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Research Article

Effectiveness of entomopathogenic fungi against peach fruit fly, *Bactrocera zonata* (Saunders) (Tephritidae: Diptera)

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ABSTRACT

Bactrocera zonata, commonly known as peach fruit fly, attacks a number of soft fruits such as peach, guava and mango. For its control, entomopathogenic fungi offer a safe and efficient substitute of insecticides, which can lead to pest resistance and environmental pollution. In the present study, four entomopathogenic fungi namely *Beauveria bassiana, Metarhizium anisopliae*, *Trichoderma harzianum* and *Verticillium lecanii* were tested against male and female adults of *B. zonata* through *in vitro* contact bioassay at concentrations of 1×10^4, 1×10^6, 1×10^8, 1×10^{10}, and 1×10^{12} cfu/ml. Mortality of *B. zonata* was recorded at 3, 7, 10 and 14-day post-exposure intervals. Results showed that as the concentration of spore/condial suspension of *B. bassiana*, *M. anisopliae, V. lecanii* and *T. harzianum* was increased from 1.0×10^4 to 1.0×10^{12} cfu/ml, LT_{50} values were decreased from 20.45 to 8.40 , 62.85 to 16.68, 62.85 to 16.68, and 34.09 to 16.42 days for female, and 23.82 to 9.1, 63.80 to 12.65, 63.80 to 12.65 and 29.55 to 13.90 days for male *B. zonata,* respectively. LC_{50} values of *B. bassiana*, *M. anisopliae, V. lecanii* and *T. harzianum* against *B. zonata* decreased from 5.17×10^{16} to 6.36×10^8, 9.33×10^{13} to 5.58×10^9, 3.02×10^{15} to $8.07 \times 10^{11}$and 4.00×10^{18} to 6.80×10^{13} cfu/ml for female, and from 2.60×10^{15} to 3.03×10^8, 2.26×10^{14} to 1.13×10^9, 3.73×10^{15} to 7.04×10^{10} and 2.67×10^{19} to 1.02×10^{12} cfu/ml for male, respectively, over a period of 3–14 days. On the 14th day, *B. zonata* males had the highest mortality (84%) due to *B. bassiana* at 1×10^{12} cfu/ml, followed by 48%, 59% and 74% mortality due to *T. harzianum, V. lecanii* and *M. anisopliae,* respectively. In general, adult female mortality was lower than the adult male mortality. With a moderate to high mortality of *B. zonata* due to different fungal species, the findings of the present study show that the tested entomopathogenic fungi are highly effective in controlling this pest. Because of their high efficacy against *B. zonata*, the two fungal species *viz. B. bassiana* and *M. anisopliae* can be included in an IPM plan to control *B. zonata*.

Keywords: *Bactrocera zonata, Beauveria bassiana*, *Metarhizium anisopliae*, *Trichoderma harzianum*, *Verticillium lecanii*, Mortality.

INTRODUCTION

Bactrocera zonata is commonly known as peach fruit fly and is a polyphagous pest worldwide that attacks different types of fruits and vegetables (Iqbal et al., 2021, et al., 2022). It has more than 50 host species and mostly attacks fleshy fruits like

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citrus, mango, guava, peaches and apricots (Boulahia-Kheder, 2021; Wang et al., 2024; Zhao et al., 2024), and increases the cost of labor in its infestation area (Agboka et al., 2024). The primary cause of the decline in guava yield and exports is the peach fruit fly causing 3–100% damage to this crop. Its destructive nature necessitates its control. Farmers generally employ synthetic pesticides that only cause harm to the environment and the fruit because they are ineffective against fruit flies due to the intricate structure of guavas (Gogi et al., 2023; Tian et al., 2024). *B. zonata* causes damage up to 89.50% in Pakistan and 10–20% in the northwestern Himalayan region (Nisar et al., 2020). In India, the pest status of *B. zonata* is similar to *B. dorsalis* and *B. cucurbitae* which also attack guava, mango, and peach (AlWahaibi, 2024; Heikal et al*.,* 2024). In Pakistan *B. zonata* is the most abundant and destructively dominant species of fruit fly causing damage up to 3–100% in a wide range of fruits and vegetables (Nisar et al., 2020). The damage to crops, fruits, and vegetables can be managed by controlling this pest and ultimately benefiting the economy (Tian et al., 2024).

Peach fruit flies exhibit a high production potential all the yearlong, leading to fruit fly generations that overlap (Sarfaraz et al*.,* 2023; Papadopoulos et al., 2024). This results in a high rate of infection, which ultimately lowers production. To manage insect pests, a wide range of insecticides has been used, leading to increased health hazards, insect resistance, and higher maximum residue levels (MRLs) in many fruits and vegetables (Iqbal et al. 2021; Gogi et al., 2023; Kubiak-Hardiman et al., 2023; Abdullah et al., 2024; Qayyum et al., 2024). Chemical pesticides have been used for a long time to control insect pests and to prevent significant yield losses, but they have a number of drawbacks, including toxicity to humans and non-target organisms, environmental toxicity, and developing resistance in the insects to these chemical pesticides. Around 586 insect species exhibit resistance to at least one of 325 available pesticides in the market (Pereira et al., 2023; Balaska et al*.,* 2024; Tossou et al., 2024). Furthermore, it is now incredibly difficult and expensive to create new pesticide compositions. Thus, there is an increasing trend of using alternative strategies including the use of biological agents for controlling pests (Ahmad et al*.,* 2022; Aziz et al*.,* 2024; Qayyum et al., 2024).

Some naturally occurring fungi can readily destroy *B. zonata* larvae and pupae in the soil (Iqbal et al., 2021). Two significant entomopathogens for Dipteran insects are *Beauveria bassiana* and *Metarhizium anisopliae* (Ali et *al.,* 2023; Ahmad et al., 2024; Sallam et al., 2024). In order to prevent the insect from ingesting the fungus, entomopathogenic fungi can be applied as biopesticides in two ways: either by surface contact or by putting them into bait (Irsad et al., 2023; Ahsan et al., 2024). In addition to ingestion and cuticle penetration, fungi can enter the body through breathing (Ma et al*.,* 2024). Numerous insect pests can be controlled by entomopathogenic fungi, which have a broad spectrum of insect hosts (Iqbal et al*.,* 2021). Numerous entomopathogenic fungal strains are highly efficient against various fruit fly species, and numerous variants have already been investigated and found to be effective against various fruit fly species (Bihal et al., 2023; Perumal et al., 2024). These days, entomopathogenic fungi are thought to be the most economical, safe, and successful method in an integrated pest management (IPM) plan (Sharma and Sharma, 2021; Abd-Elgawad, 2023). It can significantly lower fertility and fecundity in insects at every stage, from immature to mature (Qayyum *et al.,* 2024). Examining the pathogenic potential of four species of entomopathogenic fungi namely *M. anisopliae, B. bassiana, V. lecanii* and *T. harzianum* against *B. zonata* in a lab setting was the aim of this study.

MATERIAL AND METHODS

Rearing of fruit fly

B. zonata maggots were collected from a guava orchard and placed in a cage which was covered with a muslin cloth. The bottom of the box was layered with autoclaved sand (6 cm) for facilitating the pupation. The cage was checked daily until pupation. After pupation sand was sieved to separate pupae. For the emergence of adults, pupae were placed in a separate cage. After the emergence of the pupa, they were placed in a cage for rearing. After adult emergence, adults of both sexes were identified based on their morphological characteristics and were placed in separate cages. Then adults were fed on an artificial diet made up of yeast, honey, vitamins and nutrients. In addition, banana and mango pulps were also used. After 2 weeks, male and female were placed in a mating cage in a 1:1 ratio. After 24 hours, females were separated and provided with guava for egg laying. Laboratory conditions were maintained with a relative humidity of 65±5% and temperature of 27±2 °C.

Entomopathogenic fungal cultures and formulations

T. harzianum, *V. lecanii*, *B. bassiana* and *M. anisopliae* strains were acquired from USDA-USA. These Entomopathogens were cultured on dextrose agar. In one liter of distilled water, 39 g agar was mixed. On a hot plate, it was then stirred with a magnetic stirrer until a homogenous mixture was obtained. At 121 °C and 103 kPa pressure,

the media was then autoclaved for 30 min. Following sterilization of Petri dishes, autoclave media was transferred to the plates and allowed to cool for approximately half an hour until it solidified. Using scraps from the spore culture, the Petri dishes were inoculated with different fungal species. Each plate was replicated six times and placed in an incubator set at 25±2 °C. To prevent contamination, inoculation procedure was carried out in a laminar flow. Fungal conidia/spores were collected from 2-week-old cultures. To do this, we scraped the colony's top layer with a sterile needle and placed it in twin-20 (polyoxyethylene sorbitan monooleate; 1 ml/l). A magnetic shaker was then used to shake it for 10 min. It was then filtered to eliminate the undesirable components. The spores/conidia were then counted under a microscope using a hemocytometer. Distilled water was used to create the stock solution at the appropriate concentration. Following that, various concentrations were prepared by a series of dilutions. Five concentrations were prepared for *in vitro* bioassays, which were 1×10^{4}, 1×10^{6}, 1×10^{8}, 1×10^{10} and 1×10^{12} cfu/ml.

Fungal bioassay

Plastic glasses were used in the bioassays. Completely randomized design was employed for the experiment layout. The topical bioassay method was used in the experiments as the inner surface of the glasses was coated with fungal concentrations. Three replications were used for each treatment. In each treatment, twenty individuals of *B. zonata* were exposed to entomopathogenic fungi. Using an aspirator, male and female adults were placed in plastic glasses individually. The flies were placed in jars and then covered with net cloth to allow for ventilation. The fungus covered the glass surface. Along with the food, flies were unleashed. Mortality was recorded after interval of 3, 7, 10 and 14 day and converted into percentage mortality with Abbot formula:

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

Statistical analysis

Minitab software and Statistix 8.1 were used for analysis of variance to perform LC_{50} and LT_{50}. All pairwise comparison tests were conducted using the least significant difference (LSD) to statistically compare all of the data results with one another at a 5% significance level.

RESULTS

Biocontrol efficacy of *Beauveria bassiana*

The entomopathogenic fungus *B. bassiana* had a significant effect on survival of *B. zonata* at different concentrations and time intervals. Mortality of the female and male adults after 3 days at different concentrations (1×10^{4} to 1×10^{12} cfu/ml) was in the range of 6–20% and 12–27%, respectively. There was an increase in mortality percentage with the passage of time. After 7 days, mortality of female and male adults of *B. zonata* at different concentrations was 11–31% and 17–37%, respectively. Likewise, after 10- and 14-days' incubation periods, mortality of female adults of *B. zonata* was 19–54% and 30–75%, and that of male adults was 24–59% and 39–84%, respectively, at different concentrations as shown in Figure 1 A&B.

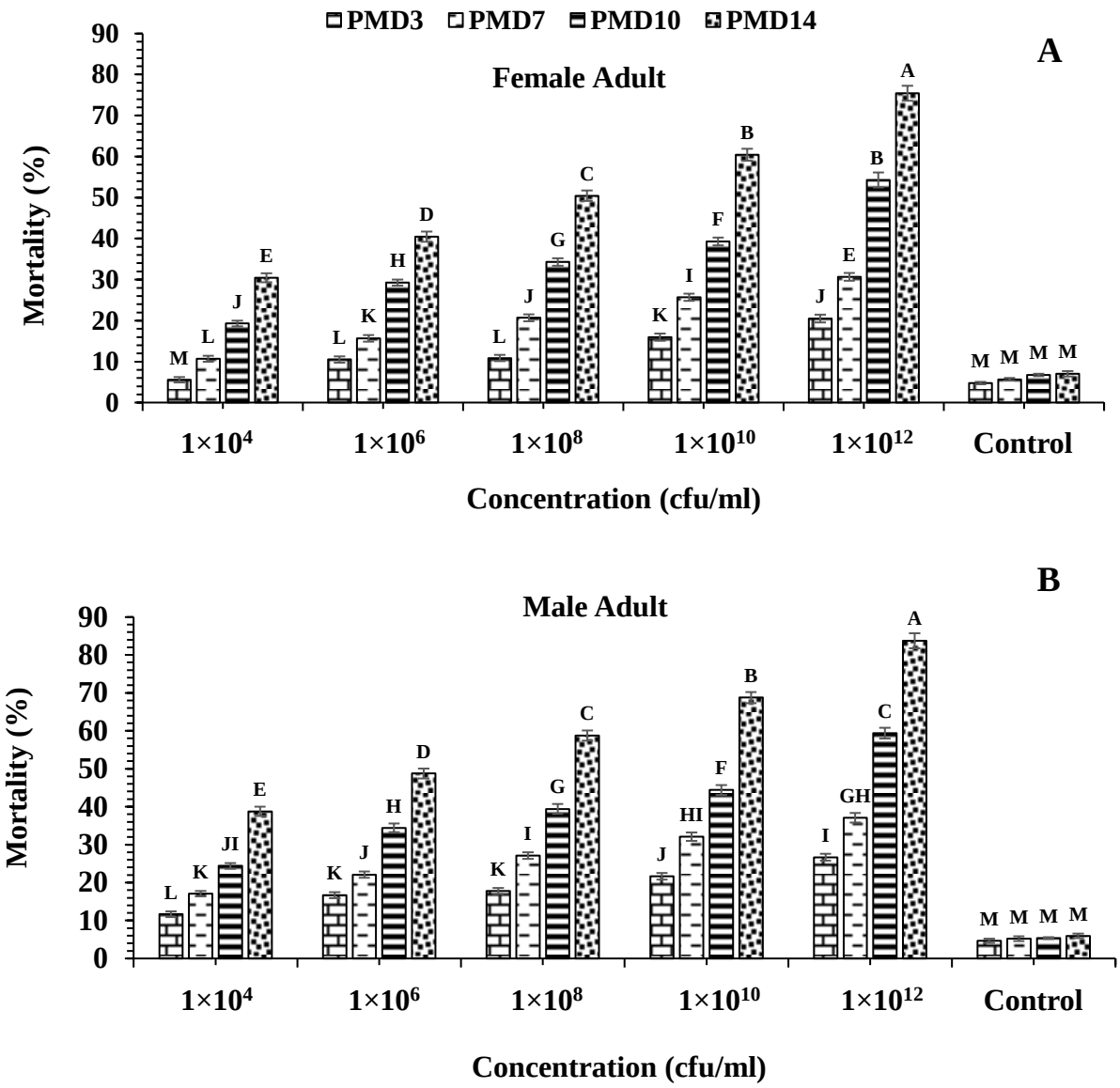

Figure 1. Effect of different concentrations of *Beauveria bassiana* on mortality of adult females and males *Bactrocera zonata* after exposure of 3, 7, 10 and 14 days. Vertical bars show standard errors of means. Bars with different letters show significant difference at P≤0.05.

Biocontrol efficacy of *Metarhizium anisopliae*

The entomopathogenic fungus *M. anisopliae* had a significant effect on mortality of *B. zonata* at different concentrations and day intervals. Mortality of female adults of *B. zonata* after 3 days at different concentrations was 5–25% while for male adults, mortality was 10–30%. Similarly, mortality of female adults of *B. zonata* after 7 days at different concentrations was 10–40% and that rof male adults was 17–47%. At higher time intervals, mortality was further increased. For female adults, mortality was 16–51% at different concentrations after 10 days while for male adults, it was 23–58%. Mortality of female adults of *B. zonata* after 14 days was 20–70% and that of male adults of *B. zonata* was 24–74% at different concentrations (Figure 2 A&B).

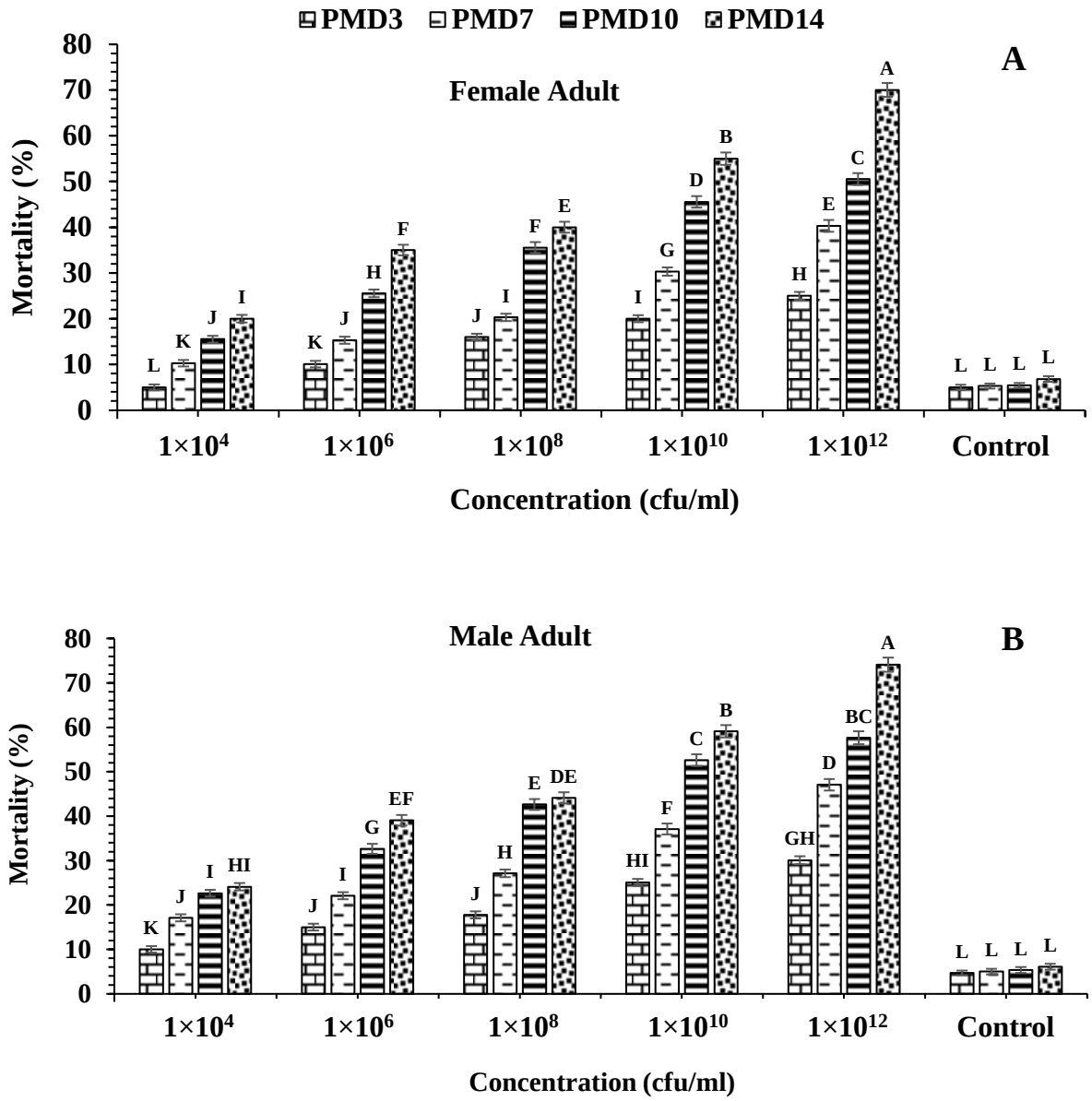

Figure 2. Effect of different concentrations of *Metarhizium anisopliae* on mortality of adult females and males *Bactrocera zonata* after exposure of 3, 7, 10 and 14 days. Vertical bars show standard errors of means. Bars with different letters show significant difference at P≤0.05.

Biocontrol efficacy of *Verticillium lecanii*

The entomopathogenic fungus *Verticillium lecanii* had significant effect on mortality of *B. zonata* on different day intervals. Percentage mortality for female adults of *B. zonata* after 3 days at different concentrations was 10–25% while for male adults of *B. zonata*, mortality was 16–31%. Mortality for female adults of *B. zonata* after 7 days at different concentrations was 15–35% as compared to 19–39% mortality in male adults. Likewise, mortality for female adults of *B. zonata* after 10 days at different concentrations was 20–43% while the mortality for male adults was 25–50% at this time interval. After 14 days, mortality for female and male adults of *B. zonata* at different concentrations was 26–56% and 29–59%, respectively (Figure 3 A&B).

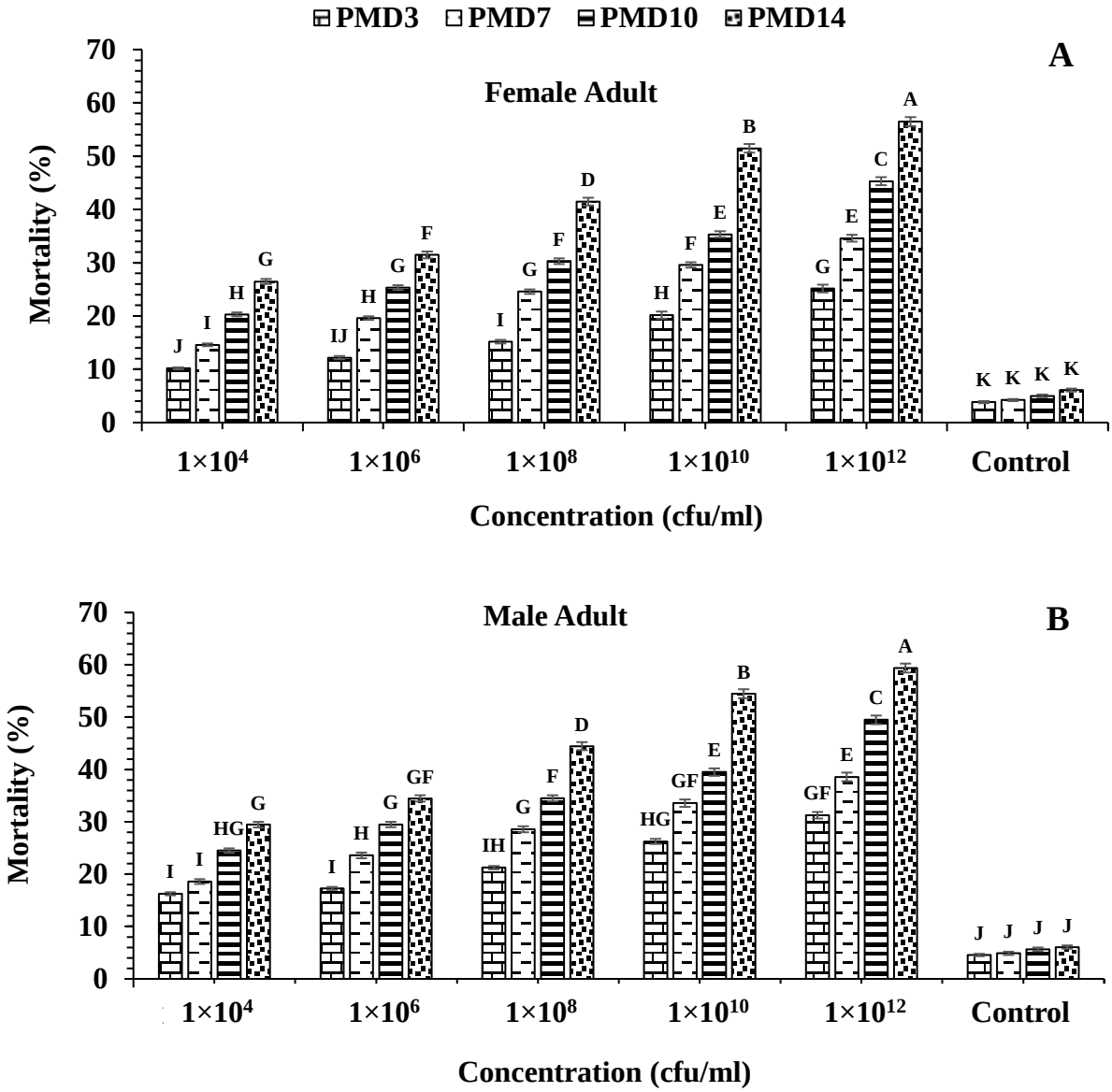

Figure 3. Effect of different concentrations of *Verticillium lecanii* on mortality of adult females and males *Bactrocera zonata* after exposure of 3, 7, 10 and 14 days. Vertical bars show standard errors of means. Bars with different letters show significant difference at P≤0.05.

Biocontrol efficacy of *Trichoderma harzianum*

The entomopathogenic fungus *Trichoderma harzianum* has a significant effect on mortality of *B. zonata* on different day intervals. Percentage mortality was calculated at day intervals of 3,7,10 and 14 days. Mortality of female and male adults of *B. zonata* after 3 days at different concentrations (1×10^4 to 1×10^{12} cfu/ml) was 6% to 17% and 11 – 22%, respectively. With increase in time, there was an increase in mortality of the insect. Mortality of female and male adults of *B. zonata* after 7 days at different concentrations was 11–31% and 15–20%, respectively. Mortality of female and male adults of *B. zonata* was further increased to 15–40% and 20–54%, respectively, after 10 days of the start of investigation. Similarly, mortality for female and male adults of *B. zonata* after 14 days at different concentrations was 25–45% and 28–48%, respectively (Figure 4 A&B).

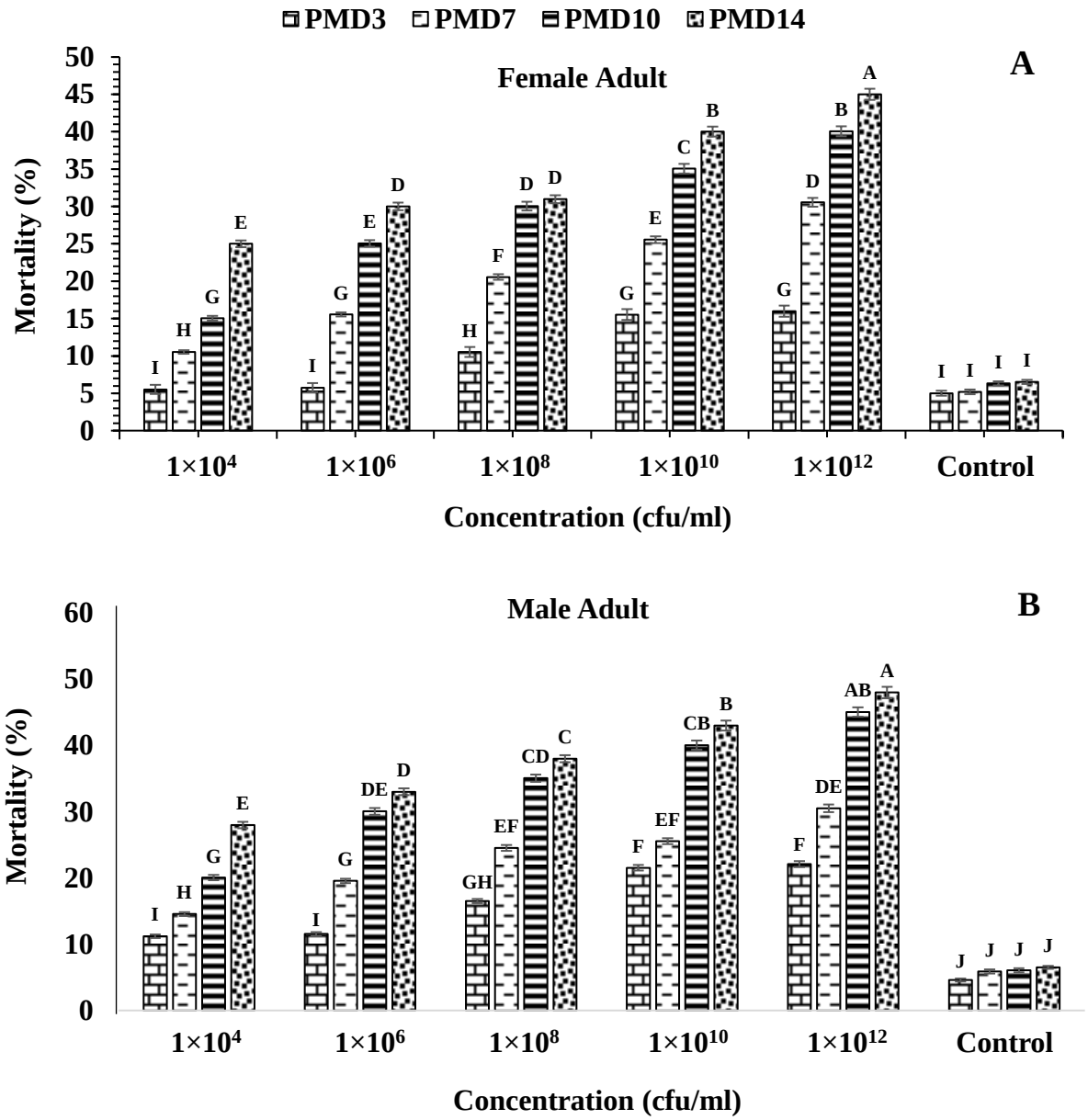

Figure 4. Effect of different concentrations of *Trichoderma harzianum* on mortality of adult females and males *Bactrocera zonata* after exposure of 3, 7, 10 and 14 days. Vertical bars show standard errors of means. Bars with different letters show significant difference at P≤0.05.

LC_{50} values of EPF against *B. zonata* at different time intervals

After applying probit analysis, we observed an increase in polyethylene imine (PEI) (d) with a decrease in values of slope. This indicates that all LC_{50} were time-dependent. LC_{50} varied significantly among the four fungi for each exposure interval as there was no overlap in their respective 95% fiducial limits. LC_{50} values of *B. bassiana*, *M. anisopliae*, *V. lecanii* and *T. harzianum* ranged from 5.17×10^{16} to 6.36×10^{8} cfu/ml; 2.60×10^{15} to 3.03×10^{8} cfu/ml; 9.33×10^{13} to 1.13×10^{9} cfu/ml and 2.26×10^{14} to 1.13×10^{9} cfu/ml for female; and 3.02×10^{15} to 8.07×10^{11} cfu/ml; 3.73×10^{15} to 7.04×10^{10} cfu/ml; 4.00×10^{18} to 6.80×10^{13} cfu/ml and 2.67×10^{19} to 1.02×10^{12} cfu/ml for male of *B. zonata,* respectively. The lowest LC_{50} value (3.03×10^{8} cfu/ml) was in case of *B. bassiana* at maximum PEI (14 d) and proved to be the most toxic for males of *B. zonata* followed by *M. anisopliae*, *V. lecanii* and *T. harzianum*. Similarly, the lowest LC_{50} and the most toxic value for females of *B. zonata* (6.36×10^{8} cfu/ml) was also in case of *B. bassiana* after 14 days followed by *M. anisopliae*, *V. lecanii* and *T. harzianum* (Table 1 to 4).

Table 1. LC_{50} (cfu/ml) values of *Beauveria bassiana* against *B. zonata* after different exposure intervals

Sex	Days	LC_{50} (cfu/ml)	FD Limit	Slope ± S.E.	χ^2	D.F.	P
Female	3	5.17×10^{16}	2.16×10^{14} - 1.50×10^{23}	0.12 ± 0.03	3.31	3	0.34
	7	1.05×10^{16}	5.31×10^{13} - 4.91×10^{21}	0.08 ± 0.01	1.19	3	0.75
	10	5.49×10^{11}	4.42×10^{10} - 3.87×10^{13}	0.07 ± 0.01	1.14	3	0.76
	14	6.36×10^{8}	1.32×10^{8} - 2.92×10^{9}	0.08 ± 0.01	0.20	3	0.97
Male	3	2.60×10^{15}	4.51×10^{13}- 1.86×10^{19}	0.12 ± 0.02	4.73	3	0.12
	7	1.35×10^{15}	1.58×10^{13} - 2.81×10^{19}	0.08 ± 0.01	0.38	3	0.94
	10	1.95×10^{11}	1.76×10^{10} - 9.70×10^{12}	0.06 ± 0.01	1.54	3	0.67
	14	3.03×10^{8}	6.14×10^{7} - 1.32×10^{9}	0.08 ± 0.01	0.008	3	1

χ^2 = Chi square value; D.F = Degree of freedom; P = Probability value; LC = Lethal Concentration; FL = Fudicial Limit; SE = Standard error

Table 2. LC_{50} (cfu/ml) values of *Metarhizium anisopliae* against *B. zonata* after different exposure intervals.

Sex	Days	LC_{50} (cfu/ml)	FD Limit	Slope ± S.E.	χ^2	D.F.	P
Female	3	9.33×10^{13}	6.82×10^{12} - 1.03×10^{16}	0.14±0.02	4.8	3	0.18
	7	3.55×10^{13}	2.35×10^{12} - 4.23×10^{15}	0.11±0.01	0.37	3	0.94
	10	1.50×10^{12}	1.24×10^{11} - 9.90×10^{13}	0.08±0.01	1.70	3	0.63
	14	5.58×10^{9}	1.03×10^{9} - 3.90×10^{10}	0.08±0.01	2.54	3	0.46
Male	3	2.26×10^{14}	8.52×10^{12} - 1.23×10^{17}	0.11±0.02	5.31	3	0.15
	7	4.11×10^{13}	1.67×10^{12} - 1.80×10^{16}	0.08±0.01	0.61	3	0.89
	10	1.61×10^{19}	2.38×10^{16} - 1.08×10^{25}	0.06±0.01	2.57	3	0.46
	14	1.13×10^{9}	2.05×10^{8} - 6.49×10^{9}	0.08±0.01	2.47	3	0.48

Table 3. LC_{50} values (cfu/ml) of *Verticillium lecanii* against *B. zonata* after different exposure intervals.

Sex	Days	LC_{50} (cfu/ml)	FD Limit	Slope ± S.E.	χ^2	D.F.	P
Female	3	3.02×10^{15}	3.71×10^{13} - 5.32×10^{19}	0.10±0.02	0.82	3	0.84
	7	6.08×10^{15}	3.57×10^{13} - 1.57×10^{21}	0.07±0.01	0.83	3	0.84
	10	1.16×10^{14}	2.44×10^{12} - 4.17×10^{17}	0.07±0.01	0.19	3	0.97
	14	8.07×10^{11}	5.75×10^{10} - 7.91×10^{13}	0.07±0.01	0.64	3	0.88
Male	3	3.73×10^{15}	3.15×10^{13} - 2.38×10^{20}	0.08±0.18	0.86	3	0.83
	7	4.45×10^{15}	2.31×10^{13} - 2.26×10^{21}	0.07±0.01	0.86	3	0.83
	10	2.78×10^{12}	1.55×10^{11} - 5.31×10^{14}	0.07±0.01	0.91	3	0.82
	14	7.04×10^{10}	7.75×10^{9} - 1.93×10^{12}	0.07±0.01	0.38	3	0.94

Table 4. LC_{50} values (cfu/ml) of *Trichoderma harzianum* against *B. zonata* after different exposure intervals.

Sex	Days	LC_{50} (cfu/ml)	FD Limit	Slope ± S.E.	χ^2	D.F.	P
Female	3	4.00×10^{18}	1.40×10^{15} - 8.49×10^{30}	0.10±0.03	4.27	3	0.23
	7	4.33×10^{17}	8.31×10^{14} - 8.79×10^{31}	0.06±0.01	1.88	3	0.59
	10	1.83×10^{14}	2.31×10^{12} - 4.50×10^{18}	0.06±0.01	1.67	3	0.64
	14	6.80×10^{13}	5.25×10^{11} - 4.68×10^{16}	0.04±0.01	0.42	3	0.93
Male	3	2.67×10^{19}	2.04×10^{15} - 5.20×10^{35}	0.06±0.02	0.88	3	0.82
	7	1.59×10^{16}	2.81×10^{13} - 2.27×10^{24}	0.05±0.01	1.69	3	0.63
	10	4.54×10^{12}	1.26×10^{11} - 9.90×10^{15}	0.05±0.01	0.83	3	0.84
	14	1.02×10^{12}	2.39×10^{10} - 7.00×10^{15}	0.04±0.01	0.48	3	0.92

LT_{50} values of EPF against *B. zonata* at different concentrations

After applying probit analysis, LT_{50} was found concentration dependent and was increased with a decrease in concentration for all the tested entomopathogenic fungi. For all the four test fungal species, LT_{50} varied significantly for each exposure interval as there was no overlap in their respective 95% fiducial limits among the tested entomopathogenic fungi. The LT_{50} values of *B. bassiana*, *M. anisopliae*, *V. lecanii* and *T. harzianum* ranged from 20.45 to 8.40; 23.82 to 9.11 d; 62.85 to 16.68 and 63.80 to 12.65 days for females; and 62.85 to 16.68; 63.80 to 12.65; 34.09 to 16.42 and 29.55 to 13.90 days for males of *B. zonata,* respectively. According to our results, the lowest LT_{50} value (3.03×10^8 cfu/ml) was higher at lower concentrations and vice versa (Table 5 to 8).

Table 5. LT_{50} (days) of *Beauveria bassiana* against *B. zonata* at different concentrations.

Sex	Conc. (cfu/ml)	LT_{50} (Days)	FD Limit	Slope ± S.E.	χ^2	D. F.	P
Female	1×10^4	20.45	16.66 - 32.35	2.35±0.50	2.65	2	0.26
	1×10^6	19.01	15.39 - 28.81	1.55±0.29	1.82	2	0.40
	1×10^8	16.11	13.47 - 22.01	1.37±0.24	1.35	2	0.50
	1×10^{10}	12.37	10.74 - 15.09	1.25±0.20	4.83	2	0.08
	1×10^{12}	8.40	7.20 - 9.69	1.04±0.77	3.73	2	0.15
Male	1×10^4	23.82	17.88 - 47.71	1.68±0.38	1.20	2	0.54
	1×10^6	18.79	14.85 - 30.09	1.23±0.24	1.37	2	0.50
	1×10^8	14.62	12.71 - 18.21	1.58±0.25	0.70	2	0.70
	1×10^{10}	12.43	10.93 - 14.81	1.40±0.21	3.15	2	0.20
	1×10^{12}	9.11	8.155 - 10.13	1.46±0.18	7.83	2	0.02

Table 6. LT_{50} (days) of *Metarhizium anisopliae* against *B. zonata* at different concentrations.

Sex	Conc. (cfu/ml)	LT_{50} (Days)	FD Limit	Slope ± S.E.	χ^2	D.F.	P
Female	1×10^4	62.85	27.15 - 97.44	0.94±0.35	1.29	2	0.52
	1×10^6	42.89	23.02 - 493.70	0.91±0.28	0.01	2	0.99
	1×10^8	28.81	18.37 - 111.03	0.82±0.23	1.12	2	0.57
	1×10^{10}	34.41	18.07 - 927.97	0.52±0.18	2.17	2	0.33
	1×10^{12}	16.68	11.93 - 41.11	0.59±0.16	2.57	2	0.27
Male	1×10^4	63.80	27.01 - 109.81	0.81±0.30	1.41	2	0.49
	1×10^6	44.49	22.58 - 862.03	0.73±0.24	0.59	2	0.74
	1×10^8	22.05	15.72 - 50.80	0.89±0.21	0.38	2	0.82
	1×10^{10}	19.27	13.18 - 59.98	0.59±0.17	2.74	2	0.25
	1×10^{12}	12.65	9.78 - 21.01	0.63±0.15	3.96	2	0.13

Table 7. LT_{50} (days) of *Verticillium lecanii* against *B. zonata* at different concentrations.

Sex	Conc. (cfu/mL)	LT_{50} (Days)	FD Limit	Slope ± S.E.	χ^2	D.F.	P
Female	1×10^4	62.85	27.15 - 99.44	0.94±0.35	1.29	2	0.52
	1×10^6	42.89	23.02 - 493.70	0.91±0.28	0.01	2	0.99
	1×10^8	28.81	18.37 - 111.03	0.82±0.23	1.12	2	0.57
	1×10^{10}	34.41	18.07 - 927.97	0.52±0.18	2.17	2	0.33
	1×10^{12}	16.68	11.93 - 41.11	0.59±0.16	2.57	2	0.27
Male	1×10^4	63.80	27.01 - 101.81	0.81±0.30	1.41	2	0.49
	1×10^6	44.49	22.58 - 862.03	0.73±0.24	0.59	2	0.74
	1×10^8	22.05	15.72 - 50.80	0.89±0.21	0.38	2	0.82
	1×10^{10}	19.27	13.18 - 59.98	0.59±0.17	2.74	2	0.25
	1×10^{12}	12.65	9.78 - 21.01	0.63±0.15	3.96	2	0.13

Table 8. LT_{50} (days) of *Trichoderma harzianum* against *B. zonata* at different concentrations.

Sex	Conc. (cfu/ml)	LT_{50} (Days)	FD Limit	Slope ± S.E.	χ^2	D.F.	P
Female	1×10^4	34.09	23.96 - 83.76	2.50±0.57	0.78	2	0.67
	1×10^6	27.11	18.70 - 72.04	1.20±0.29	0.61	2	0.73
	1×10^8	25.55	17.79 - 64.33	1.06±0.25	1.11	2	0.57
	1×10^{10}	20.17	15.13 - 38.20	1.00±0.22	1.72	2	0.42
	1×10^{12}	16.42	13.23 - 24.59	1.08±0.21	1.22	2	0.54
Male	1×10^4	29.55	19.59 - 94.47	1.19±0.30	0.39	2	0.82
	1×10^6	28.75	18.36 - 109.06	0.84±0.22	0.31	2	0.85
	1×10^8	20.19	14.87 - 40.90	0.90±0.29	0.88	2	0.64
	1×10^{10}	16.49	12.62 - 29.15	0.80±0.18	0.98	2	0.61
	1×10^{12}	13.9035	11.0652 - 21.2031	0.79±0.17	0.81	2	0.66

DISCUSSION

Numerous studies have shown how important entomopathogenic microorganisms are as bioagents against Tephritid fruit pests (Iqbal et al., 2021; Bamisile et al*.,* 2024; Heikal et al., 2024). When exposed through various ways, the entomopathogenic microorganisms show virulence against different stages (adults, pupae, and maggots) of insects (Dadaşoglu et al., 2023; Sam-On et al*.,* 2024). The pathogenicity of several EPF against *B. zonata* was assessed in the current study in order to biologically control this species. The pathogenicity of *B. bassiana*, *T. harzianum, M. anisopliae* and *V. lecanii* against *B. zonata* varied concentration and exposure duration. These outcomes are in line with the research findings of Wakil et al*.* (2024) and Li et al. (2024). Several other research works that evaluated the toxicity of EPF against different fruit fly species also highlight the varied pathogenicity of these tested entomopathogens against *B. zonata* (Shaurub, 2023; Vivekanandhan et al*.* 2024). Previous studies have also shown that local strains of *B. bassiana*, *V. lecanii* and *M. anisopliae* were effective enough in their virulence to manage various stages of *B. zonata* (Islam et al., 2021; Yadav et al., 2022; Kobisi, 2024). It was further verified that application of isolates of *B. bassiana* and *M. anisopliae* to the late third instar larvae of *B. zonata* and *B. dorsalis* in sand resulted in a considerable mortality of pupa of both species and a significant decrease in adult emergence (Murtaza et al., 2022; Qayyum et al., 2024). Additionally, all the fungal isolates caused significant mortality in emerging adults after treating them in their late third instar stage (Shaukat et al., 2023; Moreira et al*.,* 2024). According to Farrokhzadeh et al*.* (2024), *B. bassiana* and *M. anisopliae* were highly virulent against *B. dorsalis*. As the concentrations of spore suspension of *B. bassiana*, *M. anisopliae, V. lecanii* and *T. harzianum* was increased from 1.0×10^4 to 1.0×10^{12} cfu/mL, the LT_{50} values decreased from 20.45 to 8.40; 62.85 to 16.68; 62.85 to 16.68 and 34.09 to 16.42 days for female, and 23.82 to 9.11, 63.80 to 12.65; 63.80 to 12.65 and 29.55 to 13.90 days for male *B. zonata,* respectively. Similarly LC_{50} values of *B. bassiana*, *M. anisopliae, V. lecanii* and *T. harzianum* against *B. zonata* decreased from 5.17×10^{16} to 6.36×10^8, 9.33×10^{13} to 5.58×10^9, 3.02×10^{15} to 8.07×10^{11} and 4.00×10^{18} to 6.80×10^{13} cfu/ml for female, and 2.60×10^{15} to 3.03×10^8; 2.26×10^{14} to 1.13×10^9; 3.73×10^{15} to 7.04×10^{10} and 2.67×10^{19} to 1.02×10^{12} cfu/ml for male over a period of 3–14 days post adult emergence, respectively.

According to the findings of current study, all the evaluated entomopathogenic formulations showed lower LC_{50} values against male *B. zonata* making them more hazardous to males of this species than the females. Findings of present study was supported by El-Gendy et al*.* (2022) and Qayyum et al. (2024) demonstrated that *B. bassiana* was pathogenic to adults of *B. zonata*, with females of this species being less sensitive than males. Differences in fruit fly species and EPF strains could be the cause of this variation.

The variation in virulence factors, such as spore germination, hyphal growth, bacterial budding, toxins, etc., during the different growth periods of tested entomopathogens may be the cause of the varied mortality at different exposure periods in both male and female sexes of *B. zonata* caused by EPF in the current work. In this investigation, *B. zonata* was less vulnerable to *V. lecanii* than *B. bassiana*, *M. anisopliae* and *V. lecanii*. Upon testing these entomopathogens against *B. zonata*, Iqbal et al. (2021) and Yu et al*.* (2024) also noted similar outcomes.

The data showed that the pathogenicity of all the tested EPF increased as exposure intervals increased, declining at 3-day post-application interval and dramatically increased at 14-day post-application intervals. Chepkemoi et al. (2023) also confirmed these findings, determining that the highest mortality of armyworm was observed at the highest concentration (10^8 cfu/ml) and that the mortality was progressively dropped as the concentration decreased. Onsongo

et al. (2022) reported similar results, showing that the highest concentration of *B. bassiana* (2.4×10^9 cfu/ml) caused the greatest mortality of *B. cucurbitae* after 5 and 7 days of the treatment.

CONCLUSIONS

B. bassiana and *M. anisopliae* proved to the most effective species for controlling *B. zonata* followed by *V. lecanii* and *T. harzianum* in terms of% mortality, LC_{50} values, and LT_{50} values. Adult males were more vulnerable than females to entomopathogenic fungi. Since entomopathogenic fungi are safe and highly successful in controlling pests, they ought to be included in IPM programs to control *Bactrocera zonata*. For more successful fruit fly control, entomopathogenic fungi should be included in future, more comprehensive fruit fly control strategies.

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AUTHOR CONTRIBUTIONS

All authors contributed equally to this research.

COMPETING OF INTEREST

No conflicts of interest have been disclosed by the authors.

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