Pathogenicity of Entomopathogenic Fungi Beauveria bassiana Against larvae and pupae of Ceratitis capitata (Diptera: Tephritidae)

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Abstract
The use of pesticides against The Mediterranean fruit fly, Ceratitis capitata (Wiedemann) (Diptera: Tephritidae) has become an obstacle to the fresh agricultural products. The alternatives strategy based on the use of microbiological agents. The pathogenicity of the local isolate Beauveria bassiana (B53 andB100 isolates) was evaluated against immature stages and adult of C. capitata under laboratory conditions. Clear dose-dependent mortality was observed among the both different isolates of the EPFs tested against adults. There was a direct positive relationship between mortality and concentration. The highest larval mortality was 74.3 %, at the highest conidia concentration for isolate B53. The lowest LC50 against adults was observed in B53 (1.25*103 conidia mL-1. Significant differences were observed among different concentrations of isolate B53 and B100 for larvae of C.capitata at 12 days post-treatment. The highest mortality (69.7% and 63.7%) was observed at the highest conidial concentration of both isolates respectively. 50% lethal dose for larvae was 4.8*105 conidia mL-1 for isolate B53 and 4*106 conidia mL-1 for isolate B100. Conclusion: Our results indicate that B. bassiana isolate B53 was the most lethal against the larvae, pupae, and adults of C.capitata.
Keywords: entomopathogenic fungi, keratitis, pathogenicity, bioassay, Beauveria bassiana.

1. Introduction

The Mediterranean fruit fly, Ceratitis capitata Wiedemann (Diptera: Tephritidae), is the main pest in the Mediterranean region attacking more than 300 hosts. It presents the greatest threat to the production and marketing of many fruit crops, mainly fruit crops. The damage is caused by gravid females that oviposit under the skin of the fruit, followed by egg hatching and feeding of the larvae on their flesh. The fruit become rotten and inedible or drop to the ground prematurely causing large direct economic losses (Martinez-Ferrer et al., 2012). Adult control measures have largely relied on the use of broad-spectrum chemical insecticides in bait sprays and soil treatments with granulate insecticides beneath host trees to kill fruit fly larvae and puparia (Service et al., 1993). However, the continued use of chemical insecticides is associated with some deleterious effects, including environmental pollution, development of insecticide resistance, negative impacts on fruit fly parasitoids, and contamination of fruits Therefore, the development of alternatives strategies were required urgently to control this devastating fruit pest. Biological control by using entomopathogens could represent a suitable alternative strategy to chemical control of fruit flies (Butt et al., 2001). Among entomopathogenic fungi, species belonging to Beauveria and Metarhizium genera have received considerable attention as potential biological control agents of Medfly. Laboratory studies have shown the susceptibility of Medfly adults to infection by autochthonous isolates of Beauveria bassiana and Metarhizium anisopliae in Kenya (Ekesi et al., 2007),Entomopathogenic fungi, which infect their host through the cuticle, hold greater potential as biocontrol agents. They may be used against C. capitata adults by using fungus-contamination devices that attract insects to baited stations where they are contaminated with the pathogen and then escape to potentially transmit disease to no infected individuals, also may be used against prepupating larvae and puparia in the soil, which provides a favorable environment for fungal microbial control. The aims of the present study were to investigate the effectiveness of a B. bassiana strain against larvae and pupae of C. capitata under laboratory conditions,

2. Materials and methods

Insect Rearing

The adult colony arose from infected fallen citrus fruits collected from different orchards in Mada'in. Upon discharge, adults were transferred to screened plastic cages (30 cm x 30 cm x 30 cm). Adult flies were provided with water and the adult diet consisted of sugar and yeast (in a 3:1 ratio) (Sookar et al., 2014) A plastic bottle (500 ml) containing citrus juice is covered with a cap with small holes (1 mm in diameter) to collect Drosophila eggs, after which the collected eggs are transferred to an artificial diet(Quesada-Moraga et al., 2006). Larvae were

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reared on feed water (50.5%), sucrose (16.2%), bran (24.2%), torula yeast (8.0%), citric acid (0.6%), and benzoic acid (0.5%). Environmental conditions were maintained at 25 ± 1 °C and 60–70% relative humidity (RH) (Usman et al., 2021b)

3. Fungal isolate

The entomopathogenic fungi (EPF) *Beauveria bassiana*,(B53 and B100) 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.

4. Bioassays

Against Adults

The same EPF isolates tested against larvae were evaluated against the adult stage of fruit fly species. For the bioassay, 1 mL of each EPF suspension concentration of 1 × 105, 1 × 107 and 1 × 109 conidia mL-1 was applied to a glass Petri dish (9 cm diam. × 1.5 cm depth), and the control group received 1 mL of water + 0.05% tween80 in distilled water. The plates were shaken to cover the entire surface. Twenty adults of C. capitata previously cold immobilized were added to each dish, which was then covered with a lid. Three Petri plates were used for each treatment, with a total of 60 insects for each treatment. Flies were exposed to fungal conidia inside Petri dishes for 1 h (Beris et al., 2013)Then, all adults from each plate were transferred to a cage (30 cm × 30 cm × 30 cm) containing water and adult food (sugar and yeast at a 3:1 ratio). Each plate represented a single replication, adult mortality was recorded daily until 14 days post-treatment Environmental conditions were maintained at 25 ◦C with a 14:10 h (light:dark) photoperiod

5. Bioassays against Larvae

The bioassay arenas consisted of transparent plastic cups (30 mL) that contained 20 g of sterile sandy soil. The soil was autoclaved at 121 ◦C for 2 h. One mL of each fungal concentrations of 1 × $10^{5,}$ 1 × 10^{7} and 1 × 10^{9} conidia mL-1 was pipetted on the top of filter paper. After EPF application, a single third-instar larva of fruit fly species was released in each cup on top of the filter paper and the cup was again covered with a lid. Treatment effects were compared against a control that consisted of 2 mL water + 0.05% tween 80 applied. All experimental cups were placed on plastic trays and incubated at 25 ◦C with a 14:10 h (light:dark) photoperiod**(Usman et al., 2020).** Mortality was assessed on the basis of adult emergence by subtracting the total number of adults that emerged from the total number of larvae originally exposed. The bioassay was terminated four days after the first adult emergence was observed in the control group. Each treatment consisted of three replications of 20 cups each**(Usman et al., 2020)**

Bioassays against Pupae

The bioassay arena was similar to the first screening bioassay except that individual 4–5 days old pupa were buried in the soil at a depth of 3 cm. One mL (1 × 105, 1 × 107 and 1 × 109 conidia mL-1) of suspension was pipetted onto the soil surface and the soil was then mixed as described in Experiment 1. After mixing, pupae (4–5 days old) were buried individually in cups at 3-cm depth (Usman et al., 2020) and the cups were covered with lids. The control consisted of 2 mL of distilled water + 0.05% tween 80 applied to the soil surface. The rest of the procedure was the same as described above. Pupae that were unable to emerge as adult flies were considered to have died. Upon emergence, adults were transferred to cages (30 cm × 30 cm × 30 cm) and provided with water and adult food, and mortality was recorded over 10 days (Wilson et al., 2017) Adult mortality and mycosis were determined on a daily basis, and all dead individuals were removed from the cages each day. Each developmental stage (adult or pupa) was placed inside a plastic Petri dish lined with sterile and moist filter paper. The dish was wrapped with parafilm and finally incubated at 25 ◦C to observe the presence of fungal outgrowth (Quesada-Moraga et al., 2006)Before putting those into plastic Petri dishes, pupae and adults were surface sterilized with 1% sodium hypochlorite, followed by three rinses with distilled water (Wilson et al., 2017)Twenty individuals were used for each treatment replicate. There were three replicates for each treatment.

6. 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

7. Results

Bioassays against Adults

The same EPF isolates tested against larvae were evaluated against the adult stage of fruit fly species. For the bioassay, 1 mL of each EPF suspension concentration of 1 × 105, 1 × 107 and 1 × 109 conidia mL-1 was applied to a glass Petri dish (9 cm

diam. × 1.5 cm depth), and the control group received 1 mL of water + 0.05% tween80 in distilled water. The plates were shaken to cover the entire surface. Twenty adults of *C. capitata* previously cold immobilized were added to each dish, which was then covered with a lid. Three Petri plates were used for each treatment, with a total of 60 insects for each treatment. Flies were exposed to fungal conidia inside Petri dishes for 1 h **(Beris et al., 2013)**Then, all adults from each plate were transferred to a cage (30 cm × 30 cm × 30 cm) containing water and adult food (sugar and yeast at a 3:1 ratio). Each plate represented a single replication, Adult mortality was recorded daily until 14 days post-treatment (Usman et al., 2021a)Environmental conditions were maintained at 25 ◦C with a 14:10 h (light:dark) photoperiod.

Table 1: Percentage mortality (mean) of adults of Ceratitis capitata treated with different Beauveria bassiana concentration

Isolate	Concentration	3day	5 Day	7 Day	Lt50
B53	10^5	36.6a	56.6a	66.9a	3.73a
	10^7	40a	70b	70a	3.37ab
	10^9	56.7b	73.3b	74.33a	2.72b
	LC50		$1.25*10^3$		
B100		3DAY	5 DAY	7 DAY	LT50
	10^5	13.3a	33.3a	46.7a	7.45a
	10^7	33.3b	52.3b	53.3a	5.55ab
	10^9	36.7b	65.2b	68b	3.82b
	LC50	-	$6.3*10^6$		

Mean followed by same letter in the same column for each isolate are not significantly different

Bioassay against larvae

Significant differences were observed among different concentrations of isolate B53 for larvae of *C.capitata* at 12 days post-treatment. The highest mortality (69.7%) was observed at the highest conidial concentration, followed by concentration of 10^7 conidia mL^{-1} (62.2%) and then the concentration 10^5 conidia mL^{-1} (Fig.1). the same Significant differences were observed among different concentrations of isolate B100 (fig. 2) the percentage of mortality was 36.67 ,50 and 63.7% for the concentrations of 10^5 , 10^7 and 10^9 conidia mL^{-1} respectively. Probit analysis revealed that 50% lethal dose was **$4.8*10^5$** conidia mL^{-1} for isolate B53 and **$4*10^6$** conidia mL^{-1} for isolate B100 (Table 2).

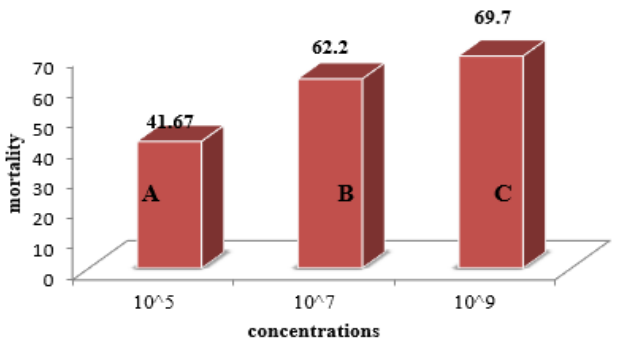

Fig1 Percentage mortality (mean) of larvae of Ceratitis capitata treated with different concentration Beauveria bassiana B53

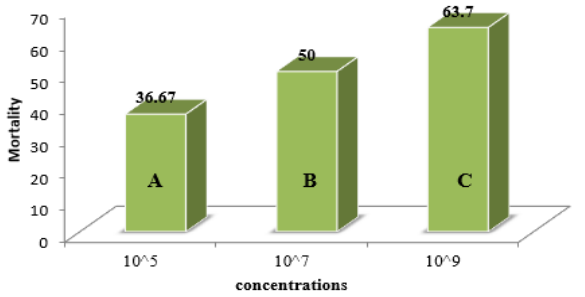

Fig2 Percentage mortality (mean) of larvae of Ceratitis capitata treated with different concentration Beauveria bassiana B100

Table 2 Probit analysis estimates of lethal concentrations required to kill 50% (LC50) of larvae of C.capitata

Isolate	LC50
B53	$4.8*10^5$
B100	$4*10^6$

Bioassay against Pupae

Pupae and emerging adults were susceptible to different concentrations. the maximum cumulative mortality for B53 isolate was caused by the higher concentration, 61.3% at the concentration 10^9 conidia mL^{-1} that was significant differences from other treatments ($p < 0.05$), followed by the concentration10^7 conidia mL^{-1} (45.2%) and 10^5 conidia mL^{-1} (37.18) (fig3) , The same significant differences was observed on B100 isolate , The highest mortality (50.3%) was observed at the highest conidial concentration, followed by concentration of 10^7 conidia mL^{-1} (40.2%) and then the concentration 10^5 conidia mL^{-1} (Fig.4). 50% lethal dose was $1.6*10^7$ conidia mL^{-1} for isolate B53 and $3*10^9$conidia mL^{-1} for isolate B100 (Table 2).

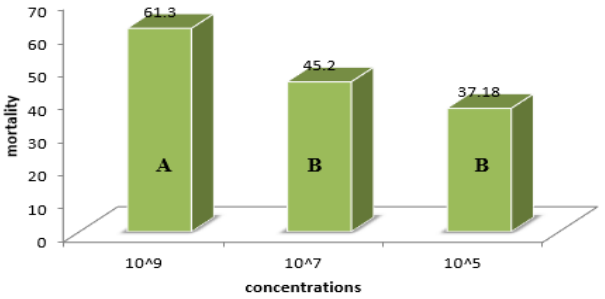

Fig3 Percentage mortality (mean) of pupae of Ceratitis capitata treated with Beauveria bassiana B53 Isolate

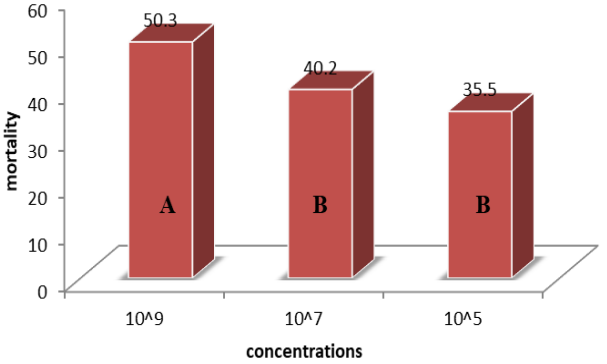

Fig4 Percent mortality (mean) of pupae of Ceratitis capitata treated with Beauveria bassiana B100 Isolate

Table 2 Probit analysis estimates of lethal concentrations required to kill 50% (LC50) of pupae of *C.capitata*	
Isolate	LC50
B53	$1.6*10^7$
B100	$3* 10^9$

8. Discussion

Laboratory work is a primary indicator of the effectiveness of control agents, including biological agents, selection of EPF isolates is one of the most important steps in a microbial control program, as the process determines which isolates are most virulent for the pest as well as their behaviour with respect to relates to mortality, sporulation and the production of harmful organisms on an artificial culture medium.The obtained results confirmed the results of(Qazzaz et al., 2015)who indicated that *B. bassiana* isolates induced significant mortality (58%-100 to adult *C. capitata* flies, depending on the isolate and inoculum concentration used. Similar results were obtained by**(Qazzaz et al., 2015**) who found that *B. bassiana* induced 85.6% mortality in a *C. capitata* population evaluated the pathogenic potential of 16 strains of *B. bassiana* against adult *C. capitata* flies and reported a mortality range of 20% - 98.7%. (Castillo et al., 2000) exhibited 100% mortality in *C. capitata* adults.

The efficacy of EPF such as *Beauveria bassiana* and *Metarhizium* anisopliae on pupae and adults of *C. capitata* has been reported by several authors (Ekesi et al., 2005, Qazzaz et al., 2015).

The present study showed that all the tested isolates of EPFs were virulent against last instar larvae and adults of C.capitata.. Differences in virulence of EPFs have been previously documented by Imoulan and Elmeziane (Imoulan and Elmeziane, 2014)who evaluated 15 isolates of B. bassiana against C. capitata and reported mortality values ranging from 65 to 95%. Variations of virulence of the tested isolates may be attributed to genetic diversity among different isolates that originated from different geographic regions (Lu and Leger, 2016), differential immune response (Chen et al., 2010)Our results indicate that B. bassiana isolate B53 was the most lethal against the larvae, pupae, and adults of C.capitata,. Therefore, these two isolates ought to be evaluated under expanded field trials. Applications should be targeted underneath tree canopies to reduce densities of the soil-dwelling stages of fruit flies. This research represents a first step toward the sustainable management of B. zonata and B. dorsalis; the model can be applied to other fruit fly pests and other pest systems.

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