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Pathogenicity of fungal and bacterial bioinsecticides against adult peach fruit fly, *Bactrocera zonata* (Saunders) (Diptera: Tephritidae) admixed with adult diet under controlled conditions

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Abstract

Background: The peach fruit fly, *Bactrocera zonata* (Saunders) (Diptera: Tephritidae), is a serious polyphagous pest of fruits and vegetables. Chemical management of *B. zonata* in fruits results in toxic residues that have adverse health effects on consumers resulting in increased demand for eco-friendly approaches. Laboratory bioassay was conducted to determine the pathogenicity of fungal and bacterial biopesticides against *B. zonata*.

Results: The pathogenicity of all tested bioinsecticides revealed that the maximum concentration (1×10^8 CFU ml^{-1}) of *Metarhizium anisopliae*, *Beauveria bassiana*, *Lecanicillium lecanii* and *Bacillus thuringiensis* caused 95.8–100%, 95.9–97.9%, 32.7–39.6%, and 20.0–22.4% mortality in *B. zonata*, respectively at 7 d post-application interval (PAI) as compared to mortality demonstrated by the same concentration at 5 d PAI. All tested microbial insecticides induced statistically similar mortality in both male and female *B. zonata* at each concentration for the same PAI. Correlation coefficient (*r*) values reveal that concentrations of each microbial insecticide had a high positive correlation with mortalities of male and female *B. zonata*. Regression parameters reveal that concentrations of tested microbial insecticides had significant linear relationship with and explained significant variability in *B. zonata* mortality ($P < 0.05$). Results also revealed that *M. anisopliae* was proved more pathogenic to males and females of *B. zonata* demonstrating the least LC_{50} values (5.48×10^3, and 6.17×10^3 CFU ml^{-1}, respectively) 7 d post-application intervals, followed by *B. bassiana* which explained LC_{50} value of 1.14×10^4 CFU and 1.15×10^5 CFU ml^{-1} for *B. zonata* males and females, respectively, at the same period of application, but less than that of *L. lecanii* (2.77×10^9 and 1.43×10^9 CFU ml^{-1}) and then *B. thuringiensis* (3.40×10^{10} and 1.39×10^{10} CFU ml^{-1}) for the males and females, respectively, at 7 d PAIs.

Conclusion: *Metarhizium anisopliae* incorporated adult diet was proved more effective against *B. zonata,* followed by *B. bassiana*, *L. lecanii*, and *B. thuringiensis*. Hence, *M. anisopliae* can be recommended for incorporating in bait-traps to develop attract-and-kill technology for *B. zonata*.

Keywords: *Bactrocera zonata*, Pathogenicity, Fungal-based formulations, Bacterial-based formulations, Diet-bioassay

Background

The peach fruit fly, *Bactrocera zonata* (Saunders) (Diptera: Tephritidae), is a serious polyphagous pest of fruits and vegetables that globally attacks over 50 cultivated

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and wild plants, mainly those with fleshy fruits including guava, mango, peach, apricot, citrus, and figs (El-Minshawy et al. 2018). *B. zonata* has globally attained the status of economic and quarantine pest. Various published reports reveal that *B. zonata* is the most dominant, devastating, and abundantly found fruit fly species in different ecological regions of Pakistan infesting variety of fruits and vegetables (Ahmad and Begum 2017). In many countries, management of *B. zonata* is difficult due to the behavioral, feeding, and biological adaptability of various life stages of fruit fly and the lack of effective broad-spectrum insecticides from markets (Dias et al. 2018).

In developing countries, management of fruit flies mostly depends upon the cover spray of synthetic insecticides because of their quick knockdown impacts (De Bon et al. 2014; Nicholson 2007). Such cover spray not only causes ecological backlashes in fruit flies against insecticides but also induces lethality to non-target beneficial arthropods and phytotoxic effects on plants (Li et al. 2018). Insecticide applications also increase the cost of production and leaves toxic residues in fruits and vegetables causing biomagnification of residues in human (Gogi et al. 2010).

The microbial agents in the form of biopesticides can be a better alternative to synthetic pesticides and an effective part of integrated pest management (IPM) strategies for the control of several agricultural insect pests (Farooq et al. 2020). Spray application and auto-dissemination (through the use of attractive materials/devices to propagate pathogens in target pest populations) (Vega et al. 2007) are used mainly for the introduction of microbial agents into an agro-ecosystem (Talaei-Hassanloui et al. 2007). Entomopathogenic fungi (EPF) typically cause infection when spores come in contact with the arthropod host (Goettel et al. 2008).

EPF have shown very promising results against various species of fruit flies (Soliman et al. 2020). Different strains of *Beauveria bassiana*, *Paecilomyces fumosoroseus*, *Lecanicillium lecanii*, and *Metarhizium anisopliae* are used for insect pest management (Lacey et al. 2001). Microbial control is a potentially useful method to inhibit fruit flies (Soliman et al. 2020). Recently, entomopathogens as natural enemies have been used to reduce the population of fruit flies, *Ceratitis capitata*, *B. oleae*, and *Z. cucurbitae* (Dias et al. 2018). Studies of some researchers confirmed that *M. anisopliae* has a very high potential in suppressing fruit flies (Dimbi 2003). Introduction of entomopathogenic bacteria (EPB), *Bacillus thuringiensis* subspecies darmadiensis, mixed with a protein diet and sugar as a bait was found very effective in killing South American fruit fly, *Anastrepha ludens* (Martinez et al. 1997).

EPF prove very effective against larvae and pupae of fruit flies when they come in contact with the treated soil (Ekesi et al. 2007). Oral and contact bioassays of *B. bassiana* and *B. brongniartii* against *B. oleae* and *C. capitata* were found effective for fruit flies (Konstantopoulou and Mazomenos 2005). EPF such as *B. bassiana*, *Isaria fumosorosea*, and *M. anisopliae* demonstrated 90–100% mortality and induced significant impact on the fecundity of European cherry fruit fly, *Rhagoletis cerasi*, while foliar application of *B. bassiana* caused 65% of infection in cherry orchards (Daniel and Wyss 2010).

Toledo et al. (2017) applied three formulations of *B. bassiana* by autoinoculation devices and sterile-male-vector technique in coffee orchards for assessing pathogenicity against *C. capitata* and concluded that application of *B. bassiana* by later technique proved more effective in the horizontal transmission of conidia to wild-population of *C. capitata*, but both techniques demonstrated >90% reduction in *C. capitata* (Flores et al. 2013). EPF can be applied in form of a bait station against fruit flies (Navarro-Llopis et al. 2015). Application of *M. anisopliae*-based attractant-contaminant device (ACD) @ 24 ACD ha^{-1} is an efficient technique for the control of *C. capitata* up to 3 months when inoculation dishes are replaced mid-season (Navarro-Llopis et al. 2015).

The present research was conducted to evaluate the fungal and bacterial bioinsecticide-based diet (*M. anisopliae*, *B. bassiana*, *L. lecanii*, and *B. thuringiensis* var *kurstaki* against both male and female flies of *B. zonata* under controlled conditions.

Methods

Mass rearing of *Bactrocera zonata*

Guava fruits infested with fruit flies were collected from different orchards in Faisalabad. *Bactrocera zonata* was identified on the basis of four morphological characteristics as described by White and Elson-Harris (1996). The infested fruits were taken into the laboratory and kept in card boxes half-filled with sieved and sterilized sand. Pupae were collected from sand by using a fine-mesh sieve after a week. The pupae were kept in the dome-shaped rearing cages till the adult emergence. The cages were provided with the spongy strips soaked with the adult diet containing honey, protein and sugar solution (1 part sugar and 9 parts water) mixed in a 1:1:9 ratio. These strips were suspended after soaking in an adult diet solution. The fresh, properly cleaned and washed guava fruits were brought to the laboratory and hanged inside the rearing cage for eggs collection. Then, after 3 d, fruits were shifted from rearing cage to card boxes having

sterilized sand for attaining the next progeny. This procedure was used to mass culture *B. zonata*.

Acquisition of fungus and bacterial-based biopesticides

Four talc-based biopesticides, *M. anisopliae* (MCC 0051) (Pacer®), *B. bassiana* (MCC 0044) (Pacer®), *L. lecanii* (MCC 0058) (Mealikil®) and *B. thuringiensis* var *kurstaki* (MCC 0089) (Lipel®) were acquired from AgriLife SOM Phytopharma (India) Limited® (www.agrilife.in). As per commercial formulation, 1 g powder of each fungal and bacterial strain contains 1×10^8 colony-forming unit/gram (CFU g^{-1}).

Fungal concentrations

Commercial formulations of *B. bassiana*, *M. anisopliae*, and *L. lecanii* were used to prepare six concentrations (1×10^8, 1×10^7, 1×10^6, 1×10^5, 1×10^4 and 1×10^3 CFU ml^{-1}) of each. As per commercial formulation, 1 g powder of each fungal and bacterial strain contains 1×10^8 CFU ml^{-1}.

Preparation and pouring of ¼ SDAY media for culturing fungal strains

A quantity of 16.25 g Saburaud dextrose agar, 11.25 g agar, and 1.25 g yeast was added in 1 liter of distilled water and autoclaved at 20 psi and 121 °C for 20 min. After autoclaving, the media was poured into Petri plates and was allowed to cool at room temperature.

Culturing of fungal strains

One gram of powder of each of the commercially available strains i.e., *B. bassiana*, *M. anisopilae* and *L. lecanii* was added in 1 ml of distilled water separately in 15-ml vortex tubes to prepare conidial suspension and covered with aluminum foil. Each vortex tube was vortexed for 1 min and then 1 ml of conidial suspension was taken and sprinkled onto a separate ¼ SDAY media plate for inoculation. After inoculation on ¼ SDAY media plates, the conidial suspension was spread on the media plate with the help of a sterile inoculating loop and then plates were incubated at 28 °C for 20–30 d.

Harvesting the conidia and preparation of different concentrations

The fungal culture was harvested by flooding 5 ml of 0.04% (vol:vol) sterile polysorbate-20 (Tween 20, Sigma-Aldrich) solution in water (0.4 ml Tween-20 in 999.6 ml H_2O, autoclaved for 20 min at 121 °C) on culture-plate and fungal conidia were harvested from media/culture-plate with the help of loop for detaching the conidia from hyphae. The resulting suspension was poured into a 15 ml sterile conical tube which was vortexed to disrupt clumping. This conidial suspension was used as a stock solution. A volume of 100 µl was taken from the stock solution and added into 900 µl of 0.04% Tween-20 in a vortex tube. Again 100 µl of this diluted stock solution was taken added to 900 µl of 0.04% Tween-20 in a vortex tube. A volume of 10 µl of second time the diluted stock solution was micropipette, spelled out on the counting chamber of hemocytometer and covered with glass cover. Then the number of conidia was counted on the counting chamber of the hemocytometer under a microscope (hemocytometer count). The conidial concentration of the stock solution was calculated by the following formula (Iqbal et al. 2020):

$$\text{Concentration of stock solution} = \text{Haemocytometer count} \times 10^4 \times \text{Dilution factor}$$

The final volume of stock solution required to prepare each concentration was determined by the following formula (Iqbal et al. 2020):

$$V_{\text{Final}} = \frac{V_{\text{Stock}} \times C_{\text{Stock}}}{C_{\text{Final}}}$$

where V_{Final} = Final volume of stock solution needed to prepare required concentration; V_{Stock} = Volume of stock solution; C_{Stock} = Concentration of stock solution; C_{Final} = Final concentration to be prepared.

Conidial viability test

Conidial viability was assessed by plating 100 µl of the second dilution of stock solution (100-fold dilution) on ¼ SDAY media. The media plates were then incubated for 24 h at 28 °C. Then, three random groups of 100 conidia were inspected. Germination of conidia was considered only when germ-tube grew longer than half of the diameter of the conidium projects from it (Parsa et al. 2013). After counting the germinating conidia, percent germination was estimated by the following formula (Iqbal et al. 2020):

$$\text{Percent germination} = \frac{\text{Germinating spores}}{\text{Total spores in group}} \times 100$$

The whole of the above-mentioned procedural protocols was used for the commercial formulation of each tested EPF to prepare their respective eleven concentrations (1×10^8, 1×10^7, 1×10^6, 1×10^5, 1×10^4 and 1×10^3 CFU ml^{-1}). In the case of each fungi > 90% conidial germination was estimated. So, the bioassay study of each EPF was conducted against *B. zonata* adults.

Bacterial culturing, harvesting, concentrations preparation, and viability test

Same procedures and protocols, as used for fungi, were used for culturing, harvesting, concentrations preparation and viability testing of bacteria. The growth media used for bacterial culturing was broth media.

Bioassay study

A solution of 1 ml of each treatment (concentration) was pipetted onto an adult diet (honey, egg yolk, protein hydrolysate, and sugar water solution) in disposable cups having lids. The solution was then admixed with fruit fly adult diet with the help of a sterilized loop. The treatment-baited adult diet was lapped partially on the walls of the treatment unit (plastic jar) as well as placed inside the treatment unit in a disposable cup. A mixed population of newly emerged 50 males and 50 females adults of *B. zonata* were aspirated from culture and released into treatment unit which was maintained at 28 °C and 70% ± 5 RH for 24 h. The flies were let to feed on a treatment-baited adult diet for 24 h. After an exposure period of 24 h, the flies were transferred to the fruit fly adult rearing unit (plastic jars) having above-mentioned normal fruit fly adults that were maintained at 28 ± 2° C for 14 d. The mortality of adult flies of *B. zonata* was recorded after 5 d and 7 d. The dead flies were placed on respective growth media to promote the growth of fungal mycelia (mycosis) from treated flies and confirm that the death of flies is caused by a fungal infection.

Data analysis

Mortality data were transformed into percent corrected mortality by Abbot Formula (Abbott 1925):

$$\text{Corrected mortality \%} = 1 - \frac{\text{Number in Treated unit after treatment}}{\text{Number in Control unit after treatment}} \times 100$$

This transformed corrected mortality data were analyzed by ANOVA 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 (HSD) test was performed to compare the mean values of significant treatments (Danho et al. 2002).

LC_{50}, LC_{75}, LC_{95}, LT_{50} and LT_{90} values and their associated significant descriptive parameters (values of degree of freedom, *P* value, fiducial limits, Chi-square, and slope) were computed for each bioinsecticides by applying probit analysis on mortality data using the Minitab Statistical Program (Finney 1971). The products were screened out for their efficacy based on their LC_{50}, LC_{95}, LT_{50} and LT_{90} values.

Linear regression and Pearson correlation analyses were also performed at α value of 5% to establish regression between *B. zonata* mortality and concentrations. The coefficient of determination (R^2), coefficient of correlation, and linear regression equation were computed to assess the nature and strength of association between concentrations of each bioinsecticide and *B. zonata* adult mortality. Scatter diagrams were also plotted for each bioinsecticide to determine the trend of the fitted simple regression line of $\hat{Y}$ (mortality) on X (concentration) of each bioinsecticide.

Results

Mortality of *Bactrocera zonata* exposed to fungal and bacterial bioinsecticides at different post-application intervals

The mortality results depict that all tested fungal and bacterial bioinsecticides demonstrated significantly different mortality against *B. zonata* at two PAIs ($P < 0.05$) (Figs. 1, 2, 3 and 4). An exposure interval and concentration-dependent mortality in both sexes of *B. zonata* was explained by all tested bioinsecticides.

Administration of *M. anisopliae*, *B. bassiana*, *L. lecanii* and *B. thuringiensis* in adult diet explained 8.0–42.0% and 6.1–38.8% (Fig. 1a); 4.0–38.0% and 0.0–40.8% (Fig. 2a); 2.0–26.0% and 0.0–22.4% (Fig. 3a); and 0.0–12% and 2.0–18% (Fig. 4a) mortality in *B. zonata* males and females, respectively, was significantly higher at higher concentration (1×10^8 CFU ml^{-1}) and lower at lower concentration (1×10^3 CFU ml^{-1}) at 5 d PAI (Figs. 1, 2, 3 and 4a). At PAI of 7 d, *M. anisopliae*, *B. bassiana*, *L. lecanii* and *B. thuringiensis* demonstrated mortality in the range of 45.8–95.8% and 39.6–100.0% (Fig. 1b); 22.4–95.9% and 31.3–97.9% (Fig. 2b); 6.1–32.7% and 1.3–39.6% (Fig. 3b); and 2.0–20.0% and 2.0–22.4% (Fig. 4b) in *B. zonata* males and females, respectively, being significantly higher at higher concentration (1×10^8 CFU ml^{-1}) and lower at lower concentration (1×10^3 CFU ml^{-1}) (Figs. 1, 2, 3 and 4b). These results also explain that all the tested microbial insecticides demonstrated more than 22% mortality at higher concentration (1×10^8 CFU ml^{-1}) at 7 d PAI; while less than 12% mortality at all concentration (1×10^3 to 1×10^8 CFU ml^{-1}) at 3 d PAI in both sexes of *B. zonata* (Figs. 1, 2, 3 and 4).

Nevertheless, the maximum concentration of *M. anisopliae*, *B. bassiana*, *L. lecanii* and *B. thuringiensis* (1×10^8 CFU ml^{-1}) caused 1.3-times and 1.6-times (Fig. 1); 1.5-times and 1.4-times (Fig. 2); 0.3-times and 0.8-times (Fig. 3); and 0.7-times and 0.2-times (Fig. 4) higher mortality in *B. zonata* males and females, respectively at 10 d PAI as compared to mortality demonstrated at the same concentration at 5 d PAI (Figs. 1, 2, 3 and 4).

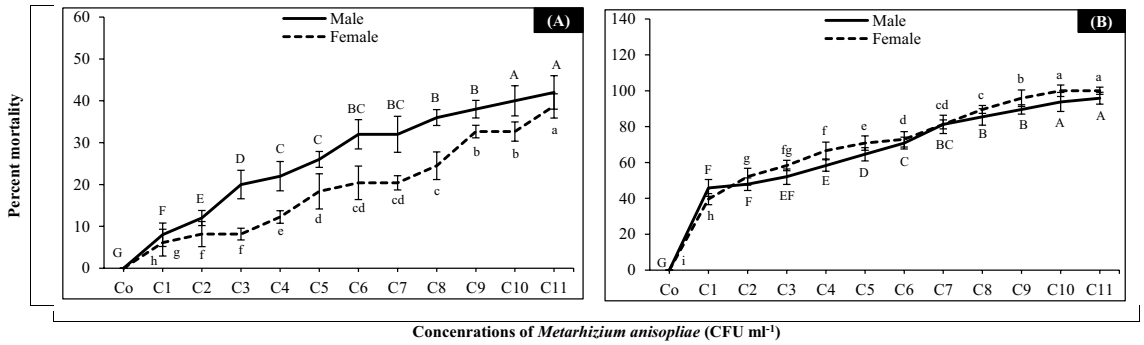

Fig. 1 Percent mortality of both sexes of *Bactrocera zonata* for different concentrations of *Metarhizium anisopliae* (solid and square-dotted lines represent mortality of male and female *Bactrocera zonata*, respectively; Capital and small letters represent mortality of male and female *Bactrocera zonata*, respectively) at 5 days post-exposure intervals[(a)] and 7 days post-exposure intervals[(b)]. Concentrations on *x-axis* represents **C0** = Control, **C1** = 1×10^3 CFU ml^{-1}, **C2** = 5×10^3 CFU ml^{-1}, **C3** = 1×10^4 CFU ml^{-1}, **C4** = 5×10^4 CFU ml^{-1}, **C5** = 1×10^5 CFU ml^{-1}, **C6** = 5×10^5 CFU ml^{-1}, **C7** = 1×10^6 CFU ml^{-1}, **C8** = 5×10^6 CFU ml^{-1}, **C9** = 1×10^7 CFU ml^{-1}, **C10** = 5×10^7 CFU ml^{-1}, **C11** = 1×10^8 CFU ml^{-1}. Error-bars indicate the ± standard error; means sharing similar style letters do not significantly differ at probability level of 5%. The mean values of lines (solid and square-dotted) bearing similar letters do not differ significantly

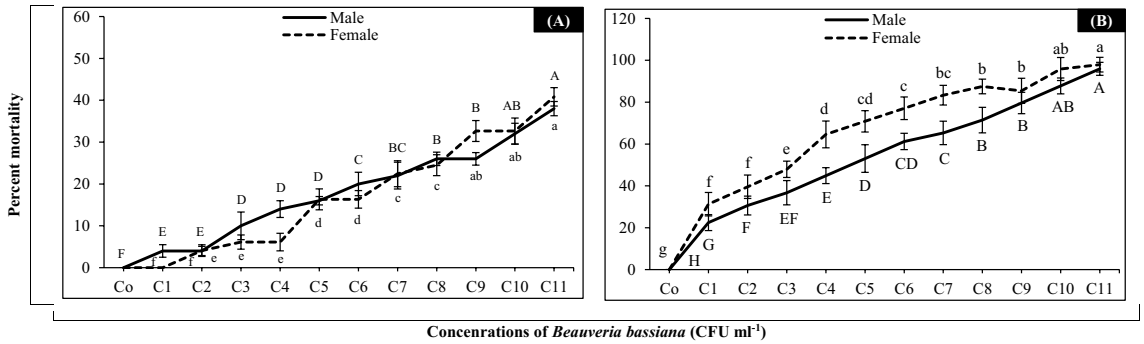

Fig. 2 Percent mortality of both sexes of *Bactrocera zonata* for different concentrations of *Beauveria bassiana* (solid and square-dotted lines represent mortality of male and female *Bactrocera zonata*, respectively; Capital and small letters represent mortality of male and female *Bactrocera zonata*, respectively) at 5 days post-exposure intervals[(a)] and 7 days post-exposure intervals[(b)]. Concentrations on *x-axis* represents **C0** = Control, **C1** = 1×10^3 CFU ml^{-1}, **C2** = 5×10^3 CFU ml^{-1}, **C3** = 1×10^4 CFU ml^{-1}, **C4** = 5×10^4 CFU ml^{-1}, **C5** = 1×10^5 CFU ml^{-1}, **C6** = 5×10^5 CFU ml^{-1}, **C7** = 1×10^6 CFU ml^{-1}, **C8** = 5×10^6 CFU ml^{-1}, **C9** = 1×10^7 CFU ml^{-1}, **C10** = 5×10^7 CFU ml^{-1}, **C11** = 1×10^8 CFU ml^{-1}. Error bars indicate the ± standard error; means sharing similar style letters do not significantly differ at probability level of 5%. The mean values of lines (solid and square-dotted) bearing similar letters do not differ significantly

The aforementioned results of present experiment explain that mortality of both sexes of *B. zonata* decreased with decreasing concentrations of each tested microbial insecticides; however, maximum mortality in male and female *B. zonata* was demonstrated by tested microbial insecticides at their highest concentration (1×10^8 CFU ml^{-1}). The results of present research also demonstrated that *M. anisopliae* and *B. bassiana* explained approximately 95 to 100% mortality in *B. zonata* at the highest concentrations (1×10^8 CFU ml^{-1}). The results of the present study also explained that *M. anisopliae*, *B. bassiana*, *L. lecanii*, and *B. thuringiensis* induced statistically similar mortality in both male and female *B. zonata* at each concentration for the same PAI (Figs. 1, 2, 3 and 4).

Regression and correlation between mortality of *Bactrocera zonata* and concentrations of fungal and bacterial bioinsecticides

The probability values for correlation ($P < 0.05$) confirm that an association existed between concentrations and mortalities of male and female flies of *B. zonata* for *M. anisopliae*, *B. bassiana*, *L. lecanii*, and *B. thuringiensis* (Figs. 5, 6, 7 and 8).

The correlation coefficient values (r) and scatter diagrams reveal that concentrations had a high positive correlation with mortalities of male and female *B. zonata* for *M. anisopliae*, *B. bassiana*, *L. lecanii*, and *B. thuringiensis* as the coefficient of correlation values were more decimated to positive one (+1) value if estimated to

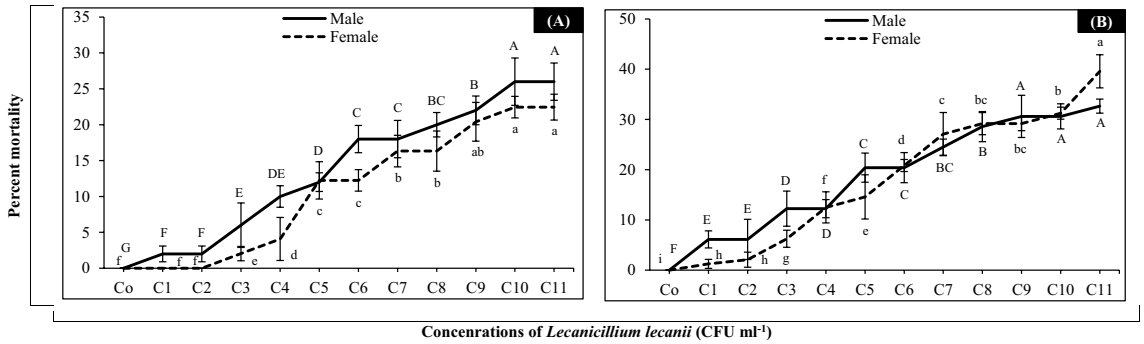

Fig. 3 Percent mortality of both sexes of *Bactrocera zonata* for different concentrations of *Lecanicillium lecanii* (solid and square-dotted lines represent mortality of male and female *Bactrocera zonata*, respectively; Capital and small letters represent mortality of male and female *Bactrocera zonata*, respectively) at 5 days post-exposure intervals[(a)] and 7 days post-exposure intervals[(b)]. Concentrations on *x-axis* represents **C0** = Control, **C1** = 1×10^3 CFU ml^{-1}, **C2** = 5×10^3 CFU ml^{-1}, **C3** = 1×10^4 CFU ml^{-1}, **C4** = 5×10^4 CFU ml^{-1}, **C5** = 1×10^5 CFU ml^{-1}, **C6** = 5×10^5 CFU ml^{-1}, **C7** = 1×10^6 CFU ml^{-1}, **C8** = 5×10^6 CFU ml^{-1}, **C9** = 1×10^7 CFU ml^{-1}, **C10** = 5×10^7 CFU ml^{-1}, **C11** = 1×10^8 CFU ml^{-1}. Error-bars indicate the ± standard error; Means sharing similar style letters do not significantly differ at probability level of 5%. The mean values of lines (solid and square-dotted) bearing similar letters do not differ significantly

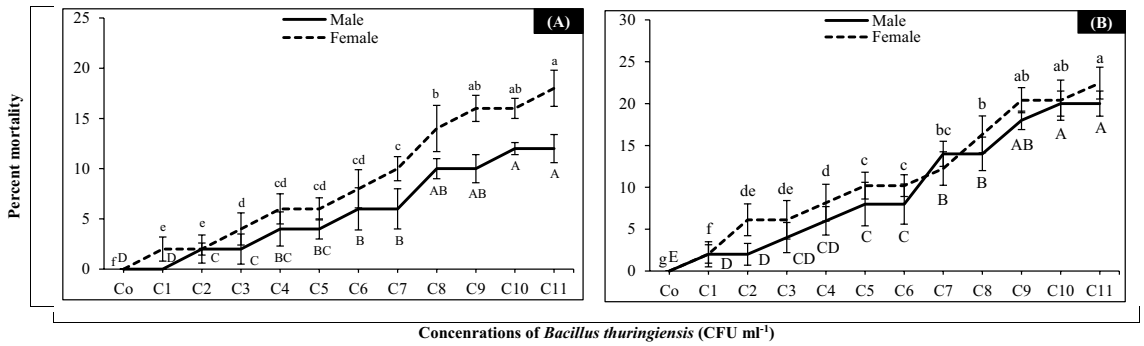

Fig. 4 Percent mortality of both sexes of *Bactrocera zonata* for different concentrations of *Bacillus thuringiensis* (solid and square-dotted lines represent mortality of male and female *Bactrocera zonata*, respectively; Capital and small letters represent mortality of male and female *Bactrocera zonata*, respectively) at 5 days post-exposure intervals[(a)] and 7 days post-exposure intervals[(b)]. Concentrations on *x-axis* represents **C0** = Control, **C1** = 1×10^3 CFU ml^{-1}, **C2** = 5×10^3 CFU ml^{-1}, **C3** = 1×10^4 CFU ml^{-1}, **C4** = 5×10^4 CFU ml^{-1}, **C5** = 1×10^5 CFU ml^{-1}, **C6** = 5×10^5 CFU ml^{-1}, **C7** = 1×10^6 CFU ml^{-1}, **C8** = 5×10^6 CFU ml^{-1}, **C9** = 1×10^7 CFU ml^{-1}, **C10** = 5×10^7 CFU ml^{-1}, **C11** = 1×10^8 CFU ml^{-1}. Error-bars indicate the ± standard error; Means sharing similar style letters do not significantly differ at probability level of 5%. The mean values of lines (solid and square-dotted) bearing similar letters do not differ significantly

significant figure and data points were found scattered close to a positively sloped line (Figs. 5, 6, 7 and 8).

The values of 95% confidence interval (CI) for correlation coefficient (r) explain that correlation between concentrations and mortalities of male and female *B. zonata* varied significantly for two PAI (5 and 10 d) for *M. anisopliae*, *B. bassiana*, *L. lecanii*, and *B. thuringiensis* as none of their 95% CI value overlap with each other (Figs. 5, 6, 7 and 8).

Regression parameters and scatter diagrams reveal that concentrations of all test bioinsecticides had a significant linear relationship and explained significant variability in mortality of male and female *B. zonata* ($P < 0.05$) (Figs. 5, 6, 7 and 8).

Coefficient of determination values ($100R^2$) depict that concentrations of *M. anisopliae* explained 30.91% and 27.42% of the total variability in mortality of *B. zonata* males; while the same attributed 50.97% and 26.67% of the total variability in mortality of *B. zonata* females at 5 and 10 d PAI, respectively (Fig. 5). The concentrations of *B. bassiana* expounded 52.14% and 42.32% of the total variation in mortality of *B. zonata* males, while the same ascribed 52.44% and 26.39% of the total variability in mortality of *B. zonata* females at 5 and 10 d PAI, respectively (Fig. 6). About 39.33% and 33.29% of

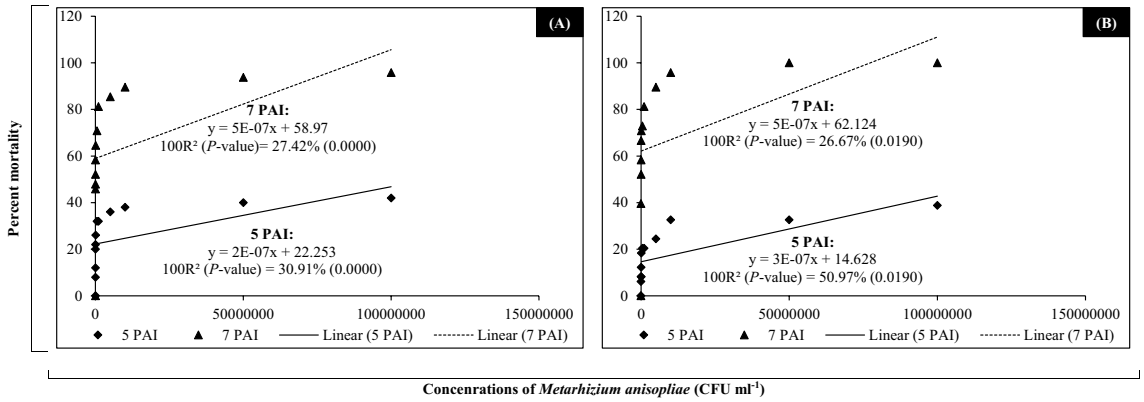

Fig. 5 Linear regression equation ($\hat{Y} = b \pm ax$), coefficient of determination ($100R^2$) and scatter diagram showing the fitted simple regression line of $\hat{Y}$ [percent mortality of adult male *Bactrocera zonata*[(a)] and adult female *Bactrocera zonata*[(b)]] on X [Concentrations of *Metarhizium anisopliae*] at different days post-application intervals

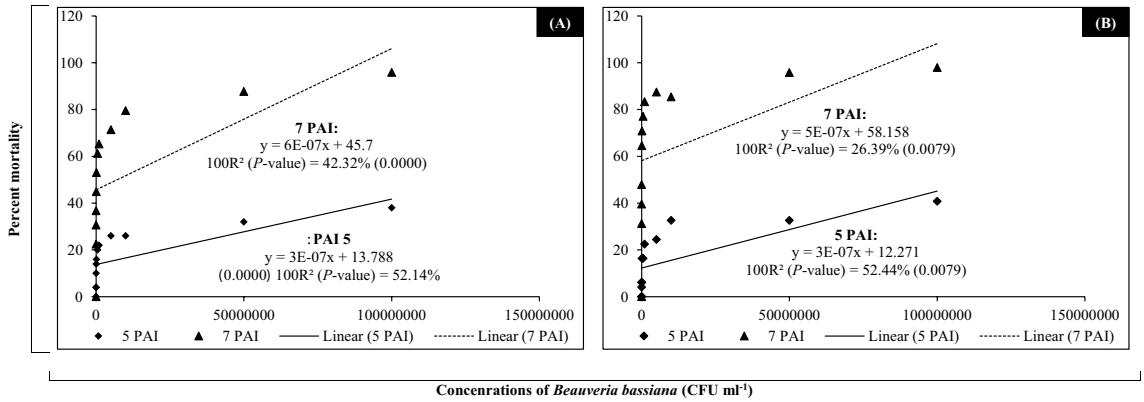

Fig. 6 Linear regression equation ($\hat{Y} = b \pm ax$), coefficient of determination ($100R^2$) and scatter diagram showing the fitted simple regression line of $\hat{Y}$ [percent mortality of adult male *Bactrocera zonata*[(a)] and adult female *Bactrocera zonata*[(b)]] on X [Concentrations of *Beauveria bassiana*] at different days post-application intervals

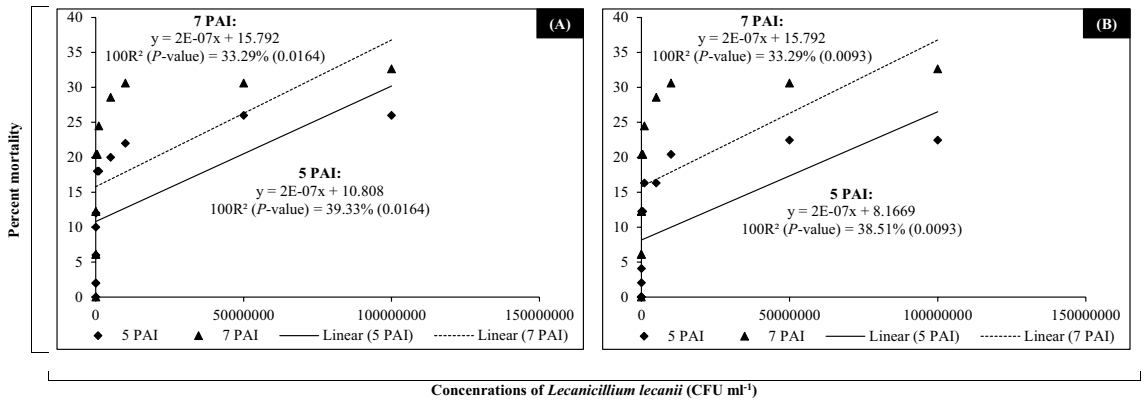

Fig. 7 Linear regression equation ($\hat{Y} = b \pm ax$), coefficient of determination ($100R^2$), and scatter diagram showing the fitted simple regression line of $\hat{Y}$ [percent mortality of adult male *Bactrocera zonata*[(a)] and adult female *Bactrocera zonata*[(b)]] on X [Concentrations of *Lecanicillium lecanii*] at different days post-application intervals

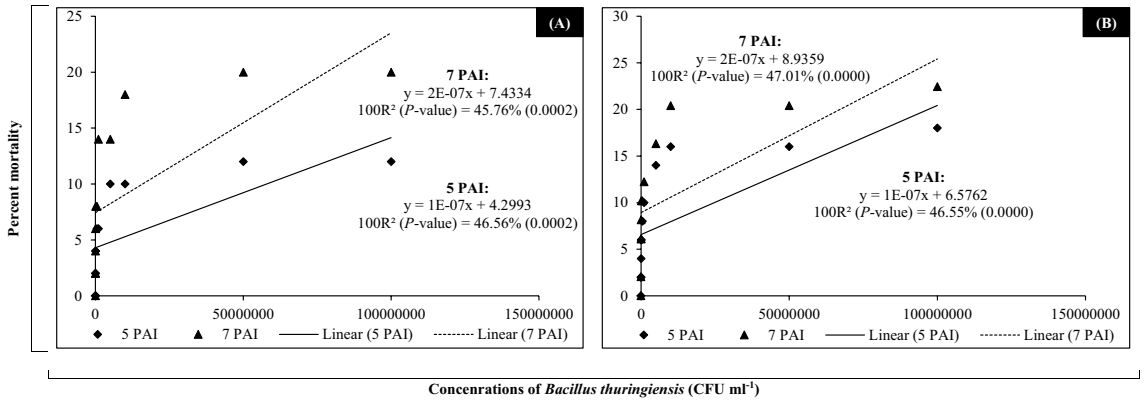

Fig. 8 Linear regression equation ($\hat{Y} = b \pm ax$), coefficient of determination ($100R^2$) and scatter diagram showing the fitted simple regression line of $\hat{Y}$ [percent mortality of adult male *Bactrocera zonata*[(a)] and adult female *Bactrocera zonata*[(b)]] on X [Concentrations of *Bacillus thuringiensis*] at different days post-application intervals

the total variation in mortality of *B. zonata* males and 38.51% and 33.29% of the total variation in mortality of *B. zonata* females was explained by different concentration of *L. lecanii* at 5 and 10 d PAI, respectively (Fig. 7). The concentrations of *B. thuringiensis* explained 46.56% and 45.76% of the total variability in mortality of *B. zonata* males, while the same attributed 46.55% and 47.01% of the total variability in mortality of *B. zonata* females at 5 and 10 d PAI, respectively (Fig. 8).

LC values of fungal and bacterial bioinsecticides against *Bactrocera zonata* exposed to different post-application intervals

The pathogenicity of all tested entomopathogens (EPs) against male and female adults of *B. zonata* varied significantly at both PAIs as the fiducially limits did not overlap with each other. Based on different LC values, *M. anisopliae* proved more pathogenic to *B. zonata* females that demonstrated the least LC_{50} (2.29×10^8 CFU ml^{-1} at 5 d PAIs; 5.48×10^3 CFU ml^{-1} at 7 d PAIs), LC_{75} (3.55×10^{10} CFU ml^{-1} at 5 d PAIs; 7.65×10^4 CFU ml^{-1} at 7 d PAIs) and LC_{95} (1.1×10^{11} CFU ml^{-1} at 5 d PAIs; 1.12×10^7 CFU ml^{-1} at 7 d PAIs) values at both PAIs (Table 1), followed by *B. bassiana* which explained LC_{50} (6.49×10^8 CFU ml^{-1} at 5 d PAIs; 1.14×10^4 CFU ml^{-1} at 7 d PAIs), LC_{75} (5.25×10^{11} CFU ml^{-1} at 5 d PAIs; 2.85×10^6 CFU ml^{-1} at 7 d PAIs) and LC_{95} (1.7×10^{12} CFU ml^{-1} at 5 d PAIs; 3.18×10^7 CFU ml^{-1} at 7 d PAIs) higher than that of *M. anisopliae* (Table 2) but less than that of *L. lecanii* and *B. thuringiensis*. *L. lecanii* demonstrated LC_{50} (5.19×10^9 CFU ml^{-1} at 5 d PAIs; 2.77×10^9 CFU ml^{-1} at 7 d PAIs) LC_{75} (6.96×10^{13} CFU ml^{-1} at 5 d PAIs; 5.76×10^{11} CFU ml^{-1} at 7 d PAIs) and LC_{95} (3.2×10^{12} CFU ml^{-1} at 5 d PAIs; 2.6×10^{11} CFU ml^{-1} at 7 d PAIs) (Table 3) less than *B. thuringiensis* and proved more effective than *B. thuringiensis* which explained LC_{50}, LC_{75} and LC_{95} values of (4.23×10^{10} and 3.40×10^{10} CFU ml^{-1}), (2.63×10^{14} and 2.03×10^{13} CFU ml^{-1}), and (2.9×10^{14} and 1.1×10^{14} CFU ml^{-1}) (at 5 and 7 d PAIs, respectively) (Table 4).

M. anisopliae proved more pathogenic to *B. zonata* males, which demonstrated the least LC_{50} (2.49×10^8 CFU ml^{-1} at 5 d PAIs; 6.17×10^3 CFU ml^{-1} at 7 d PAIs), LC_{75} (6.86×10^{11} CFU ml^{-1} at 5 d PAIs; 1.43×10^7 CFU ml^{-1} at 7 d PAIs) and LC_{95} (3.1×10^{12} CFU ml^{-1} at 5 d PAIs; 7.8×10^7 CFU ml^{-1} at 7 d PAIs) values at both PAIs (Table 1) followed by *B. bassiana* which explained LC_{50} (7.51×10^8 CFU ml^{-1} at 5 d PAIs; 1.15×10^5 CFU ml^{-1} at 7 d PAIs), LC_{75} (2.58×10^{12} CFU ml^{-1} at 5 d PAIs; 5.44×10^7 CFU ml^{-1} at 7 d PAIs) and LC_{95} (1.1×10^{13} CFU ml^{-1} at 5 d PAIs; 2.1×10^8 CFU ml^{-1} at 7 d PAIs) (Table 2) higher than that of *M. anisopliae* but less than that of *L. lecanii* and *B. thuringiensis*. *L. lecanii* demonstrated LC_{50} (3.45×10^9 CFU ml^{-1} at 5 d PAIs; 1.43×10^9 CFU ml^{-1} at 7 d PAIs), LC_{75} (4.42×10^{12} CFU ml^{-1} at 5 d PAIs; 3.28×10^{12} CFU ml^{-1} at 7 d PAIs) and LC_{95} (2.6×10^{13} CFU ml^{-1} at 5 d PAIs; 2.6×10^{13} CFU ml^{-1} at 7 d PAIs) (Table 3) less than *B. thuringiensis* and proved more effective than *B. thuringiensis* which explained LC_{50}, LC_{75} and LC_{95} values of (1.63×10^{11} and 1.39×10^{10} CFU ml^{-1}), 5.99×10^{13} and 6.61×10^{12} CFU ml^{-1}) and (2.2×10^{14} and 2.6×10^{13} CFU ml^{-1}) (at 5 and 7 d PAIs, respectively) (Table 4).

All the tested entomopathogenic formulations exhibited less LC_{50} values against *B. zonata* males and hence

Table 1 Lethal concentration (LC) (CFU ml^{-1}) values of *Metarizium anisopilae* for fifty (LC_{50}) and ninety (LC_{90}) percent mortality of *Bactrocera zonata* adults at different post-treatment intervals

Bactrocera zonata Adults	PTI (d)	LC_{25}	FL limit	LC_{50}	FD limit	LC_{75}	FD limit	LC_{95}	FL limit	Slope ± S.E	χ^2	*df*	*P* value
Males	5	3.23×10^{4}	6.11×10^{3}–9.98×10^{4}	2.49×10^{8}	6.70×10^{7}–1.90×10^{9}	6.86×10^{11}	1.51×10^{11}–5.47×10^{14}	3.1×10^{12}	8.02×10^{11}–8.1×10^{15}	0.1 ± 0.02	7.74775	9	0.56
	7	4.09×10^{0}	4.73×10^{-1}–2.04×10^{1}	6.17×10^{3}	2.38×10^{3}–1.30×10^{4}	1.43×10^{7}	7.35×10^{7}–3.29×10^{7}	7.8×10^{7}	3.40×10^{7}–2.3×10^{8}	0.2 ± 0.01	3.19323	9	0.96
Females	5	8.93×10^{5}	3.65×10^{5}–1.93×10^{6}	2.29×10^{8}	1.84×10^{8}–4.35×10^{9}	3.55×10^{10}	5.87×10^{10}–3.51×10^{13}	1.1×10^{11}	2×10^{11}–2.6×10^{14}	0.2 ± 0.02	3.44389	9	0.944
	7	1.49×10^{1}	2.78×10^{0}–5.36×10^{1}	5.48×10^{3}	2.48×10^{3}–1.03×10^{4}	7.65×10^{4}	1.71×10^{6}–5.26×10^{6}	1.12×10^{7}	6.01×10^{6}–2.45×10^{7}	0.2 ± 0.01	10.1057	9	0.342

Table 2 Lethal concentration (LC) (CFU ml^{-1}) values of *Beauveria bassiana* for fifty (LC_{50}) and ninety (LC_{90}) percent mortality of *Bactrocera zonata* adults at different post-treatment intervals

Bactrocera zonata Adults	PTI (d)	LC_{25}	FL limit	LC_{50}	FD limit	LC_{75}	FD limit	LC_{95}	FL limit	Slope ± S.E	χ^2	*df*	*P* value
Males	5	1.20×10^6	5.09×10^5–2.60×10^6	7.51×10^8	2.09×10^8–5.18×10^9	2.58×10^{12}	5.85×10^{10}–3.42×10^{13}	1.1×10^{13}	2×10^{11}–2.4×10^{14}	0.2 ± 0.02	5.67553	9	0.772
	7	3.42×10^2	9.52×10^1–9.36×10^2	1.15×10^5	6.59×10^4–1.87×10^5	5.44×10^7	2.91×10^7–1.17×10^8	2.1×10^8	9.91×10^7–5.37×10^8	0.2 ± 0.01	4.79811	9	0.852
Females	5	1.97×10^6	1.02×10^6–3.54×10^6	6.49×10^8	1.00×10^8–7.09×10^8	5.25×10^{11}	7.64×10^9–3.28×10^{11}	1.7×10^{12}	1.9×10^{10}–1.3×10^{12}	0.2 ± 0.02	12.7350	9	0.175
	7	2.46×10^1	5.03×10^0–8.39×10^1	1.14×10^4	5.46×10^3–2.09×10^4	2.85×10^6	4.45×10^6–1.46×10^7	3.18×10^7	1.65×10^7–7.18×10^7	0.2 ± 0.01	10.6486	9	0.301

Table 3 Lethal concentration (LC) (CFU ml^{-1}) values of *Lecanicillium lecanii* for fifty (LC_{50}) and ninety (LC_{90}) percent mortality of *Bactrocera zonata* adults at different post-treatment intervals

Bactrocera zonata Adults	PTI (d)	LC_{25}	FL limit	LC_{50}	FD limit	LC_{75}	FD limit	LC_{95}	FL limit	Slope ± S.E	χ^2	*df*	*P* value
Males	5	6.69×10^6	2.85×10^6–1.76×10^7	3.45×10^9	6.65×10^8–5.06×10^{10}	4.42×10^{12}	1.37×10^{11}–3.63×10^{14}	2.6×10^{13}	4.4×10^{11}–2.6×10^{15}	0.2 ± 0.02	9.1206	9	0.426
	7	7.25×10^5	2.58×10^5–1.75×10^6	1.43×10^9	2.99×10^8–1.77×10^{10}	3.28×10^{12}	1.97×10^{11}–8.24×10^{14}	2.6×10^{13}	8.1×10^{11}–8.8×10^{15}	0.1 ± 0.02	7.32804	9	0.603
Females	5	1.96×10^7	9.02×10^6–5.16×10^7	5.19×10^9	6.53×10^8–2.77×10^{10}	6.96×10^{13}	4.65×10^{10}–2.82×10^{13}	3.2×10^{12}	1.2×10^{11}–1.3×10^{14}	0.2 ± 0.03	18.5858	9	0.029
	7	1.36×10^6	6.56×10^5–2.57×10^6	2.77×10^9	5.60×10^8–3.19×10^{11}	5.76×10^{11}	1.89×10^{12}–1.61×10^{18}	2.6×10^{11}	3.7×10^{10}–4.6×10^{12}	0.1 ± 0.02	59.7803	9	0.000

proved more toxic for males than to females of *B. zonata*. The results also exhibited that pathogenicity of all the tested EPs increased with increasing exposure interval, being significantly higher at 7 d PAIs and lower at 5 d PAIs (Table 1–4).

LT values of fungal and bacterial bioinsecticides against *Bactrocera zonata* exposed to different post-application intervals

The results of lethal times (LTs) of *M. anisopliae* explained that LT_{50} and LT_{90} of *M. anisopliae* against both male and female *B. zonata* ranged between 4.47–6.34 d (LT_{50}) and 5.55–8.35 d (LT_{90}) at concentrations of 1×10^8 to 1×10^3 CFU ml^{-1}. High LT values were calculated ($LT_{50} = 6.32$ d and $LT_{90} = 8.35$ d for male and $LT_{50} = 6.34$ d and $LT_{90} = 7.86$ d for female) at the lowest concentration (1×10^3 CFU ml^{-1}), but the lowest ($LT_{50} = 4.63$ d and $LT_{90} = 5.55$ d for male and $LT_{50} = 4.47$ d and $LT_{90} = 5.73$ d for female) was recorded at high concentration (1×10^8 CFU ml^{-1}) (Table 5).

The results of LTs of *B. bassiana* demonstrated that LT_{50} and LT_{90} of *B. bassiana* against both male and female *B. zonata* ranged between 5.23–7.34 d PAIs (LT_{50}) and 6.57–9.67 d PAIs (LT_{90}) at concentrations of 1×10^8 to 1×10^3 CFU ml^{-1}. The highest LT values ($LT_{50} = 7.14$ d and $LT_{90} = 9.67$ d for male; $LT_{50} = 7.34$ d and $LT_{90} = 8.89$ d for female) were recorded at low concentration (1×10^3 CFU ml^{-1}) and the lowest values ($LT_{50} = 5.23$ d and $LT_{90} = 6.57$ d for male; $LT_{50} = 5.50$ d and $LT_{90} = 7.0$ d for female) were calculated at high concentration (1×10^8 CFU ml^{-1}) (Table 6).

The results of LTs of *L. lecanii* indicated that LT_{50} and LT_{90} of *L. lecanii* against both male and female *B. zonata* ranged between 5.33–8.47 d (LT_{50}) and 6.59–10.59 d (LT_{90}) at concentrations of 1×10^8 to 1×10^3 CFU ml^{-1}. The highest LT values ($LT_{50} = 8.47$ d and $LT_{90} = 10.59$ d for male and $LT_{50} = 5.33$ d and $LT_{90} = 6.61$ d for female) at low concentration (1×10^3 CFU ml^{-1}) and the lowest values ($LT_{50} = 5.33$ d and $LT_{90} = 6.59$ d for male and $LT_{50} = 5.39$ d and $LT_{90} = 6.61$ d for female) at high concentration (1×10^8 CFU ml^{-1}) (Table 7).

The results of LTs of *B. thuringiensis* confirmed that LT_{50} and LT_{90} of *B. thuringiensis* against both male and female *B. zonata* ranged between 6.20–9.41 d (LT_{50}) and 7.42–13.51 d (LT_{90}) at concentrations of 1×10^8 to 1×10^3 CFU ml^{-1}. High values were ($LT_{50} = 9.41$ d and $LT_{90} = 13.51$ d for male and $LT_{50} = 8.68$ d and $LT_{90} = 10.27$ d for female) at low concentration (1×10^3 CFU ml^{-1}) and low values were ($LT_{50} = 6.37$ d and $LT_{90} = 7.54$ d for male and $LT_{50} = 6.20$ d and $LT_{90} = 7.42$ d for female) at high concentration (1×10^8 CFU ml^{-1}) (Table 8).

Discussion

Many investigations demonstrate the significant role of entomopathogenic microbes as bioagents against tephritid fruit pests. The entomopathogenic microbes prove virulent against different stages (maggots, pupae, and adults) when exposed via different routes of exposure (Soliman et al. 2020). However, the pathogenicity of the entomopathogenic microbes on target insects and other arthropods varies significantly (Soliman et al. 2020).

In the present work, the pathogenicity of various EPF and bacteria was evaluated against *B. zonata* for biological control of this species. Results showed that *M. anisopliae, B. bassiana, L. lecanii*, and *B. thuringiensis* exhibited varied pathogenicity against *B. zonata* at different exposure periods. These results are in the light of findings of Iqbal et al. (2020) who studied that *B. cucurbitae* exhibited significantly varied mortality toward various EPF and EPB at various exposure intervals. Varied pathogenicity of *M. anisopliae, B. bassiana, L. lecanii*, and *B. thuringiensis* against *B. zonata* is also in the lights of various other studies in which toxicity of EPF (Soliman et al. 2020) and EPB (Cossentine et al. 2016) was assessed against various fruit fly species.

These results are in agreement with Soliman et al. (2020), who reported that local strains of *M. anisopliae* were found effective in its virulence to kill different life stages of *C. capitata*. Ekesi et al. (2003) also confirmed that isolates of *M. anisopliae* exposed to late 3rd instar larvae of *C. capitata* and *C. fasciventris* in sand and caused a significant reduction in adult emergence and a corresponding large mortality on puparia of both species. All isolates also induced large deferred mortality in emerging adults following treatment as late third instar larvae. Wang et al. (2021) found that *M. anisopliae* Ma04 presented the highest virulence against *B. dorsalis*. Results of virulence bioassay indicated that the LC_{50} values of *M. anisopliae* Ma04 against *B. dorsalis* declined from 5.2×10^{28} to 5.2×10^7 conidia ml^{-1} over a 1–10 d period post adult emergence, and the LT_{50} values decreased from 5.25 to 2.78 d with the concentrations of conidial suspension increasing from 1.0×10^8 to 1.0×10^{10} conidia ml^{-1}. Therefore, *M. anisopliae* Ma04 had a greater potential for *B. dorsalis* control.

The results of the present study revealed that at LC_{50} concentration, all the tested entomopathogenic formulations exhibited less LC_{50} values against male *B. zonata* and hence proved more toxic for males than to females of this species. However, the studies conducted by Chergui et al. (2020) showed that *B. bassiana* was

Table 4 Lethal concentration (LC) (CFU ml^{-1}) values of *Bacillus thuringiensis* for fifty (LC_{50}) and ninety (LC_{90}) percent mortality of *Bactrocera zonata* adults at different post-treatment intervals

Bactrocera zonata Aults	PTI (d)	LC_{25}	FL limit	LC_{50}	FD limit	LC_{75}	FD limit	LC_{95}	FL limit	Slope ± S.E	χ^2	*df*	*P* value
Males	5	6.15×10^8	1.12×10^8–1.74×10^{10}	1.63×10^{11}	7.65×10^9–1.11×10^{14}	5.99×10^{13}	5.97×10^{11}–1.33×10^{18}	2.2×10^{14}	1.5×10^{12}–1×10^{19}	0.2 ± 0.03	2.96383	9	0.966
	7	4.14×10^7	1.49×10^7–1.83×10^8	1.39×10^{10}	1.79×10^9–5.22×10^{11}	6.61×10^{12}	2.24×10^{11}–3.02×10^{15}	2.6×10^{13}	6.4×10^{11}–2×10^{16}	0.2 ± 0.03	4.14997	9	0.901
Females	5	9.74×10^7	2.80×10^7–7.49×10^7	4.23×10^{10}	3.52×10^9–4.71×10^{12}	2.63×10^{14}	4.83×10^{11}–6.06×10^{16}	2.9×10^{14}	1.4×10^{12}–4.9×10^{17}	0.2 ± 0.03	2.37069	9	0.984
	7	3.04×10^7	9.72×10^6–1.68×10^8	3.40×10^{10}	2.84×10^9–3.55×10^{12}	2.03×10^{13}	8.58×10^{11}–1.84×10^{17}	1.1×10^{14}	3×10^{12}–2×10^{18}	0.2 ± 0.03	2.58362	9	0.979

Table 5 Lethal time (LT) (days) of different concentrations of *Metarizium anisopilae* in oral bioassay inducing fifty (LT_{50}) and ninety (LT_{90}) percent mortality of *Bactrocera zonata* adults

Concentrations (CFU ml^{-1})	Male *Bactrocera zonata*						Female *Bactrocera zonata*					
	LT_{50} (days)	FD limit	LT_{90} (days)	FD limit	Slope ± S.E	*df*	LT_{50} (days)	FD limit	LT_{90} (days)	FD limit	Slope ± S.E	*df*
1×10^8	4.63	3.93–4.90	5.55	4.30–6.33	5.18 ± 0.63	1	4.47	4.18–4.69	5.73	4.43–7.55	4.01 ± 0.68	1
5×10^7	4.71	3.99–5.87	5.7	4.40–6.10	5.12 ± 0.61	1	4.50	4.17–4.75	5.86	4.46–7.67	4.23 ± 0.65	1
1×10^7	4.84	4.07–5.98	6.03	5.63–7.52	4.57 ± 0.60	1	4.60	4.32–4.83	6.01	5.11–7.87	5.31 ± 0.69	1
5×10^6	4.99	4.00–5.82	6.36	5.93–7.95	4.33 ± 0.64	1	4.84	4.56–5.08	6.21	5.21–7.98	5.87 ± 0.75	1
1×10^6	5.16	4.78–5.40	6.54	5.10–7.56	4.37 ± 0.66	1	4.96	4.68–5.18	6.31	5.00–8.34	5.91 ± 0.76	1
5×10^5	5.42	5.37–5.98	7.35	6.60–8.16	3.49 ± 0.69	1	5.11	4.81–5.37	6.71	5.29–8.47	5.97 ± 0.77	1
1×10^5	5.60	5.36–5.84	7.67	6.80–9.56	3.77 ± 0.74	1	5.31	5.02–5.57	6.92	5.35–8.89	5.98 ± 0.81	1
5×10^4	5.86	5.52–6.10	8.13	7.17–9.67	3.79 ± 0.79	1	5.49	5.21–6.77	7.04	6.12–9.71	6.75 ± 0.89	1
1×10^4	5.97	5.63–6.66	8.65	7.40–10.59	3.58 ± 0.82	1	5.72	6.46–7.01	7.10	6.23–9.98	6.86 ± 1.05	1
5×10^3	6.08	6.00–6.69	8.06	7.15–11.35	4.88 ± 0.99	1	5.96	6.67–7.36	7.52	6.34–10.01	7.06 ± 1.09	1
1×10^3	6.32	6.21–7.13	8.35	7.01–10.45	5.99 ± 1.16	1	6.34	6.99–8.01	7.86	6.35–10.93	7.29 ± 1.35	1

Table 6 Lethal time (LT) (days) of different concentrations of *Beauveria bassiana* in oral bioassay inducing fifty (LT_{50}) and ninety (LT_{90}) percent mortality of *Bactrocera zonata* adults

Concentrations (CFU ml^{-1})	Male *Bactrocera zonata*						Female *Bactrocera zonata*					
	LT_{50} (days)	FD limit	LT_{90} (days)	FD limit	Slope ± S.E	*df*	LT_{50} (days)	FD limit	LT_{90} (days)	FD limit	Slope ± S.E	*df*
1×10^8	5.23	4.93–5.47	6.57	6.30–6.94	5.28 ± 0.65	1	5.50	5.17–5.75	7.00	6.69–7.44	4.98 ± 0.63	1
5×10^7	5.31	4.99–5.56	6.73	6.44–7.13	5.07 ± 0.63	1	5.47	5.18–5.69	6.75	6.49–7.10	5.68 ± 0.66	1
1×10^7	5.41	5.07–5.68	7.00	6.67–7.48	4.67 ± 0.62	1	5.60	5.32–5.83	6.88	6.62–7.24	5.81 ± 0.67	1
5×10^6	5.54	5.17–5.82	7.32	6.93–7.97	4.30 ± 0.62	1	5.84	5.56–6.08	7.24	6.94–7.68	5.60 ± 0.70	1
1×10^6	5.72	5.37–6.00	7.55	7.12–8.29	4.34 ± 0.64	1	5.96	5.68–6.18	7.32	7.01–7.76	5.83 ± 0.73	1
5×10^5	5.92	5.49–6.28	8.37	7.64–10.06	3.47 ± 0.65	1	6.11	5.81–6.37	7.73	7.31–8.43	5.10 ± 0.73	1
1×10^5	6.26	5.88–6.64	8.65	7.84–10.54	3.71 ± 0.70	1	6.31	6.02–6.57	7.90	7.45–8.67	5.35 ± 0.79	1
5×10^4	6.59	6.19–7.10	8.74	8.12–11.67	3.72 ± 0.75	1	6.49	6.21–6.77	8.06	7.58–8.94	5.54 ± 0.85	1
1×10^4	6.89	6.44–7.64	9.07	8.41–13.53	3.54 ± 0.79	1	6.72	6.46–7.01	8.12	7.66–9.01	6.34 ± 1.02	1
5×10^3	7.08	6.71–7.69	9.10	8.18–11.30	4.85 ± 0.96	1	6.96	6.67–7.36	8.50	7.89–9.82	6.01 ± 1.07	1
1×10^3	7.14	6.82–7.66	9.67	8.03–10.40	5.94 ± 1.14	1	7.34	6.99–8.01	8.89	8.12–10.91	6.27 ± 1.30	1

Table 7 Lethal time (LT) (days) of different concentrations of *Lecanicillium lecanii* in oral bioassay for fifty (LT_{50}) and ninety (LT_{90}) percent mortality on different days against *Bactrocera zonata* adults

Concentrations (CFU ml^{-1})	Male *Bactrocera zonata*						Female *Bactrocera zonata*					
	LT_{50} (days)	FD limit	LT_{90} (days)	FD limit	Slope ± S.E	*df*	LT_{50} (days)	FD limit	LT_{90} (days)	FD limit	Slope ± S.E	*df*
1×10^8	5.33	5.05–5.56	6.59	6.33–6.94	5.66 ± 0.66	1	5.39	4.96–5.45	6.61	6.14–6.75	5.95 ± 0.70	1
5×10^7	5.61	5.29–5.86	7.11	6.79–7.57	5.06 ± 0.65	1	5.46	5.20–5.68	6.63	6.38–6.95	6.19 ± 0.68	1
1×10^7	5.91	5.60–6.16	7.52	7.14–8.13	4.98 ± 0.69	1	5.62	5.28–5.89	7.29	6.93–7.88	4.62 ± 0.64	1
5×10^6	6.09	5.74–6.40	8.11	7.53–9.26	4.20 ± 0.69	1	5.82	5.55–6.04	7.09	6.82–7.46	6.07 ± 0.71	1
1×10^6	6.35	6.01–6.70	8.40	7.74–9.81	4.28 ± 0.74	1	5.96	5.68–6.18	7.32	7.01–7.76	5.83 ± 0.73	1
5×10^5	6.51	6.17–6.90	8.62	7.88–10.27	4.27 ± 0.77	1	6.21	5.96–6.43	7.51	7.20–7.99	6.33 ± 0.83	1
1×10^5	6.86	6.49–7.40	9.04	8.14–11.28	4.35 ± 0.85	1	6.33	6.06–6.58	7.78	7.39–8.44	5.82 ± 0.83	1
5×10^4	7.25	6.78–8.20	9.24	8.49–13.54	4.09 ± 0.91	1	6.66	6.44–6.85	7.68	7.39–8.17	8.41 ± 1.27	1
1×10^4	7.67	7.10–9.12	9.73	8.67–15.11	4.39 ± 1.06	1	7.05	6.78–7.44	8.37	7.83–9.56	7.00 ± 1.28	1
5×10^3	7.69	7.24–8.84	10.08	8.28–12.38	6.55 ± 1.58	1	7.29	6.99–7.82	8.55	7.93–10.11	7.50 ± 1.56	1
1×10^3	8.47	7.61–12.23	10.59	8.83–20.94	5.36 ± 1.61	1	7.69	7.24–8.84	9.24	8.28–12.38	6.55 ± 1.58	1

Table 8 Lethal time (LT) (days) of different concentrations of *Bacillus thuringiensis* in oral bioassay for fifty (LT_{50}) and ninety (LT_{90}) percent mortality on different days against *Bactrocera zonata* adults

Concentrations (CFU ml^{-1})	Male *Bactrocera zonata*						Female *Bactrocera zonata*					
	LT_{50} (days)	FD limit	LT_{90} (days)	FD limit	Slope ± S.E	*df*	LT_{50} (days)	FD limit	LT_{90} (days)	FD limit	Slope ± S.E	*df*
1×10^8	6.37	4.09–9.58	7.54	5.33–9.94	6.68 ± 0.68	1	6.20	4.99–8.41	7.42	5.14–9.75	5.97 ± 0.73	1
5×10^7	6.62	4.28–9.88	8.12	5.79–10.57	6.07 ± 0.62	1	6.45	5.26–8.66	7.65	5.38–9.95	6.16 ± 0.66	1
1×10^7	6.97	4.69–9.19	8.57	6.14–11.13	6.94 ± 0.65	1	6.63	5.29–8.88	8.26	5.93–10.88	6.66 ± 0.68	1
5×10^6	7.09	4.79–10.46	9.17	6.53–11.26	7.27 ± 0.67	1	6.87	5.57–9.07	8.01	5.82–11.46	6.05 ± 0.72	1
1×10^6	7.39	5.09–10.78	9.49	6.74–11.81	7.24 ± 0.73	1	6.92	5.65–9.19	8.36	6.01–11.76	7.89 ± 0.77	1
5×10^5	7.52	5.11–10.93	9.61	6.88–12.27	7.26 ± 0.76	1	7.28	5.98–10.46	8.56	6.20–11.99	7.36 ± 0.84	1
1×10^5	7.84	5.49–11.47	10.08	7.14–13.28	8.35 ± 0.82	1	7.31	6.09–10.53	8.74	6.39–12.44	7.81 ± 0.85	1
5×10^4	8.20	5.79–12.23	10.71	7.49–13.54	8.07 ± 0.96	1	7.68	6.47–10.88	8.66	6.39–13.17	7.47 ± 1.28	1
1×10^4	8.61	6.17–12.16	11.08	7.67–14.11	8.39 ± 1.09	1	8.01	6.76–11.46	9.33	6.83–13.56	7.05 ± 1.29	1
5×10^3	8.65	6.29–12.89	12.20	7.28–15.38	8.55 ± 1.51	1	8.29	6.98–11.88	9.56	6.93–14.11	7.57 ± 1.51	1
1×10^3	9.41	6.66–13.22	13.51	7.83–16.94	8.38 ± 1.65	1	8.68	7.28–12.86	10.27	8.28–14.38	8.59 ± 1.57	1

virulent to adults of *C. capitata*, where females were less susceptible than male flies of this species in both oral and contact bioassays, which is contrary to our results. Reason for this variation might be due to difference in strains of EPF and species of fruit fly.

Varied mortality at different exposure periods in both male and female sexes of *B. zonata* caused by EPs in the present studies might be due to variation in virulence factors *i.e.,* spore germination, hyphal growth, bacterial-budding, toxins etc. during the different growth period of tested entomopathogens. In this study *B. zonata* was least susceptible to *B. thuringiensis* as compared to EPFs *i.e., B. bassiana, M. anisopliae*, and *L. lecanii*. Similar results were also observed by Iqbal et al. (2020) who tested these entomopathogens against *B. cucurbitae*.

The results also exhibited that pathogenicity of all the tested EPs increased with increasing exposure interval, being significantly higher at 7 d post-application intervals and lower at 5 d post-application intervals. These results were also supported by Ekesi et al. (2001) who figured out that maximum mortality of *B. cucurbitae* was recorded at the highest concentration (10^8 spore ml^{-1}) while mortality rate decreased gradually as concentration decreased. Similar results were also reported by Amala et al. (2013) who demonstrated that after 5 and 7 d of treatment, maximum mortality of *B. cucurbitae* was observed when treated with *Paecilomyces lilacinus* at the highest concentration (2.4×10^9 spores ml^{-1}). This variation in the highest concentration reported by Amala et al. (2013) and present results has attributed the difference in EPF used by Amala et al. (2013) and in these studies.

LC_{50} values of all tested microbial insecticides were found time-dependent and decreased with increased post-exposure interval. The LC_{50} and LC_{90} results of present experiment for male and female *B. zonata* are partially following those of Imoulan and Elmeziane (2014) who documented LC_{50} values of 2.85×10^3 and 3.16×10^3 spores ml^{-1} for male and female fruit flies, respectively. These results are not consistent with those of Alberola et al. (1999) who reported that mortality rate increased with time. Aboussaid et al. (2010) reported that adults and larvae of *C. capitata* were susceptible to different strains of *B. thuringiensis* and maximum mortality was observed after 5 to 6 d after treatment. The results regarding LT_{50} of present experiment are supported by Davidson and Chandler (2005) who reported a time-dependent infection and mortality of fungal and bacterial-based products against insects in the laboratory. The results of present experiments are partially consistent with those of various scientists (Mahmoud 2009) who documented strong potential of EPF against tephritid flies within 4–8 d after application at LC_{50} concentrations.

Conclusions

Based on the tested pathogens when incorporated in adult diets, it can be concluded that *M. anisopliae*, proved highly virulence against *B. zonata,* followed by *B. bassiana*, *L. lecanii*, and *B. thuringiensis*. Hence, *M. anisopliae* can be recommended for incorporation in *B. zonata* baits or pheromone traps to develop attract-and-kill technology.

Abbreviations

LC: Lethal concentration; LC_{50}: Lethal concentration required to kill 50% of insects; LC_{75}: Lethal concentration required to kill 75% of insects; LC_{95}: Lethal concentration required to kill 95% of insects; CFU ml^{-1}: Colony-forming unit per millimeter; EPF: Entomopathogenic fungi; EPB: Entomopathogenic bacteria; EPs: Entomopathogens; ACD: Attractant-contaminant device; IPM: Integrated pest management; CFU g^{-1}: Colony-forming unit per gram; psi: Pound per square inch; SDAY: Sabouraud dextrose agar yeast; V_{Final}: Final volume of stock solution needed to prepare required concentration; V_{Stock}: Volume of stock solution; C_{Stock}: Concentration of stock solution; C_{Final}: Final concentration to be prepared; LT: Lethal time; LT_{50}: Lethal concentration required to kill 50% of insects; LT_{75}: Lethal concentration required to kill 75% of insects; LT_{95}: Lethal concentration required to kill 95% of insects; PAIs: Post-application intervals; EPs: Entomopathogens; *r*: Correlation coefficient; CI: Confidence interval; R^2: Coefficient of determination; χ^2: Chi-square value; *df*: Degree of freedom; *P* value: Probability value; FL: Fiducial limit; SE: Standard error.

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Authors' contributions

MJN and MDG designed and conducted the experiment, collected and analyzed the data, and wrote manuscript. MDG and BA helped in apprehending the idea of this research, designing the layout of experiment and improving the write-up, format and language of this manuscript. MI reviewed the manuscript, add and improved declaration section, edited the format of the Tables according to the format of this journal. MT, RAA and WAN contributed in data setting for analysis, reviewed the final manuscript and made the format of this manuscript according to the format of this journal. 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.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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

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