# Evaluation of the efficacy of a local isolate of *Beauveria bassiana* against the peach fruit fly, *Bactrocera zonata* (Saunders) (Diptera,: Tephritidae)

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**Abstract**

Effect of entomopathogenic fungus *Beauveria bassiana* on mortality rate of the *Bactrocera zonata* (Saunders) (Diptera, Tephritidae) were concentration dependent, total mortality indicated that the *B. bassiana* at concentration of $10^9$ conidia /ml caused the maximum larval mortality (83.7%). The LC50 value was 1.4* $10^6$ conidia /ml. In the same way, the effectiveness of the fungus was recorded on pupae, as the concentration $10^9$ conidia /ml gave the highest mortality rate (71.3%). There was a significant difference between susceptibility of larvae and the pupae that depends on the cuticle's thickness and spiracles. The highest adult's mortality was observed at 10 days post-treatment (90.33%) at the highest conidial concentration. The lowest LC50 against adult *B. zonata* was 1.8 *$10^3$ conidia/ ml at 10 days post-treatment. Transmission of conidia from inoculated males to non-inoculated females was more pronounced compared to inoculated females to non-inoculated males, the first achieved 69.6 % mortality in females, the other achieved 60.1 % mortality rate in non- inoculated males.

This study indicates that *B. bassiana* has significant effects against *B. zonata* and that the conidia of the fungus could be successfully utilized as a biological insecticide.

**Keywords:** entomopathogenic fungi; horizontal transmission; *Bactrocera zonata*

## Introduction

The peach fruit fly, Bactrocera zonata (Saunders) (Order: Diptera, Family: Tephritidae) is a mong the most economically important pest species in the world (White and Elson-Harris, 1992), Its economic importance is derived from direct losses in production when female flies oviposit under the skins of fruits and vegetables. In addition to these direct losses, producer countries often lose potential markets due to quarantine restrictions imposed by importing countries to prevent entry and establishment of exotic pest species (Radonjić *et al.*, 2019) Current control methods of this pest rely heavily on the aerial application of insecticides; (Roessler 1989).These methods have a negative impact on the environment, and specifically on the populations of beneficial organisms as well as increased resistance in pest populations. There is a need to develop effective replacements for these toxic chemicals. Management of insect pests by biological control is an alternative approach that results in no risk to the environment. Among the different agents of biological control, entomopathogenic fungi (EPF) are one of them (Pell, et. Al 1993; Lacey, et al, 1994; Vega et al. 1995). The EPF, *Beauveria bassiana* is used widely because of its virulence against different insect pests. It naturally grows in soils and acts as a parasite causing the white muscardine disease in various arthropod species (Barbarin et al. 2012). This work aims to assess the susceptibility of larvae and pupae of the peach fruit fly, *B. zonata* to the

entomopathogenic fungus *Beauveria bassiana*, as a step for the application of such fungi as biological control agents against fruit flies.

## Materials and methods

### Insect colony

The laboratory insect colony of the peach fruit fly *Bactrocera zonata* was establishedfrom infected citrus fruit that were kept in plastic containers, with a layer of sterile sand under conditions of 25 ± 2 °C and 60-65% relative humidity until pupation. Adults were collected daily and transferred to rearing cages (40 x 40 x 40 cm) provided with a diet of sugar mixed with hydrolyzed protein (3:1w/w) and a wet cotton wick as water source. A transparent plastic cylindrical container (250 ml), perforated with 40 punctures 0.5-mm diameter on the wall, was used as an oviposition device, containing pieces of the citrus fruit to stimulate flies to lay eggs in the punctures. Deposited eggs were collected every 48 hours and washed with tap water, and then placed on a wet piece of thick paper towels to save moisture until they hatch. Tissue paper pieces are transferred to plastic trays (10 x 5 x 3 cm), half-filled with an artificial larval diet consisting of (500 ml distilled water, 330 g wheat bran, 82 g bread yeast, 82 granulated sugar, 3 g, benzoate Sodium, 3 g, citric acid), these trays were covered with muslin to prevent the entry of other types of insects. The diet is stirred with a spoon every 24 hours and sprinkled with distilled water as needed to maintain moisture. A layer of sterilized sand, 2 cm deep is added two days before pupation. Part of the adults is transferred to the breeding cages to start a new generation, and the other similar ones are used in the irradiation process.

### Fungal isolate

The entomopathogenic fungi (EPF) *Beauveria bassiana*, used in the following bioassays, was previously isolated from a soil sample obtained from an agricultural land in Iraq. EPF were inoculated on potato dextrose agar (PDA) in Petri plates (100 mm), sealed with parafilm, and placed inside an incubator at 25 ◦C with 14:10 h (light:dark) photoperiod for 7–10 days. After incubation, the dry conidia were harvested with a sterile scalpel and placed inside sterile tubes (50 mL) with 30 mL of 0.05% tween 80 and vortexed for 5 mints to reach homogenization. EPF concentrations were determined by pipetting 10 µL of the suspension on both sides of a hemocytometer and counting conidia under the microscope. Conidia viability was evaluated before tests.

### Pathogenicity against larvae and pupae of *B.zonata*

Pathogenicity of *B. bassiana* against larvae and pupae of *B. zonata* was estimated under laboratory conditions. Full grown larvae were infected by the fungi spore concentrations of ($1x10^5$, $1x10^7$ and $1x10^9$ conidia/ml) with 0.05 % Tween 80, larvae were sprayed carefully for 30 seconds, using a hand atomizer. The test was carried out using 20 larvae, placed in Petri dish (10 cm). The same number of full grown larvae was used as control, which were sprayed only by sterile distilled water with 0.05% Tween 80.. Each treatment was replicated 3 times.

A pathogenicity test of the fungal strains against pupae was carried out using Petri dish (10 cm) and autoclaved soil. Twenty pupae were placed about 1 cm below the soil surface. Conidial suspensions were applied by pre-mixing. Conidial suspension was prepared to the final volume of $1x10^5$, $1x10^7$ and $1x10^9$ conidia/ml. The dishs were kept under laboratory conditions

at 25.1±2°C and 70% R.H. Trials were carried out in three replicates.

Insect mortality was recorded after adult emergence, and was corrected against natural mortality that was obtained from check treatment using Abbott's formula (Abbott, 1925). All individuals failed to emerge successfully considered dead. Dead insects were kept in a Petri-dish having moist filter paper, under sterilized conditions, at 25°C to confirm the fungal infection.

**Pathogenicity against adult of *B.zonata***

Twenty newly emerged adults were placed in experimental cages (35x30x30 cm). Adults were sprayed by the fungal concentrations ($1x10^5$, $1x10^7$ and $1x10^9$ conidia/ml) for 30 seconds, using a hand atomizer. Adults' diet and water were supplied and kept under room rearing conditions. Same number of adults was used as control, which was sprayed only by sterile distilled water. Dead adults were counted at 5, 10, and 15 and days post treatment. The experiment was replicated 3 times.

**Horizontal Transmission Bioassay**

Horizontal transmission ability of *Beauveria bassiana* was assessed using adults of *B. zonata*. Four different inoculation combinations were evaluated for each isolate: (1) inoculated male + inoculated female; (2) inoculated male + non-inoculated female; (3) non-inoculated male + inoculated female; and (4) non-inoculated male + non-inoculated female (control). The experimental arenas consisted of Petri plates (9 diam. × 1.5 cm depth) applied with 1 mL solution of EPF ($1 \times 10^9$ viable conidia /ml). The plates were shaken on a rotary shaker until the suspension evaporated. Inoculated insects (either male or female) were exposed to the conidia suspension. The non-inoculated insects were exposed to a solution that contained 0.05% of tween 80. After inoculation, the different groups were released inside separate cages (30 cm × 30 cm × 30 cm) and were allowed to stay there for 24 h. After 24 h, adults were separated by gender, and placed in individual cages with adult food and water. The mortality was recorded on a daily basis for up to 14 days. Each treatment replicate consisted of 40 adults (20 male and 20 female). The experimental conditions were maintained at 25 ◦C, 50–60% RH, and a 14:10 h (light:dark) photoperiod. Dead adults were collected from cages daily to avoid cross-contamination [Sookar et. Al 2014]. The cadavers were surface-sterilized with a 1% solution of sodium hypochlorite followed by three rinses with distilled water. Then, the cadavers were placed inside plastic Petri plates lined with moist sterile filter paper and sealed with parafilm. These plates were incubated at 25 ◦C to assess fungus development or mycosis. Each inoculation method was considered as a single treatment with three replications.

**Statistical Analysis**

All statistical analyses were conducted using SPSS20. Mortality (each stage) for the treated group was corrected for control mortality by using the Abbott formula [Abbott, 1925] and then the data were subjected to analysis of variance (ANOVA). Whenever appropriate, treatment means were separated with Duncun test [51] with a significance level of 5%. Probit analysis was used to determine the LC50 and LT50 in dose response

**Results and discussion**

Effect of entomopathogenic fungus *B.bassiana* on mortality rate of the *B.zonata* larvae were concentration dependent (table1), total mortality

(corrected by using Abbot's formula) indicated that the *B. bassiana* at concentration of $10^9$ conidia /ml caused the maximum mortality (83.7%), the other treatments imposed the intermediate rates at $10^7$ conidia/ ml concentration by (54.7%) and lowest rate mortality was at concentration of $10^5$ conidia/ ml (36.7). The variations among corrected mortality percentages were significantly different at all treatments. The LC50 value was 1.4* $10^6$ conidia /ml. In the same way, the effectiveness of the fungus was recorded on pupae, as the concentration $10^9$ conidia /ml gave the highest mortality rate (71.3%) with a significant difference from the other two concentrations, which recorded a mortality rate of 42.7 and 30.33 for $10^7$ conidia/ ml and $10^5$ conidia/ ml concentrations, respectively. The results also indicated a significant difference in the effect of the fungus on the larvae and the pupae due to the difference in hardness and the presence of spiracles. The LC50 value was 4.3* $10^6$ conidia /ml.

The highest mortality pattern appeared in *B. zonata* treated with high concentration *B. bassiana* are in agreement with Hussein *et al*.,( 2018), who found that increasing *B. bassiana* conidia concentrations increased the mortality of larvae and pupae of the fruit fly, and Castillo *et al.,* (2000) and Quesada-Moraga *et al*., (2006) who reported 100 % mortality in flies of *C. capitata* and other tephritids by *B. bassiana* infection. It could be expected that when the number of adhered conidia increase, they could produce more amounts of cuticle degrading enzymes and overcome the defence action of the host stage followed by penetration and toxins production and finally death of the host (Soliman , 2020). The cuticle is the main channel of fungal penetration in insects, *B. bassiana* showed pathogenic activity against tested immature stages (full-grown larvae, and pupae) of *B. zonata* with different levels. The degree of host resistance depends on combine effects of the cuticle's thickness (David, 1969), the tensile strength imparted to the cuticle by the system of chitin lamellae and the degree of cuticle hardening by sclerotization (Hassan and Charnley, 1989). Insects have heavily sclerotized body segments are usually invaded via arthrodial membranes or spiracles (Charnley, 1989).

**Table 1: mortality of *Bactrocera zonata* larvae and pupae treated with different *Beauveria bassiana* concentrations**

| concentration | Mortality (%) | | P value |
|---|---|---|---|
| | larvae | pupae | |
| $10^9$ | 83.7 a | 71.3 a | 0.001 |
| $10^7$ | 54.7 b | 42.7 b | 0.001 |
| $10^5$ | 36.7 c | 30.33 c | 0.036 |
| LC50 | 1.4 * $10^6$ | 4.3* $10^6$ | |

Means followed by same letter in the same column are not significantly different

**Bioassay against Adults**

There were a significant differences among different concentrations of B. *bassiana* on *B. zonata* adult mortality at 5 days post-treatment (Table 2). The highest mortality was observed at 10 days post-treatment (90.33%) at the highest

conidial concentration that was achieved 78.3% mortality at 7 days post-treatment. Probit analysis revealed that mortality was dose and day dependent. Based on 95% fiducial limits, the minimum time to kill 50% of the tested population was found in concentration of $10^9$ conidia/ ml (4.2 days) , the lowest LC50 against adult *B. zonata* was 1.8 *$10^3$ conidia/ ml at 10 days post-treatment.

According to these results, the adult flies were the most susceptible stage compared to the larval or pupal stages. In accordance with our findings, Gul et al. [Castillo,et al. 200] found high susceptibility of *B. zonata* adults compared to larvae and pupae when exposed to different fungi (*B. bassiana*, *M. anisopliae*, and *Isaria fumosorosae*) . The present study showed that all the tested isolates of EPFs were virulent against last instar larvae and adults of *B. zonata* and *B. dorsalis*. These findings confirmed the susceptibility of *B. zonata* (Hussein, et al. 2018; Mahmoud, 2009).

**Table 2: mortality of adults of *Bactrocera zonata* treated with different *Beauveria bassiana* concentration**

| concentration | 5DAY | 7 DAY | 10 DAY | LT50 |
|---|---|---|---|---|
| $10^5$ | 38.6a | 62.6a | 70.9a | 5.99 |
| $10^7$ | 44.5b | 74.3b | 75.2a | 5.27 |
| $10^9$ | 59.7c | 78.3b | 90.33b | 4.2 |
| LC50 | $1.9*10^7$ | $1.5*10^4$ | $1.8*10^3$ | |

Means followed by same letter in the same column are not significantly different

**Horizontal transmission**

The highest mortality was recorded for males and females when both genders were inoculated, 89.2 and 78.3% respectively, the treatment did not differ significantly from the combination of non-inoculated females with inoculated males, 85.3 and 69.6 % for males and females respectively (Table 3). Transmission of conidia from inoculated males to non-inoculated females was more pronounced compared to inoculated females to non-inoculated males, the first achieved 69.6 % mortality in non-inoculated females, the other achieved 60.1 % mortality rate in non- inoculated males. The results also showed that there were significant differences in the mortality rates of inoculated individuals compared to non-inoculated individuals. The success of EPF as a control agent depends mainly on the possibility of transmitting the infection among conspecifics, as well as on the behavioral response of the insect species during the host-pathogen interaction (Dimbi et al., 2013). In the case of fruit flies, fungal transmission is enhanced by the mating behavior displayed by the adults. Fruit fly males form leks (i.e., groups of males calling for mating) [Shelly, 2018; Thaochan & Ngampongsai, 2018]) and compete with each other to copulate with the females present. This behavior generates several interactions that increase the chances of successful fungal transmission among conspecifics. The use of insects as vectors of biocontrol agents has been proposed as a strategy to improve biological control programs through the horizontal transmission of pathogens (Llacer et al., 2013; Diouf et al., 2022). The application of this strategy in fruit fly programs allows the release of inoculated sterile males to disseminate conidia into wild populations of *C. capitata*

(Toledo et al., 2017). Recently, Diouf et al. (2022) introduced the term “boosted SIT” to refer to the use of sterile insects as vectors of biocides to trigger an epizootic in wild populations.

**Table 3. Mortality levels (% mean) caused by horizontal transmission of entomopathogenic fungi *B.bassiana* in adults of *B. zonata*.**

| Isolate | male | female | male | female | P value |
|---|---|---|---|---|---|
| **Beauveria bassiana** | inoculated | inoculated | 89.2 a | 78.3 a | 0.189 |
| | inoculated | Non-inoculated | 85.3 a | 69.6 a | 0.023 |
| | Non-inoculated | inoculated | 60.1 b | 75.2 a | 0.021 |
| | Non-inoculated | Non-inoculated | 4.3 c | 2.4 b | 0.08 |

Means followed by same letter in the same column are not significantly different

## Conclusion

This study indicates that *B. bassiana* has significant effects against *B. zonata* and that the conidia of the fungus could be successfully utilized as a biological insecticide.

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