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Impact of Almond Oil, Amla Oil and Coconut Oil on the Growth and Virulence of Entomopathogenic Strains against *Bactrocera Zonata* Maggots

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ABSTRACT

Fruit flies, particularly *Bactrocera zonata*, are significant pests causing major agricultural losses. Chemical control methods result in pesticide residues, making alternative strategies essential. This study examines the impact of almond, amla, and coconut oils on the growth and virulence of entomopathogenic fungi (EPF) against *B. zonata* larvae. **Objectives:** To evaluate the effects of almond, amla, and coconut oils on the efficacy of nine EPF strains in controlling *B. zonata* larvae by assessing spore germination rates, lethal concentrations (LC_{50} and LC_{90}), and lethal times (LT_{50} and LT_{90}). **Methods:** The study was conducted at the University of Agriculture Faisalabad. Infested fruits were collected from citrus and guava orchards on campus. EPF strains were cultured on ¼ SDAY media with the three oils at seven concentrations (5%-35%) in IPM Laboratory of Department of Entomology UAF. Spore germination, LC_{50}, LC_{90}, LT_{50}, and LT_{90} values were assessed in the lab. **Results:** Metarhizium pinghaense (MBC709), *Lecanicillium attenuatum* (MBC807), and *Isaria farinose* (MBC389) showed significant responses to oils and concentrations. *Metarhizium anioplIae* (F52) exhibited the highest virulence with the lowest LC_{50} (5.11×10^10 cfu/ml) and LC_{90} (3.26×10^13 cfu/ml) values after 3 days. **Conclusions:** *Metarhizium aniopliae* (F52) showed the highest virulence, followed by *Isaria javanica* (MBC524) and *Beauveria brongniartii* (MBC397). *Beauveria brongniartii* (MBC397) had the shortest LT_{50}, indicating the highest mortality rate. This study highlights the potential of using EPF strains with oils for effective *B. zonata* management, offering a promising integrated pest management strategy.

INTRODUCTION

Vegetables and fruits are vital for human nutrition, offering essential vitamins, minerals, and antioxidants. Fruits like papaya, mango, peaches, and guava are particularly appealing due to their taste and visual charm [1]. However, in Asian countries, these fruits are vulnerable to various insect pests, with fruit flies being a major concern. Among these pests, the genus *Bactrocera* is notably destructive, affecting a wide range of horticultural crops due to its extensive list of host species and invasive capabilities [2]. *Bactrocera* species have spread far beyond their native Asian regions, causing significant agricultural damage and economic losses globally [3]. It is estimated that fruit flies cause about $200 million in losses each year, leading to increased use of insecticide sprays and posing quarantine threats to international trade and horticulture [4]. In Pakistan, the fruit fly *Bactrocera zonata* affects coastal

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regions of Sindh, Baluchistan, and Punjab, as well as semi-arid areas and occasionally the Peshawar valley and Islamabad foothills [5]. Another serious pest is *Bactrocera cucurbitae,* which primarily damages squash and cucurbits [6]. Entomopathogenic fungi offer a promising biological control method for managing these insect pests. These fungi, including *Metarhizium anisopliae*, *Beauveria brongniartii*, and *Beauveria bassiana*, act as natural enemies to insects [7]. Their infective spores attach to, germinate, and penetrate the insect cuticle, providing a biological control method without the need for ingestion. These fungi help regulate insect pest populations and are effective against pests like *Dacus diversus*, *Carpomyiavesuviana*, *Dacus ferrugineus*, *Myiopardalis pardalina*, *B. zonata*, and *B. cucurbitae* [8]. In Arab countries, fruit flies like Melon fruit fly (*B. cucurbitae*), Mediterranean fruit fly (*Ceratitis capitata*), Oriental fruit fly (*B. dorsalis*), Peach fruit fly (*B. zonata*), and Olive fruit fly (*Bactrocera oleae*) are significant commercial pests, though there are other less important fruit fly species as well [9, 10]. The effectiveness of entomopathogenic fungi is influenced by environmental factors such as humidity, which must exceed 95% for conidia germination, and temperature, which affects infection rates and sporulation. These fungi's ability to adapt to environmental conditions and evade host defenses is crucial for their success as biological control agents [11]. In integrated pest management (IPM) strategies, microbial control using entomopathogenic fungi can serve as an alternative to chemical insecticides, offering a sustainable solution to manage fruit fly populations [12]. Factors such as humidity, solar ultraviolet radiation, and temperature impact the effectiveness of fungal treatments. Oil-based formulations of these fungi are often more effective than water-based ones [13]. Additionally, monoterpenoids from essential oils and neem oil extracts have shown promise in controlling insect pests, affecting over 400 species, including household and agricultural pests [14].

The study aimed to evaluate the impact of almond oil, amla oil, and coconut oil on the growth and virulence of various entomopathogenic fungal strains against *Bactrocera zonata* larvae. Specifically, it designed to assess spore germination rates, lethal concentrations (LC_{50} and LC_{90}), and lethal times (LT_{50} and LT_{90}) of the fungal strains when exposed to these oils. This will help in control the potential of these oils in improving the efficiency of EPF in controlling *B. zonata.*

METHODS

Infested citrus and guava fruits were collected from orchards at the University of Agriculture Faisalabad. Fruits were placed on iron wire mesh over plastic trays with sterile sand in IPM Laboratory of Department of Entomology. Maggots dropped into the sand to pupate. Pupae were sieved, placed in rearing chambers, and provided with adult fly diet. Post-emergence, adult flies were given a diet and guava fruits for egg laying. After 48 hours, guava fruits with eggs were transferred to pupa collection boxes with sterile sand to establish fruit fly progenies. Nine entomopathogenic fungal strains were used: *Metarhizium aniopliae* (F52) , *Metarhizium pinghaense* (MBC 709), *Isaria fumosorosea* (MBC 053), *Isaria cateniannulata* (MBC 289), *Beauveria brongniartii* (MBC 397), *Beauveria bassiana* (MBC 076), *Lecanicillium attenuatum* (MBC 807), *Isaria javanica* (MBC 524), and *Isaria farinosa* (MBC 389). EPF strains were grown on ¼ SDAY media, consisting of yeast, agar, and Saborose Dextrose Agar. The media was autoclaved at 20 psi for 20 minutes. Fungal strains were inoculated with a 100 µL conidia/ml suspension and incubated at 28ºC in the dark for 10 to 14 days. Conidia were harvested using 0.04% sterile polysorbate 20 (Tween 20), separated from hyphae, and pipetted into sterile tubes. A 1:10 serial dilution was prepared and evaluated microscopically. Conidia concentrations were adjusted to $1x10^5$ -$1x10^9$ conidia/ml. Amla, almond, and coconut oils were purchased and diluted to 5%- 35% in Tween-20 using C1V1=C2V2. Media inoculation bioassay: oil concentrations were mixed in growth media, autoclaved, and inoculated with fungal spores. Plates were incubated, and germination percentages were calculated. Suspension inoculation bioassay: oil concentrations in conidial suspension were prepared, and larvae were exposed to treated diets. Mortality was recorded at multiple intervals and corrected using Henderson-Tilton's formula. Safer oil concentrations were mixed with different EPF concentrations, and mortality rates were recorded. Data were subjected to probit analysis for LC_{50}, LC_{90}, LT_{50}, and LT_{90} values and analyzed using ANOVA and Tukey HSD test. Media inoculation bioassay showed varying germination percentages based on oil concentration. Suspension inoculation bioassay indicated mortality rates at different intervals, corrected using Henderson-Tilton's formula. Mortality rates varied with oil and EPF concentration combinations. Probit analysis determined LC_{50}, LC_{90}, LT_{50}, and LT_{90} values, and ANOVA and Tukey HSD tests provided statistical significance.

RESULTS

Metarhizium anisopliae (F52) demonstrated the highest pathogenicity against *Bactrocera zonata* maggots, with the lowest LC_{50} ($5.11×10^{10}$cfu/ml) and LC_{90} ($3.26×10^{13}$cfu/ml) values, indicating its potential as an effective biocontrol agent. Conversely, *Isaria fumosorosea* (MBC053) showed the least effectiveness with the highest LC_{50} ($1.69×10^{15}$cfu/ml) and LC_{90} ($3.44×10^{22}$cfu/ml) values. The significant variation in pathogenicity among the EPF strains highlights the importance of selecting appropriate

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strains for pest management (Table 1a).

Table 1a: LC_{50} And LC_{90} of Different EPF Strains against Maggots of B. Zonatafor an Exposure Period of 3 Days.

Insecticides	LC_{50}	FD limit	LC_{90}	FD limit	Slope ± S.E	X^2	DF	P
F52	5.11×10^{10}	5.74×10^{7} , 5.50×10^{12}	3.26×10^{13}	6.70×10^{11} , 2.06×10^{17}	0.185 ± 0.037	7.41	3	0.06
MBC 709	2.66×10^{10}	1.29×10^{10} , 5.84×10^{14}	7.04×10^{14}	3.25×10^{12} , 1.08×10^{21}	0.15 ± 0.03	14.83	3	0.02
MBC053	1.69×10^{15}	3.05×10^{11} , 1.77×10^{52}	3.44×10^{22}	3.60×10^{15} , 1.35×10^{54}	0.07 ± 0.029	0.161	3	0.98
MBC 289	2.86×10^{11}	6.31×10^{11} , 6.58×10^{14}	7.35×10^{14}	3.37×10^{12} , 1.15×10^{21}	0.15 ± 0.035	0.52	3	0.015
MBC397	3.01×10^{12}	1.34×10^{10} , 8.72×10^{14}	1.15×10^{15}	4.32×10^{12} , 3.57×10^{21}	0.145 ± 0.034	12.23	3	0.07
MBC076	2.46×10^{11}	1.25×10^{10} , 4.34×10^{14}	6.59×10^{14}	3.24×10^{12} , 7.20×10^{20}	0.15 ± 0.035	10.96	3	0.012
MBC807	1.26×10^{12}	8.53×10^{7} , 7.45×10^{13}	3.09×10^{14}	2.26×10^{12} , 6.07×10^{19}	0.15 ± 0.03	11.96	3	0.08
MBC524	1.49×10^{11}	9.45×10^{7} , 1.11×10^{14}	3.58×10^{14}	2.42×10^{12} , 9.90×10^{19}	0.15 ± 0.034	11.43	3	0.010
MBC389	1.76×10^{11}	9.83×10^{7} , 2.12×10^{14}	7.08×10^{13}	3.50×10^{12} , 6.05×10^{20}	0.145 ± 0.03	13.53	3	0.004

Metarhizium anisopliae (F52) was the most effective EPF strain against *Bactrocera zonata* maggots over a 5-day exposure, with the lowest LC_{50} (4.24×10^{6}cfu/ml) and LC_{90} (1.99×10^{5}cfu/ml) values. *Isaria cateniannulata* (MBC289) and *Isaria fumosorosea* (MBC053) were the least effective, with the highest LC_{50} (7.55×10^{10}cfu/ml) and LC_{90} (2.52×10^{19}cfu/ml) values, respectively (Table 1b).

Table 1b: LC_{50} and LC_{90} of Different EPF Strains against Maggots of *B. Zonata* for an Exposure Period of 5 Days.

Insecticides	LC_{50}	FD limit	LC_{90}	FD limit	Slope ± S.E	X^2	DF	P
F52	4.24×10^{6}	6.18×10^{5} , 2.25×10^{10}	1.99×10^{5}	3.74×10^{12} , 9.56×10^{22}	0.078 ± 0.019	0.22	3	0.973
MBC 709	8.99×10^{6}	9.16×10^{5} , 2.87×10^{11}	2.95×10^{16}	1.29×10^{13} , 3.89×10^{15}	0.07 ± 0.019	0.62	3	0.89
MBC053	3.74×10^{10}	3.38×10^{6} , 1.62×10^{15}	1.66×10^{12}	2.72×10^{15} , 9.04×10^{16}	0.041 ± 0.019	0.92	3	0.819
MBC 289	7.55×10^{10}	1.27×10^{7}, 2.32×10^{19}	4.58×10^{14}	$3.008\times1^{01}4$, 1.54×10^{12}	0.059 ± 0.020	0.30	3	0.958
MBC397	1.42×10^{7}	6.94×10^{6} , 8.16×10^{14}	2.52×10^{19}	1.46×10^{14} , 3.91×10^{11}	0.050 ± 0.018	0.36	3	0.946
MBC076	5.61×10^{6}	7.10×10^{5}, 5.79×10^{10}	5.92×10^{15}	6.14×10^{12} , $6,03\times10^{14}$	0.074 ± 0.019	0.247	3	0.970
MBC807	3.25×10^{7}	2.61×10^{6} , 3.28×10^{12}	3.43×10^{16}	1.53×10^{3} , 3.13×10^{13}	0.074 ± 0.020	0.861	3	0.835
MBC524	1.85×10^{8}	1.86×10^{8} , 2.01×10^{9}	1.10×10^{16}	9.12×10^{12} , 2.95×10^{25}	0.076 ± 0.020	0.33	3	0.953
MBC389	2.46×10^{10}	7.31×10^{6}, 2.99×10^{16}	9.03×10^{18}	1.82×10^{14} , 1.86×10^{13}	0.063 ± 0.020	0.89	3	0.826

Metarhizium anisopliae (MBC389) exhibited the highest effectiveness against *Bactrocera zonata* maggots over a 7-day exposure, with the lowest LC_{50} (1.05×10^{3}cfu/ml) and LC_{90} (1.43×10^{11}cfu/ml) values. In contrast, *Isaria cateniannulata* (MBC289) and *Lecanicillium attenuatum* (MBC807) were the least effective, with the highest LC_{50} (1.22×10^{7}cfu/ml) and LC_{90} (5.68×10^{20}cfu/ml) values, respectively (Table 1c).

Table 1c: LC_{50} and LC_{90} of Different EPF Strains against Maggots of *B. Zonatafor an* Exposure Period of 7 Days

Insecticides	LC_{50}	FD limit	LC_{90}	FD limit	Slope ± S.E	X^2	DF	P
F52	4.68×10^{4}	6.58×103 , 2.19×10^{5}	1.03×10^{13}	1.68×10^{11} , 1.51×10^{13}	0.082 ± 0.017	0.129	3	0.98
MBC 709	3.33×10^{4}	1.96×103 , 6.99×10^{5}	5.98×10^{17}	1.69×10^{13}, 3.05×10^{12}	0.046 ± 0.016	0.084	3	0.99
MBC053	1.08×10^{5}	8.39×103 ,1.34×10^{7}	4.31×10^{15}	2.97×10^{12} , 3.52×10^{15}	0.060 ± 0.017	1.019	3	0.79
MBC 289	1.22×10^{7}	5.23×105 , 1.58×10^{14}	1.32×10^{20}	2.37×10^{14} , 1.77×10^{15}	0.047 ± 0.018	0.725	3	0.86
MBC397	1.26×10^{5}	4.16×105 , 3.49×10^{5}	1.43×10^{11}	1.72×10^{10} , 5.32×10^{12}	0.128 ± 0.018	11.32	3	0.01
MBC076	5.87×10^{3}	7.12×103 , 9.56×10^{4}	4.14×10^{17}	1.07×10^{13}, 2.03×10^{16}	0.04 ± 0.016	1.13	3	0.77
MBC807	9.80×10^{3}	7.71 ×103 , 8.38×10^{3}	5.68×10^{20}	6.02×10^{13} , 7.08×10^{12}	0.031 ± 0.015	0.008	3	1
MBC524	8.12×10^{4}	0.009×104 , 1.93×10^{4}	4.09×10^{16}	8.04×10^{12} , 4.67×10^{13}	0.041 ± 0.015	0.52	3	0.91
MBC389	1.05×10^{3}	0.03×105 , 3.16×10^{5}	1.63×10^{19}	2.95×10^{13} , 2.67×10^{14}	0.036 ± 0.015	0.22	3	0.97

Lecanicillium attenuatum (MBC807) exhibited the highest effectiveness against *Bactrocera zonata* maggots over a 14-day exposure, with the lowest LC50 (1.04×10^{3}cfu/ml) and LC90 (6.72×10^{10}cfu/ml) values. Conversely, *Isaria farinosa* (MBC389) and *Metarhizium anisopliae* (F52) were the least effective, with the highest LC50 (9.30×10^{3}cfu/ml) and LC90 (9.37×10^{12}cfu/ml) values, respectively (Table 2a).

Table 2a: LC_{50} and LC_{90} of Different EPF Strains against Maggots of *B. Zonatafor* an Exposure Period of 14 Days

Insecticides	LC_{50}	FD limit	LC_{90}	FD limit	Slope ± S.E	X^2	DF	P
F52	1.47×10^{3}	1.30×10^{3} , 6.47×10^{3}	2.42×10^{13}	3.01×10^{8} , 1.01×10^{12}	0.09 ± 0.015	0.32	3	0.95
MBC 709	2.21×10^{3}	0.008×10^{3} , 1.25×10^{3}	9.07×10^{11}	1.10×10^{11} , 9.64×10^{17}	0.054 ± 0.014	0.70	3	0.872

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MBC053	2.36×10^{3}	0.06×10^{5} , 3.36×10^{4}	6.42×10^{11}	7.02×10^{7} , 4.33×10^{11}	0.049 ± 0.014	0.42	3	0.93
MBC 289	1.76×10^{3}	1.78×10^{3} , 1.81×10^{3}	1.10×10^{11}	5.71×10^{7} , 7.51×10^{13}	0.076 ± 0.015	2.48	3	0.47
MBC397	1.43×10^{3}	0.924×10^{3} , 8.23×10^{3}	2.54×10^{11}	5.18×10^{7} , 2.36×10^{11}	0.056 ± 0.014	0.869	3	0.08
MBC076	5.37×10^{3}	1.64×10^{4} , 1.93×10^{3}	9.37×10^{12}	2.69×10^{14} , 1.60×10^{24}	0.044 ± 0.014	0.22	3	0.974
MBC807	1.04×10^{3}	0.43×10^{4} , 2.43×10^{3}	9.55×10^{10}	3.67×10^{7} , 3.16×10^{14}	0.065 ± 0.015	2.12	3	0.54
MBC524	2.03×10^{3}	0.009×10^{4} , 8.51×10^{4}	$6.72\ 10^{10}$	2.45×10^{8} , 4.12×10^{14}	0.061 ± 0.014	1.92	3	0.58
MBC389	9.30×10^{3}	0.01×10^{3} , 1.70×10^{3}	2.05×10^{11}	3.37×10^{14} , 1.92×10^{17}	0.050 ± 0.014	0.34	3	0.95

Lecanicillium attenuatum (MBC807) exhibited the highest effectiveness against *Bactrocera zonata* maggots over a 14-day exposure, with the lowest LC_{50} (1.83×10^{3}cfu/ml) and LC_{90} (4.95×10^{4}cfu/ml) values. Conversely, *Isaria farinosa* (MBC389) and *Metarhizium pinghaense* (MBC709) were the least effective, with the highest LC50 (6.34×10^{6}cfu/ml) and LC90 (5.61×10^{7}cfu/ml) values, respectively (Table 2b).

Table 2b: LC_{50} and LC_{90} of Different EPF Strains against Maggots of *B. Zonatafor* an Exposure Period of 21 Days

Insecticides	LC_{50}	FD limit	LC_{90}	FD limit	Slope ± S.E	X^2	DF	P
F52	7.16×10^{3}	3.72×10^{3} , 4.23×10^{3}	3.84×10^{7}	1.06×10^{7}, 2.73×10^{7}	0.11 ± 0.015	1.22	3	0.74
MBC 709	5.85×10^{3}	2.30×10^{3} , 3.86×10^{3}	5.61×10^{7}	1.39×10^{7}, 5.08×10^{8}	0.10 ± 0.015	1.75	3	0.62
MBC053	3.73×10^{3}	0.005×10^{4} , 3.40×10^{4}	1.81×10^{7}	2.93×10^{3}, 5.88×10^{7}	0.067 ± 0.015	3.03	3	0.38
MBC 289	0.10×10^{4}	6.4×10^{4}, 7.84×10^{3}	1.45×10^{7}	1.03×10^{6}, 2.74×10^{12}	0.05 ± 0.013	0.61	3	0.89
MBC397	0.22×10^{4}	1.01×10^{7}, 5.97×10^{3}	4.96×10^{5}	7.94×10^{4} , 1.17×10^{7}	0.057 ± 0.014	1.28	3	0.73
MBC076	2.92×10^{3}	2.59×10^{4} , 1.45×10^{4}	1.12×10^{7}	1.38×10^{7}, 8.94×10^{11}	0.068 ± 0.014	2.12	3	0.54
MBC807	1.83×10^{3}	3.59×10^{4} , 1.75×10^{3}	4.95×10^{4}	9.52×10^{4}, 7.03×10^{6}	0.070 ± 0.014	0.35	3	0.94
MBC524	4.05×10^{4}	2.08×10^{3} , 3.14×10^{3}	1.09×10^{6}	1.34×10^{4}, 1.01×10^{4}	0.05 ± 0.014	2.40	3	0.49
MBC389	6.34×10^{6}	1.96×10^{3} ,3.35×10^{4}	5.35×10^{4}	7.59×10^{4}, 1.96×10^{8}	0.05 ± 0.014	1.64	3	0.64

Beauveria brongniartii (MBC397) was the most effective EPF strain against *Bactrocera zonata* maggots with the lowest LT_{50} (1.04 days) and LT_{90} (27.37 days) values. In contrast, *Metarhizium anisopliae* (F52) was the least effective, with the highest LT_{50} (1.73 days) and LT_{90} (36.41 days) values. The significant differences in efficacy highlight the importance of selecting appropriate EPF strains for pest control (Table 3a).

Table 3a: LT_{50} and LT_{90} of Different EPF Strains against Maggots of *B. Zonatafor an* Exposure Period of 3 Days

Insecticides	LT_{50}	FD limit	LT_{90}	FD limit	Slope ± S.E	X^2	DF	P
F52	1.73	1.46 , 2.16	36.41	39.90 , 98.23	1.02 ± 0.13	21.99	3	0
MBC 709	1.39	1.20 , 1.63	33.82	33.13 , 66.21	1.04 ± 0.11	31.2	3	0
MBC053	1.05	9.24 , 1.21	33.40	26.66 , 46.16	1.05 ± 0.12	86.02	3	0.1
MBC 289	1.20	1.06 , 1.35	31.52	26.06 , 41.15	1.24 ± 0.12	26.42	3	0
MBC397	1.04	9.32 , 1.17	27.37	23.16 , 34.27	1.25 ± 0.11	25.98	3	0
MBC076	1.16	1.02 , 1.31	32.03	26.38 , 42.15	1.18 ± 0.11	28.3	3	0
MBC807	1.20	1.06 , 1.37	34.10	27.54 , 46.45	1.15 ± 0.12	34.42	3	0
MBC524	1.06	9.51 , 1.18	26.36	22.65 , 32.31	1.33 ± 0.12	27.14	3	0
MBC389	1.05	9.40 , 1.17	25.91	22.13 , 31.17	1.33 ± 0.11	25.7	3	0

The EPF strain MBC397 exhibited the lowest LT_{50} of 1.06 days, indicating the highest efficacy, while F52 had the highest LT_{50} of 1.73 days, showing the least effectiveness. For LT_{90}, MBC807 was the most effective with the lowest value of 21.43 days, while F52had the highest LT_{90} of 56.41 days. The pathogenicity ranking for LT50 was MBC397 > MBC389 > MBC053 > MBC524 > MBC076 > MBC807 > MBC289 > MBC709 > F52, and for LT_{90}, it was MBC807 > MBC524 > MBC397 > MBC289 > MBC076 > MBC053 > MBC709 > F52 (Table 3b).

Table 3b: LT_{50} and LT_{90} of Different EPF Strains against Maggots of *B. Zonatafor* an Exposure Period of 5 Days

Insecticides	LT_{50}	FD limit	LT_{90}	FD limit	Slope ± S.E	X^2	DF	P
F52	1.06	9.02 , 1.27	41.60	34.30 , 76.79	0.82 ± 0.10	8.21	3	0.042
MBC 709	8.25	6.91 , 9.64	32.52	24.67 , 34.34	0.82 ± 0.09	5.23	3	0.15
MBC053	8.11	6.99 , 9.25	25.68	26.20 , 39.54	1.003 ± 0.10	3.50	3	0.3
MBC 289	8.33	7.18 , 9.51	21.78	28.26 , 38.71	0.99 ± 0.10	7.02	3	0.071
MBC397	7.29	6.24 , 8.30	25.38	18.98 , 39.06	1.01 ± 0.09	3.78	3	0.28
MBC076	7.73	6.56 , 8.91	26.85	23.29 , 36.91	0.91± 0.09	6.75	3	0.08

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MBC807	7.54	6.49 , 8.58	21.43	25.02 , 36.13	1.02 ± 0.09	6.58	3	0.08
MBC524	7.48	6.42 , 8.53	28.45	28.03 , 32.17	1.01 ± 0.09	4.43	3	0.21
MBC389	7.44	6.43 , 8.42	27.27	15.92 , 28.85	1.07 ± 0.10	4.08	3	0.25

For a 7-day exposure period, F52 demonstrated the lowest LT_{50} value of 5.41 days, making it the most effective strain against *Bactrocera zonata* maggots, while MBC289 had the highest LT_{50} value of 6.05 days, indicating the least effectiveness. For LT_{90}, MBC524 had the lowest value of 15.63 days, showing the highest efficacy, whereas MBC076 exhibited the highest LT_{90} of 18.07 days, reflecting the least effectiveness (Table 4a).

Table 4a: LT_{50} and LT_{90} of Different EPF Strains against Maggots of *B. Zonata* for an Exposure Period of 7 Days

Insecticides	LT_{50}	FD limit	LT_{90}	FD limit	Slope ± S.E	X^2	DF	P
F52	5.41	4.54 , 6.23	17.61	15.01 , 21.62	1.01 ± 0.09	5.37	3	0.15
MBC 709	5.87	5.002 , 6.69	18.01	15.53 , 21.81	1.06 ± 0.09	3.17	3	0.36
MBC053	5.94	5.12 , 6.71	16.76	14.61 , 19.95	1.15 ± 0.10	3.95	3	0.26
MBC 289	6.05	5.19 , 6.86	17.88	15.48 , 21.47	1.10 ± 0.09	6.77	3	0.079
MBC397	5.61	4.81 , 6.37	16.27	14.17 , 19.38	1.12 ± 0.09	6.41	3	0.09
MBC076	5.63	4.75 , 6.46	18.07	15.48 , 22.10	1.03 ± 0.09	8.23	3	0.04
MBC807	5.68	4.89 , 6.43	16.01	14.09 , 18.03	1.15 ± 0.10	3.53	3	0.316
MBC524	5.59	4.81 , 6.32	15.63	13.69 , 18.44	1.16 ± 0.100	5.22	3	0.15
MBC389	5.86	5.04 , 6.62	16.59	14.46 , 19.75	1.15 ± 0.10	2.36	3	0.50

For a 14-day exposure period, MBC397 had the lowest LT50 of 6.44 days (most effective), while MBC289had the highest LT50 of 7.18 days (least effective). MBC389 showed the lowest LT90 of 19.07 days (most effective), and F52had the highest LT90 of 23.66 days (least effective) (Table 4b).

Table 4b: LT_{50} and LT_{90} of Different EPF Strains against Maggots of *B. Zonata* for an Exposure Period of 14 Days

Insecticides	LT_{50}	FD limit	LT_{90}	FD limit	Slope ± S.E	X^2	DF	P
F52	6.53	5.48 , 7.53	23.66	19.49 , 30.95	0.93 ± 0.09	7.17	3	0.07
MBC 709	6.78	5.76 , 7.76	22.96	19.12 , 29.46	0.98 ± 0.96	4.20	3	0.24
MBC053	6.90	5.96 , 7.81	20.68	17.66 , 25.44	1.09 ± 0.01	2.11	3	0.54
MBC 289	7.18	6.14 , 8.20	23.84	19.82 , 30.69	1.02 ± 0.09	7.31	3	0.063
MBC397	6.44	5.52 , 7.32	19.82	16.93 , 24.39	1.06 ± 0.09	4.55	3	0.20
MBC076	6.82	5.79 , 7.81	23.27	19.33 , 30	0.97 ± 0.09	4.20	3	0.24
MBC807	6.63	5.71 , 7.52	19.98	17.10 , 24.51	1.08 ± 0.09	4.65	3	0.19
MBC524	6.56	5.62 , 7.46	20.40	17.37 , 25.19	1.05 ± 0.09	5.28	3	0.15
MBC389	6.64	5.75 , 7.49	19.07	17.07 , 24.09	1.13 ± 0.10	3.66	3	0.3

For a 21-day exposure period, MBC524was the most effective EPF strain against B. zonata maggots, with the lowest LT_{50} of 5.59 days and LT_{90} of 15.63 days. In contrast, MBC709 had the highest LT_{50} of 6.78 days, indicating the lowest effectiveness, while F52showed the highest LT_{90} of 23.66 days, demonstrating the least effectiveness. The pathogenicity order was MBC524 > MBC397 > MBC076 > MBC807 > MBC389 > MBC053 > MBC289 > F52 >MBC709, with significant variations in effectiveness among the strains (Table 5).

Table 5: LT_{50} and LT_{90} of Different EPF Strains against Maggots of *B. Zonata* for an Exposure Period of 21 Days

Insecticides	LT_{50}	FD limit	LT_{90}	FD limit	Slope ± S. E	X^2	DF	P
F52	6.53	5.48 , 7.53	23.66	19.49 , 30.95	0.93 ± 0.09	7.17	3	0.067
MBC 709	6.78	5.76 , 7.76	22.96	19.12 , 29.46	0.98 ± 0.09	4.20	3	0.24
MBC053	5.94	5.12 , 6.71	16.76	14.61 , 19.95	1.15 ± 0.10	3.95	3	0.26
MBC 289	6.05	5.19 , 6.86	17.87	15.48 , 21.47	1.10 ± 0.09	6.78	3	0.07
MBC397	5.61	4.81 , 6.37	16.27	14.17 , 19.38	1.12 ± 0.09	6.41	3	0.09
MBC076	5.63	4.75 , 6.46	18.07	15.48 , 22.10	1.03 ± 0.09	8.22	3	0.04
MBC807	5.68	4.89 , 6.43	16.01	14.98 , 18.97	1.15 ± 0.10	3.53	3	0.316
MBC524	5.59	4.81 , 6.32	15.63	13.69 , 18.44	1.16 ± 0.1	5.22	3	0.15
MBC389	5.86	5.04 , 6.62	16.59	14.46 , 19.75	1.15 ± 0.10	2.36	3	0.50

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DISCUSSION

The present study aimed to evaluate the virulence of nine entomopathogenic fungi (EPFs) against larvae of Bactrocera zonata. The EPFs tested were *Metarhizium anisopliae* (F52), *Metarhizium pinghaense* (MBC 709), *Isaria fumosorosea* (MBC 053), *Isaria cateniannulata* (MBC 289), *Beauveria brongniartii* (MBC 397), *Beauveria bassiana* (MBC 076), *Lecanicillium attenuatum* (MBC 807), Isaria javanica (MBC 524), and *Isaria farinosa* (MBC 389). Under laboratory conditions (25±1°C and 61±5% RH), *B. zonata* was reared on an artificial diet and guava fruits for oviposition. Results showed varying virulence among EPF strains, with *M. anisopliae* (F52), *I. fumosorosea*, and *B. bassiana* demonstrating significant pathogenic effects. These findings align with previous studies showing that EPFs effectively control fruit flies. For instance, *B. bassiana* has been reported to cause 10-100% mortality in Ceratitis capitata and Ceratitis rosa adults [15] and 22-98.7% mortality in *C. capitata* adults [16]. Similarly, *M. anisopliae* and *B. bassiana* were effective against fruit flies, with mortality rates up to 99% [17]. Our study revealed that while *M. anisopliae* (F52) showed high virulence under laboratory conditions, its field efficacy was less pronounced, highlighting that laboratory results may not always translate to field conditions [18]. We observed that the larval stage of *B. zonata* lasted 6-10 days, consistent with previous studies [19, 20]. Younger stages of *B. zonata* were more susceptible to EPFs, with newly emerged pupae showing 25.8% mortality with *B. bassiana* and 35.0% with *M. anisopliae.* This variability underscores the importance of considering developmental stages when evaluating EPF efficacy [21]. Effectiveness was measured using LC50 and LC90 values, with MBC 389 and MBC 397 showing the lowest values after 7 days, while MBC 807 and MBC 524 proved effective after 14 and 21 days, respectively [22]. Additionally, *I. fumosorosea* and *M. anisopliae* have been established as pathogens for other fruit fly species [23, 24]. However, neem oil significantly reduced spore germination and vegetative growth of both *M. anisopliae* and *B. bassiana* [25-27]. In conclusion, various EPFs show potential for controlling *B. zonata* larvae. While laboratory trials are promising, field efficacy remains crucial for practical pest management. Future research should optimize these EPFs for field use and explore their interactions with environmental factors.

CONCLUSIONS

This study demonstrated that several entomopathogenic fungi, including *Metarhiziumanisopliae*, *Beauveria bassiana*, and *Isaria fumosorosea*, are highly effective against *Bactrocera zonata* larvae under laboratory conditions. The most virulent strains exhibited significant mortality rates, with notable effects on larval development and pupation. However, translating these results to field efficacy remains a challenge. Future research should aim to optimize these fungi for practical field applications and further investigate their interactions with environmental factors to enhance their pest control potential.

Authors Contribution

Conceptualization: MS
Methodology: MS
Formal analysis: MDG, AK, SF
Writing, review and editing: SS, SK, RAH, AA, AN

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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