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BIOLOGICAL CONTROL POTENTIAL OF ENTOMOPATHOGENIC FUNGAL STRAINS AGAINST *BACTROCERA DORSALIS* (TEPHRITIDAE: DIPTERA): A LABORATORY STUDY

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ABSTRACT

Fruit flies, specifically *Bactrocera dorsalis* (Diptera: Tephritidae), are recognized as significant pests in Pakistani orchards. The present study aimed to evaluate the efficacy of different entomopathogenic fungi (EPF) against male and female adults of *B. dorsalis*. Four entomopathogens namely *Trichoderma harzianum*, *Isaria cateniannulata*, *Beauveria bassiana* and *Metarhizium anisopliae* were assessed for their biocontrol potential. Efficiency of these entomopathogens was tested at five different concentrations *viz.* 1×10^5, 1×10^6, 1×10^7, 1×10^8 and 1×10^9 cfu/mL. The mortality of flies was recorded after 2, 4, 6, 8, 10 and 12 days. *Beauveria bassiana* (MBC 076) was found to be more efficient biocontrol agent than other EPF. Mortality of both male and female adults of the fruit flies was increased with time intervals. Maximum mortality (90%) of the flies was recorded after 12 days where fruit flies were treated with *B. bassiana* (MBC 076) at a concentration of 1×10^9 cfu/mL. *M. anisopliae* caused the lowest mortality (75%) at the highest concentration. This study demonstrates that inclusion of entomopathogenic fungi in integrated pest management plans can lead to the effective and environmentally safe management of fruit flies.

Keywords: *Bactrocera dorsalis*, Entomopathogenic fungi, Fruit flies, Mortality, Virulence.

INTRODUCTION

Agriculture plays an important role in boosting the economy of Pakistan (Hena *et al*., 2019). It is the backbone of the economy of the country (Chandio *et al*., 2019). Unfortunately, this economy is badly affected by internal political problems and a fast increase in population (Sheikh *et al*., 2012; Chandio *et al*., 2019). Guava is a very common fruit in Pakistan. It occupies the third position in terms of area and production in Pakistan (Yousaf *et al*., 2020). Pests are causing serious threats to fruits and vegetables all over the world due to climate change. The members of family Tephritidae are causing very serious effects on fruits. Its subfamily Bactrocera is of great importance including its species *Bactrocera dorsalis* and *Bactrocera zonata* (Verghese *et al*., 2002; Saeed *et al*., 2022; Sarwar *et al*., 2023). The family Tephritidae contains about 4000 species. Of these, 250 species are economically significant. They are the most abundant at below 30 ^{o}C temperature and with relative humidity between 60 and 70%. Females of fruit flies place their eggs 2 to 4 mm deep. Larvae emerge from these eggs and bore into the fruit pulp. They are very harmful as they destroy our fruits by the infestation. Pupae develop 0.5 to 15 cm deep in the soil (McQuate and Liquido, 2017; Bonanomi *et al*., 2021).

Fruit flies are the major problem in the production of fruit and vegetables in the world, especially in developing countries (Papadopoulos, 2014). Mediterranean fruit fly and oriental fruit fly are the two fruit fly species that are very significant and economic (Dionne and Schneider, 2008; Saeed *et al*., 2022; Sarwar *et al*., 2023). They cause significant losses to fruits and vegetables which are about 200 million dollars in Pakistan (Saeed *et al*., 2022; Sarwar *et al*., 2023). Mass trapping is used on a large scale in different regions of the world to control fruit flies. Different types of colors, shapes, and designs of traps are used to capture large numbers of fruit flies (Dominiak *et al*., 2016; Dias *et al*., 2018). Insecticides are sprayed in fruits and vegetables to control fruit flies but these are causing environmental pollution and health problems that is a risk for people and animals (Kankam, 2021; Jayaprakas *et al*., 2023). A high level of insecticide resistance has been reported in China, therefore, some chemicals that are extracted from plants are used as attractants for fruit flies. The best example of an attractant is methyl eugenol, which is used

to attract male *Bactrocera dorsalis*. This method is very helpful to monitor the population of fruit flies in the field (Liu *et al*., 2022; He *et al*., 2023).

In addition to insecticide, other techniques such as male inhalation techniques, cultural practices, and the release of sterile insects are also used to control the population of fruit flies. However, insecticides should not be used to control flies due to resistance issues. Therefore, the potential use of entomopathogens has been explored to combat the population of fruit flies (Ahmad *et al.,* 2022, 2024; Shaurub, 2023; Iqbal *et al.,* 2025). *Beauveria bassiana* and *Metarhizium* spp. are helpful to reduce the population of fruit flies (Aatif *et al.,* 2019; Iqbal *et al*., 2021). *Beauveria bassiana* and *Metarhizium anisopliae* are the important entomopathogenic fungi of fruit flies (Iqbal *et al*., 2021; Wakil *et al*., 2022). *Beauveria bassiana* and *M. anisopliae* are highly efficient against the larvae, pupae and adults of Mediterranean fruit fly by using different ways of exposure (Quesada-Moraga *et al*., 2006; Prince *et al*., 2024). Keeping in view the importance of entomopathogenic fungi in controlling the population of fruit flies, the present study aimed to evaluate the biocontrol efficacy of four species of these fungi against male and females adult fruit flies under laboratory conditions.

MATERIALS AND METHODS

Morphological identification of *Bactrocera dorsalis*

For the collection of samples, methyl eugenol traps were put in guava orchards (Fan *et al.,* 2022). To separate the samples, traps were transported to the laboratory. To identify the samples, they were placed on slides and examined under a light microscope.

Collection of fruit fly

Different locations in the University of the Punjab, Lahore were chosen for sampling fruit flies by using traps. Twenty plastic jars were taken from the market and transformed into traps. To allow fruit flies to enter, two holes were drilled onto the opposing edges of the jars. Jars were strung up by being knotted with rope. Cotton was dipped in Methyl eugenol which was then placed inside the jar. Under the cover of the trees, jars were hung on guava trees, 1.5 meters or more above the ground. Pheromone traps were placed in guava orchards to catch fruit flies. After 24 hours, the samples were collected and taken to the lab. Jars and cotton swabs that had been soaked in methyl eugenol were replaced while the samples were brought in the jars.

A large jar was taken to rear the population of fruit flies. A plastic bottle (500 mL) containing mango juice, having small holes in it and covered with a lid, also placed in the jar to collect eggs of fruit flies (Zhang *et al.,* 2015). An artificial diet was given to the collected eggs (Quesada-Moraga *et al.,* 2006). Until the third larval instar, larvae fed on the diet and pupate under the layer of sand. A thin layer of sand was also placed inside the jar for the pupation of larvae. Pupation occurred inside the sand and adults were formed. The lab conditions were maintained at 25 ^{o}C and 60–70% relative humidity (Usman *et al.,* 2021). After emergence, adult flies were placed in screened plastic cages. Water was provided to adult flies and an adult diet that comprised sugar and enzymatic yeast (3:1 ratio) was also provided to adult flies (Sookar *et al.,* 2014). Fresh guava fruits were also placed inside the jar as a food source for adult flies.

Fungal bioassay

Four entomopathogenic fungi namely *T. harzianum, I. cateniannulata* (MBC 289), *B. bassiana* (MBC 076) and *M. anisopliae* were obtained from the Integrated Pest Management Laboratory of the Faculty of Agricultural Sciences, Punjab University, Lahore to check their efficacy against fruit fly *B. dorsalis.* Inoculation of these entomopathogenic fungi (EPF) on Petri dishes were carried out and left for one week. A small amount of these EPF was taken from every Petri dish and a stock solution was made. Five different concentrations of entomopathogenic fungi *viz.* 1×10^5, 1×10^6, 1×10^7, 1×10^8 and 1×10^9 cfu/mL were made to check their efficacy against fruit flies.

Five small jars were taken for each entomopathogenic fungus. Entomopathogens were applied on the walls of jars and 20 fruit flies were put in these jars. Hibernation of fruit flies was done for five minutes in the refrigerator to easily put in jars. A small piece of mesh banana was also put in a jar for diet purpose. Jars were closed by a thin piece of muslin cloth with the help of a rubber band. Data were taken after every two days for twelve days. The experiment was carried out in a completely randomized design with 3 replications.

Statistical analysis

All the data were subjected to ANOVA (analysis of variance) and means were compared using LSD. Analysis of all the data was computed using Statistix 8.1 software (McGraw-Hill, 2008).

RESULTS

Data regarding the pathogenicity of four selected entomopathogenic fungi against males and females of *B. dorsalis* at different intervals of time after treatments is shown in the Fig. 1 to 4. As the concentration of EPF increased, increase in mortality of fruit fly was observed. The efficacy of entomopathogens was time and concentration-dependent. The efficacy of entomopathogens was increased as the number of days increased. Maximum mortality was observed by all entomopathogens after 12 days at the concentration of 1×10^9 cfu/mL. *T. harzianum* caused 81% mortality at the concentration of 1×10^9 cfu/mL after 12 days while *B. bassiana* caused 89% mortality at the same concentration and time interval. *I. cateniannulata* and *M. anisopliae* were found comparatively less effective as their inoculation caused 78% and 73%mortality, respectively, at the concentration of 1×10^9 cfu/mL after 12 days.

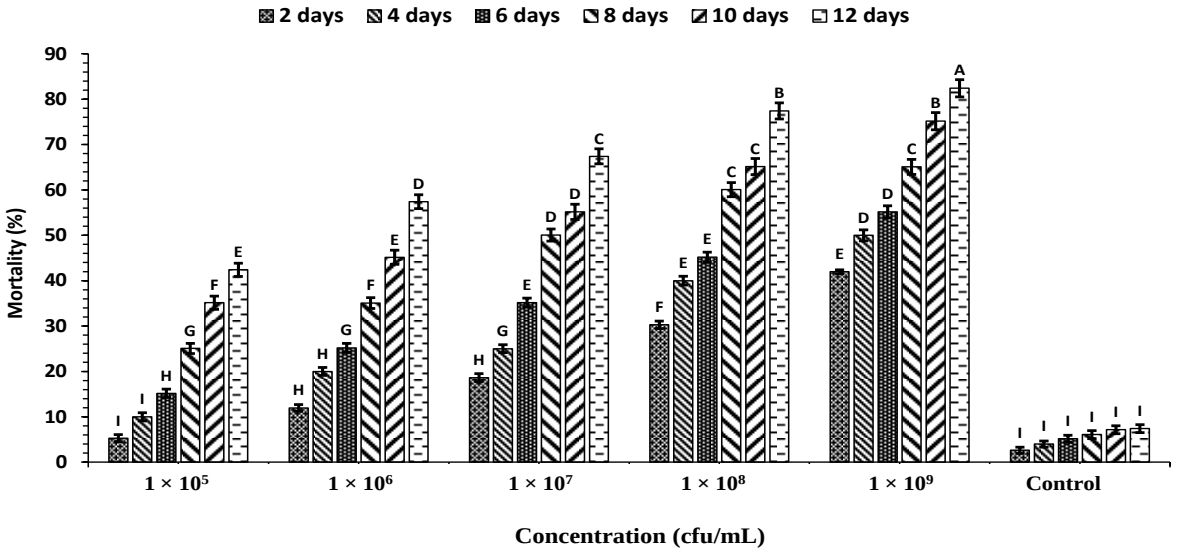

Fig. 1. Data of mortality of male and female adults of *Bactrocera dorsalis* by using different concentrations of *Trichoderma harzianum* after different intervals of days. Vertical bars show standard errors of means of three replicates. Different letters on the bars show significant difference as determined by LSD test at P≤0.05.

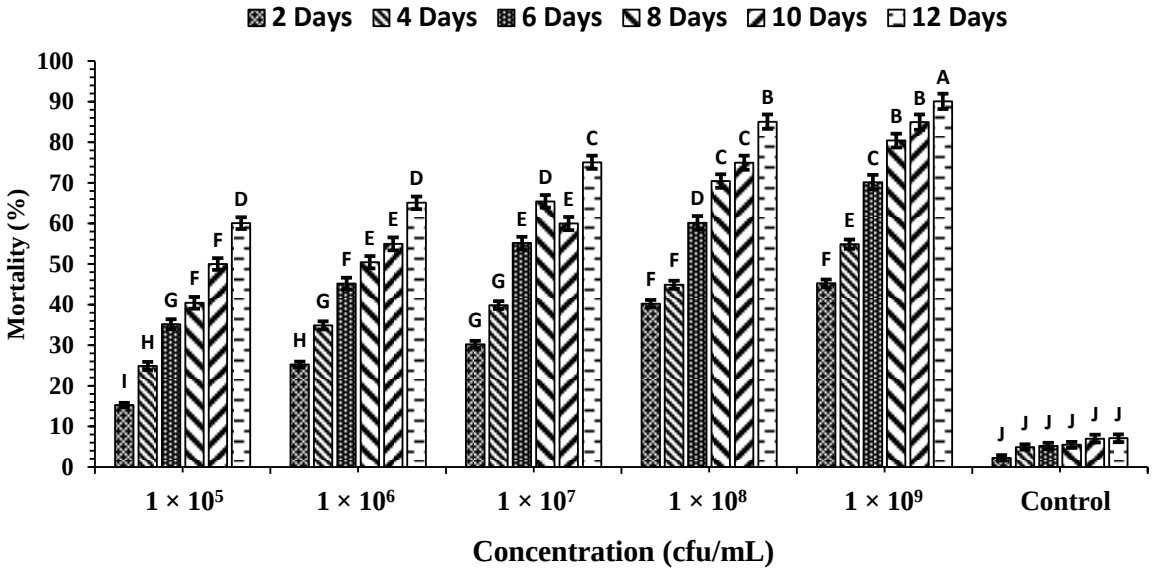

Fig. 2. Data of mortality of male and female adults of *Bactrocera dorsalis* by using different concentrations of *Beauveria bassiana* (MBC 076) after different intervals of days. Vertical bars show standard errors of means of three replicates. Different letters on the bars show significant difference as determined by LSD test at P≤0.05.

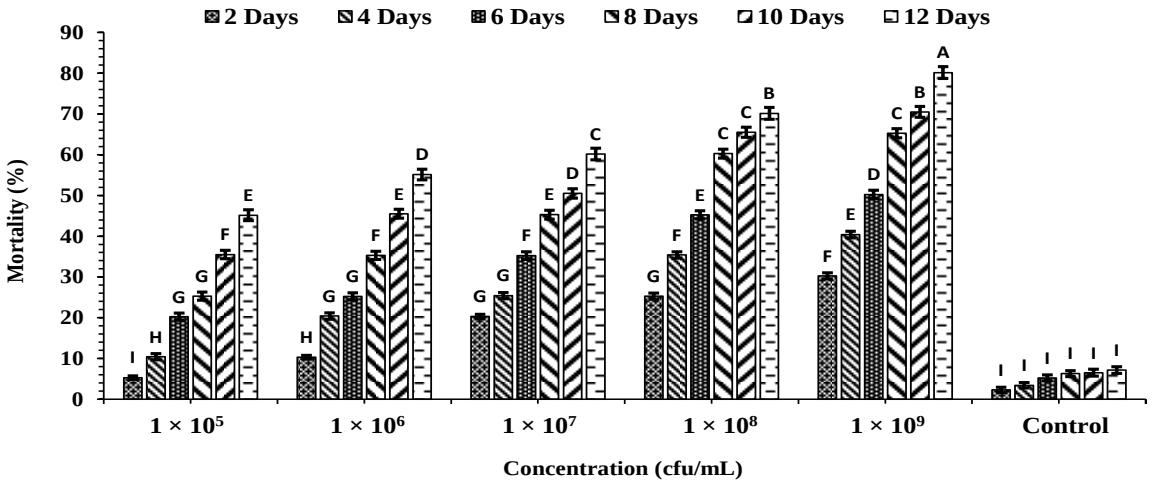

Fig. 3. Data of mortality of male and female adults of *Bactrocera dorsalis* by using different concentrations of *Isaria cateniannulata* (MBC 289) after different intervals of days. Vertical bars show standard errors of means of three replicates. Different letters on the bars show significant difference as determined by LSD test at P≤0.05.

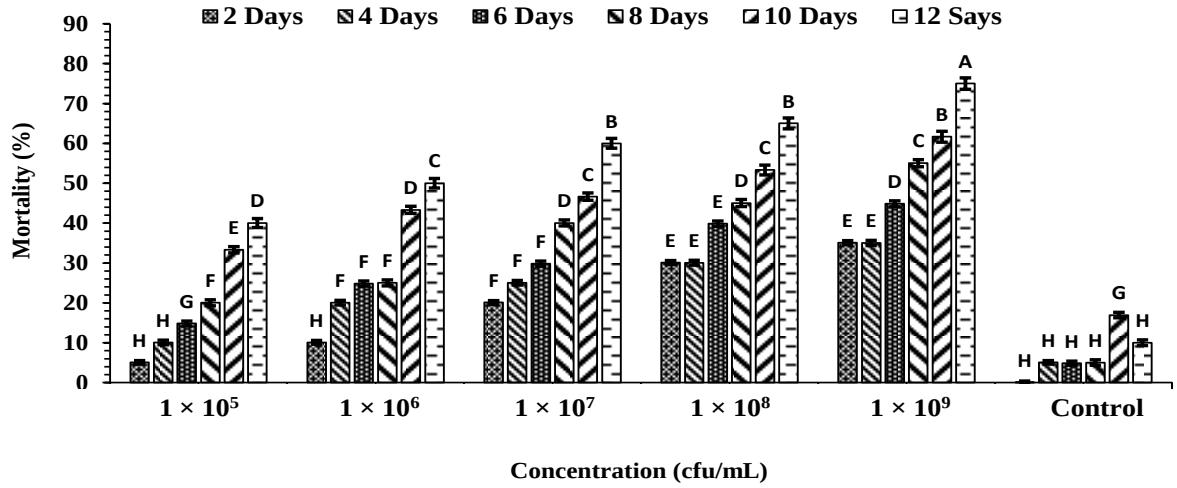

Fig. 4. Data of mortality of male and female adults of *Bactrocera dorsalis* by using different concentrations of *Metarhizium anisopliae* after different intervals of days. Vertical bars show standard errors of means of three replicates. Different letters on the bars show significant difference as determined by LSD test at P≤0.05.

EPF screening bioassays against adults of fruit flies

LC_{50} values for different concentrations of *T. harzianum* against male adults of fruit fly after 2, 4, 6, 8, 10 and 12 days is shown in the Table 1. LC_{50} values of different concentration of *T. harzianum* were 1.99×10^9 after 2 days of post-treatment application and was significantly different than values after 4, 6, 8, 10 and 12 days, which were 1.36×10^9, 5.69×10^8, 9.33×10^7, 1.48×10^7 and 1.93×10^6, respectively. LC_{50} value of different concentration of *T. harzianum* against female adults was 9.34×10^8 after 2 days of post treatment application and was significantly different than values at 4, 6, 8, 10 and 12 days, which were 6.99×10^8, 4.68×10^8, 1.43×10^8, 2.51×10^7 and 1.20×10^6.

LC_{50} value of different concentrations of *B. bassiana* was 1.06×10^9 after 2 days of post-treatment application that was significantly higher than the values after 4, 6, 8, 10 and 12 days, which were 2.56×10^8, 9.6×10^6, 1.16×10^6, 6.06×10^5 and 2.72×10^{-4}, respectively. Likewise, LC_{50} values of different concentration of *B. bassiana* against female adults were 2.02×10^9 after 2 days, 7.97×10^8 after 4 days, 8.50×10^6 after 6 days, 1.25×10^6 after 8 days, 3.32×10^5 after 10 days and 1.56×10^{-4} after 12 days of post treatment application (Table 2).

Table 1. LC_{50} values of *Trichoderma harzianum* by using different concentrations against male and female adults of oriental fruit fly *Bactrocera dorsalis*.

Sex	Days	LC_{50} (cfu/ml)	FD Limit	Slope ± S.E.	χ^2	D.F.	P
Male	2nd day	1.99×10^9	8.08×10^8 to 7.83×10^9	0.28 ± 0.03	4.61	3	0.202
	4th day	1.36×10^9	5.08×10^8 to 6.07×10^9	0.22 ± 0.03	4.33	3	0.228
	6th day	5.69×10^8	2.18×10^8 to 2.29×10^9	0.18 ± 0.02	1.15	3	0.763
	8th day	9.33×10^7	4.05×10^7 to 2.50×10^8	0.16 ± 0.02	3.30	3	0.347
	10th day	1.48×10^7	5.39×10^6 to 3.75×10^7	0.14 ± 0.02	0.65	3	0.88
	12th day	1.93×10^6	5.59×10^5 to 4.77×10^6	0.14 ± 0.01	2.94	3	0.401
Female	2nd day	9.34×10^8	4.89×10^8 to 2.22×10^9	4.45 ± 9.23	3.13	3	0.0.372
	4th day	6.99×10^8	2.84×10^8 to 2.54×10^9	0.21 ± 0.02	3.16	3	0.367
	6th day	4.68×10^8	1.72×10^8 to 2.04×10^9	0.17 ± 0.02	1.52	3	0.677
	8th day	1.43×10^8	5.08×10^7 to 5.92×10^8	0.13 ± 0.02	4.32	3	0.22
	10th day	2.51×10^7	9.69×10^6 to 6.92×10^7	0.14 ± 0.02	1.83	3	0.708
	12th day	1.20×10^6	2.87×10^5 to 3.24×10^7	0.13 ± 0.01	2.54	3	0.467

Table 2. LC_{50} values of *Beauveria bassiana* (MBC 076) by using its different concentrations against male and female adults of oriental fruit fly *Bactrocera dorsalis*.

Sex	Days	LC_{50} (cfu/ml)	FD Limit	Slope ± S.E.	χ^2	D.F.	P
Male	2nd day	1.06×10^9	2.73×10^8 to 1.16×10^{10}	0.4 ± 0.02	0.68	3	0.87
	4th day	2.56×10^8	7.12×10^7 to 2.06×10^9	0.11 ± 0.02	2.10	3	0.55
	6th day	9.6×10^6	2.96×10^6 to 2.61×10^7	0.12 ± 0.02	1.26	3	0.737
	8th day	1.16×10^6	2.47×10^5 to 3.30×10^6	0.12 ± 0.01	1.33	3	0.72
	10th day	6.06×10^5	1.13×10^5 to 1.80×10^6	0.13 ± 0.01	1.77	3	0.62
	12th day	2.72×10^{-4}	1.37×10^{-3} to 1.46×10^5	0.11 ± 0.01	1.05	3	0.789
Female	2nd day	2.02×10^9	4.83×10^8 to 2.71×10^{10}	0.15 ± 0.02	2.19	3	0.53
	4th day	7.97 x 10^8	1.66×10^8 to 1.71×10^{10}	0.11 ± 0.02	3.76	3	0.28
	6th day	8.50×10^6	2.35×10^6 to 2.46×10^7	0.11 ± 0.02	4.27	3	0.23
	8th day	1.25×10^6	2.69×10^5 to 3.56×10^6	0.12 ± 0.01	1.05	3	0.789
	10th day	3.32×10^5	3.95×10^{-4} to 1.19×10^6	0.11 ± 0.02	0.63	3	0.88
	12th day	1.56×10^{-4}	3.76×10^{-2} to 1.08×10^5	0.10 ± 0.02	0.52	3	0.91

Table 3. LC_{50} values of *Isaria cateniannulata* (MBC 289) by using its different concentrations against male and female adults of oriental fruit fly *Bactrocera dorsalis*.

Sex	Days	LC_{50} (cfu/mL)	FD Limit	Slope ± S.E.	χ^2	D.F.	P
Male	2nd day	1.02×10^{10}	1.98×10^{9} to 2.34×10^{11}	0.19 ± 0.03	4.47	3	0.214
	4th day	4.96×10^{9}	1.17×10^{9} to 6.69×10^{10}	0.18 ± 0.03	1.59	3	0.660
	6th day	1.08×10^{9}	3.02×10^{8} to 9.43×10^{9}	0.18 ± 0.02	0.41	3	0.93
	8th day	7.81×10^{7}	3.27×10^{7} to 2.16×10^{8}	0.16 ± 0.02	3.42	3	0.331
	10th day	1.29×10^{7}	4.60×10^{6} to 3.27×10^{7}	0.13 ± 0.02	1.83	3	0.60
	12th day	1.16×10^{6}	2.08×10^{5} to 3.56×10^{6}	0.11 ± 0.01	1.34	3	0.71
Female	2nd day	1.43×10^{10}	2.17×10^{9} to 6.17×10^{11}	0.16 ± 0.03	6.61	3	0.08
	4th day	1.51×10^{9}	4.80×10^{8} to 9.69×10^{9}	0.25 ± 0.02	3.60	3	0.308
	6th day	3.87×10^{8}	1.37×10^{8} to 1.80×10^{9}	0.16 ± 0.02	0.16	3	0.98
	8th day	1.03×10^{8}	3.79×10^{7} to 3.77×10^{8}	0.14 ± 0.02	0.37	3	0.94
	10th day	1.05×10^{7}	3.25×10^{6} to 2.91×10^{7}	0.12 ± 0.01	1.09	3	0.77
	12th day	8.28×10^{5}	1.55×10^{5} to 2.48×10^{6}	0.12 ± 0.01	1.72	3	0.63

Table 4. LC_{50} values of *Metarhizium anisopliae* by using its different concentrations against male and female adults of oriental fruit fly *Bactrocera dorsalis*.

Sex	Days	LC_{50} (cfu/mL)	FD Limit	Slope ± S.E.	χ^2	D.F.	P
Male	2nd day	4.03×10^{9}	1.34×10^{9} to 2.42×10^{10}	0.27 ± 0.04	6.17	3	0.104
	4th day	1.46×10^{10}	2.00×10^{9} to 1.02×10^{12}	0.15 ± 0.03	2.44	3	0.46
	6th day	2.40×10^{9}	5.25×10^{8} to 4.13×10^{10}	0.14 ± 0.02	1.51	3	0.68
	8th day	6.32×10^{8}	2.01×10^{8} to 3.88×10^{9}	0.15 ± 0.02	1.61	3	0.65
	10th day	3.85×10^{7}	1.37×10^{7} to 1.19×10^{8}	0.13 ± 0.02	0.93	3	0.81
	12th day	3.68×10^{6}	9.53×10^{5} to 1.00×10^{7}	0.12 ± 0.01	0.44	3	0.93
Female	2nd day	5.25×10^{9}	1.64×10^{9} to 3.73×10^{10}	0.27 ± 0.04	0.89	3	0.82
	4th day	3.83×10^{9}	9.25×10^{8} to 4.86×10^{10}	0.17 ± 0.03	3.41	3	0.33
	6th day	9.47×10^{9}	2.79×10^{8} to 7.10×10^{9}	0.15 ± 0.02	1.93	3	0.58
	8th day	4.06×10^{8}	1.39×10^{8} to 1.02×10^{9}	0.15 ± 0.02	1.75	3	0.62
	10th day	8.54×10^{7}	2.40×10^{7} to 5.02×10^{8}	0.10 ± 0.02	0.13	3	0.36
	12th day	3.14×10^{6}	7.69×10^{5} to 8.71×10^{6}	0.12 ± 0.01	1.27	3	0.73

LC_{50} value of different concentration of *I. cateniannulata* was 1.02×10^{10} after 2 days of treatment application, which were significantly different than values at other time intervals. Similarly, LC_{50} value of different concentration of this fungus against female adults was 1.43×10^{10} after 2 days of treatment application and the values were significantly different at intervals of 4, 6, 8, 10 and 12 days that is 1.51×10^{9}, 3.87×10^{8}, 1.03×10^{8}, 1.05×10^{7} and 8.28×10^{5}, respectively (Table 3).

LC_{50} value of different concentration of *M. anisopliae* was 4.03×10^{9} after 2 days of post-treatment application that were markedly reduced after 4, 6, 8, 10 and 12 days *i.e.* 1.46 x 10^{10}, 2.40×10^{9}, 6.32×10^{8}, & 3.85×10^{7} and 3.68×10^{6}, respectively. In case of female fruit flies, LC_{50} value of different concentration of *M. anisopliae* was 5.25×10^{9} after 2 days, 3.83×10^{9} after 4 days, 9.47×10^{9} after 6 days, 4.06×10^{8} after 8 days, 8.54×10^{7} after 10 days and 3.14×10^{6} after 12 days of treatments applications (Table 4).

DISCUSSION

Four entomopathogenic fungi were used to control the population of adults of *B. dorsalis*. These fungi showed variable effects on the mortality of adults of fruit flies. The concentrations of fungi and mortality of fruit flies were correlated to each other. Maximum temperature and humidity were shown to be correlated positively. The peak population was observed during the hot season. The same results were observed by Sharma *et al.* (2015) that studied a positive correlation with weather patterns. Similarly, Zamora-Ros *et al.* (2011) investigated seasonal high population months for fruit flies. Their research also revealed that fruit fly invasion reached its peaks in the warmer months. Khan *et al.* (2017) observed similar effects when they conducted experiment with the installation of traps with methyl eugenol. The high population was recorded at higher temperature and humidity. The same results were observed by Laskar and Chatterjee (2010). They reported that fruit fly infestation was more in rainy and warmer months than in dry months of the year.

In the current study, the pathogenicity of the entomopathogenic fungi namely *T. harzianum, I. cateniannulata, B. bassiana* and *M. anisopliae* were evaluated unde laboratory conditions. Male and female adults of fruit flies were exposed to different concentrations for 12 days. Data of mortality was recorded after every two days. *B. bassiana* was found highly efficient causing maximum mortality of both male and female adults of the fruit fly. The highest concentration (1×10^{9}) was proved the most effect as maximum mortality (90%) was observed due to this concentration in case of *B. bassiana* treatment. The similar results were also shown by Mahmoud (2009), Sookar *et al*. (2014) and Rashad *et al.* (2015) who conducted different experiments to check the efficacy of the pathogenicity of fungi against peach fruit fly. The current study can also be compared with the study conducted by Beris *et al.* (2013) who tested the efficacy of *B. bassiana, I. fumosorosea* and *M. anisopliae* against *Ceratitis capitata*. Results showed that adults were very susceptible to exposure to entomopathogenic fungi. In the present study, *M. anisopliae* showed the lowest mortality of 75% at the concentration of 1×10^{9}, while *B. bassiana* caused the highest mortality of flies (90%) at a concentration of 1×10^{9}. Results were also in line with the findings of Ros *et al.* (2002) carried out an experiment on Mexican fruit flies in which the larvae, pupae, and adults of the flies were exposed to eight distinct strains of *B. bassiana*. Adult fly mortality was at its highest.

Conclusion

Given the high adult mortality that was noted during this investigation, *B. bassiana* can be used as an effective biocontrol agent against the targeted fruit fly. Adults were highly vulnerable to exposure to this entomopathogenic fungus. Additionally, it is self-sustaining, allowing us to build it in the field.

Availability of data and materials

All the data and materials are available and can be provided on request.

REFERENCES

Aatif, H.M., M.S. Hanif, M. Ferhan, M. Raheel, Q. Shakeel, W. Ashraf and S. Ali (2019). Assessment of the entomopathogenic nematodes against maggots and pupae of the oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae), under laboratory conditions. *Egyptian Journal of Biological Pest Control*, 29(1): 51.

Ahmad, S., A. Sarwar, A. Shoaib, A. Javaid, M.S. Hanif and Q. Ali (2022). Sustainable management of guava fruit fly, *Bactrocera zonata* (Tephritidae: Diptera) by entomopathogenic fungi. *Fresenius Environmental Bulletin*, 31(6): 5522-5527.

Ahmad, A., M. Iqbal, A. Javaid, M.B. Chattha, M. Ashfaq, T. Hussain and S. Ashraf (2024). Plant-derived oils enhance the effectiveness of entomopathogenic fungi in controlling melon fruit fly maggots. *Pakistan Journal of Weed Science Research*, 30(4): 162-177.

Beris, E.I., D.P. Papachristos, A. Fytrou, S.A. Antonatos and D.C. Kontodimas (2013). Pathogenicity of three entomopathogenic fungi on pupae and adults of the Mediterranean fruit fly, *Ceratitis capitata* (Diptera: Tephritidae). *Journal of Pest Science,* 86: 275-284.

Bonanomi, G., G. Jesu, M. Zotti, M. Idbella, G. d'Errico, S. Laudonia, F. Vinale and A. Abd-ElGawad (2021). Biochar-derived smoke-water exerts biological effects on nematodes, insects, and higher plants but not fungi. *Science of the Total Environment*, 750: 142307.

Boudjelida, H. and N. Soltani (2011). Pathogenicity of *Metarhizium anisopliae* (Metsch) on *Ceratitis capitata* L. (Diptera: Tephritidae). *Annals of Biological Research,* 2(2): 104-110.

Chandio, A.A., A.A. Mirani and R.U. Shar (2019). Does agricultural sector foreign direct investment promote economic growth of Pakistan? Evidence from cointegration and causality analysis. *World Journal of Science, Technology and Sustainable Development*, 16(4): 196-207.

Destéfano, R.H.R., I.J. Bechara, C.L. Messias and A.E. Piedrabuena (2005). Effectiveness of *Metarhizium anisopliae* against immature stages of Anastrepha fraterculus fruitfly (Diptera: Tephritidae). *Brazilian Journal of Microbiology*, 36: 94-99.

Dias, N.P., M.J. Zotti, P. Montoya, I.R. Carvalho, and D.E. Nava (2018). Fruit fly management research: A systematic review of monitoring and control tactics in the world. *Crop Protection*, 112: 187-200.

Dionne, M.S. and D.S. Schneider (2008). Models of infectious diseases in the fruit fly Drosophila melanogaster. *Disease Models & Mechanisms*, 1(1): 43-49.

Dominiak, B.C., J. Ekman and S. Broughton (2016). Mass trapping and other management option for mediterranean fruit fly and Queensland fruit fly in Australia. *General and Applied Entomology*, 44: 1-8.

He, Y., Y. Xu and X. Chen (2023). Biology, ecology and management of tephritid fruit flies in China: A review. *Insects*, 14(2): 196-201.

Hena, S., L. Jingdong, A. Rehman, and O. Zhang (2019). A comparative analysis of agricultural development and modernization between China and Pakistan. *International Journal of Advanced and Applied Sciences,* 6(4): 81-94.

Iqbal, M., M.D. Gogi, B. Atta, M.J. Nisar, M.J. Arif and N. Javed (2021). Assessment of pathogenicity of *Beauveria bassiana*, *Metarhizium anisopliae*, *Verticillium lecanii* and *Bacillus thuringiensis* var. Kurstaki against *Bactrocera cucurbitae* Coquillett (Diptera: Tephritidae) via diet-bioassay technique under controlled conditions. *International Journal of Tropical Insect Science*, 41: 1129-1145.

Iqbal, M., S. Ahmad, Ibrar ul Haq, A. Javaid and M.B. Chattha (2025). Virulence of entomopathogenic fungi on different life stages of pink bollworm, *Pectinophora gossypiella*. *International Journal of Biology and Biotechnology*, 22(1): In press.

Jayaprakas, C.A., J. Tom and S. Sreejith (2023). Impact of insecticides on man and environment. In: *Biomedical Applications and Toxicity of Nanomaterials*. IntechOpen. pp. 751-768).

Kankam, F. (2021). Causes and management of pesticides contamination in agriculture: A review. *Ghana Journal of Science, Technology and Development*, 7(2): 103-118.

Laskar, N. and H. Chatterjee (2010). The effect of meteorological factors on the population dynamics of melon fly, *Bactrocera cucurbitae* (Coq.) (Diptera: Tephritidae) in the foot hills of Himalaya. *Journal of Applied Sciences and Environmental Management,* 14(3): 53-58.

Liu, H., D.D. Wang, L. Wan, Z.Y. Hu, T.T. He, J.B. Wang, S.Z. Deng and X.S. Wang (2022). Assessment of attractancy and safeness of (E) coniferyl alcohol for management of female adults of oriental fruit fly, *Bactrocera dorsalis* (Hendel). *Pest Management Science*, 78(3): 1018-1028.

Mahmoud, M.F. (2009). Susceptibility of the peach fruit fly Bactrocera zonata (Saunders), (Diptera: Tephritidae) to three entomopathogenic fungi. *Egyptian Journal of Biological Pest Control,* 19(2): 169-175.

McQuate, G.T. and N.J. Liquido (2017). Host plants of invasive tephritid fruit fly species of economic importance. *International Journal of Plant Biology & Research,* 5: 1072.

Papadopoulos, N.T. (2014). Fruit fly invasion: historical, biological, economic aspects and management. In: Shelly, T., N. Epsky, E. Jang, J. Reyes-Flores, R. Vargas (eds) *Trapping and the Detection, Control, and Regulation of Tephritid Fruit Flies. Springer, Dordrecht.* pp. 219-252.

Prince, M., A.C. McKinnon, D. Leemon, T. Sawbridge and J.P. Cunningham (2024). *Metarhizium* spp. isolates effective against Queensland fruit fly juvenile life stages in soil. *Plos One*, 19(1): 297-301.

Quesada-Moraga, E., A. Ruiz-García and C. Santiago-Alvarez (2006). Laboratory evaluation of entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* against puparia and adults of *Ceratitis capitata* (Diptera: Tephritidae). *Journal of Economic Entomology,* 99(6): 1955-1966.

Rashad, M.M., A. El-Heneidy, K. Djelouah, N. Hassan and S.A. Shaira (2015). On the pathogenicity of entomopathogens to the peach fruit fly, *Bacterocera zonata* (Saunders) (Diptera: Tephritidae). *Egyptian Journal of Biological Pest Control, 25*(3).

Ros, J., E. Wong, J. Olivero, and E. Castillo (2002). Mejora de los mosqueros, atrayentes y sistemas de retención contra la mosca mediterránea de la fruta *Ceratitis capitata* Wied. Como hacer de la Técnica del Trampeo Masivo una buena herramienta para controlar esta plaga. *Boletin de Sanidad Vegetal Plagas,* 28: 591-597.

Saeed, M., T. Ahmad, M. Alam, L.A. Al-Shuraym, N. Ahmed, M.A. Alshehri, H. Ullah and S.M. Sayed (2022). Preference and performance of peach fruit fly (*Bactrocera zonata*) and Melon fruit fly (*Bactrocera Cucurbitae*) under laboratory conditions. *Saudi Journal of Biological Sciences*, *29*(4): 2402-2408.

Sarwar, M., B. Rasool, M.M. Shah and N. Ahmad (2023). Effects of environmental variables and role of food attractants for management of *Bactrocera zonata* (Saunders, 1842) and *Bactrocera dorsalis*, (Hendel, 1912) (Diptera: Tephritidae). *Journal of the Entomological Research Society*, *25*(3): 563-578.

Sharma, K., R. Sharma, S. Chander and V. Jilu (2015). Effects of weather parameters on guava fruit fly (*Bactrocera zonata*) population at IARI, New Delhi. *Journal of Agrometeorology,* 17(2): 227-229.

Shaurub, E.S.H. (2023). Review of entomopathogenic fungi and nematodes as biological control agents of tephritid fruit flies: current status and a future vision. *Entomologia Experimentalis Applicata*, 171(1): 17-34.

Sheikh, S.M., M. Ahmed, S. Shahan and M.Z. Khan (2012). Importance of agricultural sector in Pakistan. *Interdisciplinary Journal of Contemporary Research in Business,* 3(12): 421.

Sookar, P., M. Alleck, N. Ahseek and S. Bhagwant (2014). Sterile male peach fruit flies, *Bactrocera zonata* (Saunders) (Diptera: Tephritidae), as a potential vector of the entomopathogen *Beauveria bassiana* (Balsamo) Vuillemin in a SIT programme. *African Entomology,* 22(3): 488-498.

Usman, M., W. Wakil, J.C. Piñero, S. Wu, M.D. Toews and D.I. Shapiro-Ilan (2021). Evaluation of locally isolated entomopathogenic fungi against multiple life stages of *Bactrocera zonata* and *Bactrocera dorsalis* (Diptera: Tephritidae): Laboratory and field study. *Microorganisms,* 9(8): 1791.

Verghese, A., H. Madhura, P.K. Jayanthi and J.M. Stonehouse (2002). Fruit flies of economic significance in India, with special reference to *Bactrocera dorsalis* (Hendel). In: *Proceedings of 6th International Fruit Fly Symposium, 6-10 May 2002, Stellenbosch, South Africa,* pp. 317-324.

Wakil, W., M. Usman, J.C. Pinero, S. Wu, M.D. Toews and D.I. Shapiro-Ilan (2022). Combined application of entomopathogenic nematodes and fungi against fruit flies, *Bactrocera zonata* and *B. dorsalis* (Diptera: Tephritidae): Laboratory cups to field study. *Pest Management Science*, 78(7): 2779-2791.

Yousaf, A.A., K.S. Abbasi, A. Ahmad, I. Hassan, A. Sohail, A. Qayyum and M.A. Akram (2020). Physico-chemical and nutraceutical characterization of selected indigenous guava (*Psidium guajava* L.) cultivars. *Food Science and Technology*, 4: 47-58.

Zamora-Ros, R., V. Knaze, L. Lujan-Barroso, N. Slimani, I. Romieu, M. Touillaud and S. Grioni (2011). Estimation of the intake of anthocyanidins and their food sources in the European prospective investigation into cancer and Nutrition (EPIC) study. *British Journal of Nutrition,* 106(7): 1090-1099.

Zhang, R., E.B. Jang, S. He and J. Chen (2015). Lethal and sublethal effects of cyantraniliprole on *Bactrocera dorsalis* (Hendel)(Diptera: Tephritidae). *Pest Management Science,* 71(2): 250-256.

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