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Virulence effect of *Metarhizium anisopliae* (Met.) and *Beauveria bassiana* (Bals.) fungi against the peach fruit fly, *Bactrocera zonata* (Saunders) (Diptera: Tephritidae)

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Abstract

Background: The peach fruit fly, *Bactrocera zonata* (Saunders) (Diptera: Tephritidae), is a key pest of fruits in Egypt. Insect-pathogenic fungi are one of the biocontrol agents that increasingly substitute the traditional pesticides to overcome pesticide risks. Therefore, the present study aims to assess the fungal virulence of *Beauveria bassiana* (Balsamo) and *Metarhizium anisopliae* (Metchnikoff) against *B. zonata* pupae. Also, extended pathogenicity effect of these fungi on adult flies was studied.

Results: The results showed that *M. anisopliae* fungus had more pathogenicity to *B. zonata* pupae on the 2nd, 3rd, and 5th days post-treatment than *B. bassiana*. Pathogenicity fungal effects of treated larvae extended to the surviving adults. Fungal concentration and post-exposure interval reversely impacted the pupae by 63.88 and 63.59% mortality in the case of *M. anisopliae* and *B. bassiana*, respectively. The lethal concentration of treated fly by *M. anisopliae* ($LC_{50} = 9.5 \times 10^6$ conidia/ml and $LC_{90} = 9.9 \times 10^7$ conidia/ml) was lower than that of *B. bassiana* ($LC_{50} = 5.1 \times 10^7$ conidia/ml and $LC_{90} = 1.9 \times 10^9$ conidia/ml). Median lethal time (LT_{50}) value was fungal species-dependent, and concentration. *Metarhizium anisopliae* was more virulent than *B. bassiana*; the lowest LT_{50} value was 9.48 days by *M. anisopliae* and 13.33 days by *B. bassiana*, depending on the fungal tested concentration of 2.3×10^6 conidia/ml.

Conclusions: The tested entomopathogenic fungi could be considered promising biocontrol agents against *B. zonata* and could be used for fly suppression through soil application in IPM programs.

Keywords: Entomopathogenic fungi, Fruit flies, Microbial control

Background

Tephritid fruit flies are a group of economic insect pests that attack fruits and certain vegetables worldwide, causing direct and indirect economic injury (Lysandrou 2009). The genus *Bactrocera* has a wide range of hosts and invasive capabilities of certain species that are considered a severe threat to horticultural crops (Clarke et al. 2005). The peach fruit fly, *Bactrocera zonata* (Saunders) (Diptera: Tephritidae), is one of the significant economic pests present in West Asia, North Africa, and Southern Europe (Eppo 2010). It attacks over 40 host plants (Delrio and Cocco 2012) between fruits and vegetables. Furthermore, according to the European and Mediterranean Plant Protection Organization, it is classified as a quarantine A1 pest (Eppo 2010).

Extensive applications of conventional pesticides against fruit flies generate major environmental problems (Magaña et al. 2007), such as resistance development of field strain of the fly. Several efforts have been carried out to limit the usage of toxic pesticides to manage this pest,

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such as employing abiotic factors, soil water content, soil compaction, and biopesticides alternative to traditional insecticides (El-Gendy and AbdAllah 2020; El-Gendy et al. 2021).

Over the last decades, entomopathogenic fungi (EPF) have played a significant role in the natural biological control of many insects (Burges 1981). *Beauveria bassiana* (Balsamo) Vuillemin (Hypocreales: Clavicipitaceae) and *Metarhizium anisopliae* (Metchnikoff) Sorokin (Hypocreales: Clavicipitaceae) have an essential role in insect pests' control. *B. bassiana* is the only insect pathogen that can directly penetrate into its hosts through their cuticles and provides good prospects for managing *B. zonata* puparia in the soil (soil inocula) (Garrido-Jurado et al. 2011). *B. bassiana* is a safe biocontrol organism on non-target insects and mammals, including people (Zimmermann 2007). *B. zonata* adults and pupae were found susceptible to the EPF, such as *M. anisopliae* and *B.* bassiana (Rashad et al. 2015). Extended pathogenicity of *B. bassiana* and *M. anisopliae* to surviving adult flies treated in the larval stage is limited. Therefore, the present trial aimed to assess the pathogenicity of the EPF, *B. bassiana* and *M. anisopliae*, against *B. zonata* pupae and their extended potential effect on the adult flies under laboratory conditions.

Methods

Target pest

The peach fruit fly, *B. zonata*, was obtained from the Eradiation of Peach Fruit Fly Laboratory at Damanhour, El-Beheira Governorate, Egypt, which was reared according to El-Gendy (2002).

Fungi used

Two commercial fungus formulations of *B. bassiana* and *M. anisopliae* (WP 2.5%, 2.3×10^8 conidia/gm) were tested against *B. zonata* in the present study. *B. bassiana* strain was originally isolated from the red palm weevil, *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae), in Ismailia governorate, while *M. anisopliae* was isolated from the cotton whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), in Sharkiah governorate, and both were identified in the fungal center of Faculty of Science, Assiut University, Egypt (Ibrahim 2006). Fungal formulations were gained from Bioinsecticides Production Unit, Plant Protection Research Institute, Agricultural Research Center, Giza, Egypt.

Virulence assay of EPF, *M. anisopliae* and *B. bassiana*, against *B. zonata*

Laboratory assay of the EPF, *M. anisopliae* and *B. bassiana*, was carried out against the 3rd larval instar of *B. zonata* (full-grown larvae) under laboratory conditions of $25 \pm 2^{\circ}$ C, and 70–80% RH. The experiments were applied in plastic cups (250 cm^3) closed at the top with a cloth net (1 mm mesh). The cups contained 75 gm of sterilized sand, autoclaved at 105°C for 24 h. The fungi were dissolved in tap water. Each fungus was assayed in 5 concentrations, one with a recommended concentration (6×10^5 conidia/ml), and the others were above and below the recommended concentration (2.0×10^5, 4.0×10^5, 1.2×10^6, and 2.3×10^6 conidia/ml), in addition to the control treatment (water only).

One hundred larvae were used per each soil treatment (concentration) in 5 replicates, 20 larvae each. Ten milliliters of fungal suspension per replication was dropped on the soil by the plastic pipette 3 ml. Larvae, full-grown, were transferred to the cups and freely allowed to pupate in the soil. After 2, 3, and 5 days of treatment, the treatments were inspected, and the dead pupae were recorded. On the 7th day, the remained pupae were transferred to Petri dishes until flies' emergence. The newly emerged flies were transferred into plastic cups (250 cm^3) covered with a cloth net, supplied with a source of food and water, as previously described. Treatments were checked daily, and the dead flies were removed to Petri dishes containing wet filter papers to confirm that death was caused by the fungal infection.

Determination of the lethal concentration for 50% (LC_{50}) and 90% (LC_{90}) of the individuals

The concentration–mortality relationship, the lethal concentration for 50 and 90% of the fly's individuals (LC_{50} and LC_{90}), was determined for the tested fungal concentrations (2.0×10^5, 4.0×10^5, 6×10^5, 1.2×10^6, and 2.3×10^6 conidia/ml) of *M. anisopliae* and *B. bassiana* against *B. zonata* pupae 72 h post-treatment.

Determination of the lethal time for 50% (LT_{50}) and 90% (LT_{90}) of the individuals

Time-mortality (median time values associated with 50 and 90% mortality of flies, LT_{50} and LT_{90}) of *B. zonata* exposed to the above-mentioned fungal concentrations of *M. anisopliae* and *B. bassiana* was determined.

Statistical analysis

Mortality percentages of *B. zonata* were subjected to two-way analysis of variance (ANOVA), using CoStat Software (2008) Version 6.4. Means were compared by the Tukey–Kramer test at the 5% probability level. LC_{50} and LC_{90} and LT_{50} and LT_{90} values were determined by probit analysis (Finney 1952), using the Ldp-Line program (Bakr 2007).

Results

Virulence of *B. bassiana* and *M. anisopliae* against *B. zonata* pupae and adult stages

The cumulative mortality of B. zonata pupae of various time intervals

As mentioned above, the EPF, *B. bassiana* and *M. anisopliae*, were assayed on the full-grown larvae of *B. zonata* in 5 fungal concentrations: 2.0×10^5, 4.0×10^5, 6.0×10^5, 1.2×10^6 and 2.3×10^6 conidia/ml, using soil treatment application. It was indicated that no fly's mortality was recorded during the experimental period, either during the pupal or adult stages in the control treatment. As shown in Fig. 1a, b, pupal mortality was dependent on fungal species and concentration, as well as post-treatment time. No fungal effect was detected on *B. zonata* pupae after two days of treatment with low concentrations of *M. anisopliae* and *B. bassiana* (2.0×10^5 and 4.0×10^5 conidia/ml, respectively). Pupal mortality began at the concentration of 6×10^5 conidia/ml, with 5 and 2.5% mortality, respectively, by *M. anisopliae* and *B. bassiana*. Mortality of pupae increased gradually, with increasing the fungal concentration of 1.2×10^6 and 2.3×10^6 conidia/ml, to achieve 7.5 and 12.5% mortality by *M. anisopliae* and 5 and 7.5% by *B. bassiana*, respectively. These mortalities varied significantly among fungal concentrations based on fungus species (*M. anisopliae*: $F=7.68$, $P=0.005$ and *B. bassiana*: $F=2.88$, $P=0.04$).

On the 3rd day of pupal age, the treatments exhibited a low mortality percentage (2.5%) at the treatment with 4.0×10^5 conidia/ml of both fungi. Mortality rates increased significantly to 7.5–20 and 7.5–12.5%, respectively, to the fungal concentrations (6.0×10^5–2.3×10^6 conidia/ml) of *M. anisopliae* ($F=11.2$, $P=0.0001$) and *B. bassiana* ($F=9.13$, $P=0.0002$). On the 5th day, the pupal mortality rate increased significantly as the fungal concentration increased. Pupal mortality rates ranged from 12.5 to 27.5% for *M. anisopliae* ($F=7.51$, $P=0.0006$) and from 5 to 17.5% for *B. bassiana* ($F=8.49$, $P=0.0003$), with their respective fungal concentrations that ranged from 4.0×10^5 to 2.3×10^6 conidia/ml. In parallel, pupal mortality rates were significantly correlated with fungal species and fungal concentration [*M. anisopliae*: $r=0.81$ (at the 1-day old pupa), 0.87 (3-days) and 0.84 (5-days), while for *B. bassiana*: $r=0.64$ (1-days), 0.86 (3-days) and 0.85 (5-days)].

Pupal mortality was affected by the fungal concentration and post-exposure time by 63.88% with *M. anisopliae* and 63.59% with *B. bassiana* of the total factors affected pupal mortality, according to determination coefficient (r^2).

Cumulative mortality of adult fly of *B. zonata* at various time intervals

The effect of EPF continued to the adult fly stage. *M. anisopliae* was more effective than *B. bassiana* based on mortality rates that appeared in the adult stage (Fig. 2a, b). On the adult's 5th day, fly mortality rate was 50% ($F=19.11$, $P=0.0000$) after treating *M. anisopliae* and 27.5% ($F=14.73$, $P=0.0000$) after treating with *B. bassiana*. As both fly age and fungal concentration increased, the fly mortality rate increased. Fungal treatments of *M. anisopliae* (2.0×10^5–2.3×10^6 conidia/ml) achieved significant mortality effects of 20 to 57.5% ($F=21.15$, $P=0.0000$), 35–65% ($F=26.93$, $P=0.000$),

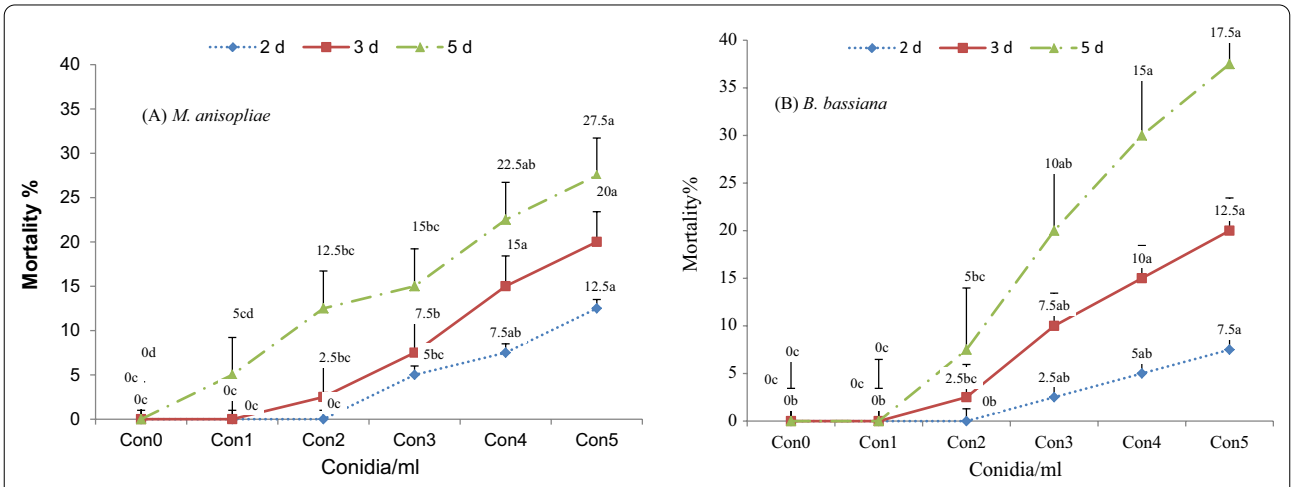

Fig. 1 Accumulated mortality percentages of *Bactrocera zonata* pupae at three time intervals, treated in the larval stage with different concentrations of *Metarhizium anisopliae* (**A**) and *Beauveria bassiana* (**B**) fungus under laboratory conditions. Concentrations on x-axis represent Con0 = Control, Con1 = 2.0×10^5-, Con2 = 4.0×10^5-, Con3 = 6.0×10^5-, Con4 = 1.2×10^6-, Con5 = 2.3×10^6-Conidia/ml. Error bars indicate the ± standard error; means sharing similar style letters do not significantly differ at a probability level of 5%

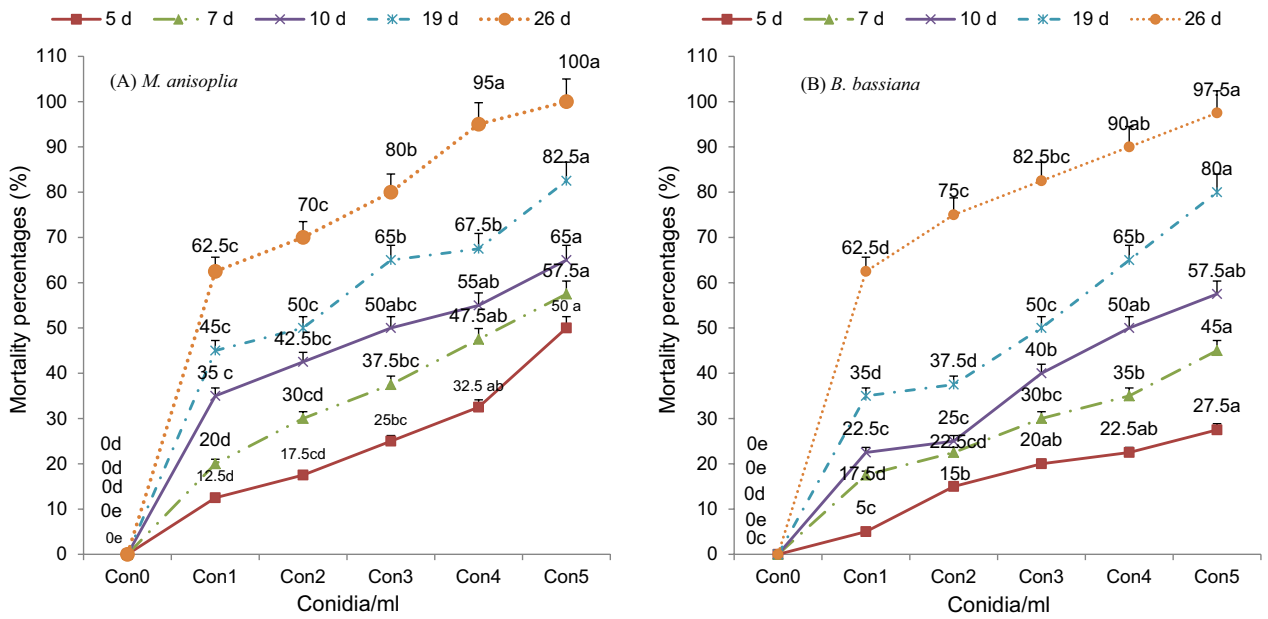

Fig. 2 Accumulated mortality percentages of *Bactrocera zonata* adults at five time intervals, treated in the larval stage with different concentrations of *Metarhizium anisopliae* (**A**) and *Beauveria bassiana* (**B**) fungus under laboratory conditions. Concentrations on x-axis represent Con0 = Control, Con1 = 2.0×10^5-, Con2 = 4.0×10^5-, Con3 = 6.0×10^5-, Con4 = 1.2×10^6-, Con5 = 2.3×10^6-Conidia/ml. Error bars indicate the ± standard error; means sharing similar style letters do not significantly differ at a probability level of 5%

45–82.5% ($F = 51.29$, $P = 0.000$), and 62.5–100% ($F = 121.8$, $P = 0.000$) with respective of adult fly age of the 7th, 12th, 19th, and 26th, while the treatments by *B. bassiana* achieved mortalities of 17.5–45.5% ($F = 31.75$, $P = 0.0000$), 22.5–57.5% ($F = 30.17$, $P = 0.000$), 35–80% ($F = 81.71$, $P = 0.000$), and 62.5–97.5% ($F = 124.49$, $P = 0.000$) with respective tested days. The mortality rate of the adult fly was significantly different based on the fungal species and fly age [*M. anisopliae*; $r = 0.93$ (at 5-days old fly), 0.91 (7-days), 0.89 (10-days), 0.92 (19-days) and 0.95 (26-days), and *B. bassiana*; $r = 0.88$ (5-days), 0.93 (7-days), 0.93 (10-days), 0.96 (19-days) and 0.95 (26-days)].

Table 1 Lethal concentration values of LC_{50} and LC_{90} (conidia/ml) of *Metarhizium anisopliae* and *Beauveria bassiana* for *Bactrocera zonata* fly at 72 h of treatments under laboratory conditions

Lethal concentration (conidia/ml)		χ^2	χ^2 tabulated	*P*	Slope ± SE
LC_{50}	LC_{90}				
M. anisopliae					
9.5×10^6	9.9×10^7	0.79	6.0	0.69	0.59 ± 0.244
B. bassiana					
5.1×10^7	1.9×10^9	0.69	6.0	0.71	0.82 ± 0.50

LC_{50}—lethal concentration of 50% of the tested insect, χ^2—Chi-square, *P*—probability level of 5%, SE—standard error

LC_{50} and LT_{50} values of EPF

The concentration–mortality correlation revealed that LC_{50} and LC_{90} values were fungus species-dependent, where the fly pupae were more sensitive to *B. bassiana* than *M. anisopliae* fungus. The estimated LC_{50} value for pupal fly, 72 h post-treatment, of *B. bassiana* was ($LC_{50} = 5.1 \times 10^7$ conidia/ml) 5.4-fold higher than that of *M. anisopliae* ($LC_{50} = 9.5 \times 10^6$ conidia/ml), while the LC_{90} value of *B. bassiana* was ($LC_{90} = 1.9 \times 10^9$ conidia/ml) about 37 times higher than that of *M. anisopliae* ($LC_{90} = 9.9 \times 10^7$conidia/ml) (Table 1).

The LT_{50} values of *B. zonata* were fungal species- and concentration-dependent. The fungal impact of *M. anisopliae* was 1.41 times faster than *B. bassiana* at the lowest LT_{50} values, which were 9.48 and 13.33 days for *M. anisopliae* and *B. bassiana* fungi, respectively, at 2.3×10^6 conidia/ml. LT_{50} values were inversely related to the fungal concentration; it reached 28.58 and 30.03 days of treatment at the lowest fungal concentration (2.0×10^5 conidia/ml) of *M. anisopliae* and *B. bassiana*, respectively (Table 2).

Table 2 Lethal time values (LT_{50} and LT_{90} (day)) for *Bactrocera zonata* fly treated with different fungal concentrations of *Metarhizium anisopliae* and *Beauveria bassiana* under laboratory conditions

Fungus concentration (conidia/ml)	LT_{50} (day)	LT_{90} (day)	χ^2	χ^2 tabulated	*p*	Slope ± SE
M. anisopliae						
2.3×10^6	9.48	42.99	8.16	12.6	0.23	1.95 ± 0.20
1.2×10^6	13.69	65.95	8.29	12.6	0.39	1.88 ± 0.21
6.0×10^5	18.27	81.58	6.38	12.6	0.38	1.97 ± 0.24
4.0×10^5	24.37	98.90	5.53	11.1	0.35	2.11 ± 0.32
2.0×10^5	28.58	100.1	3.84	9.5	0.43	2.65 ± 0.46
B. bassiana						
2.3×10^6	13.33	51.64	9.52	12.6	0.23	2.18 ± 0.23
1.2×10^6	17.55	65.34	12.00	12.6	0.09	2.42 ± 0.26
6.0×10^5	22.44	99.75	11.58	12.6	0.07	1.98 ± 0.25
4.0×10^5	28.99	103.99	10.11	11.1	0.49	2.31 ± 0.36
2.0×10^5	30.03	124.52	3.76	7.8	0.39	3.76 ± 0.65

LT_{50}—Median time needs to achieve 50% mortality of the tested individuals, χ^2—Chi-square, *P*—probability level of 5%, SE—standard error

Discussion

Entomopathogenic fungi (EPF) have received growing interest as microbial agents to control insect pests during the last decades. The pathogenicity of EPF, *B. bassiana* and *M. anisopliae*, was assayed on the full-grown larvae of *B. zonata* in 5 concentrations: 2.0×10^5, 4.0×10^5, 6.0×10^5, 1.2×10^6, and 2.3×10^6 conidia/ml, using soil treatment application. The findings revealed that mortality rates of *B. zonata* pupae were significantly related to the fungal concentration and fungal species. These findings were confirmed by *B. zonata* and *B. cucurbitae* (Coquillett) adult flies, i.e., as the mortality rates of the pests varied based on fungus species and its isolated race (Sookar et al. 2008). The variation in mortality rates of the tested fly stages between both fungal species revealed that *B. zonata* pupae were much tolerant to the fungal insecticide than in the adult fly stage. They were also more tolerant to *B. bassiana* than *M. anisopliae.* In a similar vein, Mahmoud (2009) recorded fairly susceptible of *B. zonata* pupae by the soil treatment of *M. anisopliae* than *B. bassiana* fungus.

On the other hand, fungal mortality rates for *B. zonata* pupae increased as the fungal concentration and exposure-time interval increased, with higher mortality rates at *M. anisopliae* than *B. bassiana*. In parallel with the results of Rashad et al. (2015), the pupal mortality rates of *B. zonata* increased significantly by increasing the concentration of *M. anisopliae* and *B. bassiana* fungi at the same time of exposure. Similar results were recorded on the Medfly, *Ceratitis capitata* (Wiedemann), as the *M. anisopliae* fungus was superior pathogenically to the pupae than *B. bassiana* at the same fungal concentration along with different tested times (Soliman et al. 2020).

Fungal mortality effects of *M. anisopliae* and *B. bassiana* on *B. zonata* pupae appeared on the 2nd day of treatment with little effects to be increased with time post-treatment increased to 27.5% by treatment of *M. anisopliae* and 17.5% by *B. bassiana* on the 5th day of pupal age. Obtained results showed differences in pupal mortality rates during the different test periods and between fungal species. However, mortality rates may be different according to the fruit fly species and race. In parallel, Attia (2018) reported 37.75–55.02% mortality in the pupal stage of *B. zonata* treated with 1–4gm/l of *B. bassiana*. On *C. capitata* pupae, *M. anisopliae* and *B. bassiana* resulted in about 94% mortality (Ekesi et al. 2002). However, no fungal effect of *B. bassiana* was detected on *Anastrepha ludens* (Loew) pupae (Aluja 1993).

Pathogenicity effect of *M. anisopliae* and *B. bassiana* extended to the adult fly stage; *M. anisopliae* fungus was more fatality than *B. bassiana*. In parallel with the present results, in earlier studies on *B. zonata* fly, *M. anisopliae* fungus was superior in the fatality rates on the fly than *B. bassiana* fungus (Ibrahim et al. 2014). However, Hussein et al. (2018) reported that *B. bassiana* was more effective than *M. anisopliae* on the immature stages and adult flies of *B. zonata*. Furthermore, Rashad et al. (2015) found a significant influence of the soil application of *B. Bassiana* fungus on mortality rates in adult flies *B. zonata*, compared to *M. anisopliae* fungus.

Mortality rates of the adult fly of *B. zonata* were significantly related to the concentrations of fungi and according to adult stage and fungus species. Similar to the present results, the correlation coefficient values of microbial-insecticide concentrations of *B. bassiana* and

M. anisopliae were positively correlated with mortalities of *B. cucurbitae* fly (Iqbal et al. 2021).

According to the present study's findings, the median LT_{50} values on the fly were fungal species- and concentration-dependent. The *M. anisopliae* fungus affected the fly faster than *B. bassiana*. Results of LT_{50} values ranged from 28.57 to 9.475 days by *M. anisopliae* and from 30.03 to 13.33 days by *B. bassiana* fungus, respectively, depending on fungal concentration. These results are consistent with the results of Mahmoud (2009), whereby the LT_{50} values for *B. zonata* males and females treated with *M. anisopliae* fungus were less than those with *B. bassiana*, and LT_{50} values were 8.93–10.89 days for males and 12.69–14.39 days for females with the respective fungi.

Conclusions

This study concluded that the appreciable lethal effects of *M. anisopliae*, and *B. bassiana* fungi were set at the end of the pupal stage of *B. zonata* with 27.5 and 17.5% mortalities, respectively, which persisted during the adult fly stage, achieving 100 and 97.5% mortality with, respectively, tested fungi at the highest concentration. *M. anisopliae* fungus was more effective on both pupal and adult stages of *B. zonata* than *B. bassiana*. *M. anisopliae* was more virulent than *B. bassiana*; *M. anisopliae*, causing 50% mortality of individuals (LT_{50} value) in 9.475 and 13.33 days, respectively, at the highest tested fungal concentration. *M. anisopliae* and *B. bassiana* had potent effects in *B. zonata* control under laboratory conditions. This information may help in the pest management of the pest and in integrated and organic production systems.

Abbreviations

ANOVA: Analysis of variance; IPM: Integrated pest management; LC: Lethal concentration; LT: Lethal time; *P*: Probability level; SE: Standard error; χ^2: Chi square.

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Author contributions

IR reared *B. zonata*, and IR and MFZ put strategy to achieve this work. IR, MFZ, and MI achieved this investigation. IR, MFZ, and MI are the contributors in writing the manuscript. IR and MFZ revised the manuscript. All authors read and approved the final manuscript.

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All data and materials are available.

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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