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Research Article

ENTOMOPATHOGENICITY OF BEAUVERIA BASSIANA AGAINST TWO BACTROCERA SPECIES (TEPHRITIDAE: DIPTERA) UNDER LABORATORY CONDITION

Muhammad Umair Shahzad[1], Mirza Abdul Qayyum*[1], Muhammad Ishtiaq[1], Rao Muhammad Ikram[2], Muhammad Asad Saleem[1]
[1]*Institute of Plant Protection, MNS University of Agriculture Multan Pakistan*
[2]*Department of Agronomy, MNS University of Agriculture Multan Pakistan*
**Corresponding author: qayyum.mirza@mnsuam.edu.pk*

Abstract
Fruit fly *Bactrocera* spp. (Tephritidae: Diptera) are the anxious pest of different vegetables, fruit crops and fruit orchards. The present study was carried out to evaluate the virulence of *B. Bassiana* against larvae of fruit fly species (*B. zonata* and *B. dorsalis*). Three concentrations [Bb1 (1×10^3), Bb-2 (1×10^5), Bb-3 (1×10^7) spores/ml] of *B. bassiana* were tested against *B. zonata* and *B. dorsalis*. It was recorded that *B. bassiana* provided significant morality, Bb-1 (54.54%), Bb-2 (63.25%) and Bb-3 (82.76%) against larvae of *B. zonata*. However, For the larvae of *B. dorsalis* all tested concentrations were provided significant mortality (49.05%, 59.47% and 93.56%). For untreated insects, lowest (2.27%) mortality was recorded. This study is indicative of the potential of using *B. bassiana* isolate against both species of fruit fly thus providing a novel alternative to chemical application.

Keywords: **Fruit fly, *B. zonata, B. dorsalis,* EPF, *B. bassiana***

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Introduction
Fruit fly is the destructive pest of different vegetables and fruits in all over the world. The genus *Bactrocera* is most diverse species up to 50 species to be known (Vargas *et al.*, 2015). Tephritid fruit flies are the most damaging species causing economic losses and deteriorate the quality of fruits (Choudhary *et al.*, 2018). It has been reported that about 10% economically important fruit fly species were found in tropical and subtropical as well as temperate regions of the world and it is very dangerous pest of various crops (Singh, 2003).

The third instar larvae of fruit fly are damaging stage that comes out from fruit and reaches to ground for pupation in soil (White and Elson-Harris, 1992). Most of the researchers evaluated that tephritid species also effected by abundance diversity of hosts (cultivated and local), temperature, altitude and rainfall (Rwomushana *et al.*, 2008, Mwatawala *et al.*, 2009). New emerging larvae enter the new healthy tissues and introduce the pathogens which cause fruit decomposition.

Many control strategies have been developed for the control of fruit fly population. Farmer relay on the use of insecticides which are harmful to our natural fauna as well as excessive use of chemicals may cause resistance problem in insects. So, it is need to develop eco-friendly approach for the management fruit fly. Entomopathogenic fungi are promising biological control agent which are safe for environment and non-target species. *Beauveria bassiana* (Bals.) considered promising entomopathogens against the dipteran insects (Salvatore *et al.*, 2009; Boudjelida and Soltani, 2011). Climatic factors play an important role towards the survival of EPF and ability to cause disease (Inglis *et al.,* 2001). Both under laboratory and field conditions, entomopathogenic fungi particularly *B. bassiana* and *Metarhizium anisopliae* are potential candidate with notable pathogenicity against adults and pupae of fruit fly (Quesada *et al.*, 2006; Almeida *et al.*, 2007). Evaluating the pathogenicity against different stages of *B. zonata* is the need of hour as a reliable tool to cater this insect that has put trade of fruits and vegetables at risk.

Material and Methods

Fungal isolates and conidial suspension

Fungal isolate (*B. bassiana*) were analyzed to observed the potential against larvae of *B. zonata*.

Potato Dextrose Agar (39g/L) was used for cultivation of EPF's. Fungi were placed at 4°C as stock culture. The development of conidia on MA at 25°C from the stock culture was used for bio-assays. *B. bassiana* was grown on MA plate and was incubated at 25°C for the time period of 7-10 days for first screening. Spores were collected with Tween 80 (0.1%) as spore suspension in germ-free distill water and determination of spore concentration were held by Haemacytometer. Three *B. bassiana* concentrations Bb-1, Bb-2 and Bb-3 (1×10^3, $1\times10^{5,}$ 1×10^7 spores/ml) were used in bioassay.

Insect Rearing and Maintenance:

Adults of *B. zonata* and *B. dorsalis* were collected from its host crops and maintained in rearing cage in Insect Rearing Laboratory, Institute of Plant Protection, MNS University of Agriculture, Multan during 2017 (2.5×2.5 ft). Adults were placed separately in rearing cages for mating and egg laying. The harvested eggs were collected and placed in plastic jars supplied with soft paper as nappy liner. Artificial diet provided to emerging larva (Methyl-p-hydroxy benzoate 0.1%, sodium benzoate (0.1%), sugar (12.0%), dried yeast (3.6%), wheat bran (26.0%), HCl (0.2%), and water (58.0%). Laboratory condition were for the study were 27±1°C temperature maintained with 65±5% relative humidity (RH).

Bioassay

Last instar (3^{rd}) larvae which was near to pupate used in bioassays. The 12 larvae of both species were used in each treatment and replicated 4 times. Larvae were sprayed with fungal suspension for 60s at tested concentrations. Pupation and adult emergence were observed after application and effects of fungal suspension also observed. For control group, distilled water with Tween80 @ 0.1% was applied.

RESULTS

Virulence of *B. Bassiana* on larvae of *B. zonata*

Three *B. bassiana* concentrations [Bb-1 (1×10^3, Bb-2 (1×10^5), Bb-3 (1×10^7) spores/ml] were tested against the larvae of *B. zonata*. It was recorded that Bb-1 provided 8.33% mortality after application of 24h, Bb-2 provided 16.85% and Bb-3 provided 25.18% mortality after 24h. Similar trends was observed after application of 48h, all three tested concentrations provided

27.81%, 33.90 and 48.67% mortality for larvae of *B. zonata*. However, after 72h of treatment 54.54%, 63.25 and 82.76% morality were recorded at tested concentrations. For the control group mortality was lowest (4.54%) as compared to tested treatments (Table 1).

Table 1: Mean mortality (%±SE) of larvae of *B. zonata* and *B. dorsalis* treated with *B. bassiana* at different concentrations [Bb-1 (1×10^3, Bb-2 (1×10^5), Bb-3 (1×10^7) spores/ml].

B. bassiana	Mean mortality 24h		Mean mortality 48h		Mean mortality 72h	
Concentration	*B. zonata*	*B. dorsalis*	*B. zonata*	*B. dorsalis*	*B. zonata*	*B. dorsalis*
Bb-1	8.33b	14.58b	27.84b	25.37b	54.54b	49.05c
Bb-2	16.85ab	20.83ab	33.90b	34.09b	63.25b	59.47b
Bb-3	25.18a	29.16a	48.67a	53.03a	82.76a	93.56a
Untreated	2.27b	0.00c	2.27c	2.27c	4.54c	2.27d
$F_{3,15}$	6.68	38.1	35.1	69.3	113	241
P value	<0.00	<0.00	<0.00	<0.00	<0.00	<0.00

Virulence of *B. Bassiana* on larvae of *B. dorsalis*

For the larvae of *B. dorsalis* all tested concentrations were provided significant mortality. After 24 h of treatment Bb-1 provided 14.58%, Bb-2 gave 20.83% and Bb-3 provided 29.16% mortality after applied concentrations. However, at the same concentrations, mortality were 25.37%, 34.09% and 53.03% after 48 of applications for the larvae of *B. dorsalis*. *B. dorsalis* mortality was highest after 72h of applications at same tested concentrations which was 49.05%, 59.47% and 93.56% for larvae of fruit fly. For the untreated insects, mortality was lowest (2.27%) as compared to treated insects (Table 1).

Growth and development of *B. zonata* and *B. dorsalis*

Growth and development of *B. zonata* and *B. dorsalis* was affected by the different treatments of *B. bassiana*. When last instar larvae exposed to *B. bassiana*, significant differences were recorded for pupal and adult durations. Percent pupation and adult emergence from surviving insects was found inversely correlated to toxic of microbial agents in last instar larvae. The highest concentrations of *B. bassiana* Bb-3 (1×10^7 spores/ml) effect on development as compared to lowest concentration. For the untreated insects, maximum pupation for *B. zoanata* and *B. dorsalis* (92.41% and 94.2%) and adult emergence (89.53% and 91.92%) were recorded (Figure 1,2).

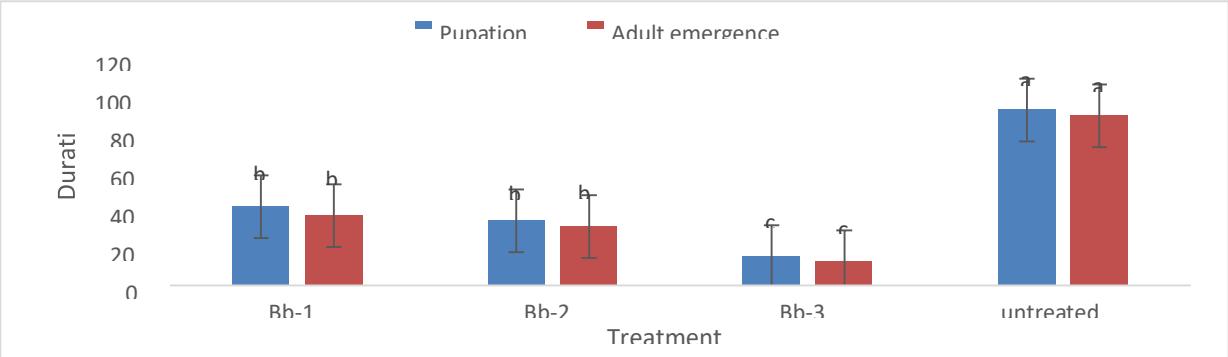

Figure 1: Pupation and Adult emergence (%±SE) of *B. zonata* when last intsar (3rd) larvae were exposed to *B. bassiana* at different concentrations [Bb-1 (1×10^3, Bb-2 (1×10^5), Bb-3 (1×10^7) spores/ml].

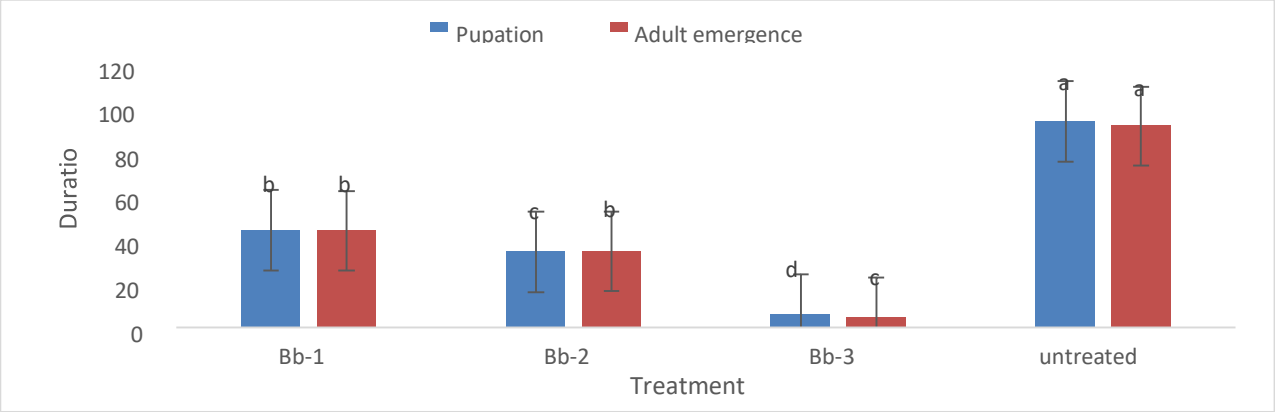

Figure 2: Pupation and Adult emergence (%±SE) of *B. dorsalis* when last intsar (3rd) larvae were exposed to *B. bassiana* at different concentrations [Bb-1 (1×10^3, Bb-2 (1×10^5), Bb-3 (1×10^7) spores/ml].

Statistical analysis

Data recorded for larval mortality, pupation and adult emergence under laboratory conditions were subjected to ANOVA in Minitab software (Minitab, 2007). Means were separated for significance using Tukey's HSD test at α=5 (Sokal and Rohlf, 1995).

Discussion

The present study showed that three concentrations [Bb-1 (1×10^3, Bb-2 (1×10^5),Bb-3 (1×10^7) spores/ml] of *B. bassiana* provided significant results. However, the highest concentrations provided maximum mortality as compared to other concentrations. The highest pupal and adult emergence was observed at lowest concentrations and for control group. Munoz (2000) observed that 98.7% mortality was recorded with fungus application. Similar observations were recorded by Campos (2000) who observed that mortality was found 82 to 100% by the application of *B. bassiana.* Gul *et al.* (2015) observed that *B. bassiana* and *M. aniopliae* provided effect result against the *B. zonata*. Both fruit fly species showed susceptibility against *B. bassiana*.

In the present study, highest concentrations provided minimum pupation and adult emergence. Mortality was increased with increasing the concentration of *B. bassiana*. In various countries, EPF were tested against many agricultural pests and their products available in market (Jackson, 1999). Due to their host specificity and safe for non target organisms, EPF considering promising biological control agents, helpful in IPM programs (Ekesi et al., 1999). Our study showed that *B. bassiana* isolates provided effective results and have pathogenicity against *B. zonata*. Our results agrred with Castillo *et al.* (2000) who recorded 100 % mortality against *C. capitata*. Similar findings obtained by many other researchers Daniel and Wyss, (2008) and Daniel (2009) who reported *B. zonata* susceptibility against EPF. Entomopathogenic fungus are helpful for the control of insect pests of fruit orchards and vegetables. Integration of EPF with other control methods could be reliable and give better control. This initiative is alternative to

insecticides which are harmful to our environment and non-target organisms. EPF integrate with IPM strategies could helpful for break down the resistant problem in insects and give effective results against many insect species especially voracious fruit flies.

Conclusion

Our results suggest that entomopathogenic fungi present locally, could be integrated for the control of *B. zonata* and *B. dorsalis*. *B. bassiana* is an alternate to chemical application for the control of fruit fly. This eco-friendly approach helpful in integrated pest management and reduce the risk of exposing natural enemies to chemicals.

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