Total number of fruit flies

$$\%\mathrm{T_{SFF}}\ \mathrm{after}\ x\ \mathrm{day} = \frac{\mathrm{C_{SFF}}}{\mathrm{M_{SFF}}} \times 100$$

where T_{SFF} = Target spores per fruit fly; x = Specific time duration; C_{SFF} = Counted spores per fruit fly; M_{SFF} = Maximum spores per fruit fly

$$\%\mathrm{M_{FF}}\ \mathrm{after}\ x\ \mathrm{day} = \frac{\mathrm{N_{DFF}}}{\mathrm{N_{FF}}} \times 100$$

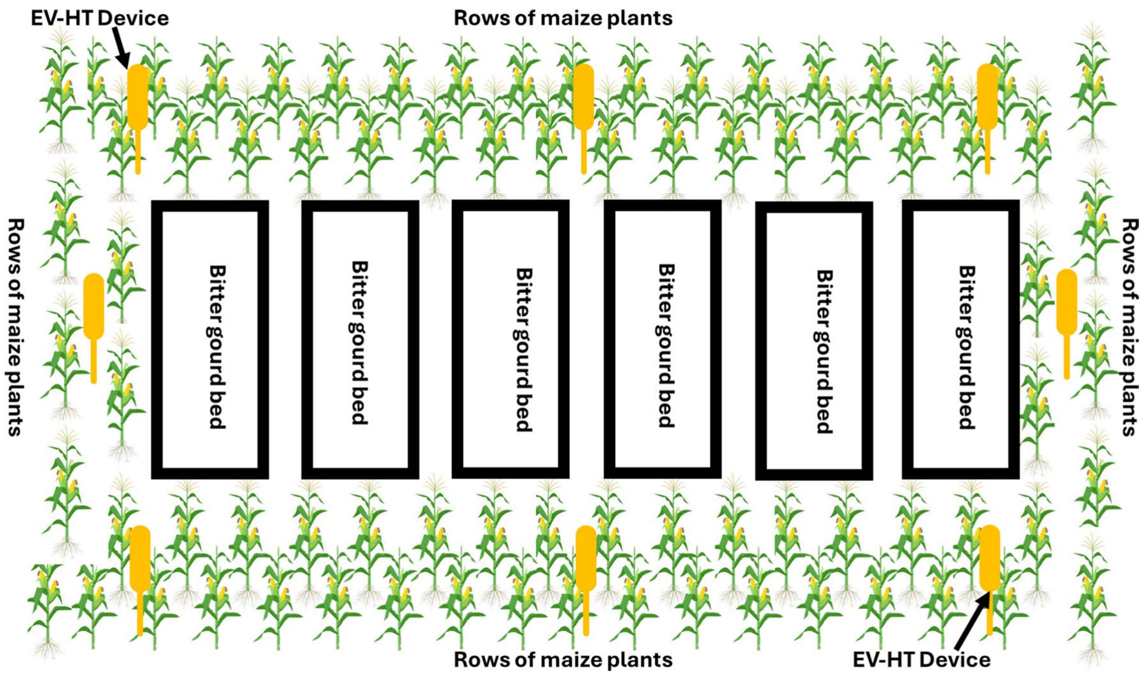

Fig. 2 The picture illustrates an experimental setup where a variety of bitter gourd was grown on a one-acre plot surrounded by four rows of maize serving as border crops. These maize rows were strategically utilized as both resting places for fruit flies and as locations for installing entomo-vectored horizontal transmission devices (EV-HTD). Each acre had 8 EV-HTD devices installed, with specific treatments: 2 devices baited with Butanone acetate and dusted with *Beauveria bassiana*, 2 with Butanone acetate and dusted with *Metarhizium anisopliae*, 2 with GF-120 and dusted with *Beauveria bassiana*, and 2 with GF-120 and dusted with *Metarhizium anisopliae*

where M_{FF} = Mortality of fruit flies; x = Specific time duration; N_{DFF} = Number of dead fruit flies; N_{FF} = Total number of fruit flies

$$YL_P \text{ after } x \text{ day} = FW_I - FW_F$$

where YL_P = Yield loss per plant; x = Specific time duration; FE_I = Initial fruit weight; FW_F = Final fruit weight

$$FY_{MP} \text{ after } x \text{ day} = FW_F$$

where FY_{MP} = Marketable fruit yield per plant; x = Specific time duration; FW_F = Final fruit weight

$$MY_H \text{ after } x \text{ day} = \frac{FY_{MP} \text{ after } x \text{ day } \times N_{PH}}{1000}$$

where MY_H = Marketable yield per hectare; x = Specific time duration; FY_{MP} = Marketable fruit yield per plant; N_{PH} = Number of plants per hectare

$$YL_H \text{ after } x \text{ day} = \frac{YL_P \text{ after } x \text{ day } \times N_{PH}}{1000}$$

where YL_H = Yield loss per hectare; x = Specific time duration; YL_P = Yield loss per plant; N_{PH} = Number of plants per hectare.

Results

Effect of *Metarhizium anisopliae* and *Beauveria bassiana* treatments combined with GF-120 and Butanone acetate on the percentage of entomo-vectored *Bactrocera cucurbitae* over time intervals

After 5-day time interval, maximum percentage of entomo-vectored *B. cucurbitae* was observed in plots treated with Butanone acetate + *B. bassiana* based EV-HTD (62.96%), followed by plots treated with GF-120 + *B. bassiana* based EV-HTD (59.74%), Butanone acetate + *M. anisopliae* based EV-HTD (56.38%) and GF-120 + *M. anisopliae* based EV-HTD (51.09%). Furthermore, Butanone acetate + *B. bassiana* based EV-HTD and GF-120 + *B. bassiana* based EV-HTD were statistically at par with each other. While negligible percentage of entomo-vectored *B. cucurbitae* was recorded in

Table 1 Effect of *Metarhizium anisopliae* and *Beauveria bassiana* treatments combined with GF-120 and Butanone acetate on the percentage of entomo-vectored *Bactrocera cucurbitae* over time intervals

Treatments	Time intervals												
	5-day	10-day	15-day	20-day	25-day	30-day	35-day	40-day	45-day	50-day	55-day	60-day	65-day
GF-120 + *Metarhizium anisopliae* based HTD	51.09 d	42.72 d	52.25 c	55.27 c	60.98 d	58.42 c	61.99 d	44.20 d	57.94 d	51.95 d	57.25 d	62.51 d	59.63 d
GF-120 + *Beauveria bassiana* based HTD	59.74 ab	53.91 b	58.92 b	63.66 b	70.69 b	65.98 b	72.57 b	55.12 b	71.01 b	65.48 b	69.36 b	72.16 b	75.22 b
GF-120 + Control based HTD	4.87 e	3.90 e	4.68 d	4.02 d	4.55 e	5.38 d	4.38 e	4.51 e	5.80 e	6.21 e	6.13 e	4.41 e	3.68 e
Butanone acetate + *Metarhizium anisopliae* based HTD	56.38 bc	46.14 c	54.16 c	58.44 c	64.74 c	64.85 b	65.79 c	48.98 c	63.73 c	57.86 c	62.76 c	66.94 c	66.00 c
Butanone acetate + *Beauveria bassiana* based HTD	62.96 a	57.79 a	61.87 a	67.16 a	76.44 a	71.15 a	76.18 a	62.30 a	77.52 a	70.81 a	74.15 a	76.05 a	78.79 a
Butanone acetate + Control based HTD	6.06 e	4.72 e	5.60 d	5.28 d	5.06 e	6.28 d	5.55 e	6.93 e	6.21 e	7.04 e	6.23 e	6.21 e	4.47 e
LSD	3.52	2.81	2.92	3.47	2.83	3.53	3.49	3.59	3.79	3.65	4.06	3.50	2.96

Means with the same lowercase letters are not significantly different at $\alpha = 0.05$ (LSD test)

control treatment in Butanone acetate + Control based EV-HTD (6.06%) and GF-120 + Control based EV-HTD (4.87%) and both were statistically at par with each other (Table 1).

Following a 10-day time interval, maximum percentage of entomo-vectored *B. cucurbitae* was observed in plots treated with Butanone acetate + *B. bassiana* based EV-HTD (57.79%), followed by plots treated with GF-120 + *B. bassiana* based EV-HTD (53.91%), Butanone acetate + *M. anisopliae* based EV-HTD (46.14%) and GF-120 + *M. anisopliae* based EV-HTD (42.72%), respectively. While negligible percentage of entomo-vectored *B. cucurbitae* was recorded in control treatment in GF-120 + Control based EV-HTD (3.90%) and in Butanone acetate + Control based EV-HTD (4.72%) and both were statistically at par with each other (Table 1).

At 15-day time interval, maximum percentage of entomo-vectored *B. cucurbitae* was observed in plots treated with Butanone acetate + *B. bassiana* based EV-HTD (61.87%), followed by plots treated with GF-120 + *B. bassiana* based EV-HTD (58.92%), Butanone acetate + *M. anisopliae* based EV-HTD (54.16%) and GF-120 + *M. anisopliae* based EV-HTD (52.25%), respectively. Furthermore, Butanone acetate + *M. anisopliae* based EV-EV-HTD and GF-120 + *M. anisopliae* based EV-EV-HTD were statistically at par with each other. While negligible percentage of entomo-vectored *B. cucurbitae* was recorded in control treatment in GF-120 + Control based EV-EV-HTD (4.68%) and in Butanone acetate + Control based EV-HTD (5.60%) and both were statistically at par with each other (Table 1).

After 20-day time interval, maximum percentage of entomo-vectored *B. cucurbitae* was observed in plots treated with Butanone acetate + *B. bassiana* based EV-HTD (67.16%), followed by plots treated with GF-120 + *B. bassiana* based EV-HTD (63.66%), Butanone acetate + *M. anisopliae* based EV-HTD (58.44%) and GF-120 + *M. anisopliae* based EV-HTD (55.27%), respectively. Furthermore, Butanone acetate + *M. anisopliae* based EV-HTD and GF-120 + *M. anisopliae* based EV-HTD were statistically at par with each other. While negligible percentage of entomo-vectored *B. cucurbitae* was recorded in control treatment in GF-120 + Control based EV-HTD (4.02%) and in Butanone acetate + Control based EV-HTD (5.28%) and both were statistically at par with each other (Table 1).

Following a 25-day time interval, maximum percentage of entomo-vectored *B. cucurbitae* was observed in plots treated with Butanone acetate + *B. bassiana* based EV-HTD (76.44%), followed by plots treated with GF-120 + *B. bassiana* based EV-HTD (70.69%), Butanone acetate + *M. anisopliae* based EV-HTD (64.74%) and GF-120 + *M. anisopliae* based EV-HTD (60.98%), respectively. While negligible percentage of entomo-vectored *B. cucurbitae* was recorded in control treatment in GF-120 + Control based EV-HTD (4.55%) and in Butanone acetate + Control based EV-HTD (5.06%) and both were statistically at par with each other (Table 1).

At 30-day time interval, maximum percentage of entomo-vectored *B. cucurbitae* was observed in plots treated with Butanone acetate + *B. bassiana* based EV-HTD (71.15%), followed by plots treated with GF-120 + *B. bassiana* based EV-HTD (65.98%), Butanone acetate + *M. anisopliae* based EV-HTD (64.85%) and GF-120 + *M. anisopliae* based EV-HTD (58.42%), respectively. Furthermore, GF-120 + *B. bassiana* based EV-HTD and Butanone acetate + *M. anisopliae* based EV-HTD were statistically at par with each other. While negligible percentage of entomo-vectored *B. cucurbitae* was recorded in control treatment in GF-120 + Control based EV-HTD (5.38%) and in Butanone acetate + Control based EV-HTD (6.28%) and both were statistically at par with each other (Table 1).

After 35-day time interval, maximum percentage of entomo-vectored *B. cucurbitae* was observed in plots treated with Butanone acetate + *B. bassiana* based EV-HTD (76.18%), followed by plots treated with GF-120 + *B. bassiana* based EV-HTD (72.57%), Butanone acetate + *M. anisopliae* based EV-HTD (65.79%) and GF-120 + *M. anisopliae* based EV-HTD (61.99%), respectively. While negligible percentage of entomo-vectored *B. cucurbitae* was recorded in control treatment in GF-120 + Control based EV-HTD (4.38%) and in Butanone acetate + Control based EV-HTD (5.55%) and both were statistically at par with each other (Table 1).

Following a 40-day time interval, maximum percentage of entomo-vectored *B. cucurbitae* was observed in plots treated with Butanone acetate + *B. bassiana* based EV-HTD (62.30%), followed by plots treated with GF-120 + *B. bassiana* based EV-HTD (55.12%), Butanone acetate + *M. anisopliae* based EV-HTD (48.98%) and GF-120 + *M. anisopliae* based EV-HTD (44.20%), respectively. While negligible percentage of entomo-vectored *B. cucurbitae* was recorded in control treatment in GF-120 + Control based EV-HTD (4.51%) and in Butanone acetate + Control based EV-HTD (6.93%) and both were statistically at par with each other (Table 1).

At 45-day time interval, maximum percentage of entomo-vectored *B. cucurbitae* was observed in plots treated with Butanone acetate + *B. bassiana* based EV-HTD (77.52%), followed by plots treated with GF-120 + *B. bassiana* based EV-HTD (71.01%), Butanone acetate + *M. anisopliae* based EV-HTD (63.73%) and GF-120 + *M. anisopliae* based EV-HTD (57.94%), respectively. While negligible percentage of entomo-vectored *B. cucurbitae* was recorded in control treatment in GF-120 + Control based EV-HTD (5.80%) and in Butanone acetate + Control based EV-HTD (6.21%) and both were statistically at par with each other (Table 1).

After 50-day time interval, maximum percentage of entomo-vectored *B. cucurbitae* was observed in plots treated with Butanone acetate + *B. bassiana* based EV-HTD (70.81%), followed by plots treated with GF-120 + *B. bassiana* based EV-HTD (65.48%), Butanone acetate + *M. anisopliae* based EV-HTD (57.86%) and GF-120 + *M. anisopliae* based EV-HTD (51.95%), respectively. While negligible percentage of entomo-vectored *B. cucurbitae* was recorded in control treatment in GF-120 + Control based EV-HTD (6.21%) and in Butanone acetate + Control based EV-HTD (7.04%) and both were statistically at par with each other (Table 1).

Following a 55-day time interval, maximum percentage of entomo-vectored *B. cucurbitae* was observed in plots treated with Butanone acetate + *B. bassiana* based EV-HTD (74.15%), followed by plots treated with GF-120 + *B. bassiana* based EV-HTD (69.36%), Butanone acetate + *M. anisopliae* based EV-HTD (62.76%) and GF-120 + *M. anisopliae* based EV-HTD (57.25%), respectively. While negligible percentage of entomo-vectored *B. cucurbitae* was recorded in control treatment in GF-120 + Control based EV-HTD (6.13%) and in Butanone acetate + Control based EV-HTD (6.23%) and both were statistically at par with each other (Table 1).

At 60-day time interval, maximum percentage of entomo-vectored *B. cucurbitae* was observed in plots treated with Butanone acetate + *B. bassiana* based EV-HTD (76.05%), followed by plots treated with GF-120 + *B. bassiana* based EV-HTD (72.16%), Butanone acetate + *M. anisopliae* based EV-HTD (66.94%) and GF-120 + *M. anisopliae* based EV-HTD (62.51%), respectively. While negligible percentage of entomo-vectored *B. cucurbitae* was recorded in control treatment in GF-120 + Control based EV-HTD (4.41%) and in Butanone acetate + Control based EV-HTD (6.21%) and both were statistically at par with each other (Table 1).

After 65-day time interval, maximum percentage of entomo-vectored *B. cucurbitae* was observed in plots treated with Butanone acetate + *B. bassiana* based EV-HTD (78.79%), followed by plots treated with GF-120 + *B. bassiana* based EV-HTD (75.22%), Butanone acetate + *M. anisopliae* based EV-HTD (66.00%) and GF-120 + *M. anisopliae* based EV-HTD (59.63%), respectively. While negligible percentage of entomo-vectored *B. cucurbitae* was recorded in control treatment in GF-120 + Control based EV-HTD (3.68%) and in Butanone acetate + Control based EV-HTD (4.47%) and both were statistically at par with each other (Table 1).

Effect of various treatments, combined with GF-120 and Butanone acetate, on spore count dynamics (spores/cm^2) of *Metarhizium anisopliae* and *Beauveria bassiana* in *Bactrocera cucurbitae* over time intervals

After 5-day time interval, the number of spores per *B. cucurbitae* revealed that maximum spores were recorded in Butanone acetate + *B. bassiana* based EV-HTD (8.20 spores/cm^2) and GF-120 + *B. bassiana* based EV-HTD (7.00 spores/cm^2) and both were statistically at par with each other but significantly different with Butanone acetate + *M. anisopliae* based EV-HTD (6.40 spores/cm^2) and GF-120 + *M. anisopliae* based EV-HTD (5.60 spores/cm^2). While minimum number of spores was observed in control treatment GF-120 + Control based EV-HTD (1.40 spores/cm^2) and Butanone acetate + Control based EV-HTD (1.60 spores/cm^2) (Table 2).

Following a 10-day time interval, the number of spores per *B. cucurbitae* revealed that maximum spores were recorded in Butanone acetate + *B. bassiana* based EV-HTD (8.00 spores/cm^2) and GF-120 + *B. bassiana* based EV-HTD (7.00 spores/cm^2) and both were statistically at par with each other but significantly different with Butanone acetate + *M. anisopliae* based EV-HTD (6.20 spores/cm^2) and GF-120 + *M. anisopliae* based EV-HTD (5.40 spores/cm^2). While minimum number of spores was observed in control treatment GF-120 + Control based EV-HTD (1.00 spores/cm^2) and Butanone acetate + Control based EV-HTD (1.40 spores/cm^2) (Table 2).

At 15-day time interval, the number of spores per *B. cucurbitae* revealed that maximum spores were recorded in Butanone acetate + *B. bassiana* based EV-HTD (7.20 spores/cm^2) and GF-120 + *B. bassiana* based EV-HTD (7.00 spores/cm^2) and both were statistically at par with each other but significantly different with Butanone acetate + *M. anisopliae* based EV-HTD (5.80 spores/cm^2) and GF-120 + *M. anisopliae* based EV-HTD (5.60 spores/cm^2). While minimum number of spores was observed in control treatment GF-120 + Control based EV-HTD (1.00 spores/cm^2) and Butanone acetate + Control based EV-HTD (1.20 spores/cm^2) (Table 2).

After 20-day time interval, the number of spores per *B. cucurbitae* revealed that maximum spores were recorded in Butanone acetate + *B. bassiana* based EV-HTD (8.00 spores/cm^2), followed by plots treated with GF-120 + *B. bassiana* based EV-HTD (6.80 spores/cm^2) and Butanone acetate + *M. anisopliae* based EV-HTD (6.40 spores/cm^2), respectively. Furthermore, GF-120 + *B. bassiana* based EV-HTD and Butanone acetate + *M. anisopliae* based EV-HTD were statistically at par with each other but significantly different with GF-120 + *M. anisopliae* based EV-HTD (5.40 spores/cm^2). While minimum number of spores was observed in control treatment

Table 2 Effect of various treatments, combined with GF-120 and Butanone acetate, on spore count dynamics (spores/cm^2) of *Metarhizium anisopliae* and *Beauveria bassiana* in *Bactrocera cucurbitae* over time intervals

Treatments	Time intervals												
	5-day	10-day	15-day	20-day	25-day	30-day	35-day	40-day	45-day	50-day	55-day	60-day	65-day
GF-120 + *Metarhizium anisopliae* based HTD	5.60 c	5.40 c	5.60 c	5.40 c	5.20 c	5.00 c	5.20 c	4.80 c	5.20 c	5.20 c	5.00 bc	5.20 b	5.60 c
GF-120 + *Beauveria bassiana* based HTD	7.00 ab	7.00 ab	7.00 ab	6.80 b	7.20 ab	6.40 b	6.80 b	6.60 ab	7.20 ab	6.60 b	6.80 ab	6.60 ab	6.80 b
GF-120 + Control based HTD	1.40 d	1.00 d	1.00 d	0.80 d	1.00 d	1.00 d	1.00 d	1.00 d	1.20 d	1.20 d	1.20 c	1.00 c	1.00 d
Butanone acetate + *Metarhizium anisopliae* based HTD	6.40 bc	6.20 b	5.80 bc	6.40 b	6.20 bc	5.80 bc	5.80 c	6.00 bc	5.80 bc	5.80 bc	5.60 b	5.60 b	5.80 bc
Butanone acetate + *Beauveria bassiana* based HTD	8.20 a	8.00 a	7.20 a	8.00 a	8.00 a	7.80 a	7.80 a	7.80 a	8.00 a	7.80 a	7.20 a	7.40 a	8.20 a
Butanone acetate + Control based HTD	1.60 d	1.40 d	1.20 d	1.40 d	1.20 d	1.20 d	1.40 d	1.60 d	1.60 d	1.40 d	1.40 c	1.20 c	1.20 d
LSD	1.34	1.02	1.36	0.90	1.24	0.98	1.12	1.33	1.54	1.26	0.99	1.40	1.16

Means with the same lowercase letters are not significantly different at $\alpha = 0.05$ (LSD test)

GF-120 + Control based EV-HTD (0.80 spores/cm^2) and Butanone acetate + Control based EV-HTD (1.40 spores/cm^2) (Table 2).

Following a 25-day time interval, the number of spores per *B. cucurbitae* revealed that maximum spores were recorded in Butanone acetate + *B. bassiana* based EV-HTD (8.00 spores/cm^2) and GF-120 + *B. bassiana* based EV-HTD (7.20 spores/cm^2) and both were statistically at par with each other but significantly different with Butanone acetate + *M. anisopliae* based EV-HTD (6.20 spores/cm^2) and GF-120 + *M. anisopliae* based EV-HTD (5.20 spores/cm^2). While minimum number of spores was observed in control treatment GF-120 + Control based EV-HTD (1.00 spores/cm^2) and Butanone acetate + Control based EV-HTD (1.20 spores/cm^2) (Table 2).

At 30-day time interval, the number of spores per *B. cucurbitae* revealed that maximum spores were recorded in Butanone acetate + *B. bassiana* based EV-HTD (7.80 spores/cm^2), followed by plots treated with GF-120 + *B. bassiana* based EV-HTD (6.40 spores/cm^2) and Butanone acetate + *M. anisopliae* based EV-HTD (5.80 spores/cm^2), respectively. Furthermore, GF-120 + *B. bassiana* based EV-HTD and Butanone acetate + *M. anisopliae* based EV-HTD were statistically at par with each other but significantly different with GF-120 + *M. anisopliae* based EV-HTD (5.00 spores/cm^2). While minimum number of spores was observed in control treatment GF-120 + Control based EV-HTD (1.00 spores/cm^2) and Butanone acetate + Control based EV-HTD (1.20 spores/cm^2) (Table 2).

After 35-day time interval, the number of spores per *B. cucurbitae* revealed that maximum spores were recorded in Butanone acetate + *B. bassiana* based EV-HTD (7.80 spores/cm^2), followed by plots treated with GF-120 + *B. bassiana* based EV-HTD (6.80 spores/cm^2), Butanone acetate + *M. anisopliae* based EV-HTD (5.80 spores/cm^2) and GF-120 + *M. anisopliae* based EV-HTD (5.00 spores/cm^2), respectively. Furthermore, GF-120 + *B. bassiana* based EV-HTD and Butanone acetate + *B. bassiana* based EV-HTD were statistically at par with each. While minimum number of spores was observed in control treatment GF-120 + Control based EV-HTD (1.00 spores/cm^2) and Butanone acetate + Control based EV-HTD (1.40 spores/cm^2) (Table 2).

Following a 40-day time interval, the number of spores per *B. cucurbitae* revealed that maximum spores were recorded in Butanone acetate + *B. bassiana* based EV-HTD (7.80 spores/cm^2) and GF-120 + *B. bassiana* based EV-HTD (6.60 spores/cm^2) and both were statistically at par with each other but significantly different with GF-120 + *M. anisopliae* based EV-HTD (6.00 spores/cm^2) and Butanone acetate + *M. anisopliae* based EV-HTD (4.80 spores/cm^2). While minimum number of spores was observed in control treatment GF-120 + Control based EV-HTD (1.00 spores/cm^2) and Butanone acetate + Control based EV-HTD (1.60 spores/cm^2) (Table 2).

At 45-day time interval, the number of spores per *B. cucurbitae* revealed that maximum spores were recorded in Butanone acetate + *B. bassiana* based EV-HTD (8.00 spores/cm^2) and GF-120 + *B. bassiana* based EV-HTD (7.20 spores/cm^2) and both were statistically at par with each other but significantly different with GF-120 + *M. anisopliae* based EV-HTD (5.80 spores/cm^2) and Butanone acetate + *M. anisopliae* based EV-HTD (5.20 spores/cm^2). While minimum number of spores was observed in control treatment GF-120 + Control based EV-HTD (1.20 spores/cm^2) and Butanone acetate + Control based EV-HTD (1.60 spores/cm^2) (Table 2).

After 50-day time interval, the number of spores per *B. cucurbitae* revealed that maximum spores were recorded in Butanone acetate + *B. bassiana* based EV-HTD (7.80 spores/cm^2), followed by plots treated with GF-120 + *B. bassiana* based EV-HTD (6.60 spores/cm^2), Butanone acetate + *M. anisopliae* based EV-HTD (5.80 spores/cm^2) and GF-120 + *M. anisopliae* based EV-HTD (5.20 spores/cm^2), respectively. Furthermore, the plots treated with GF-120 + *B. bassiana* based EV-HTD and Butanone acetate + *M. anisopliae* based EV-HTD were statistically at par with each. While minimum number of spores was observed in control treatment GF-120 + Control based EV-HTD (1.20 spores/cm^2) and Butanone acetate + Control based EV-HTD (1.40 spores/cm^2) (Table 2).

Following a 55-day time interval, the number of spores per *B. cucurbitae* revealed that maximum spores were recorded in Butanone acetate + *B. bassiana* based EV-HTD (7.20 spores/cm^2), followed by plots treated with GF-120 + *B. bassiana* based EV-HTD (6.80 spores/cm^2), Butanone acetate + *M. anisopliae* based EV-HTD (5.60 spores/cm^2) and GF-120 + *M. anisopliae* based EV-HTD (5.00 spores/cm^2), respectively. Furthermore, the plots treated with Butanone acetate + *B. bassiana* based EV-HTD and Butanone acetate + *B. bassiana* based EV-HTD were statistically at par with each and similarly plots treated with Butanone acetate + *M. anisopliae* based EV-HTD and GF-120 + *M. anisopliae* based EV-HTD were also non-significant with each other. While minimum number of spores was observed in control treatment GF-120 + Control based EV-HTD (1.20 spores/cm^2) and Butanone acetate + Control based EV-HTD (1.40 spores/cm^2) (Table 2).

At 60-day time interval, the number of spores per *B. cucurbitae* revealed that maximum spores were recorded in Butanone acetate + *B. bassiana* based EV-HTD (7.40 spores/cm^2), followed by plots treated with GF-120 + *B. bassiana* based EV-HTD (6.60 spores/cm^2), Butanone

Table 3 Effect of *Metarhizium anisopliae* and *Beauveria bassiana* treatments combined with GF-120 and Butanone acetate on mortality percentage of *Bactrocera cucurbitae* over time intervals

Treatments	Time intervals												
	5-day	**10-day**	**15-day**	**20-day**	**25-day**	**30-day**	**35-day**	**40-day**	**45-day**	**50-day**	**55-day**	**60-day**	**65-day**
GF-120 + *Metarhizium anisopliae* based HTD	65.02 c	57.14 c	56.82 c	62.91 d	66.61 d	65.36 c	69.94 d	56.22 c	62.37 c	62.18 c	64.97 d	71.37 d	72.69 c
GF-120 + *Beauveria bassiana* based HTD	72.14 b	64.17 b	63.52 b	72.63 b	76.87 b	72.93 b	84.32 b	64.83 b	82.70 a	71.18 b	76.53 b	83.45 b	89.81 a
GF-120 + Control based HTD	0.00 d	0.00 d	0.00 d	0.00 e	0.00 e	0.00 d	0.00 e	0.00 d	0.00 d	0.00 d	0.00 e	0.00 e	0.00 d
Butanone acetate + *Metarhizium anisopliae* based HTD	71.64 b	61.11 b	59.57 c	68.46 c	72.15 c	70.53 b	72.03 c	62.06 bc	68.59 b	67.88 b	70.42 c	76.14 c	77.91 b
Butanone acetate + *Beauveria bassiana* based HTD	76.21 a	68.67 a	67.91 a	76.12 a	80.99 a	79.51 a	87.36 a	70.77 a	87.02 a	78.86 a	87.69 a	90.23 a	92.56 a
Butanone acetate + Control based HTD	0.00 d	0.00 d	0.00 d	0.00 e	0.00 e	0.00 d	0.00 e	0.00 d	0.00 d	0.00 d	0.00 e	0.00 e	0.00 d
LSD	3.03	3.60	4.08	3.28	3.43	3.83	2.82	4.49	5.79	3.79	3.07	3.42	4.04

Means with the same lowercase letters are not significantly different at $\alpha = 0.05$ (LSD test)

acetate + *M. anisopliae* based EV-HTD (5.60 spores/cm^2) and GF-120 + *M. anisopliae* based EV-HTD (5.20 spores/cm^2), respectively. Furthermore, the plots treated with Butanone acetate + *B. bassiana* based EV-HTD and Butanone acetate + *B. bassiana* based EV-HTD were statistically at par with each and similarly plots treated with Butanone acetate + *M. anisopliae* based EV-HTD and GF-120 + *M. anisopliae* based EV-HTD were also non-significant with each other. While minimum number of spores was observed in control treatment GF-120 + Control based EV-HTD (1.00 spores/cm^2) and Butanone acetate + Control based EV-HTD (1.20 spores/cm^2) (Table 2).

After 65-day time interval, the number of spores per *B. cucurbitae* revealed that maximum spores were recorded in Butanone acetate + *B. bassiana* based EV-HTD (8.20 spores/cm^2), followed by plots treated with GF-120 + *B. bassiana* based EV-HTD (6.80 spores/cm^2), Butanone acetate + *M. anisopliae* based EV-HTD (5.80 spores/cm^2) and GF-120 + *M. anisopliae* based EV-HTD (5.600 spores/cm^2), respectively. Furthermore, the plots treated with GF-120 + *B. bassiana* based EV-HTD and Butanone acetate + *M. anisopliae* based EV-HTD were statistically at par with each. While minimum number of spores was observed in control treatment GF-120 + Control based EV-HTD (1.00 spores/cm^2) and Butanone acetate + Control based EV-HTD (1.20 spores/cm^2) (Table 2).

Effect of *Metarhizium anisopliae* and *Beauveria bassiana* treatments combined with GF-120 and Butanone acetate on mortality percentage of *Bactrocera cucurbitae* over time intervals

After 5-day time interval, percentage mortality of *B. cucurbitae* revealed that maximum mortality was observed in Butanone acetate + *B. bassiana* based EV-HTD (76.21%), followed by GF-120 + *B. bassiana* based EV-HTD (72.14%) and Butanone acetate + *M. anisopliae* based EV-HTD (71.64%) treated plots and these treatments were statistically at par with each other and significantly different with GF-120 + *M. anisopliae* based EV-HTD (65.02%) mortality (Table 3).

Following a 10-day time interval, percentage mortality of *B. cucurbitae* revealed that maximum mortality was observed in Butanone acetate + *B. bassiana* based EV-HTD (68.67%), followed by GF-120 + *B. bassiana* based EV-HTD (64.17%) and Butanone acetate + *M. anisopliae* based EV-HTD (61.11%) treated plots and these treatments were statistically at par with each other and significantly different with GF-120 + *M. anisopliae* based EV-HTD (57.14%) mortality (Table 3).

At 15-day time interval, percentage mortality of *B. cucurbitae* revealed that maximum mortality was observed in Butanone acetate + *B. bassiana* based EV-HTD (67.91%), followed by GF-120 + *B. bassiana* based EV-HTD (63.52%), Butanone acetate + *M. anisopliae* based EV-HTD (59.57%) and GF-120 + *M. anisopliae* based EV-HTD (56.82%) treated plots, respectively. Furthermore, Butanone acetate + *M. anisopliae* based EV-HTD and GF-120 + *M. anisopliae* based EV-HTD were statistically at par with each other (Table 3).

After 20-day time interval, percentage mortality of *B. cucurbitae* revealed that maximum mortality was observed in Butanone acetate + *B. bassiana* based EV-HTD (76.12%) treated plot, followed by GF-120 + *B. bassiana* based EV-HTD (72.63%), Butanone acetate + *M. anisopliae* based EV-HTD (68.46%) and GF-120 + *M. anisopliae* based EV-HTD (62.91%) treated plots, respectively (Table 3).

Following a 25-day time interval, percentage mortality of *B. cucurbitae* revealed that maximum mortality was observed in Butanone acetate + *B. bassiana* based EV-HTD (80.99%), followed by GF-120 + *B. bassiana* based EV-HTD (76.87%), Butanone acetate + *M. anisopliae* based EV-HTD (72.15%) and GF-120 + *M. anisopliae* based EV-HTD (66.61%) treated plots, respectively (Table 3).

At 30-day time interval, percentage mortality of *B. cucurbitae* revealed that maximum mortality was observed in Butanone acetate + *B. bassiana* based EV-HTD (79.51%) treated plot, followed by GF-120 + *B. bassiana* based EV-HTD (72.93%), Butanone acetate + *M. anisopliae* based EV-HTD (70.53%) and GF-120 + *M. anisopliae* based EV-HTD (65.36%) treated plots, respectively. Furthermore, GF-120 + *B. bassiana* based EV-HTD and Butanone acetate + *M. anisopliae* based EV-HTD were statistically at par with each other (Table 3).

After 35-day time interval, percentage mortality of *B. cucurbitae* revealed that maximum mortality was observed in Butanone acetate + *B. bassiana* based EV-HTD (87.46%) treated plot, followed by GF-120 + *B. bassiana* based EV-HTD (84.72%), Butanone acetate + *M. anisopliae* based EV-HTD (72.03%) and GF-120 + *M. anisopliae* based EV-HTD (69.94%) treated plots, respectively (Table 3).

Following a 40-day time interval, percentage mortality of *B. cucurbitae* revealed that maximum mortality was observed in Butanone acetate + *B. bassiana* based EV-HTD (70.77%) treated plot, followed by GF-120 + *B. bassiana* based EV-HTD (64.83%), Butanone acetate + *M. anisopliae* based EV-HTD (62.06%) and GF-120 + *M. anisopliae* based EV-HTD (56.22%) treated plots, respectively. Furthermore, GF-120 + *B. bassiana* based EV-HTD and Butanone acetate + *M. anisopliae* based EV-HTD were statistically at par with each other (Table 3).

At 45-day time interval, percentage mortality of *B. cucurbitae* revealed that maximum mortality was observed in plots treated with Butanone acetate + *B. bassiana* based EV-HTD (87.027%) and GF-120 + *B. bassiana* based EV-HTD (82.70%) and both were statistically at par with each other but significantly different with, Butanone acetate + *M. anisopliae* based EV-HTD (68.59%) and GF-120 + *M. anisopliae* based EV-HTD (62.37%) treated plots, respectively (Table 3).

After 50-day time interval, percentage mortality of *B. cucurbitae* revealed that maximum mortality was observed in Butanone acetate + *B. bassiana* based EV-HTD (78.86%) treated plot, followed by GF-120 + *B. bassiana* based EV-HTD (71.18%), Butanone acetate + *M. anisopliae* based EV-HTD (67.88%) and GF-120 + *M. anisopliae* based EV-HTD (62.18%) treated plots, respectively. Furthermore, GF-120 + *B. bassiana* based EV-HTD and Butanone acetate + *M. anisopliae* based EV-HTD were statistically at par with each other (Table 3).

Following a 55-day time interval, percentage mortality of *B. cucurbitae* revealed that maximum mortality was observed in Butanone acetate + *B. bassiana* based EV-HTD (87.69%) treated plot, followed by GF-120 + *B. bassiana* based EV-HTD (76.53%), Butanone acetate + *M. anisopliae* based EV-HTD (70.42%) and GF-120 + *M. anisopliae* based EV-HTD (64.97%) treated plots, respectively (Table 3).

At 60-day time interval, percentage mortality of *B. cucurbitae* revealed that maximum mortality was observed in Butanone acetate + *B. bassiana* based EV-HTD (90.23%) treated plot, followed by GF-120 + *B. bassiana* based EV-HTD (83.45%), Butanone acetate + *M. anisopliae* based EV-HTD (76.14%) and GF-120 + *M. anisopliae* based EV-HTD (71.37%) treated plots, respectively (Table 3).

After 65-day time interval, regarding percentage mortality of *B. cucurbitae* revealed that maximum mortality was observed in Butanone acetate + *B. bassiana* based EV-HTD (92.56%) and GF-120 + *B. bassiana* based EV-HTD (89.81%) treated plots and these treatments were statistically at par with each other and significantly different with plots treated with Butanone acetate + *M. anisopliae* based EV-HTD (77.91%) and GF-120 + *M. anisopliae* based EV-HTD (72.69%) (Table 3).

Impact of entomo-vectoring *Metarhizium anisopliae* and *Beauveria bassiana*, combined with GF-120 and Butanone acetate, on fruit infestation percentage by *Bactrocera cucurbitae* over time intervals

After 15-day time interval, percentage fruit infestation by *B. cucurbitae* was ranged from 35.00 to 11.00%. Maximum percentage of fruit infestation was observed in control treatment in GF-120 + Control based EV-HTD (35.00%) and in Butanone acetate + Control based EV-HTD (33.00%) and both were statistically at par with each other. While minimum fruit infestation was recorded in plots treated with Butanone acetate + *B. bassiana* based EV-HTD (11.00%), followed by plots treated with GF-120 + *B. bassiana* based EV-HTD (14.00%), Butanone acetate + *M. anisopliae* based EV-HTD (17.00%) and GF-120 + *M. anisopliae* based EV-HTD (19.00%), respectively. Furthermore, Butanone acetate + *M. anisopliae* based EV-HTD, GF-120 + *B. bassiana* based EV-HTD and GF-120 + *M. anisopliae* based EV-HTD were statistically at par with each other (Table 4).

Following a 30-day time interval, percentage fruit infestation by *B. cucurbitae* was ranged from 33.00 to 8.00%. Maximum percentage of fruit infestation was observed in control treatment in GF-120 + Control based EV-HTD (33.00%) and in Butanone acetate + Control based EV-HTD (32.00%) and both were statistically at par with each other. While minimum fruit infestation was recorded in plots treated with Butanone acetate + *B. bassiana* based EV-HTD (8.00%) followed by plots treated with GF-120 + *B. bassiana* based EV-HTD (11.00%), Butanone acetate + *M.*

Table 4 Impact of entomo-vectoring *Metarhizium anisopliae* and *Beauveria bassiana*, combined with GF-120 and Butanone acetate, on fruit infestation percentage by *Bactrocera cucurbitae* over time intervals

Treatments	Time intervals			
	15-day	30-day	45-day	60-day
GF-120 + *Metarhizium anisopliae* based HTD	19.00 b	15.00 b	18.00 b	14.00 b
GF-120 + *Beauveria bassiana* based HTD	14.00 bc	11.00 bc	13.00 cd	9.00 c
GF-120 + Control based HTD	35.00 a	33.00 a	32.00 a	36.00 a
Butanone acetate + *Metarhizium anisopliae* based HTD	17.00 bc	13.00 b	15.00 bc	11.00 bc
Butanone acetate + *Beauveria bassiana* based HTD	11.00 c	8.00 c	10.00 d	7.00 c
Butanone acetate + Control based HTD	33.00 a	32.00 a	30.00 a	34.00 a
LSD	5.16	4.84	3.67	4.84

Means with the same lowercase letters are not significantly different at α = 0.05 (LSD test)

anisopliae based EV-HTD (13.00%) and GF-120 + *M. anisopliae* based EV-HTD (15.00%), respectively. Furthermore, Butanone acetate + *M. anisopliae* based EV-HTD, GF-120 + *B. bassiana* based EV-HTD and GF-120 + *M. anisopliae* based EV-HTD were statistically at par with each other (Table 4).

At 45-day time interval, percentage fruit infestation by *B. cucurbitae* was ranged from 32.00 to 10.00%. Maximum percentage of fruit infestation was observed in control treatment in GF-120 + Control based EV-HTD (32.00%) and in Butanone acetate + Control based EV-HTD (30.00%) and both were statistically at par with each other. While minimum fruit infestation was recorded in plots treated with Butanone acetate + *B. bassiana* based EV-HTD (10.00%) followed by plots treated with GF-120 + *B. bassiana* based EV-HTD (13.00%), Butanone acetate + *M. anisopliae* based EV-HTD (15.00%) and GF-120 + *M. anisopliae* based EV-HTD (18.00%), respectively. Furthermore, Butanone acetate + *M. anisopliae* based EV-HTD, and GF-120 + *M. anisopliae* based EV-HTD were statistically at par with each other (Table 4).

After 60-day time interval, percentage fruit infestation by *B. cucurbitae* was ranged from 36.00 to 7.00%. Maximum percentage of fruit infestation was observed in control treatment in GF-120 + Control based EV-HTD (36.00%) and in Butanone acetate + Control based EV-HTD (34.00%) and both were statistically at par with each other. While minimum fruit infestation was recorded in plots treated with Butanone acetate + *B. bassiana* based EV-HTD (7.00%) and GF-120 + *B. bassiana* based EV-HTD (9.00%) and both were statistically at par with each other and significantly different with Butanone acetate + *M. anisopliae* based EV-HTD (11.00%) and GF-120 + *M. anisopliae* based EV-HTD (14.00%), respectively (Table 4).

Impact of entomo-vectoring *Metarhizium anisopliae* and *Beauveria bassiana*, combined with GF-120 and Butanone acetate, on yield loss per plant (g) by *Bactrocera cucurbitae* over time intervals

After 15-day time interval, the minimum yield loss per plant by *B. cucurbitae* were recorded in plots treated with Butanone acetate + *B. bassiana* based EV-HTD (81.50 g/plant), followed by GF-120 + *B. bassiana* based EV-HTD (103.86 g/plant), Butanone acetate + *M. anisopliae* based EV-HTD (118.58 g/plant) and GF-120 + *M. anisopliae* based EV-HTD (140.88 g/plant), respectively. Furthermore, the plots treated with GF-120 + *B. bassiana* based EV-HTD, Butanone acetate + *M. anisopliae* based EV-HTD and GF-120 + *M. anisopliae* based EV-HTD were statistically at par with each other. While maximum yield losses were recorded in control treatment in GF-120 + Control based EV-HTD (274.67 g/plant) and in Butanone acetate + Control based EV-HTD (273.07 g/plant) and both treatments were also non-significant with each other (Table 5).

Following a 30-day time interval, minimum yield loss per plant by *B. cucurbitae* were recorded in plots treated with Butanone acetate + *B. bassiana* based EV-HTD (59.24 g/plant), followed by GF-120 + *B. bassiana* based EV-HTD (81.56 g/plant), Butanone acetate + *M. anisopliae* based EV-HTD (96.36 g/plant) and GF-120 + *M. anisopliae* based EV-HTD (111.21 g/plant), respectively. Furthermore, the plots treated with GF-120 + *B. bassiana* based EV-HTD, Butanone acetate + *M. anisopliae* based EV-HTD and GF-120 + *M. anisopliae* based EV-HTD were statistically at par with each other. While maximum yield losses were recorded in control treatment in GF-120 + Control based EV-HTD (245.01 g/plant) and in Butanone acetate + Control based EV-HTD (237.05 g/plant) and both treatments were non-significant with each other (Table 5).

Table 5 Impact of entomo-vectoring *Metarhizium anisopliae* and *Beauveria bassiana*, combined with GF-120 and Butanone acetate, on yield loss per plant (g) by *Bactrocera cucurbitae* over time intervals

Treatments	Time intervals			
	15-day	30-day	45-day	60-day
GF-120 + *Metarhizium anisopliae* based HTD	140.88 b	111.21 b	133.47 b	103.81 b
GF-120 + *Beauveria bassiana* based HTD	103.86 bc	81.56 bc	96.41 cd	66.74 c
GF-120 + Control based HTD	259.78 a	245.01 a	237.52 a	267.27 a
Butanone acetate + *Metarhizium anisopliae* based HTD	118.58 bc	96.36 b	111.17 bc	81.53 bc
Butanone acetate + *Beauveria bassiana* based HTD	81.50 c	59.24 c	74.05 d	51.86 c
Butanone acetate + Control based HTD	244.46 a	237.05 a	222.25 a	251.89 a
LSD	40.49	35.91	27.18	36.05

Means with the same lowercase letters are not significantly different at α = 0.05 (LSD test)

At 45-day time interval, minimum yield loss per plant by *B. cucurbitae* were recorded in plots treated with Butanone acetate + *B. bassiana* based EV-HTD (74.05 g/plant), followed by GF-120 + *B. bassiana* based EV-HTD (96.41 g/plant), Butanone acetate + *M. anisopliae* based EV-HTD (111.17 g/plant) and GF-120 + *M. anisopliae* based EV-HTD (133.47 g/plant), respectively. Furthermore, the plots treated with Butanone acetate + *M. anisopliae* based EV-HTD and GF-120 + *M. anisopliae* based EV-HTD were statistically at par with each other. While maximum yield losses were recorded in control treatment in GF-120 + Control based EV-HTD (237.52 g/plant) and in Butanone acetate + Control based EV-HTD (222.25 g/plant) and both treatments were non-significant with each other (Table 5).

After 60-day time interval, minimum yield loss per plant by *B. cucurbitae* were recorded in plots treated with Butanone acetate + *B. bassiana* based EV-HTD (51.86 g/plant), followed by GF-120 + *B. bassiana* based EV-HTD (66.74 g/plant), Butanone acetate + *M. anisopliae* based EV-HTD (81.53 g/plant) and GF-120 + *M. anisopliae* based EV-HTD (103.81 g/plant), respectively. Furthermore, the plots treated with Butanone acetate + *B. bassiana* based EV-HTD and GF-120 + *B. bassiana* based EV-HTD were statistically at par with each other and similarly plots treated with Butanone acetate + *M. anisopliae* based EV-HTD and GF-120 + *M. anisopliae* based EV-HTD were also non-significantly with each other. While maximum yield losses were recorded in control treatment in GF-120 + Control based EV-HTD (267.27 g/plant) and in Butanone acetate + Control based EV-HTD (251.89 g/plant) and both treatments were statistically at par with each other (Table 5).

Impact of entomo-vectoring *Metarhizium anisopliae* and *Beauveria bassiana*, combined with GF-120 and Butanone acetate, on marketable fruit yield/plant (g) by *Bactrocera cucurbitae* over time intervals

After 15-day time interval, maximum marketable fruit yield per plant by *B. cucurbitae* was recorded in Butanone acetate + *B. bassiana* based EV-HTD (659.03 g/plant), GF-120 + *B. bassiana* based EV-HTD (637.86 g/plant) and Butanone acetate + *M. anisopliae* based EV-HTD (622.54 g/plant) and all these treatments were statistically at par with each other and significantly different with plots treated with GF-120 + *M. anisopliae* based EV-HTD (600.54 g/ plant). While minimum yield was observed in control treatments in GF-120 + Control based EV-HTD (482.53 g/plant) and in Butanone acetate + Control based EV-HTD (496.37 g/plant) (Table 6).

Following a 30-day time interval, maximum marketable fruit yield per plant by *B. cucurbitae* was recorded in plots treated with Butanone acetate + *B. bassiana* based EV-HTD (681.29 g/plant) and GF-120 + *B. bassiana* based EV-HTD (660.16 g/plant) and both were statistically at par with each other and significantly different with plots treated with Butanone acetate + *M. anisopliae* based EV-HTD (644.77 g/plant) and GF-120 + *M. anisopliae* based EV-HTD (630.21 g/plant). While minimum yield was observed in control treatments in GF-120 + Control based EV-HTD (497.30 g/plant) and in Butanone acetate + Control based EV-HTD (503.78 g/plant) (Table 6).

At 45-day time interval, maximum marketable fruit yield per plant by *B. cucurbitae* was recorded in plots treated with Butanone acetate + *B. bassiana* based EV-HTD (666.48 g/plant) and GF-120 + *B. bassiana* based EV-HTD (645.30 g/plant) and both were statistically at par with each other and significantly different with plots treated with Butanone acetate + *M. anisopliae* based

Table 6 Impact of entomo-vectoring *Metarhizium anisopliae* and *Beauveria bassiana*, combined with GF-120 and Butanone acetate, on marketable fruit yield/plant (g) by *Bactrocera cucurbitae* over time intervals

Treatments	Time intervals			
	15-day	30-day	45-day	60-day
GF-120 + *Metarhizium anisopliae* based HTD	600.54 b	630.21 b	607.95 c	637.61 b
GF-120 + *Beauveria bassiana* based HTD	637.86 ab	660.16 ab	645.30 ab	674.97 ab
GF-120 + Control based HTD	482.53 c	497.30 c	504.79 d	475.04 c
Butanone acetate + *Metarhizium anisopliae* based HTD	622.54 ab	644.77 b	629.96 bc	659.60 ab
Butanone acetate + *Beauveria bassiana* based HTD	659.03 a	681.29 a	666.48 a	688.68 a
Butanone acetate + Control based HTD	496.37 c	503.78 c	518.58 d	488.94 c
LSD	38.21	36.33	27.49	35.43

Means with the same lowercase letters are not significantly different at α = 0.05 (LSD test)

EV-HTD (629.96 g/plant) and GF-120 + *M. anisopliae* based EV-HTD (607.95 g/plant). While minimum yield was observed in control treatments in GF-120 + Control based EV-HTD (504.79 g/plant) and in Butanone acetate + Control based EV-HTD (518.58 g/plant) (Table 6).

After 60-day time interval, maximum marketable fruit yield per plant by *B. cucurbitae* was recorded in plots treated with Butanone acetate + *B. bassiana* based EV-HTD (688.68 g/plant) followed by GF-120 + *B. bassiana* based EV-HTD (674.97 g/plant) and Butanone acetate + *M. anisopliae* based EV-HTD (659.60 g/plant) and all these treatments were statistically at par with each other and significantly different with plots treated with GF-120 + *M. anisopliae* based EV-HTD (637.61 g/plant). While minimum yield was observed in control treatments in GF-120 + Control based EV-HTD (475.04 g/plant) and in Butanone acetate + Control based EV-HTD (488.94 g/plant) (Table 6).

Impact of entomo-vectoring *Metarhizium anisopliae* and *Beauveria bassiana*, combined with GF-120 and Butanone acetate, on marketable yield/ha (kg) by *Bactrocera cucurbitae* over time intervals

After 15-day time interval, maximum marketable yield by *B. cucurbitae* was recorded in the plots treated with Butanone acetate + *B. bassiana* based EV-HTD (2169.00 kg/ha), followed by plots treated with GF-120 + *B. bassiana* based EV-HTD (2099.30 kg/ha) and Butanone acetate + *M. anisopliae* based EV-HTD (2048.60 kg/ha) and all these treatments were statistically at par with each other and significantly different with GF-120 + *M. anisopliae* based EV-HTD (1976.50 kg/ha). While minimum marketable yield was observed in control treatment in GF-120 + Control based EV-HTD (1587.90 kg/ha) and in Butanone acetate + Control based EV-HTD (1633.60 kg/ha) and both treatments were non-significant with each other (Table 7).

Following a 30-day time interval, maximum marketable yield by *B. cucurbitae* was recorded in the plots treated with Butanone acetate + *B. bassiana* based EV-HTD (2242.30 kg/ha) and GF-120 + *B. bassiana* based EV-HTD (2172.70 kg/ha) and both were statistically at par with each other and significantly different with plots treated with Butanone acetate + *M. anisopliae* based EV-HTD (2122.10 kg/ha) and GF-120 + *M. anisopliae* based EV-HTD (2074.10 kg/ha). While minimum marketable yield was observed in control treatment in GF-120 + Control based EV-HTD (1636.70 kg/ha) and in Butanone acetate + Control based EV-HTD (1658.00 kg/ha) and both treatments were non-significant with each other (Table 7).

At 45-day time interval, maximum marketable yield by *B. cucurbitae* was recorded in the plots treated with Butanone acetate + *B. bassiana* based EV-HTD (2193.50 kg/ha) and GF-120 + *B. bassiana* based EV-HTD (2123.80 kg/ha) and both were statistically at par with each other and significantly different with plots treated with Butanone acetate + *M. anisopliae* based EV-HTD (2073.30 kg/ha) and GF-120 + *M. anisopliae* based EV-HTD (2000.90 kg/ha). While minimum marketable yield was observed in control treatment in GF-120 + Control based EV-HTD (1661.40 kg/ha) and in Butanone acetate + Control based EV-HTD (1706.80 kg/ha) and both treatments were non-significant with each other (Table 7).

After 60-day time interval, maximum marketable yield by *B. cucurbitae* was recorded in the plots treated with Butanone acetate + *B. bassiana* based EV-HTD (2266.60 kg/ha), followed by GF-120 + *B. bassiana* based EV-HTD (2221.50 kg/ha) and Butanone acetate + *M. anisopliae* based EV-HTD (2170.50 kg/ha) and all these treatments were statistically at par with each other and significantly different with plots treated with GF-120 + *M. anisopliae* based EV-HTD (2098.50 kg/ha). While minimum

Table 7 Impact of entomo-vectoring *Metarhizium anisopliae* and *Beauveria bassiana*, combined with GF-120 and Butanone acetate, on marketable yield/ha (kg) by *Bactrocera cucurbitae* over time intervals

Treatments	Time intervals			
	15-day	**30-day**	**45-day**	**60-day**
GF-120 + *Metarhizium anisopliae* based HTD	1976.50 b	2074.10 b	2000.90 c	2098.50 b
GF-120 + *Beauveria bassiana* based HTD	2099.30 ab	2172.70 ab	2123.80 ab	2221.50 ab
GF-120 + Control based HTD	1587.90 c	1636.70 c	1661.40 d	1563.50 c
Butanone acetate + *Metarhizium anisopliae* based HTD	2048.60 ab	2122.10 b	2073.30 bc	2170.50 ab
Butanone acetate + *Beauveria bassiana* based HTD	2169.00 a	2242.30 a	2193.50 a	2266.60 a
Butanone acetate + Control based HTD	1633.60 c	1658.00 c	1706.80 d	1606.50 c
LSD	125.77	119.56	90.47	116.60

Means with the same lowercase letters are not significantly different at α = 0.05 (LSD test)

marketable yield was observed in control treatment in GF-120 + Control based EV-HTD (1563.50 kg/ha) and in Butanone acetate + Control based EV-HTD (1606.50 kg/ha) and both treatments were non-significant with each other (Table 7).

Impact of entomo-vectoring *Metarhizium anisopliae* and *Beauveria bassiana*, combined with GF-120 and Butanone acetate, on yield loss/ ha (kg) by *Bactrocera cucurbitae* over time intervals

After 15-day time interval, the minimum yield loss by *B. cucurbitae* was observed in plots treated with Butanone acetate + *B. bassiana* based EV-HTD (268.22 kg/ha), followed by plots treated with GF-120 + *B. bassiana* based EV-HTD (341.82), Butanone acetate + *M. anisopliae* based EV-HTD (390.28 kg/ha) and GF-120 + *M. anisopliae* based EV-HTD (463.68 kg/ha), respectively. Among these treatments' plots treated with GF-120 + *B. bassiana* based EV-HTD, Butanone acetate + *M. anisopliae* based EV-HTD and GF-120 + *M. anisopliae* based EV-HTD were statistically at par with each other. While maximum yield losses were recorded in control treatment in GF-120 + Control based EV-HTD (854.98 kg/ha) and in Butanone acetate + Control based EV-HTD (804.57 kg/ha) (Table 8).

Following a 30-day time interval, the minimum yield loss by *B. cucurbitae* was observed in plots treated with Butanone acetate + *B. bassiana* based EV-HTD (194.97 kg/ha), followed by plots treated with GF-120 + *B. bassiana* based EV-HTD (268.42), Butanone acetate + *M. anisopliae* based EV-HTD (317.12 kg/ha) and GF-120 + *M. anisopliae* based EV-HTD (366.02 kg/ha), respectively. Among these treatments' plots treated with GF-120 + *B. bassiana* based EV-HTD, Butanone acetate + *M. anisopliae* based EV-HTD and GF-120 + *M. anisopliae* based EV-HTD were statistically at par with each other. While maximum yield losses were recorded in control treatment in GF-120 + Control based EV-HTD (806.38 kg/ha) and in Butanone acetate + Control based EV-HTD (780.17 kg/ha) (Table 8).

At 45-day time interval, the minimum yield loss by *B. cucurbitae* was observed in plots treated with Butanone acetate + *B. bassiana* based EV-HTD (243.72 kg/ha), followed by plots treated with GF-120 + *B. bassiana* based EV-HTD (317.32 kg/ha), Butanone acetate + *M. anisopliae* based EV-HTD (365.88 kg/ha) and GF-120 + *M. anisopliae* based EV-HTD (439.28 kg/ha), respectively. Among these treatments' plots treated with Butanone acetate + *M. anisopliae* based EV-HTD and GF-120 + *M. anisopliae* based EV-HTD were statistically at par with each other. While maximum yield losses were recorded in control treatment in GF-120 + Control based EV-HTD (781.73 kg/ha) and in Butanone acetate + Control based EV-HTD (731.43 kg/ha) (Table 8).

After 60-day time interval, the minimum yield loss by *B. cucurbitae* was observed in plots treated with Butanone acetate + *B. bassiana* based EV-HTD (170.67 kg/ha), followed by plots treated with GF-120 + *B. bassiana* based EV-HTD (219.66 kg/ha), Butanone acetate + *M. anisopliae* based EV-HTD (268.32 kg/ha) and GF-120 + *M. anisopliae* based EV-HTD (341.67 kg/ha), respectively. Among these treatments' plots treated with Butanone acetate + *B. bassiana* based EV-HTD and GF-120 + *B. bassiana* based EV-HTD were statistically at par with each other and similarly Butanone acetate + *M. anisopliae* based EV-HTD and GF-120 + *M. anisopliae* based EV-HTD were non-significant with each other. While maximum yield losses were recorded in control treatment in GF-120 + Control based EV-HTD (879.63 kg/ha) and in Butanone acetate + Control based EV-HTD (829.02 kg/ha) (Table 8).

Table 8 Impact of entomo-vectoring *Metarhizium anisopliae* and *Beauveria bassiana*, combined with GF-120 and Butanone acetate, on yield loss/ ha (kg) by *Bactrocera cucurbitae* over time intervals

Treatments	Time intervals			
	15-day	30-day	45-day	60-day
GF-120 + *Metarhizium anisopliae* based HTD	463.68 b	366.02 b	439.28 b	341.67 b
GF-120 + *Beauveria bassiana* based HTD	341.82 bc	268.42 bc	317.32 cd	219.66 c
GF-120 + Control based HTD	854.99 a	806.38 a	781.73 a	879.63 a
Butanone acetate + *Metarhizium anisopliae* based HTD	390.28 bc	317.12 b	365.88 bc	268.32 bc
Butanone acetate + *Beauveria bassiana* based HTD	268.22 c	194.97 c	243.72 d	170.67 c
Butanone acetate + Control based HTD	804.57 a	780.17 a	731.46 a	829.02 a
LSD	133.27	118.18	89.45	118.66

Means with the same lowercase letters are not significantly different at α = 0.05 (LSD test)

Table 9 Cost–Benefit analysis of entomo-vectoring *Metarhizium anisopliae* and *Beauveria bassiana* in combination with GF-120 and Butanone acetate against *Bactrocera cucurbitae* over time intervals

Treatments	Time intervals			
	15-day	30-day	45-day	60-day
GF-120 + *Metarhizium anisopliae* based HTD	1:1.24	1:1.27	1:1.20	1:1.34
GF-120 + *Beauveria bassiana* based HTD	1:1.29	1:1.30	1:1.25	1:1.39
Butanone acetate + *Metarhizium anisopliae* based HTD	1:1.30	1:1.31	1:1.30	1:1.38
Butanone acetate + *Beauveria bassiana* based HTD	1:1.33	1:1.35	1:1.33	1:1.41

Cost–Benefit analysis of entomo-vectoring *Metarhizium anisopliae* and *Beauveria bassiana* in combination with GF-120 and Butanone acetate against *Bactrocera cucurbitae* over time intervals

In the present investigation, the results revealed that the plot treated with Butanone acetate + *B. bassiana* based EV-HTD was found most economical having maximum cost benefit ratio (14.99), followed by plots treated with Butanone acetate + *M. anisopliae* based EV-HTD (14.1), GF-120 + *B. bassiana* based EV-HTD (14.5) and GF-120 + *M. anisopliae* based EV-HTD (13.73) after first picking of bitter gourd. Similar kind of trend was observed in 2nd and 3rd picking. Maximum cost benefit ratio was found in 4th picking where Butanone acetate + *B. bassiana* based EV-HTD was found most economically beneficial having maximum cost benefit ratio (14.99) followed by plots treated with GF-120 + *B. bassiana* based EV-HTD (14.5), Butanone acetate + *M. anisopliae* based EV-HTD (1:1.38) and GF-120 + *M. anisopliae* based EV-HTD (1:1.34) (Table 9).

Mean value of different picking of bitter gourd revealed that the plot treated with Butanone acetate + *M. anisopliae* based EV-HTD was found most economically beneficial having maximum cost benefit ratio (1:1.35) followed by plots treated with Butanone acetate + *M. anisopliae* based EV-HTD (1:1.32), GF-120 + *B. bassiana* based EV-HTD (1:1.31) and GF-120 + *M. anisopliae* based EV-HTD (1:1.26) (Table 9).

Discussion

The results of present study provide compelling evidence of the effectiveness of EV-HTD in controlling *B. cucurbitae* infestations in bitter gourd fields. In particular, Butanone acetate-based EV-HTD, especially when combined with *B. bassiana*, showed significant efficacy in mitigating fruit infestation, reducing *B. cucurbitae* mortality, and minimizing yield losses. These findings highlight the potential of Butanone acetate (chemical compound) as a vector of EPF, potentially improving their spread and effectiveness in pest control applications (Salem et al. 2023).

Upon closer inspection, a high average percentage of the entomo-vectored *B. cucurbitae* was observed in plots treated with Butanone acetate + *B. bassiana* based EV-HTD, indicating the increased attractiveness and infectivity of this combination to the target pests. Furthermore, the increase in spore numbers and mortality in these plots suggests a role for Butanone acetate in promoting fungal spore dispersal and adhesion, resulting in increased mortality and reduced infection levels (Chen et al. 2021).

Furthermore, the observed yield losses and changes in marketable yield provide practical insights into the implications of these findings for bitter gourd cultivation. Butanone acetate + *B. bassiana* based EV-HTD produced the minimum yield losses and the maximum marketable yield, highlighting its possible economic benefits for growers. Generally, these results demonstrate the possibility of incorporating EV-HTD into IPM approaches, providing a sustainable and environmentally friendly substitute to traditional pesticide-based tactics.

By comparing the results of this study with previous ones, important general information and understanding can be gained about the effectiveness of EPF in controlling *B. cucurbitae* infections. These findings are consistent with previous studies showing that *B. bassiana* can effectively control *B. cucurbitae* population. As evidence, using *B. bassiana*-based formulation, Hamzah et al. (2021) revealed similar patterns of mortality in *B. cucurbitae*. According to Zhao et al. (2020), *B. bassiana* BC-B1 strain can contribute to the control of *Zeugodacus cucurbitae*. Faleh et al. (2017) revealed that *B. bassiana* Bb 100 strain showed the greatest reduction in adult emergence rate, excellent pathogenicity, and maximum efficacy in suppressing male and female *Dacus ciliates*.

Although, the present study continues earlier investigations assessed the influence of various carriers, such as Butanone acetate and GF-120, on the effectiveness of EPF. Although both vectors have been used for pest control in the past, there has been little research on how they improve the spread and efficiency of EPF in controlling *B. cucurbitae* (Gogi et al. 2023). The results suggested that Butanone acetate may be a better vector for *B. bassiana* than GF-120, possibly due to differences in its volatility, pest attractiveness, or compatibility with pathogenic fungi.

The mode of action, persistence, and interactions between the vector and EPF are some of the factors that may contribute to the differences in reported efficacy among treatments (Mannino et al. 2019). As a carrier, Butanone acetate can enhance the attachment and spread of fungal spores to target pests, thereby reducing infestation levels and increasing mortality. The increased virulence and persistence of this fungus may be the result of a unique interaction between Butanone acetate and *B. bassiana*, which may also contribute to the superior efficacy of this treatment.

Furthermore, the preference of *B. cucurbitae* for Butanone acetate compared to GF-120 may affect treatment efficacy. It was observed that Butanone acetate is more attractive to *B. cucurbitae* than GF-120, which may lead to high uptake and subsequent fungal infection (Iqbal et al. 2020). Furthermore, the observed differences in treatment efficiency may be due to differences in spore production and viability of *B. bassiana* and *M. anisopliae*.

Although the results of the present study are encouraging, it is important to recognize that there are a number of limitations that may affect the results. First, because this study was conducted in a well-controlled experimental setting, it may not accurately capture the subtle relationships and diversity found in real field settings. In real-world agricultural environments, variables such as natural enemy abundance, crop management techniques, and weather patterns can have a significant impact on the effectiveness of EV-HTD. Furthermore, the study focused only on *B. cucurbitae* infections in bitter gourd fields, which further limits the applicability of the findings to other crops and pest species. Further studies should examine the effectiveness of EV-HTD against a wider range of crop and pest combinations to assess its adaptability and applicability to various agricultural environments. Moreover, this study does not fully address the scalability and economic feasibility of deploying EV-HTD in large-scale agricultural settings. Although Butanone acetate and *B. bassiana* based treatments showed encouraging economic results, further economic studies and field trials are needed to determine the long-term viability and practicality of this treatment for farmers.

To improve our understanding of EV-HTD and their potential use in pest management, future research should focus on a number of important topics. First, expanded field trials are needed to evaluate the durability and effectiveness of EV-HTD under various cropping systems and environmental conditions. This will provide valuable information on the robustness and reliability of EV-HTD in real-life agricultural environments. In addition, to evaluate the overall ecological sustainability and potential hazards of EV-HTD, it is necessary to study its effects on non-target organisms, soil microbiota, and ecosystem dynamics. Understanding the broader ecological impacts of EV-HTD use can help reduce unintended consequences and ensure the approach is consistent with sustainable agricultural approaches. Moreover, the effectiveness and adaptability of IPM systems can also be improved by studying the synergistic effects of combining EPF with other biological control agents, such as parasitoids or predators. By utilizing a variety of biocontrol agents, integrated technologies can reduce the need for synthetic pesticides and minimize negative environmental impacts, while providing more resilient and long-lasting pest control solutions.

Conclusions

The study concluded that using Butanone acetate as a vector in EV-HTD can effectively control *B. cucurbitae* infestation in bitter gourd fields. This is especially true when combined with the *B. bassiana*. Butanone acetate-based EV-HTD had better efficacy than GF-120, resulting in a higher percentage of entomo-vectored *B. cucurbitae*, more spores per insect and higher pest mortality. Furthermore, *B. bassiana* had better performance than *M. anisopliae* in inhibiting *B. cucurbitae*. According to economic studies, treatment with Butanone acetate + *B. bassiana* also minimized yield losses and provided the most marketable yields. With an optimal cost–benefit ratio, this treatment had the potential to be an effective and economically sustainable pest control technology. These results highlight the use of the *B. bassiana* in IPM strategies for the control of *B. cucurbitae* in bitter gourd cultivation in a sustainable and environmentally friendly manner. Further research and field testing are needed to verify the long-term effectiveness and scalability of this strategy in real agricultural settings.

Abbreviations

EV-HTD	Entomo-vectored horizontal transmission device
EPF	Entomopathogenic fungi
SDAY	Sabouraud dextrose yeast agar
GLM	General linear model
ANOVA	Analysis of variance
EVFF	Entomo-vectored fruit flies
x	Specific time duration
N_{EVFF}	Total number of entomo-vectored fruit flies
N_{FF}	Total number of fruit flies
T_{SFF}	Target spores per fruit fly
C_{SFF}	Counted spores per fruit fly
M_{SFF}	Maximum spores per fruit fly
M_{FF}	Mortality of fruit flies
N_{DFF}	Number of dead fruit flies
YL_P	Yield loss per plant
FE_I	Initial fruit weight
FW_F	Final fruit weight
FY_{MP}	Marketable fruit yield per plant
MY_H	Marketable yield per hectare

N_{PH} Number of plants per hectare
YL_H Yield loss per hectare

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Author contributions
MDG and AM conceptualized the study and recorded the data; MDG, BA and MJA statistical analyzed the data; MDG, AM and BA wrote Introduction section of the manuscript; MDG, BA, MJN and MAA wrote methodology section of the manuscript; MDG, AM, BA and AMS wrote Results and Discussion section of the manuscript; MDG and BA edited the format of the manuscript according to the format of this journal. All the authors read and approved the manuscript.

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

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