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Pathogenicity of *Beauveria bassiana* (Balsamo-Crivelli) and *Metarhizium anisopliae* (Metschnikoff) isolates against life stages of *Zeugodacus cucurbitae* (Coquillett) (Diptera: Tephritidae)

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Abstract

Background Entomopathogenic fungi are primary pathogens that naturally affect insect pests by suppressing their populations and considered as an ecofriendly agents. The present study aimed to evaluate in vitro activity of different isolates of *Beauveria bassiana* and *Metarhizium anisopliae* against the development of larval stages of the Cucurbit fruit fly, *Zeugodacus cucurbitae* (Coquillett) (Diptera: Tephritidae).

Results Larval mortality was significantly high with *B. bassiana* isolate Bb337 (5.82–21.70%) and with the lowest in *M. anisopliae* isolate MaD (1.49–6.33%). Pupal mortality rate was comparatively higher with more than 50%. The cadavers of all host instars produced conidia (sporulation). Sporulated dead larvae were significantly higher in Bb337 (61.10%) than at the least in MaD (18.60%) at 10^5 conidia/ml. At 10^8 conidia/ml, MaD induced the highest pupal cadavers with mycosis (32.42%). Regardless of applied fungal species, host instars mortality significantly increased with increasing concentration of *B. bassiana* isolates, suggesting a concentration-dependent response of *Z. cucurbitae*.

Conclusion The tested isolates demonstrated their pathogenicity through vertical transmission of mycosis from one instar to another, regardless of the concentrations used.

Keywords Cucurbit fruit fly, *Zeugodacus cucurbitae*, Entomopathogenic fungi, *Metarhizium anisopliae*, *Beauveria bassiana*

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Background

The Cucurbit fruit fly, *Zeugodacus cucurbitae* (Coquillett) (Diptera: Tephritidae), represents one of the most popular vegetable pests from the cucurbit family, especially on watermelon, *Citrullus lanatus*, Cucurbitaceae (De Meyer et al. 2015). The average yield loss due to damage by cucurbit fruit flies' species was estimated at 53% for vegetables such as pumpkin (*Cucurbita pepo*, Cucurbitaceae) and tomatoes (*Solanum lycopersicum*, Solanaceae) (Kambura et al. 2018). Several management strategies including chemical control, monitoring with attractants, cultural control, soil inoculation, release of natural enemies, release of sterile insects, and male annihilation technique have been implemented (Vargas et al. 2015). The use of insecticides as the only and major method to control pests in fruits and vegetables induced many side and non-target effects, especially environmental pollution, pest resurgence and resistance and health hazards (Fadiji and Babalola 2020).

Given the above side effects, alternative control methods by the use of entomopathogenic fungi (EPFs) have become attractive option to chemical pesticides for sustainable agriculture (Bamisile et al. 2021). Fungal agents are one of the promising groups worldwide developed to control several insects of different feeding guilds including aphids, locusts, thrips, grubs, moths, mites, mosquitoes, whiteflies, and tephritid fruit flies. The use of EPFs in tephritid fruit fly IPM (Integrated Pest Management) includes aerial applications targeting adults and soil inoculation targeting both pupating larvae and pupae (Hallouti et al. 2020). The effectiveness of EPFs according to a modern approach should be applied in localized spots and their dissemination would rely on the ability of conidia to be transmitted from infected to uninfected hosts (FAO/IAEA 2019). The inherent habit of tephritid fruit flies to spend part of their life cycle in the soil as larvae and pupae is an advantage since the soil is known to be an excellent environment for fungal growth as it protects conidia from UV irradiation and extreme temperatures (Ekesi et al. 2016).

The present work focused on assessing the toxicity effect of some *B. bassiana* and *M. anisopliae* isolates on various *Z. cucurbitae* life stages under laboratory conditions.

Methods

Study site

All experiments were conducted at the Pathology and Entomology laboratories of the International Institute of Tropical Agriculture (IITA), Benin-Station in Cotonou (06°25,002 N; 002°19,842 E) at a temperature of 26 ± 1 °C and 75 ± 5% RH and 12-h light-12-h dark photoperiod.

Establishment of *Zeugodacus cucurbitae* rearing colony

Initial colony of *Z. cucurbitae* originated from flies' population reared on magda-zucchini. They were maintained for several generations for laboratory adaptation at the Entomology Laboratory of IITA. A couple of 200 males and 200 females of 12 days old were placed in a Plexiglas cage of (20 × 20 × 20 cm), having one side with a sleeve attached through, which handling manipulation was done. Flies were fed with a mixture of brown sugar and yeast at a 3:1 ratio. Water was provided through an entomological vial soaked with cotton wool. Magda-zucchini were introduced inside the cages for 72 h and infested fruits were incubated in plastic containers of (1500 cm^3). The whole incubation unit was covered with a mesh scarf maintained with an elastic band. Pupae were collected 10 days later and placed in new cages until adult emergence. Larvae, pupae, and adults form of *Z. cucurbitae* were used for bioassay.

Origin and production of *B. bassiana* and *M. anisopliae* isolates

Fungal isolates used were obtained from the fungal bank of IITA-Benin. Six viable isolates of *B. bassiana* including Bb13, Bb14, Bb337, Bb338, Bb339, Bb353, and one isolate of *M. anisopliae* Ma31 were selected from 45 isolates originally stored on silica gel or in powder form. An isolate of *M. anisopliae* (MaD) was collected from the Crop Protection Service of Dakar in Senegal. Fungi were initially grown on Potato dextrose agar (PDA) media. Petri dishes with conidia were incubated for 15 days. For the eight isolates, germination rates after 24 h of incubation at 26 ± 1 °C were 97.76, 96.73, 95.76; 95.54, 92.67, 91.30 and 90.91% for Bb338, Bb353, Bb13; Bb337, Bb339, Bb14, MaD and Ma31, respectively. All isolates were sub-cultured on PDA and harvested for conditioning tests.

Conditioning of fungal isolates using *Z. cucurbitae*

Suspensions of conidia of different isolates (Bb13, Bb14, Bb337, Bb338, Bb339, Bb353, Ma31, and MaD) were prepared by scraping slightly from the surface of the fungal culture in a sterile liquid solution of 0.1% Tween80. Third instar larvae of *Z. cucurbitae* were placed in plastic boxes of 3.5 cm × 4 cm. Using a micropipette, 2×10^{10} µl conidia of each suspension was applied topically onto each larva at the pronotum. Each treatment was replicated 10 times. The boxes were placed inside a humid transparent container and maintained inside the incubation room. They were checked daily for 12 days. Dead larvae were removed and placed on a humidified sterile Whatman No 5-filter paper in Petri dishes, after drying for 24 h. All Petri dishes were kept in the inoculation

room and cadavers were monitored for 15 days for evidence of fungal growth on the surface of infected larval instars. Conidia from sporulated dead larvae were mass-cultured and used for bioassays on *Z. cucurbitae*.

Insecticidal effect of *B. bassiana* and *M. anisopliae* on *Z. cucurbitae* life stages

Six concentrations, 10^5, 10^6, 10^7, 10^8, 10^9 and 10^{10} conidia/ml, of each isolate (MaD, Bb13, Bb14, Bb337, Bb338, Bb339 and Bb353) were prepared. Suspensions were used to inoculate third instar larvae (5–7 days), fresh pupae (1–3 days), and adults (5 day old) of *Z. cucurbitae*. Two controls were set, untreated groups and groups treated with 0.1% Tween in distilled water. Treatments were replicated five times in a completely randomized block. Ten larvae, 10 fresh pupae, and pairs of four adults of *Z. cucurbitae* were introduced into sterile a separate plastic box of 3.5 cm × 4 cm. Using a micropipette, 2 µl of each concentration per isolate was applied topically onto larvae and pupae instars. The plastic box was prior sprayed with the conidia's suspension before the adults were aspirated with a hoover and placed in the box. Treated larvae, pupae, and adults were checked daily for recording mortality. All dead host instars larvae were removed from the boxes and placed on a humidified sterile Whatman No 5-filter paper, after drying, to check for fungal growth for 2 weeks. Petri dishes were sealed with para-film and labeled with the corresponding cage code, date of death, and number of cadavers. The number of enclosed pupae and/or adults from treated larvae and pupae was also recorded and incubated. Non-emerged pupae, after 10 days post-inoculation, were considered dead and the number recorded. Emerged flies were followed up until dead and then incubated.

A pair of five male and female adults of *Z. cucurbitae* of 12 days old were placed in a container and sprayed with the conidia's suspension of each isolate of the EPFs, then aspirated with a hoover and placed in plastic boxes. Flies were provided with food and water as described above. Punctured pieces of magda-zucchini were placed inside each box for 48 h. Control was treated with 0.1% Tween in distilled water and another group were not exposed to fungal conidia solution but fed in the same way as infected flies. Five replications were done per concentration. Three ovipositional punctures per fruit were dissected to monitor eggs laid. The remaining infested fruits were incubated in a plastic box of 150 cm^3 and covered with a mesh scarf maintained with an elastic band. Eggs' hatching rate and progression through life stages were monitored and recorded per isolate.

Data analysis

Data collected were summarized using excel. A generalized linear model (GLM) was used to analyze larval, pupae, and adult mortality rates. Data on larval mortality and percent sporulated cadavers were processed by applying the Analysis of Variance (ANOVA). The multiple comparisons of means (Tukey Contrast) was used to separate means when ANOVA revealed significant differences. Chi-square were judged at $P=0.05$. All the analyses were performed using $R \times 64$ (version 3.2.5, R Development Core Team) statistical software.

Results

Host instars and pupal mortality rate after treatment with different isolates of *B. bassiana* and *M. anisopliae*

In general, mortality rates were low for all isolates used to inoculate *Z. cucurbitae* larvae. Highest mortality rate was recorded at Bb337 (21.71%), followed by Bb339 (17.14%) and Bb338 (16.72%) at 10^{10} conidia/ml with the lowest in MaD (Fig. 1). On the other hand, MaD showed higher pupal mortality rate than isolates of *B. bassiana*. Significant mortality rate was observed after 15 days of treatment in all tested concentrations. MaD ranked first with 63.10% mortality, when applied at 10^{10} conidia/ml and was significantly higher than those of Bb338 (49.17%) and Bb337 (47.49%). Similarly, at 10^5 conidia/ml, pupal mortality rate was in order MaD (49.29%), Bb338 (35.48%) and Bb337 (33.95%). The mean percent pupal mortality rate decreased with a decrease in conidia concentrations for all isolates (Fig. 2). Pupal mortality rate was significantly different among isolates ($P=1.097 \times 10^{-15}$) and concentrations ($P=7.246 \times 10^{-16}$).

All isolates showed high mortality rates when applied to pupae of *Z. cucurbitae* at any concentration, except for Bb337 (53.52–57.42%), Bb338 (52.60–56.53%) and MaD (46.80–50.75%). However, all isolates caused more than 50% mortality rates at very low concentrations, regardless to the isolates tested (Fig. 3). Pupal mortality rates were significantly different among isolates ($P=1.097 \times 10^{-15}$) and concentrations ($P=7.246 \times 10^{-16}$).

Sporulation rate of dead larvae, pupae, and adults after treatment with *B. bassiana* and *M. anisopliae* isolates

Percent sporulated host instars cadavers of *Z. cucurbitae* after treatment with different isolates at different concentrations are presented in (Fig. 4). Percent sporulated dead larvae after treatment with *B. bassiana* isolates Bb337 and Bb338 were significantly higher than the other isolates. These rates were consistent and decreased only slightly with the concentrations. However, there was an increase in the percent sporulated dead larvae when

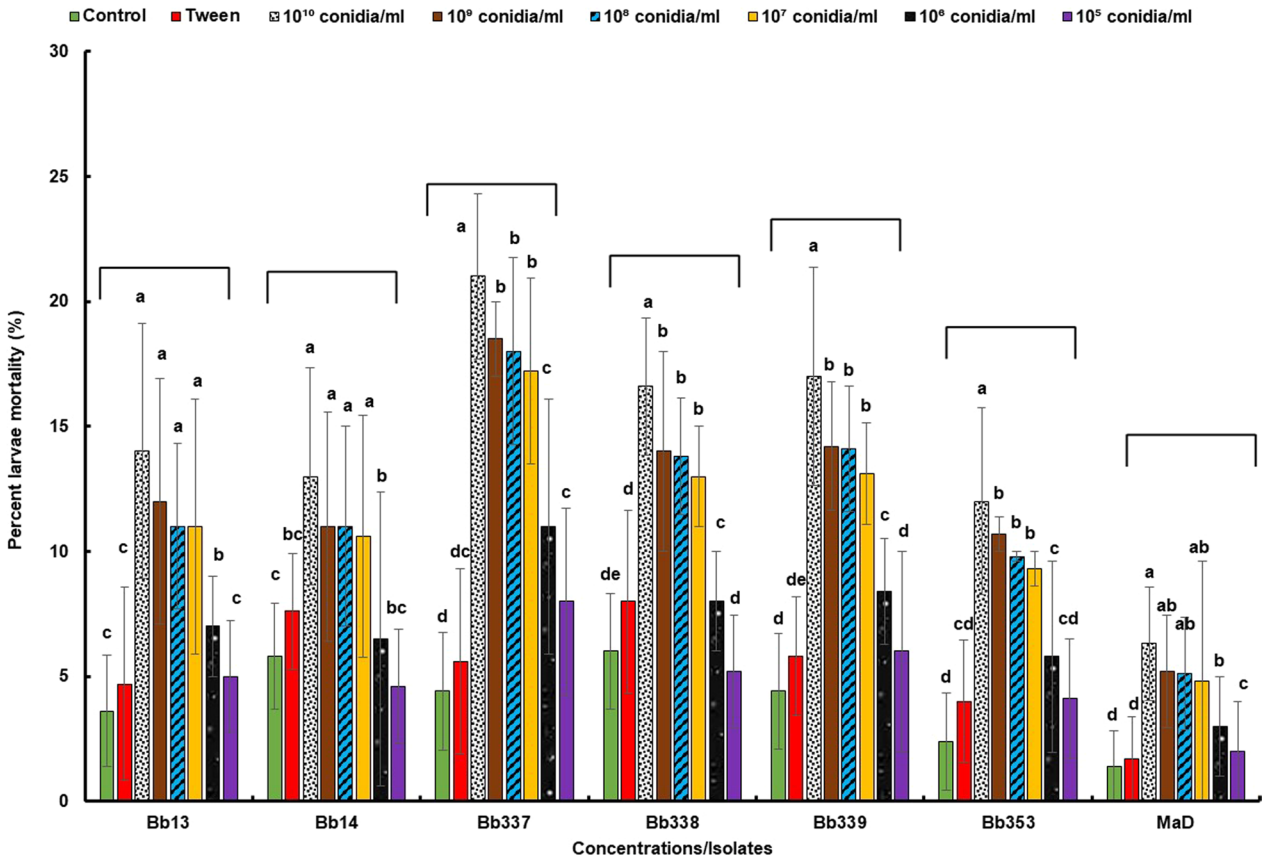

Fig. 1 Mortality of larvae of *Zeugodacus cucurbitae* treated with different concentrations of isolates of *Beauveria bassiana* and *Metarhizium anisopliae* in larval inoculation method

using Bb337 (61.10%) than those obtained with Bb338 (30.80%) applied at 10^5 conidia/ml per insect. Percent larvae cadavers with mycosis were significantly different among concentrations ($P=0.0009$) but not among isolates ($P=0.0683$).

The effect of isolates on enclosed pupae showed high percent sporulated dead pupae for all isolates when applied at 10^{10} conidia/ml per insect. These rates were in order Bb353 (18.65%), Bb338 (16.99%), Bb339 (15.41%), Bb337 (14.99%), Bb13 (13.97%), Bb14 (9.44%) and MaD (7.35%). The lowest percent of sporulated dead pupae was recorded when applying 10^5 conidia/ml per insect (Fig. 5). However, significant differences were observed among concentrations ($P=2\times10^{-16}$) and isolates ($P=8.431\times10^{-15}$). However, the highest percentage of sporulated dead adults was recorded with Bb338 (18.61%), followed by Bb337 (16.43%) at 10^{10} conidia/ml per insect with non-significant difference between both isolates (Fig. 6).

Sporulated dead pupae and adults after pupal treatment using different *B. bassiana* and *M. anisopliae* isolates

Dead pupae after treatment with MaD showed the highest percent sporulated pupae of 20.27% at 10^5 conidia/ml and 32.42% at 10^8 conidia/ml. Isolates Bb337 and Bb338 showed also good performances with percent sporulated dead pupae of 11.87% and 20.27% applied at 10^5 conidia/ml respectively (Fig. 8). For dead sporulated adults, the highest rates were recorded at Bb337 (37.93%) and Bb338 (29.60%) at 10^9 conidia/ml. However, dead adults emerged from inoculated pupae with Bb339 and MaD did not show fungal growth (Fig. 7). There was a significant difference between isolates and concentrations ($P=2.2\times10^{-16}$). When adults were directly inoculated with different isolates, the highest percentage of sporulated dead adults was recorded with Bb338 (36.00%), followed by Bb337 (28.84%) when applied at 10^{10} conidia/ml (Fig. 8). Significant differences were observed between isolates ($P=2.2\times10^{-16}$) and concentrations ($P=1.15\times10^{-10}$).

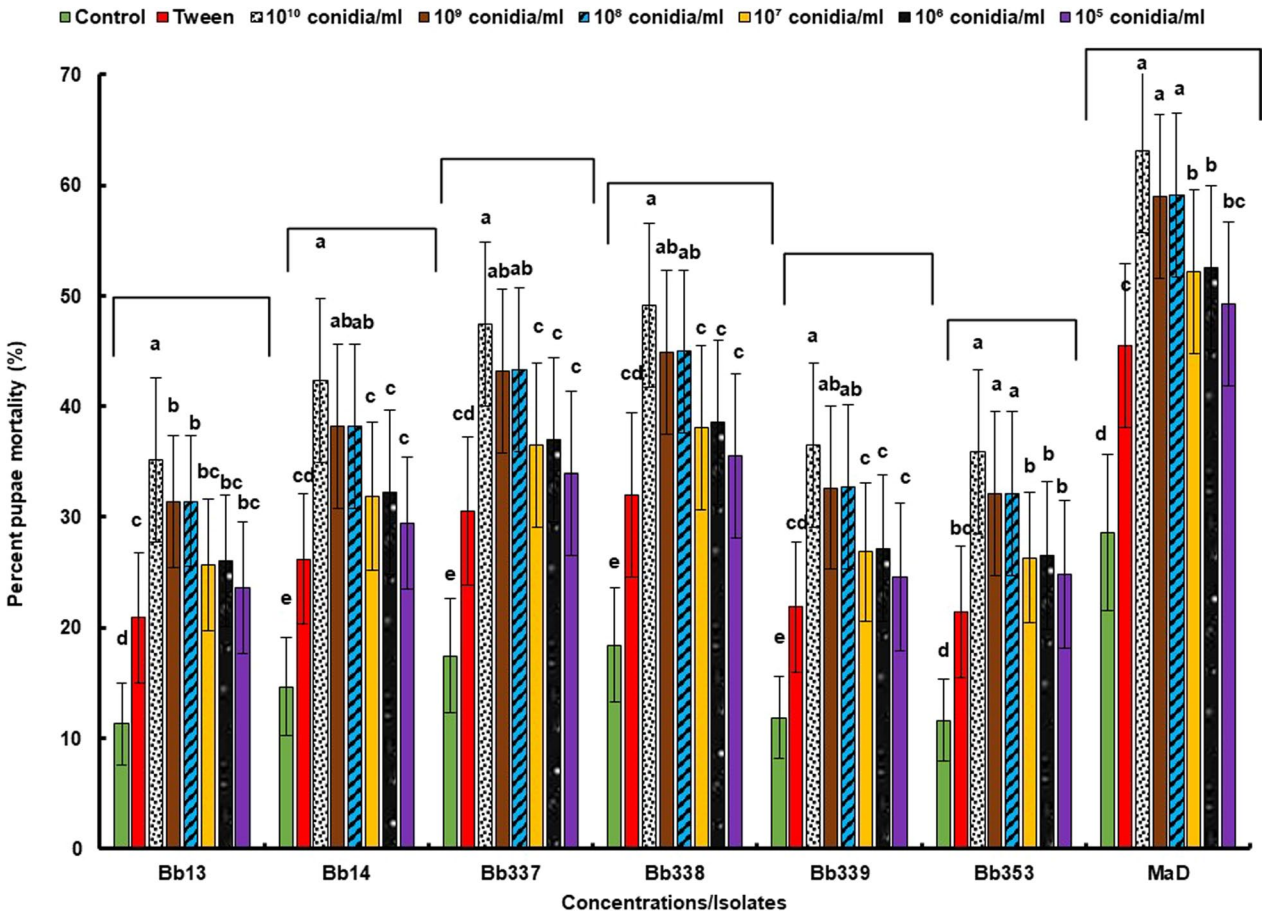

Fig. 2 Mortality of pupae of *Zeugodacus cucurbitae* treated with different concentrations of isolates of *Beauveria bassiana* and *Metarhizium anisopliae* in larval inoculation method

Effect of *B. bassiana* and *M. anisopliae* on fruit flies fecundity using adults' inoculation method

Fecundity of *Z. vertebratus* was not significantly affected by fungal isolates ($P=0.6568$). The highest number of pupae was recorded in the control 3.79 ± 1.36 pupae, while at the treatment with a Tween80 solution; it was 1.15 ± 0.46 pupae. At high concentrations of conidia per ml, the number of pupae was reduced than adults infected with the lowest concentrations of conidia of each isolate (Table 1). The mean number of collected pupae from fruits infested by adults inoculated with different isolates showed significant differences among concentrations ($P=2.855 \times 10^{-10}$).

Lethal concentration value after treatment of the third instar larvae of *Z. cucurbitae* with various concentrations of *B. bassiana* and *M. anisopliae*

The Cox regression analysis demonstrated that the different fungal doses used were significant indicators of *Z. cucurbitae* larval mortality ($P=0017$). Isolates Bb337, Bb338 and Bb13 induced lesser than 50% mortality of the tested larvae. The death ratio curve decreased with an increase of fungal concentration (Fig. 9). However, the death ratio increased with an increase in conidia suspensions concentration showing the existence of a concentration-mortality response. The required concentrations to kill 50% of third instar larvae of *Z. cucurbitae* ranged between 10^5 and 10^6 conidia/ml for isolate Bb13, while it felt between 10^6 and 10^7 conidia/ml for isolates Bb339, Bb353, and MaD (Fig. 10 and 11).

Discussion

Entomopathogenic microorganisms were successfully used as an alternative to chemical pesticides (Gulzar et al. 2021). The present study showed that all the tested isolates of EPFs, *B. bassiana* (Bb337 and Bb338) and *M. anisopliae* (MaD) were virulent against third instar larvae pupae and adults of *Z. cucurbitae*. All isolates had larvicidal, pupicidal and adulticidal effects against *Z.*

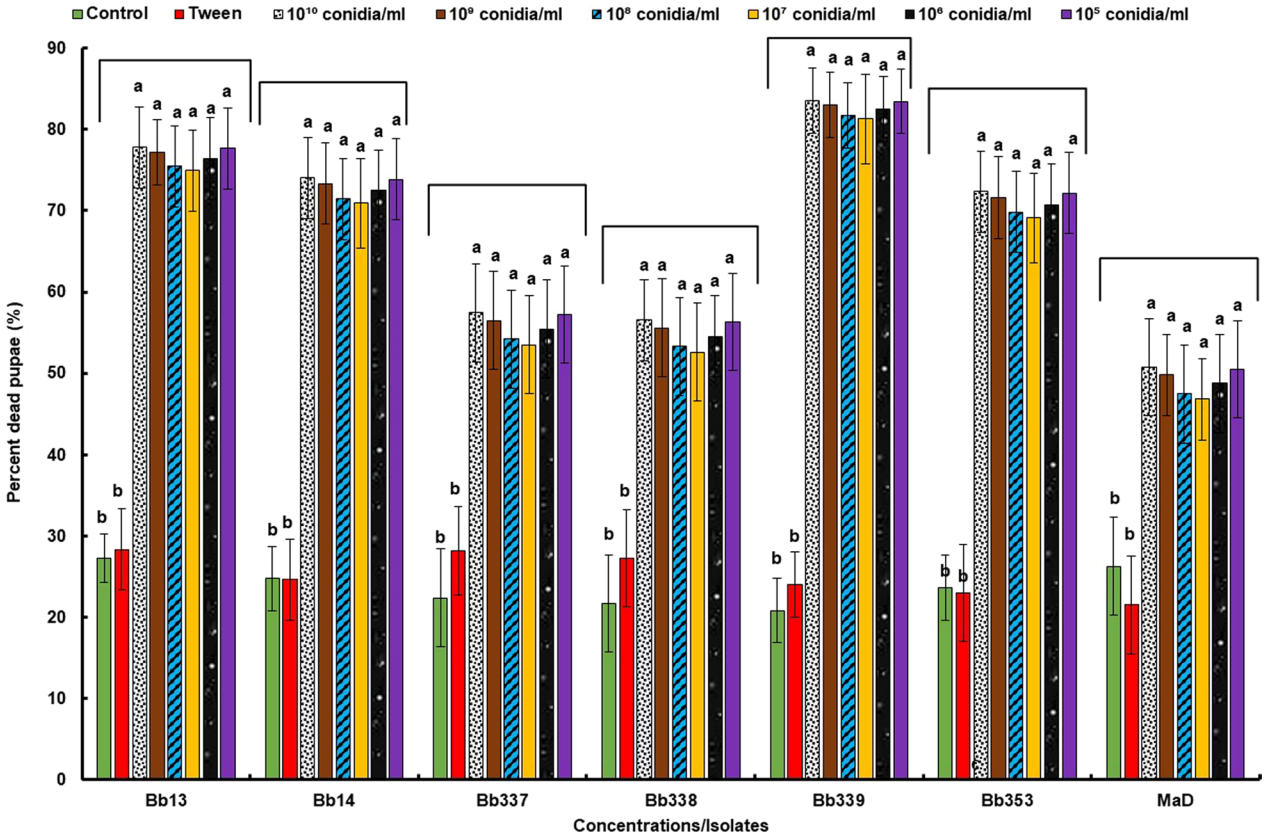

Fig. 3 Mortality of *Zeugodacus cucurbitae* pupae after treatment with different concentrations of isolates of *Beauveria bassiana* and *Metarhizium anisopliae* in pupal inoculation method

cucurbitae. These findings confirmed the susceptibility of others species of fruit flies in the genera *Bactrocera* to EPFs infection in previous reports (Hussein et al. 2018). Tested isolates varied in their levels of virulence (sporulation) toward different developmental stages of *Z. cucurbitae*, confirming our hypothesis. Thus, the susceptibility of fruit flies to EPFs has been confirmed as reported by many workers (FAO/IAEA 2019).

In larval inoculation methods, isolates Bb337, Bb338 and Bb339 caused up to 20% larval mortality while the lowest mortality was recorded with isolate MaD. However, the relatively low larval mortality was compensated by a high pupal mortality with different treatments. High adult mortality was recorded after emergence from treated pupae in this study. These results agree with findings from other studies showing a high adult mortality rate from infected pupae in *Ceratitis capitata* and other insect pests of other species (Goble et al. 2011). This result showed the virulence of Bb337, Bb338, and Bb339 which varied depending on fungal species, host instars stages, and applied concentrations as compared to other treatments.

The screening and concentration response bioassays demonstrated that the morality rates in host instars were dependent on the concentrations of conidia applied. These results are in concordance with findings by Soliman et al. (2020) with the fungus *M. anisopliae* causing the highest percentage of larval mortality in late third larval instar, ranged 15–60% at the 1×10^6 and 1×10^{10} conidia/ml, respectively. The cadavers of all host instars produced conidia (sporulation), which can help regulate the pest population through the production of secondary infection, and may also increase pathogen's persistence in the environment (Thomas 1996). The sporulation rates after treatments were also concentration-dependent with about 60% and over 40% of larval cadavers infected by Bb337 and Bb338 showing fungal activity through sporulation, respectively. This confirmed the virulence of those isolates to the larvae of *Z. cucurbitae* and showed that mortality was caused by fungi tested.

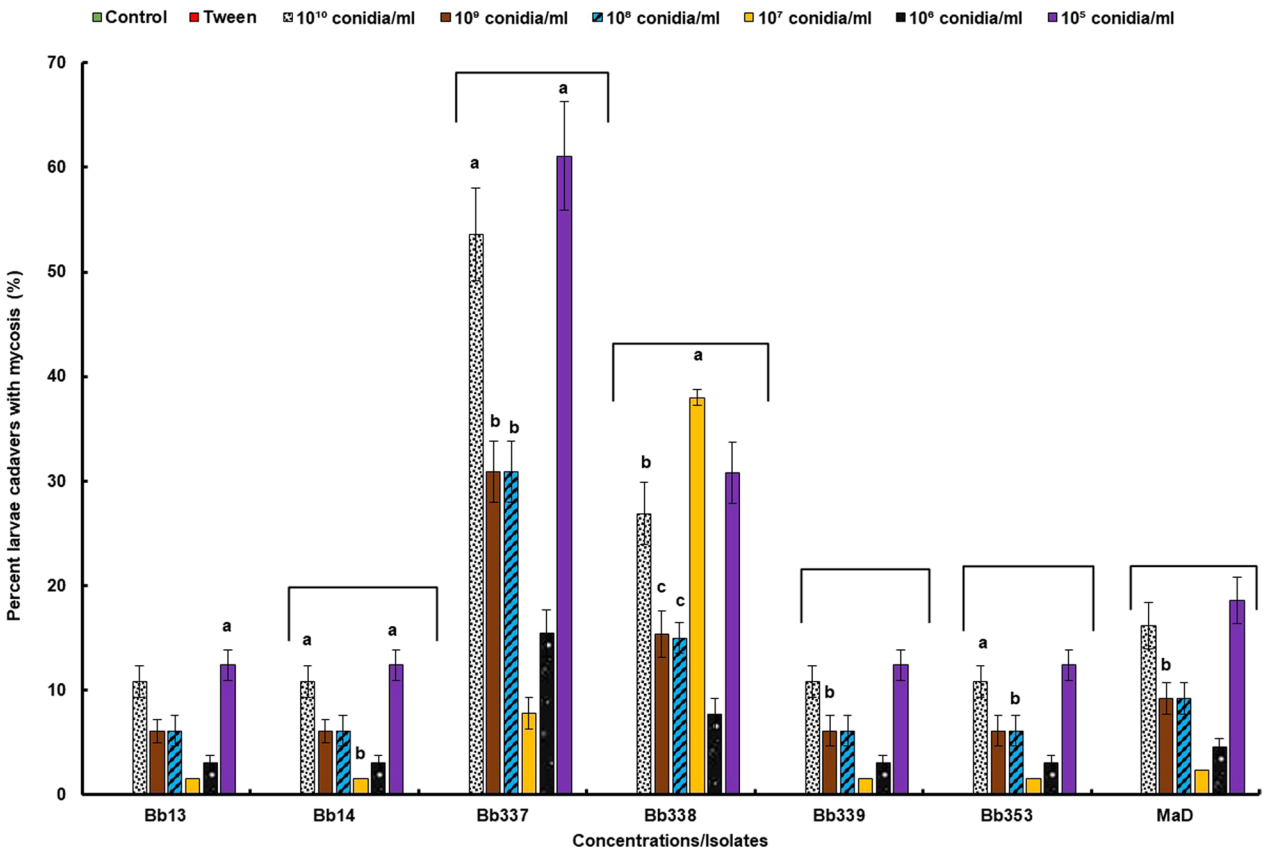

Fig. 4 Cadavers of *Zeugodacus cucurbitae* larvae with fungal growth after treatment different concentrations of isolates of *Beauveria bassiana* and *Metarhizium anisopliae* in larval inoculation method

Enclosed pupae filled to emerge within 15 days after pupation and were fully covered with white mycelium after incubation, the inoculum picked up during the larval stage was enough to kill the insect within the mentioned time. A similar persistence of EPFs infection along the developmental stages persisted also in the pupae leading to 100% mortality of treated medfly, as well as for other insect species (Hallouti et al. 2020). Again, the highest emergence rate was observed in the controls. Isolates significantly reduced subsequent adult emergence. Sporulation could be due to the relative duration of the fungal infection process regarding the insect cycle and could have practical side, increasing then the total effect of the fungal isolate (Beris et al. 2013). It was noticed that emerged adult flies from enclosed pupae died within 3–5 days and that dead adults showed fungal growth (up to 18% sporulation rate with Bb338) after incubation. However, most of the fruit fly's species acquired sexual maturity. This demonstrated that emerged adults might have not copulated within that time to produce eggs for the next generation.

When pupae were treated with different isolates, more than 50% sporulation rate was recorded, when treated with Bb337, Bb338, and MaD. Isolates Bb337 and Bb338 demonstrated an effect on adults of *Z. cucurbitae* when applied directly with a sporulation rate of 28.84 and 36% respectively. The results were following what Dimbi et al. (2003) found adult mortality in *Ceratitis capitata* (Weidemann) and *C. rosa* var. *fasciventris* (Karsch) treated with different isolates of *B. bassiana* and *M. anisopliae* were 7–100% and 11.4–100%, respectively, at 4 days post-inoculation. These results were confirmed by Quesada-Moraga et al. (2006) who reported 30–100% mortality after 20 days, while testing 10 isolates of *B. bassiana* and five isolates of *M. anisopliae* against adult fruit fly, *C. capitata*. Sookar et al. (2008) reported the pathogenicity of seven isolates of *M. anisopliae*, five isolates of *B. bassiana* and two isolates of *Paecilomyces fumosoroseus* (Wise) in adults of *Bactrocera zonata* (Saunders) and *B. cucurbitae* (Coquillett). The vulnerability of adult tephritid flies to EPFs was also confirmed by results reported by (Usman et al. 2021).

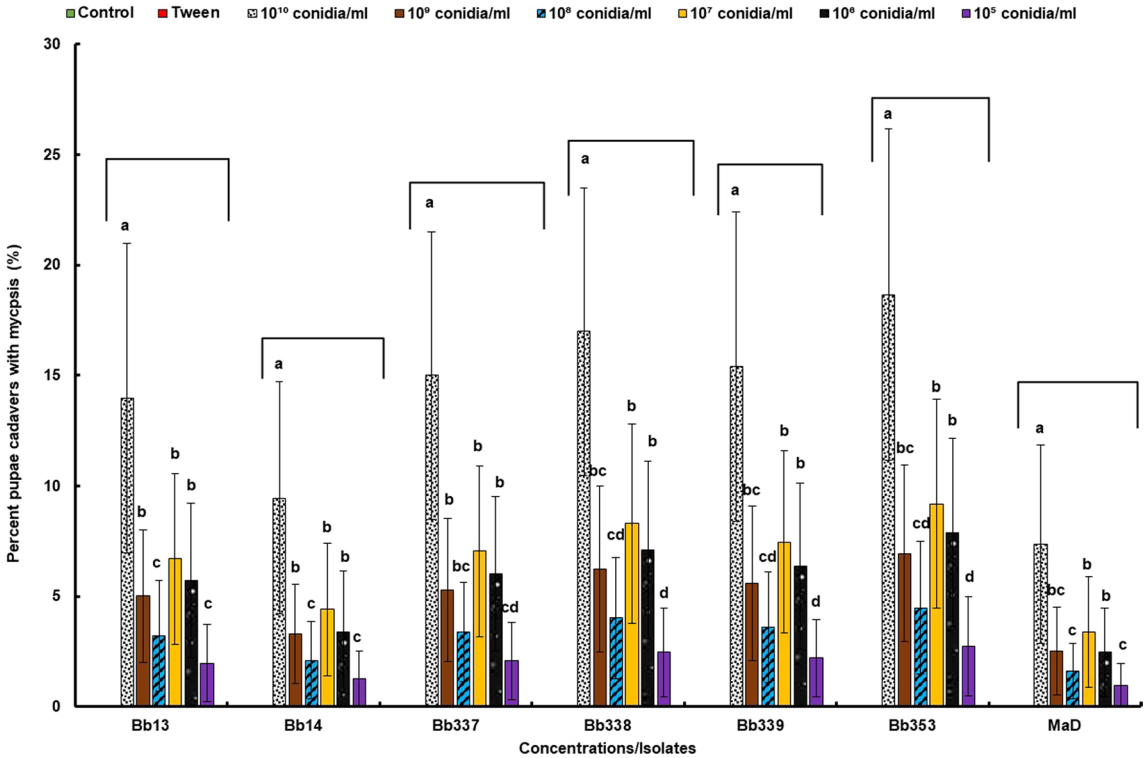

Fig. 5 Cadavers of *Zeugodacus cucurbitae* pupae with fungal growth after treatment different concentrations of isolates of *Beauveria bassiana* and *Metarhizium anisopliae* in larval inoculation method

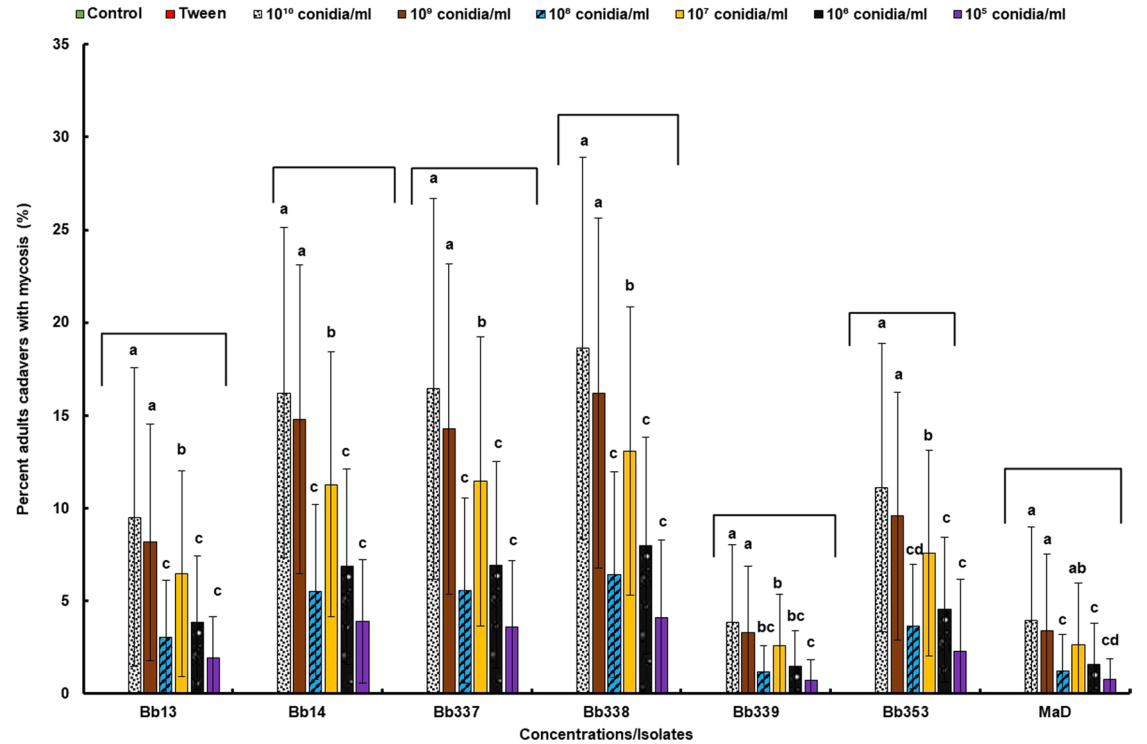

Fig. 6 Cadavers of *Zeugodacus cucurbitae* adults emerged from enclosed pupae with fungal growth after treatment different concentrations of isolates of *Beauveria bassiana* and *Metarhizium anisopliae* in larval inoculation method

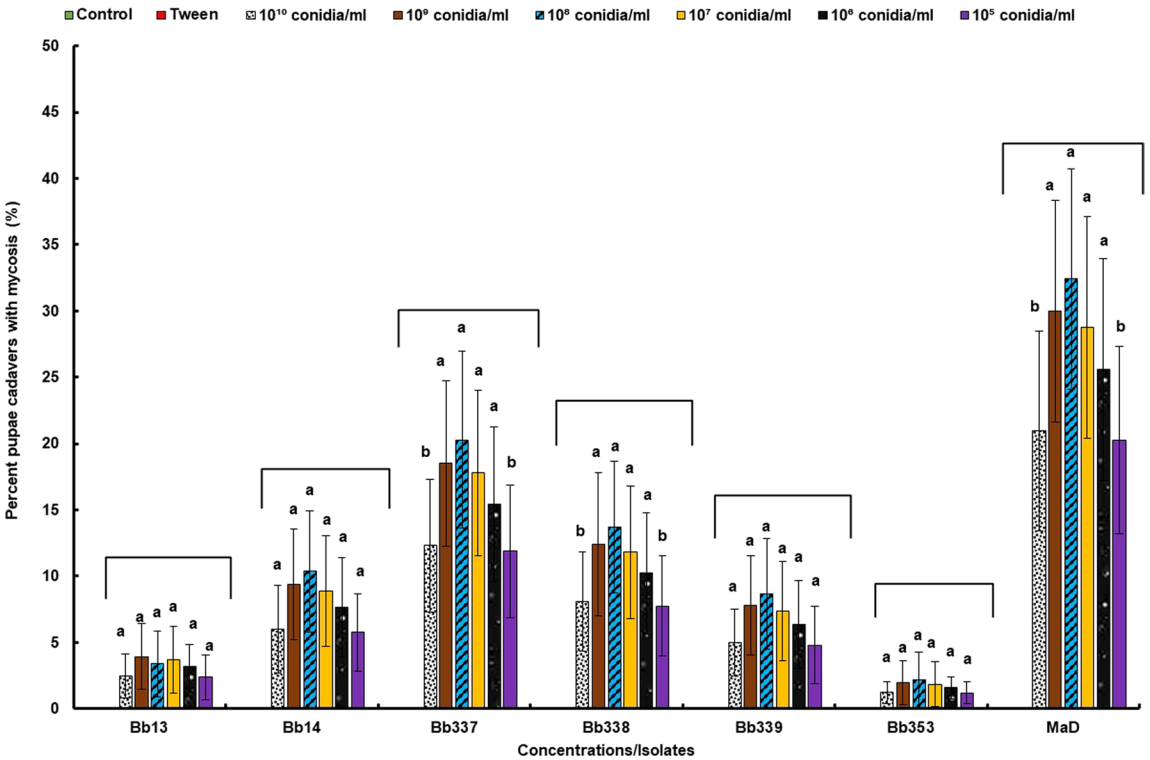

Fig. 7 Cadavers *Zeugodacus cucurbitae* pupae with fungal growth after treatment with different concentrations of isolates of *Beauveria bassiana* and *Metarhizium anisopliae* in pupal inoculation method

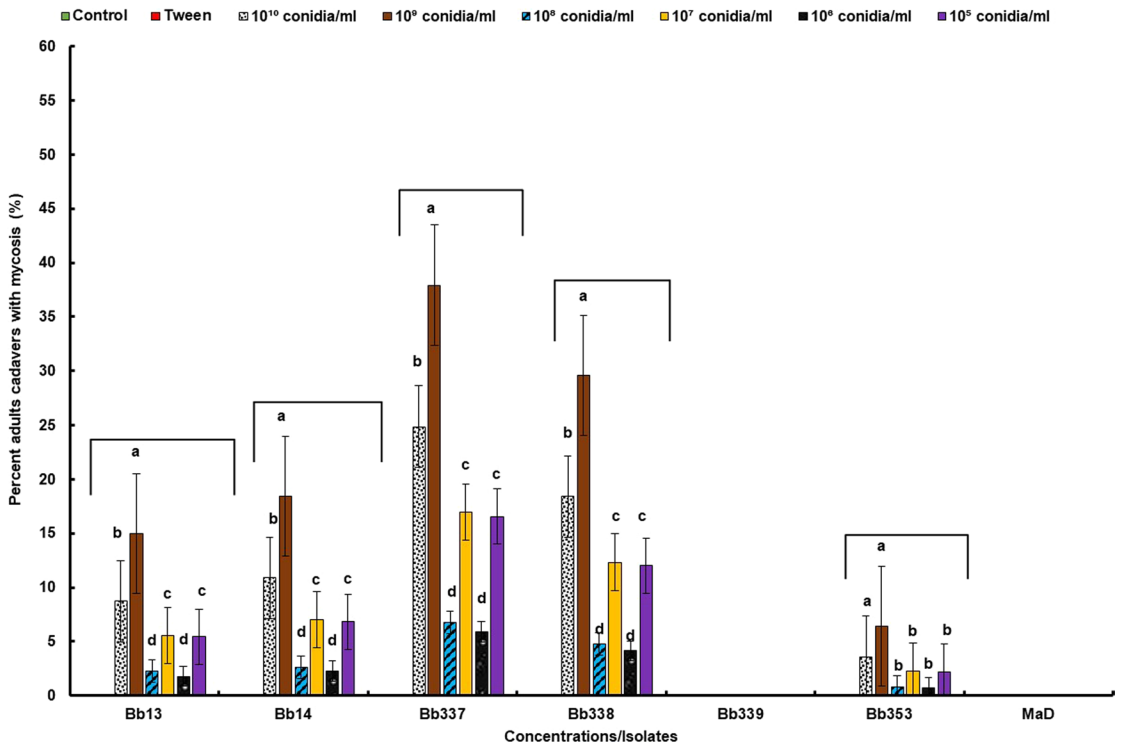

Fig. 8 Cadavers *Zeugodacus cucurbitae* adults with fungal growth after treatment with different concentrations of isolates of *Beauveria bassiana* and *Metarhizium anisopliae* in pupal inoculation method

Table 1 Number of collected pupae from infected adults

Treatments	Bb13	Bb14	Bb337	Bb338	Bb339	Bb353
Control	2.64 ± 0.98b	3.79 ± 1.36b	2.17 ± 0.83b	2.13 ± 0.81b	3.74 ± 1.34b	1.98 ± 0.76b
Tween80	0.81 ± 0.33a	1.15 ± 0.46a	0.66 ± 0.28a	0.65 ± 0.27a	1.14 ± 0.45a	0.61 ± 0.27a
10^{10} conidia/ml	0.45 ± 0.20a	0.64 ± 0.28a	0.37 ± 0.17a	0.35 ± 0.16a	0.63 ± 0.27a	0.33 ± 0.15a
10^{9} conidia/ml	0.16 ± 0.09a	0.22 ± 0.13a	0.13 ± 0.08a	0.13 ± 0.07a	0.22 ± 0.12a	0.12 ± 0.07a
10^{8} conidia/ml	0.91 ± 0.37a	1.30 ± 0.51a	0.74 ± 0.31a	0.73 ± 0.30a	1.28 ± 0.50a	0.68 ± 0.28a
10^{7} conidia/ml	0.16 ± 0.09a	0.23 ± 0.13a	0.13 ± 0.08a	0.22 ± 0.13a	0.12 ± 0.07a	0.12 ± 0.07a
10^{6} conidia/ml	0.43 ± 0.19a	0.61 ± 0.26a	0.35 ± 0.16a	0.34 ± 0.15a	0.60 ± 0.26a	0.32 ± 0.15a
10^{5} conidia/ml	1.19 ± 0.47b	1.70 ± 0.65a	0.98 ± 0.39a	0.96 ± 0.39a	1.68 ± 0.64a	0.89 ± 0.36a

*Means within rows followed by the same letters are non-significantly different at $\alpha = 0.05$

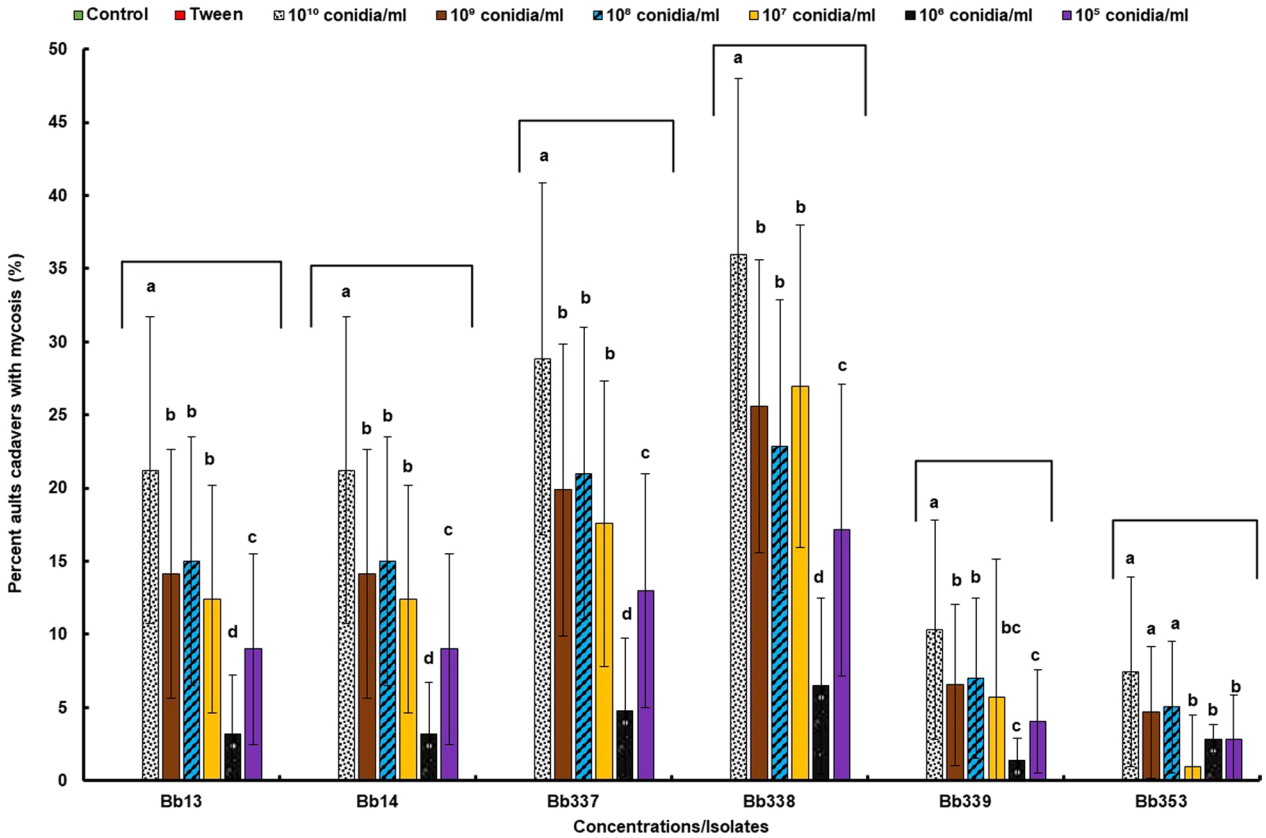

Fig. 9 Cadavers *Zeugodacus cucurbitae* adults with fungal growth after treatment with different concentrations of isolates of *Beauveria bassiana* and *Metarhizium anisopliae* in adult inoculation method

Several studies have confirmed the ability of EPFs to be transmitted horizontally. Examples include *A. ludens (*Ekesi et al. 2002), *C. capitata* (Beris et al. 2013), *B. zonata*, and *B. cucurbitae* (Sookar et al. 2014). In the present study, infected males of both species were highly infectious to females. This is in line with Quesada Moraga et al. (2004) which observed that *B. bassiana* and *M. anisopliae* reduced fertility and fecundity rates and delayed initial oviposition in *C. capitata*; maximum reductions in fertility and fecundity were detected when *C. capitata* adults were treated with *B. bassiana.* Yee and Lacey (2005) detected that *M. anisopliae* treated *R. indifferens* females laid fewer eggs between 3 and 7 days post-inoculation. This type of information is scarce in tephritid flies (Usman et al. 2021).

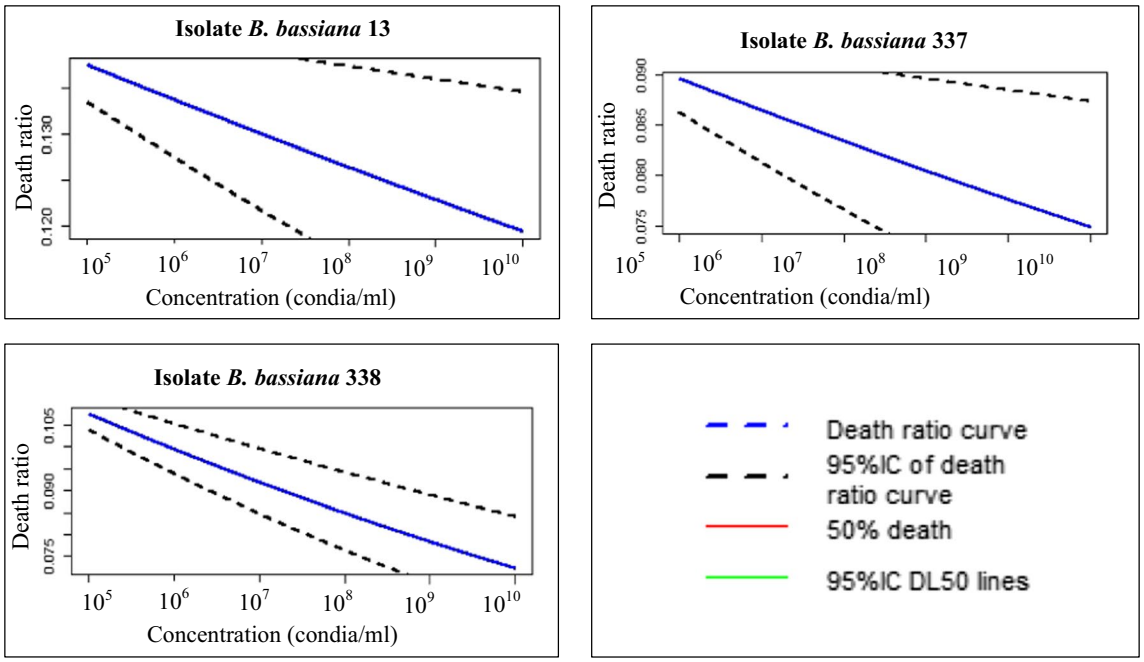

Fig. 10 LC50 values after treatment of the larvae of *Zeugodacus cucurbitae* to various concentrations of Bb13, Bb337 and Bb338

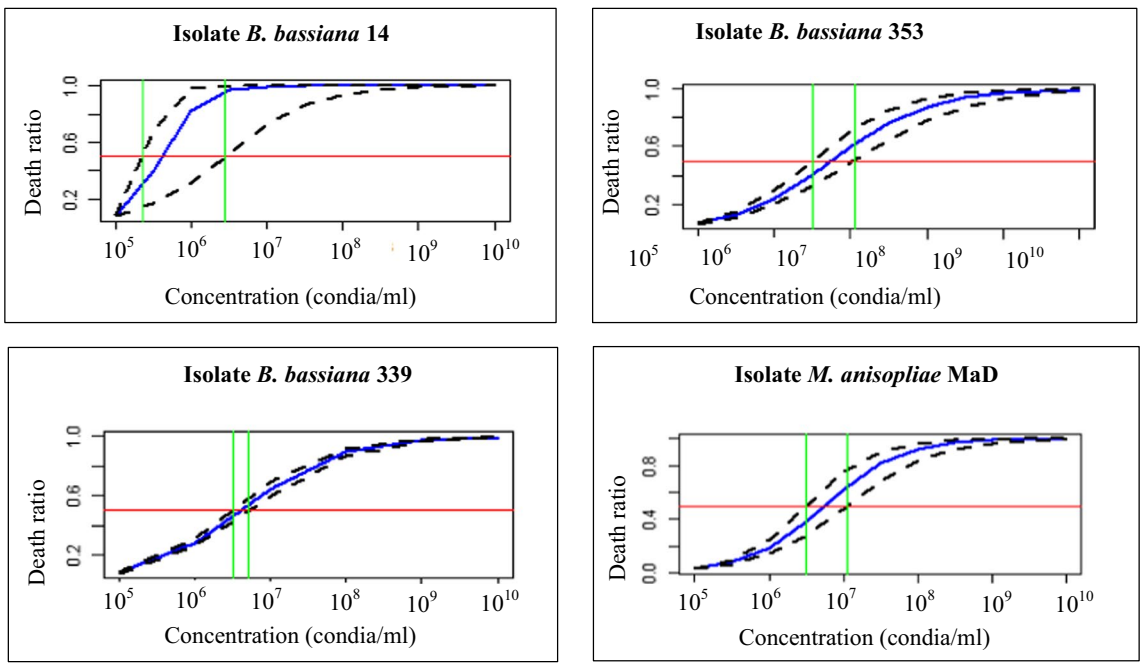

Fig. 11 LC50 values after treatment of larvae of *Zeugodacus cucurbitae* to various concentrations of Bb14, Bb353, Bb339 and MaD

Conclusions

The present study proved that three isolates of *B. bassiana* and *M. anisopliae* (MaD, Bb337, and Bb338) had larvicidal, pupicidal, and adulticidal effects on *Z. cucurbitae*. They induced mortality in all *Z. cucurbitae* host instars development at different concentrations. Therefore, they demonstrated their efficacy against this insect pest under laboratory conditions. Also, it was found out that once the larval stage of the fruit fly is infected, it was likely to cause mortality in the following host

instar developmental stages reducing considerably the population of the pest at any level of its development. In addition to the efficacy of those EPF isolates, they have proved to have no toxic effects regardless of the concentrations used.

Abbreviations

Bb337	*Beauveria bassiana* 3337
Bb338	*Beauveria bassiana* 338
Bb14	*Beauveria bassiana* 14
Bb13	*Beauveria bassiana* 13
PDA	Potato Dextrose Agar
MaD	*Metarhizium anisopliae*
Ma31	*Metarhizium anisopliae* 31
RH	Relative humidity

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Author contributions
The methodology was developed and designed by MVH, OKDK, AAO, AHB-G, RD, and MT. MVH and OKDK conceptualized the ideas, the formulation and evolution of overarching research goals and aims. AAO, AHB-G and MT supervised the work and validated the research goals and aims. MVH, AAO, OKDK, AHB-G, MT and RD validated the methodology by verifying whether as a part of the activity or separate, of the overall replication of results/experiments and other research outputs. OKDK, MFK, DR, AHB-G, MT and RD afforded experimental conditions, facilities and advised in experiments specifically study materials, reagents, material, laboratory samples, insects, instrumentation, computing resources, revision of study data. MVH conducted the research assays and investigation process, performed the experiments, statistical analyses, designed the figures and tables, and wrote the manuscript draft. OKDK supervised all experimental set-ups and assisted in statistical analyses and writing/improving the manuscript. OKDK, AAO, AHB-G, MFK, RD and MT provided specific critical review, commentary and revision. AHB-G and MT provided the financial support for the project leading to this publication. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request. All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no conflict of interest.

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