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RESEARCH ARTICLE

Pathogenicity of an entomopathogenic fungus against mango fruit flies under controlled conditions

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Abstract

Management of the insect pests is mainly done through synthetic insecticides which have resulted in residual effects in fruits as well environmental hazards. The current research was conducted to examine the biopesticidal impact of an entomopathogenic fungus *Beauveria bassiana* on two species of mango fruit flies, namely *Bactrocera dorsalis* and *Bactrocera zonata*. Virulence of the fungus was assessed at three dose rates under laboratory conditions. Three dosages of *B. bassiana* isolate viz. 3×10^8, 6×10^8, and 9×10^8 conidia/ml, were assessed for their larvicidal efficacy. The bioassays were conducted in an incubator set at 30°C with 65% relative humidity and devoid of lighting. The mortality of the introduced larvae was recorded 5 and 10 days' post-exposure to the respective dosages. Results of mortality bioassays revealed that maximum mortality (50%) of *B. dorsalis* was due to 9×10^8 conidia/ml whereas the minimum mortality (38%) was noted at 3×10^8 conidia/ml by exposure period of 10 days. In case of *B. zonata*, the highest mortality (41%) was noted down at the highest conidial dose rate i.e. 9×10^8 conidia by exposure period of 10 days whereas the lowest mortality value (33%) was noted at 3×10^8 conidial/ml dose of *B. bassiana*. Outcomes of mycosis revealed that maximum mycosis (85.34%) was recorded at 9×10^8 conidial/ml dose by exposure period of 10 days in case of *B. dorsalis* whereas the comparatively low mycosis (67.56%) was enumerated at 3×10^8 conidial/ml. Similarly, maximum sporulation maximum (152.31 conidial/ml) was recorded at dose rate of 9×10^8 conidia and the lowest (110.65 conidia/ml) at dose rate of 3×10^8 conidia. Comparatively low values of mycosis and sporulation were recorded in case of *B. zonata*. Overall results disclosed that mortality, mycosis and sporulation responses were found dose as well as exposure period dependent and *B. dorsalis* showed comparatively more susceptibility towards *B. bassiana* compared with *B. zonata*. Further research is warranted to assess the efficacy of these entomopathogenic fungi in controlling fruit flies within both controlled laboratory environments and natural field conditions. This necessitates the evaluation of more virulent strains and isolates of entomopathogenic fungi, the development of appropriate formulations, and the standardization of application methods.

Keywords: *Conidia,* Exposure period, IPM, Mortality, Mycosis, Sporulation

Introduction

Mango (*Mangifera indica* L.) is among the most notorious and vital fruit crops among all fruits being grown worldwide due to their seductive aroma, great taste, stunning color, natural interaction and aromatic flavor. Hence, termed as "King of fruits" (Boulahia-Kheder et al., 2021; Yadav & Paudel, 2022). The yield of mango fruits is being hindered by a range of climatic factors. Among these, insect pest infestation occupies the supreme position (Atif et al., 2019; Aarthi et al., 2024; Junaid and Gokce, 2024; Subramaniam et al., 2024). Tephritid flies (*Diptera: Tephritidae*) are known as the most devastating insect pests resulting in massive losses in monetary terms in agriculture sector, mainly in a diversity of flowers, fruits and vegetables (Usman et al., 2021; Ganesh et al., 2023; Li et al., 2024; Rehman et al., 2020). *Bactrocera* is a fruit fly genus of high economic importance with 50 species known to be main insect pests, most of which are polyphagous in nature (Bhatti et al., 2023; Javed et al., 2022; Vargas et al., 2015; Papadopoulos et al., 2024). Mango, guava, bitter gourd musk melon and Apple are the main hosts of fruit flies observed in Pakistan (Kausar et al., 2022; Nazir et al., 2022). In Pakistan, around 11 species of fruit flies have been documented and among them, *B. dorsalis* and *B. zonata*, are of huge economic importance (Kausar et al., 2022; Wakil et al., 2022; Sarwar et al., 2023; Shah et al., 2024).

Fruit flies are responsible for causing direct harm to fruit. Upon oviposition, female fruit flies deposit their eggs just beneath the surface of the fruit. Following hatching, the newly emerged larvae commence feeding on the pulp of the fruit. This process ultimately results in premature fruit drop and a subsequent reduction in fruit yield (Nazir et al., 2021; Li et al., 2024). Importing countries may incur significant economic losses when trade is suspended due to the discovery of maggots within the consignment. This can lead to the imposition of quarantine restrictions on the exporting countries (Quesada-Moraga et al., 2006; Hoskins et al., 2023 Papadopoulos et al., 2024). The control of problematic fruit flies involves the application of residual foliar sprays or baits (Boulahia-Kheder et al., 2021; Khan et al., 2023). Furthermore, the eradication of fruit fly larvae and pupae is achieved through the application of insecticidal treatments to the soil beneath tree canopies. Moreover, Integrated Pest Management (IPM) strategies, such as cultural practices, parasitoid utilization, male annihilation technique, and sterile insect technique, have been implemented in various countries, including the Pacific region, Hawaii, Africa, and Japan (Vargas et al., 2015; Vreysen et al., 2021). However, recurrent and unjudicial use of the synthetic insecticides have resulted in resistance development (Haider et al., 2011) and health-hazards to human and mortality of non-target organisms (Magana et al., 2007). The limitations associated with synthetic insecticides have underscored the urgent requirement for biologically-based pest control strategies to facilitate the eco-friendly management of insect pests (Gulzar et al., 2021; Bacelar et al., 2024; Chen et al., 2024).

Entomopathogenic microorganisms have garnered considerable attention in contemporary contexts. Among these, Entomopathogenic Fungi (EPFs) have been investigated as a bio-based strategy for the management of various insect pests, encompassing fruit flies (Rehman et al., 2019; Wakil et al., 2020; Kannan et al., 2023; Hudson et al., 2024; Mushtaq et al., 2024). *Bacillus thuringiensis* strains were proved slightly operative nevertheless not appropriate for the control of Tephritid pests owing to the concealed habit of the insect (Aarthi et al., 2024). The failure of protozoa and baculovirus against the olive fruit flies (Yousef et al., 2013) has prompted the researchers to emphasis on use of entomopathogenic fungi (Usman et al., 2020). In past, many research trials have assessed EPFs against *B. oleae* (Rossi) (Yousef et al., 2018), European cherry fruit fly, *Rhagoletis cerasi* L. (Daniel et al., 2009), Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) (Ekesi et al., 2003, 2005, Toledo-Hernández et al., 2019), Mexican fruit fly *Anastrepha ludens* (Loew) (Toledo-Hernández et al., 2019), western cherry fruit fly *R. indifferens* Curran (Cossentine et al., 2010) and *R. pomonella* (Walsh) (Usman et al., 2020). Nevertheless, very few research trials have been EPF has been executed on *B. (Zeugodacus) cucurbitae* (Coquillett) (Sookar et al., 2008), *B. dorsalis* (Purwar et al., 2013; Hudson et al., 2024) and *B. zonata* (Sookar et al., 2014; Hudson et al., 2024). Whereas EPF are undoubtedly virulent against Tephritid fruit flies, noteworthy difference among different species of EPF. The variability in virulence among species can be ascribed to the specific host from which they were derived, their genetic makeup, and their co-evolution with hosts within a particular geographic region (Quesada-Moraga et al., 2006; Hudson et al., 2024; Horgan, 2024; Peng et al., 2024). A very few case studies have been reported on the effectiveness of native *B. bassiana* against *B. dorsalis* and *B. zonata* is scarce.

Materials and Methods

Preparation of different dose rates of fungal conidia

A solution of 1.0 ml of each treatment (concentration) was pipetted onto an adult diet (honey, egg yolk, protein hydrolysate and sugar water solution) in disposable cups having lids. The solution was then admixed with fruit fly adult diet with the help of a sterilized loop. The treatment-baited adult diet was lapped partially on the walls of the treatment units (plastic jars) as well as placed inside the treatment unit in a disposable cup (Iqbal et al., 2021).

Bioassays-1: Pathogenicity (%) of *Beauveria bassiana* against the two selected species of mango fruit fly

A mixed population of newly emerged 100 adults of *B. zonata* and *B. dorsalis* were aspirated from culture and released into treatment units which were maintained at 28°C ± 2°C and 70% ± 5% RH for 24 h. the flies were allowed to feed on a treatment-baited adult diet for 24 h. After an exposure period of 24 h, the flies were transferred to the fruit fly adult rearing unit (plastic jars having above mentioned normal fruit fly adults that were maintained at 28°C ± 2°C for 14 d. The mortality of adult flies of *B. zonata* and *B. dorsalis* were recorded after 5 d and 7 d (Nisar et al., 2021). The dead flies were placed on respective growth media to promote the growth of fungal mycelia (mycosis) from treated flies and confirm that the death of flies was caused by a fungal infection. *B. bassiana* was applied separately at three conidial dose rates; $Bb_1 = 3 \times 10^8$ conidia, $Bb_2 = 6 \times 10^8$ conidia, $Bb_2 = 9 \times 10^8$ conidia.

Data collection

Mortality data was transformed into percent corrected mortality by Abbot Formula (Abbott., 1925):

$$\text{Corrected mortality (\%)} = 1 - \frac{number\ in\ treated\ unit\ after\ treatment}{number\ in\ control\ unit\ after\ treatment} \times 100$$

Probit analysis serves as a method for examining data derived from bioassay tests, wherein diverse concentrations of insecticide are administered to eliminate varying proportions of insects. The outcomes of probity analysis are typically presented as the duration necessary to eradicate a specific percentage of test insects, as exemplified by the LT_{50} (Finney, 1971).

Bioassays-2 Growth inhibition (%) potential of *B. bassiana* against the two selected species of mango fruit fly

Disease and infestation free mango fruits were brought to laboratory and adults of the both fruit flies were allowed to oviposit on the fruits, placed in separate chambers. Field observations have revealed that first two instars of the fruit flies remain inside the egg while 3rd instars larvae of the fruit flies usually come out from the host plant and drop to soil for pupation (Susanto et al., 2022). Therefore, 3rd instars larvae the both selected fruit fly species were taken, released into small plastic jars, bottoms of which were provided with different conidia dose rates of each selected fungal sp. Data for pupae and adult inhibition of the fruit fly sp. were recorded till the emergence of pupae and adult stage.

Mycosis and sporulation

For this purpose, cadavers of *B. dorsalis* and *B. zonata* were collected from the fungal treated experimental units, positioned on a disinfected petri dish and refrigerated at 4°C in small vials. Later on all the cadavers were superficial decontaminated by 0.05% sodium hypochlorite solution for a period of 2 min-3 min trailed by 2-3 washing through distilled water? Afterwards, cadavers were positioned on Potato Dextrose Agar (PDA) petri-dishes and incubated at 26°C ± 1°C; 75% ± 5% R. H. for seven days. The examination of insect cadavers exhibiting external fungal proliferation was conducted under microscopic observation. To assess sporulation, the mycosed insect cadavers from each replication were amalgamated in 20 ml of distilled water with a drop of Tween-80 and homogenized for a duration of ten minutes. The

enumeration of conidia/ml was then performed utilizing a hemocytometer under microscopic scrutiny (Riasat et al., 2011; Baek et al., 2022).

Statistical analysis

This transformed corrected mortality data were analyzed by STATISTICA-10 software. The data analysis entailed the utilization of ANOVA to ascertain the variance among means, employing Tukey's multiple range test with an honestly significant difference (HDS 0.5%). Additionally, measures were taken to ensure homogeneity of variances and normality of the data (Lozano-Tovar et al., 2015; Resquín-Romero et al., 2016; Khanday et al., 2018).

Results

Pathogenicity of fungal bio insecticides against *Bactrocera zonata* and *Bactrocera dorsalis*

Tab. 1 shows pathogenicity of *B. bassiana* revealed that maximum mortality (50.23%) of *B. dorsalis* was enumerated at dose rate of 9×10^8 conidia/ml while the lowest mortality (38.05%) at 3×10^8 conidia by exposure period of 10 DAT. Comparatively low mortality values were observed by exposure period of 5 DAT. In case of *B. zonata*, maximum mortality (41.23%) was recorded at highest conidial dose rate (i.e. 9×10^8 conidia) by exposure period of 10 DAT while the low mortality value (33.05%) was noted at 3×10^8 conidial dose rate of *B. bassiana*.

Table 1. Pathogenicity of *Beauveria bassiana* (Bb) against *Bactrocera dorsalis* adults.

Fruit flty sp.		Mortality (%)	
		5 DAT	10 DAT
Bactrocera dorsalis	Bb_1	27.29 bc ± 2.12 bc	38.05 ± 1.10 d
	Bb_2	30.47 b ± 2.21b	41.17±1.92 cd
	Bb_3	35.68 a ± 1.78 a	50.23 a ±1.10 a
Bactrocera zonata	Bb_1	19.86 c ± 2.18 c	33.05 d ± 1.10d
	Bb_2	26.47 bc ± 2.61 bc	40.92 cd ± 1.89cd
	Bb_3	32.68 ab ± 1.83ab	38.23 b ± 1.34b
		F=9.85, df=2	F=292.17, df=2

Sp.=Species, F=F-test, df=degree of freedom, Bb_1=3 × 10^8conidia, Bb_2=6 × 10^8 conidia, Bb_2=9 × 10^8 conidia. Treatments means sharing similar lettering within a column were not significantly different from each other at α=5%. DAT=Days after treatment.

LT values of fungal isolates against *Bactrocera zonata* and *Bactrocera dorsalis*

Tab. 2 reveals that maximum LT_{50} (14.3 days) that decreased to 10.3 days as the concentrations of the *B. bassiana* got increased against *B. dorsalis*. Similarly, maximum LT_{50} (15.6 days) was recorded for *B. zonata* when treated with (Bb_1 = 3 × 10^8 conidia/ml) Comparatively greater LT_{50} values were recorded against *B. dorsalis* and *B. zonata* indicating that *B. bassiana* proved less lethal against. Overall results showed that the more exposure period shows more lethality of treatment.

Table 2. LT50 of at different conidial dose rats of the *Beauveria bassiana* against the tow studied entomopathogenic fungi.

Fruit fly sp.	LT_{50} (Days)		
	Bb_1	Bb_2	Bb_3
Bactrocera dorsalis	14.2	11.5	10.3
Bactrocera zonata	15.6	13.8	12.6

Pupae and adult inhibition (%) impact of *Beauveria bassiana* against the two fruit fly species

Tab. 3 shows growth inhibition impacts of *B. bassiana* against the both fruit fly species revealed that maximum pupae inhibition (59.83%) of *B. dorsalis* was enumerated at dose rate of 9 × 10^8 conidia whereas comparatively low inhibition (47.84%) at 3 × 10^8 conidia. In case of *B. zonata*, the values were 63.46 and 40.19% at highest and lowest conidial dose rates of *B. bassiana*. Highest adult inhibition (54.20%) was recorded in case of *B. dorsalis* at conidial dose rate of 9 × 10^8 conidia while the lowest adult inhibition (36.95%) was recorded in case of *B. zonata* at 3 × 10^8 conidia conidial dose rate of *B. bassiana*.

Table 3. Mean effect of *Beauveria bassiana* (Bb) on pupation and adult emergence (% ± SE) of *Bactrocera dorsalis* and *Bactrocera zonata*.

		B. dorsalis	
Fruit fly spp.	**Treatments**		
		Pupae inhibition (%)	**Adult inhibition (%)**
	Bb_1	47.84 cd ±1.92 cd	42.70 c ± 1.23 c
Bactrocera dorsalis	Bb_2	55.43 b ± 2.10 b	45.31 bc ± 3.10 bc
	Bb_3	59.83 a ± 2.30 a	54.20 bc ± 2.11 a
	Bb_1	40.19 d ± 1.28 d	36.95 d ± 1.72 d
Bactrocera zonata	Bb_2	51.84 b ± 2.43 b	47.32 b ± 2.52 b
	Bb_3	63.46 a ± 3.10 a	50.92 ab ± 2.34 ab
		F = 65.12, df = 2	F=14.32, df=2

Mycosis (%) and sporulation of *Beauveria bassiana* (Bb) on cadavers of *Bactrocera dorsalis* larvae

Variations in mean values of mycosis were statistically significant ($p<0.05$) and ranged as 58.10%-85% throughout the study period (Fig. 1). Maximum mycosis (85%) was recorded at 9 × 10^8 conidial/ml dose rate by exposure period of 10 DAT while the comparatively low mycosis (67%) was enumerated at 3 × 10^8 conidia/ml. The values of mycosis were comparatively lower with exposure period of 5 DAT. Sporulation was lowest (110.65 conidia/ml) at dose rate of 3 × 10^8 conidia which reached to maximum (152.31 conidia/ml) at dose rate of 9 × 10^8 conidia (Fig. 2) discovered that. Overall results depicted that sporulation response was found dose as well as exposure period dependent.

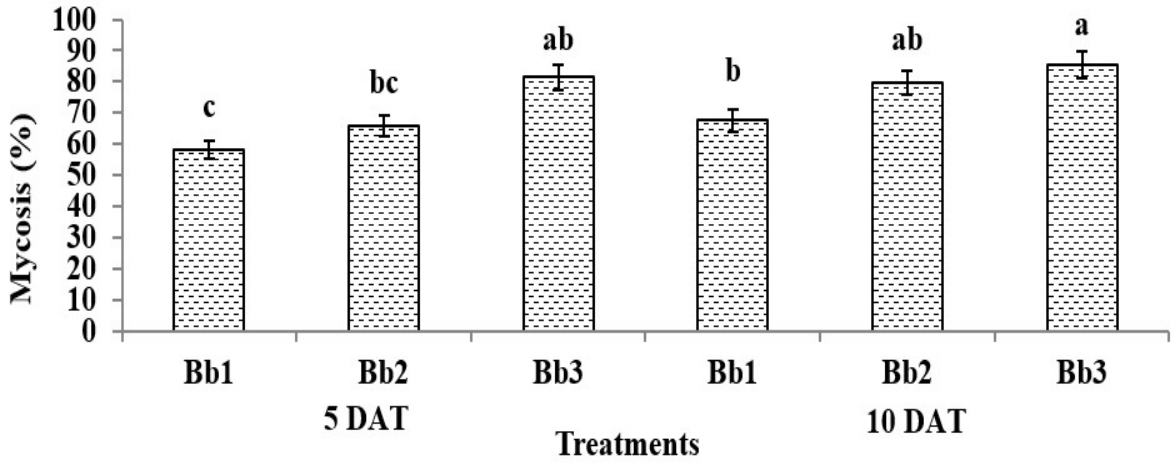

Figure 1. Mycosis (%) of *Beauveria bassiana* (Bb) against of larvae of *Bactrocera dorsalis*.

Impact of the different concentrations of *Beauveria bassiana* (Bb) on (%) Mycosis in cadavers of 3rd instar larvae of *Bactrocera dorsalis* (Bars with the same letters are not significantly different at 5% significance level. Vertical bars reflect SE. Bb1: 3 × 10^8 conidia/ml, Bb2: 6 × 10^8 conidia/ml, Bb3: 9 ×10^8 conidia/ml

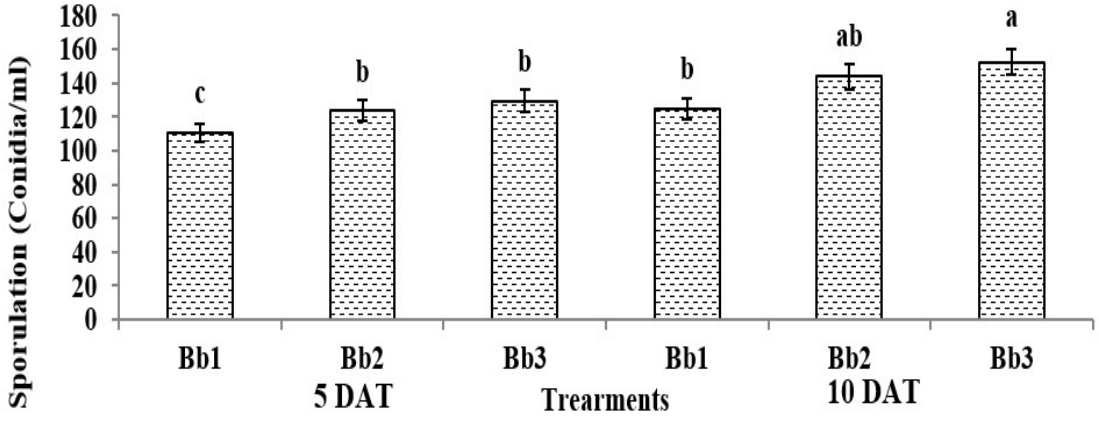

Figure 2. Sporulation (conidia/ml) of *Beauveria bassiana* (Bb) against of larvae of *Bactrocera dorsalis*.

Impact of different concentrations of *Beauveria bassiana* (Bb) on sporulation in cadavers of 3rd instar larvae of *Bactrocera dorsalis*. Bars with the same letters are not significantly different at 5% significance level. Vertical bars reflect SE. Bb1: 3 × 10^8 conidia/ml, Bb2: 6 × 10^8 conidia/ml, Bb3: 9 × 10^8 conidia/ml.

Efficacy of different concentrations of *Beauveria bassiana* (Bb) on (%) mycosis and sporulation in 3rd instar larvae of *Bactrocera zonata*

Mycosis varied: 39.56%-71.80% and variations were statistically significant ($p<0.05$). Highest mycosis (71.80%) was noted at 9 × 10^8 conidial dose rate by exposure period of 10 DAT followed by 6 × 10^8 conidial dose rate (68.16%) while comparatively low mycosis (65.12%) was noted down at 3 × 10^8 conidial (Fig. 3). In case of 5 DAT, the values were 60.78%, 52.90% and 39.56%, respectively. Maximum sporulation (96.15 conidia/ml) at dose rate of 3 × 10^8 conidia which reached to maximum (138.15%) at 9 × 10^8 conidia/ml dose rate by exposure period of 10 DAT whereas the relatively low mycosis was enumerated at 3 × 10^8 conidia/ml in case of the both exposure periods (Fig. 4). However, values of mycosis were relatively lower with exposure period of 5 DAT.

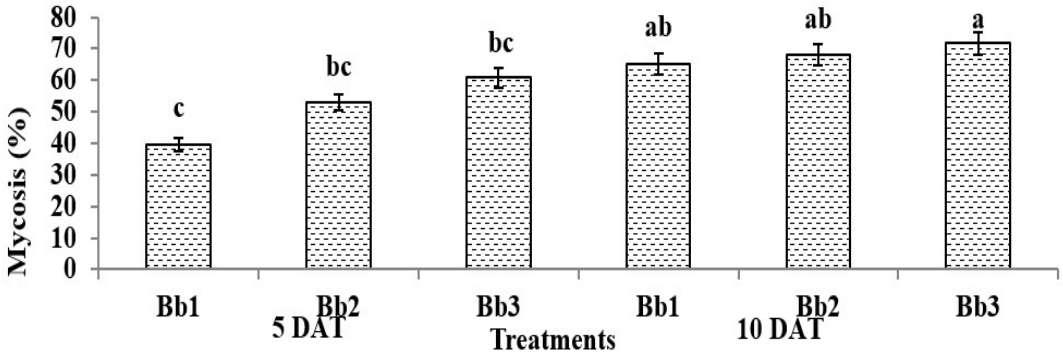

Figure 3. Mycosis (%) of *Beauveria bassiana* (Bb) against of larvae of *Bactrocera zonata*.

Impact of the different cncentrations of *Beauveria bassiana* (Bb) on (%) Mycosis in cadavers of 3rd instar larvae of *Bactrocera zonata*. Bars with the same letters are not significantly different at 5% significance level. Vertical bars reflect SE. Bb1: 3 × 10^8 conidia/ml, Bb2: 6 × 10^8 conidia/ml, Bb3: 9 × 10^8 conidia/ml.

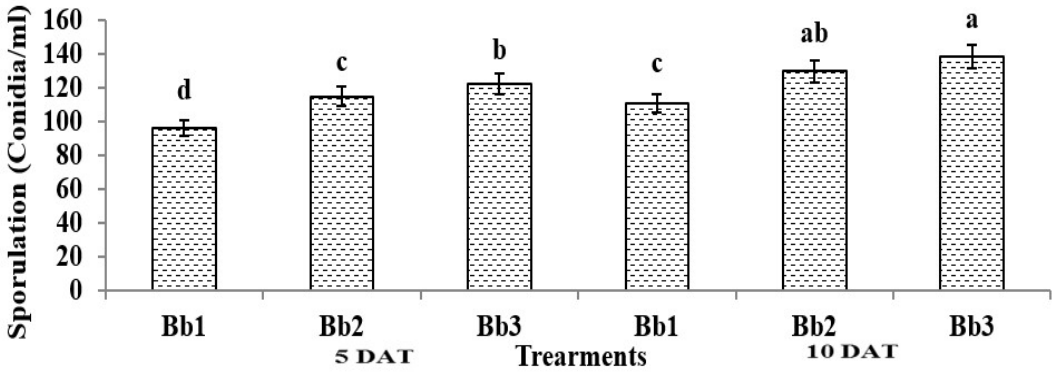

Figure 4. Sporulation (conidia/ml) of *Beauveria bassiana* (Bb) against of larvae of *Bactrocera zonata*.

Impact of different concentrations of *Beauveria bassiana* (Bb) on sporulation in cadavers of 3rd instar larvae of *Bactrocera zonata*. Bars with the same letters are not significantly different at 5% significance level. Vertical bars reflect SE. Bb1: 3×10^8 conidia/ml, Bb2: 6×10^8 conidia/ml, Bb3: 9×10^8 conidia/ml.

Discussion

Mango fruit occupies supreme position among the all fruits due to its specific taste, aroma and dietary values. It equips human body with vitamin C and many other nutrients. In recent years, the yield of mangoes and other fruits has been significantly impacted by both biotic and abiotic factors. Of particular concern among the biotic factors is the infestation of insect pests (Naz et al., 2016). Fruit flies have gain tremendous importance in recent few years due to rejection of export in Pakistan. The main cause of this dilemma is the fruit fly infestation. Management of the fruit flies through traditionally used synthetic insecticides have resulted in many problems including residue effects in fruits and resistance development in fruit flies. Hence, the present research work was executed to control the fruit with newer and bio derived insecticides. Owing to greater stress on application of bio-control for the environment pleasant control of stored commodities insect pests and field crops, the EP fungi have gained huge consideration in recent few years (Rice 1999; Rehman et al., 2020). These are target specific and have no residual effects in stored commodities. Entomopathogenic Fungi (EPF) exhibit a broad host range, and their mechanisms of action may vary. In contrast to chemical insecticides, EPF pose no threat to human safety (Zimmermann, 2007). Given their distinctive pest control attributes, these fungi represent a promising biocontrol agent and can be effectively integrated into organic farming practices (Litwin et al., 2020). These EP fungi have natural source and are less toxic to human beings. The *Beauveria bassiana* (Balsamo) *Vuillemin* (Ascomycota: Hypocreales: *Clavicipitaceae*) has been extensively studied in this respect. The fungus forms *appressorium* when comes to in contact with cuticle of insect's body. The structure then penetrates deep into the hemolymph and enter into gut leading to germination of fungal hyphae. Later the fungal spore formation occurs which protrude our and visible as colonies of hyphae of specific color according to the species character of each fungus. In this investigation, the maximum pathogenicity was observed at the highest concentration of 1×10^8 conida. This finding is consistent with the research conducted by (Mar & Lumyong 2012). The study utilized three concentrations (10^6 CFU/ml, 10^7 CFU/ml, and 10^8 CFU/ml), and their effectiveness was evaluated over a period of 2 days up to 2 weeks in a laboratory experiment. The highest concentration demonstrated the most promising results. In a research work, (Trinh et al. 2020) employed equivalent concentration levels in their study on aphids, yielding results that supported the notion of increased efficacy of EPF with escalating concentrations. Their findings also underscored the significance of time intervals in EPF efficacy, as the highest mortality rate was recorded at 14 DAT. This outcome aligns with the observations made by (Nazir et al. 2019) who documented a rise in insect mortality following the application of EPF over time. Many researchers have explored the pathogenicity of *B. bassiana* and some other Entomopathogenic Fungi (EPFs) against stored grains as well as the field crops insect pests. Many researchers have explored the pathogenicity of EPFs against different insect pests like for control of stored grains (Ahmad and Shakeel, 2024; Riast et al., 2011; Rehman et al., 2019; Wakil et al., 2020; Sharma et al., 2023; Adegbola et al., 2024) and field crops insect pests (Yousef et al., 2013; Nazir et al., 2022; Susanto et al., 2022). All the studies revealed a dose as well as time depended response of pathogenicity of EPFs against insect pests as was

recorded in our research work. However, difference in mortality, mycosis and sporulation values noted in our research work may be diversity in insect species, dose rate and experimental conditions of research trials.

Conclusions

From the research work, it could be concluded that *B. bassiana* possessed huge entomopathogenic potential against *B. dorsalis* and *B. zonata*. Repeated use of synthetic insecticides of same mode of action and residue effects in fruits have lost the interest of growers on these synthetic insecticides. Use of *B. bassiana* and other EPFs could be effective in the Integrated Management of pests but moisture and other conditions should be kept in mind before applying the EPFs to achieve the better results.

Conflict of Interest

The authors of this manuscript have no conflict of interest.

Data Availability

Data will be available anytime on a realistic request.

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