International Journal of Entomology Research
ISSN: 2455-4758
Impact Factor: RJIF 5.24
www.entomologyjournals.com
Volume 2; Issue 6; November 2017; Page No. 23-26

Pathogenicity of *Beauveria bassiana* against cucurbit fruit fly *Dacus ciliates* (loew) Diptera: Tephritidea

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Abstract
The current study was conducted to evaluate the efficiency of three local isolates of *Beauveria bassiana* on two stages (larvae and adults) of cucurbit fruit fly *Dacus cilliatus* under laboratory conditions. The results showed that both stages were sensitive to the concentrations of 10^5, 10^7 and 10^9 spores / ml. Depending on the LC_{50} values the highest efficiency on females was achieved by the isolate Bb100, it was 1×10^2 spores\ml. followed by Bb 17 isolate with the median lethal concentration of 1.1×10^5 spores\ml. The most severe isolates was Bb 100 depending on LT_{50} that was 4.15 days followed by Bb47 (4.5 days). According to LC_{50} values, Bb100 isolate was the most severe on males, the LC_{50} value was 8.3×10^3 spores\ml. and then Bb 17 (2.7×10^5 spores\ml.) and BE47 (5×10^6 spores\ml.). The results of median lethal time, which expresses on virulence, showed superior of Bb100 with LT50 2.5 days, then the isolate of BE47 (5.5 days) and Bb17 (9 days) at concentration of 10^9 spores / ml. Females were greater sensitivity than males when exposed to the suspension of all isolates. The effect of *B. bassiana* isolates on the cucurbits fruit fly larvae was measured depending on the adult's emergence rates. The reduction rate of adults emergence was increased with increasing of concentration, the highest was at concentration of 10^9 spores\ml. (75%) for the isolate Bb 100 that significantly differed from other isolates, in both of them the reduction rate reached to 66%.

Keywords: *beauveria bassiana, dacus cilliatus*

Introduction
Cucurbits crops (family: Cucurbitaceae) are a major horticultural crops cultivated for fruits, that characterized as high nutritional value and economic importance. They are exposed to a wide variety of insect pests, one of the most serious is the cucurbit fruit fly, *Dacus ciliatus* Loew (Diptera: Tephritidae) (White, and Elson-Harris, (1994 [17]; Hancock, 2012) [9]. Its economic importance refer to the direct damage caused by females that oviposit into the host fruit and larvae feeding on the fruit flesh. In addition to direct losses, severe quarantine policies are imposed by importing countries to avoid importation and establishment of exotic pests. Because of the environmental impact of chemical pesticides and by the nature feed of their larvae inside the fruit, the chemical pesticides was ineffective strategy to control this pest, so it was recommended to find out safer alternative control strategies such as use of microbial control agents. Fungal agents belong to the most promising group of biological control agents against insect pests. *Beauveria bassiana* and *Metarhizium anisopliae* are two of the most important entomopathogenic fungi currently used against a wide range of arthropod, mainly insect pests, and the most common species developed as mycopesticides (Butt *et al.* 2001; Zimmerman 2007a, b) [2, 19, 20]. Different strains of *B. bassiana or M. anisopliae* cause different rate of mortality in their pest, due to genetic variability among their isolates (De La Rosa *et al.*, 2002; Garcia *et al.*, 1984). The objective of this study was to evaluate the pathogenicity of some isolates of *B. bassiana* and the mortality response of *D.ciliatus* under laboratory conditions.

Materials and methods
Insect rearing
The infected fruits with eggs of cucurbits fly *D. ciliatus* were placed in cages of Plexiglas (40×40×40 cm) and were reared in the laboratory under optimal conditions (temperature 25–29 C; relative humidity 75–85%; photoperiod 16:8 h L: D) the larvae feed after eggs hatching on the fruit pulp, then leave the fruit after complete their growth and go down to the ground corn for pupate. Pupae were collected in Glass jar (14 cm high and 9 cm diameter), covered with cloth, and placed in incubators under similar conditions of rearing room, except it is without lighting. all emerging adult were transferred to mating cages (20× 20×20 cm) equipped with yeast solution as a source of protein for females and to improve the activity mating of males, and sugar solution 5% besides dry sugar as well as pieces of cotton moistened with water in a small Petri dish (Keiser & Schneider, 1969, Keiser, *et al.* 1972 and Drew, 1987). Mating cages were provided with fruits of ground as a natural medium to lay eggs, placed under the conditions of rearing room, these fruits have been replaced frequently.

Fungal spore suspension preparation
Three isolates of *Beauveria bassiana* (Bb100, Bb47 and Bb17) were grown on 9 cm Petri dishes containing Potato dextrose Agar PDA (39 g l^{-1}) and incubation at 25°c in darkness until

colonies fully occupied the dishes then stored at 4ºc for further use. The fungal suspension was prepared by adding 5 ml sterile distal water SDW mixed with 500 µl of tween 80 and gently scraped with sterile scalpel. The suspensions were stirred vigorously for 5 min to break up the spores from the conidiophores and the hyphal debris were removed by passing the suspension through fabric cloths. The concentration was determined by the aid of haemocytometer. The viability of spore was determined as in Lacey (1997).

Effects of different spore suspension concentrations on the adults.

Three concentration of each fungal isolates 1 x 10^5, 1 x 10^7 and 1x 10^9 spores\ml. were used to measure the isolates effectiveness on adults, each concentration was sprayed (2ml) on adults at age of 3 days (5 males and 5 female) in plastic container 3 cm x 12 cm (diameter x high) with opened ends that were closed with fabric clothes. Control was sprayed with SDW mixed with 0.05% tween 80. The percentage of mortality was measured daily. All dead insects were transfer into 9 cm Petri dishes containing wet filter paper at 22ºc allowing fungi to grow. The experiment was designed to randomized complete design with four replicates.

Effects of fungal isolates on larval instar

Last instar larvae were dipped in the suspension of each isolates at concentrations of 1 x 10^5, 1 x 10^7 and 1x 10^9 spores\ml. for five second, where the control was by dipping the larvae in the distilled water and tween 80. After that all larvae were placed in tubes 46x 42 cm (diameter x high) containing 40g sterile sand to pupate. Adult's emergence was measured as indicator on efficacy of fungal isolate against larvae. All dead larvae and pupa were transfer into 9 cm Petri dishes containing wet filter paper at 22ºc allowing fungi to grow. The experiment was designed to randomized complete design with four replicates (10 larvae / replicate).

Statistical analysis

Data were statistically analyzed by ANOVA (SPSS software 20 edition), means were separated using Duncan's multiple range test (DMRT) at the 0.05 level of significance. LT50 and LC50 were calculated by probit analysis (Finney 1971) [8].

Results and discussion

Evaluation of local fungal isolates in the control of adult grapefruit fly

The effectiveness of different concentrations of local fungal isolates Bb 100, Bb 47 and Bb17 of *B. bassiana* on adults of *D.ciliatus* showed increasing of mortality rates with increasing of concentration (Table 1), after 5 days of females treatment, significant differences was recorded to the concentrations of 10^7 and 10^9 spores\ml. from concentration of 10^5 spores / ml. Their mortality rate were 60 and 63 and 53% respectively. The same statistical differences were continues after 10 days of treatment with mortality rates of 70, 70% and 63% respectively. 100% mortality were achieved by all concentrations after 15 days of treatment. The isolate Bb47 of *B. Bassiana* gave varied mortality rates according to concentration, the highest after 5 days of treatment was 60% at concentration of 10^9 spores / ml compared with the other concentrations (10^5 and 10^7 spores \ml.) in them the mortality rates were 0.0% and 34% and became 46 and 54%, respectively after 10 days. Bb17 isolates achieved mortality after 10-days their percentages were 50, 56 and 63% for concentrations of $10^{5,}$ 10^7 and 10^9 spores \ml. respectively. Comparing the efficacy of fungal isolates depending on the LC50 values demonstrated high effect for the isolate Bb100, it was 1 × 10^2 spores\ml. followed by Bb 17 with the median lethal concentration of 1.1 × 10^5 spores\ml. The most severe isolates was Bb 100 depending on LT50 that was 4.15 days followed by Bb47 (4.5 days). The results of the male treatment (Table 2) showed a significant correlation between the mortality rate and the concentrations, after 15 days of treatment the isolate Bb 100 achieved 70, 86 and 100% mortality for concentrations 10^5, 10^7 and 10^9 spores/ ml of fungal suspension. Mortality rate due to Bb47 isolate at mentioned concentration on day 15 were 37, 53 and 67%, respectively, while mortality rates were 50, 60 and 63% for Bb17.

The comparison among fungal isolates according to LC50 values, Bb100 isolate was the most severe, its LC50 value was 8.3 x 10^3 spores\ml. and then Bb 17 (2.7 x 10^5 spores\ml.) and of BE47 (5 × 10^6 spores\ml.). The results of median lethal time, which expresses on virulence, showed superior of Bb100 with LT50 2.5 days, then the isolate of BE47 (5.5 days) and Bb17 (9 days) at concentration of 10^9 spores / ml.

The results of comparing the sensitivity of males and females (Fig. 1) showed greater sensitivity to females than males when exposed to the suspension of all isolates. The difference between isolates of the same species is due to genetic variability among isolates. These differences have been reported in many studies on the isolates of *Beauveria bassiana* and *Metarhizium anisopliae* (De La Rosa *et al.*, 2002, Garcia *et al.*, 1984). Similar results were reported by Ekesi *et al.* (2001) [7] that find out the highest rates of mortality at highest concentrations (10^8Spore ml^{-1}) and the mortality rate decreased when concentration decreased. Dose–mortality responses have also been reported on many other pests, *Bactrocera cucurbitae* infected with *Paecilomyces lilacinus*, the highest mortality occurring at (2.4x10^9) after 5 and 7 days of treatment (Amala. 2013) [1]. Laboratory study for six concentrations of fungal isolates of *Beauveria bassiana*, *Metarhizium anisopliae*. *Verticillium lecanii*, *Hirsutella thompsonii* and *Cladosporium oxysporum* on adult aphids *Aphis craccivora* showed the highest concentration (10^8 Spore ml^{-1}) resulting in highest mortality rate (Saranya *et al.* 2010). These laboratory assays were important in identifying and selecting the isolates that were worth to test under field conditions. Pathogenicity is the most important indicator when determining the effectiveness of pathogenic fungi against pests. The differences in the effectiveness of fugal isolates towards the pest has been mention in several studies. Quesada, *et al.* (2006) evaluated effectiveness of *M. anisopliae* against pupae of Mediterranean fruit fly, he found that mortality levels between 30-100% during 6.5- 8.6 day period time. Sirinun (2007) [16] evaluated 12 strains of *B. bassiana*, 7 of *M. anisopliae* and one of *Hirsutella citriformis* against *Bactrocera dorsali* was treated with 1 x 10^8 spore $ml^{-1}$and found one strain of *B. bassiana*was virulent achieving mortality up to 68%. Similarly, Munos (2000), testing 16

isolates of *B. bassiana* for the control of Mediterranean fruit fly reported a mortality range between 20-98.7%. Regarding to the difference in the susceptibility of males and females to entompathogenic fungi, many studies have reported the same results. Dimbi *et al.* (2003) [5] found that females were more susceptible than males when treated with *M. anisopliae*. *Whereas, Carsewell et al.* (1998), found the mortality between females and males was not considerably different.

Table 1: Mortality rates, LC 50 value and LT 50 of the adult's cucurbits fruit fly *Dacus ciliatus* (female) treated with different isolates of *Beauveria bassiana*

Fungal isolates	Conc.	% mortality after 5 days of treatment	% mortality after 10 Days of treatment	% mortality after 15 days of treatment	LC50	x^2	P Values	LT50	x^2	P Values
Bb100	10^5	53a	63a	100	10^2	7.3	0.4	5.35	84	0.001
	10^7	60b	70b	100				4.52	73	0.000
	10^9	63b	76b	100				4.15	46	0.002
Bb47	10^5	0a	46 a	66 a	7×10^5	8.1	0.32	11.7	33	0.000
	10^7	34 b	54 a	80 b				7.8	12.7	0.079
	10^9	c 60	73 b	100 c				4.5	52	0.000
Bb 17	10^5	27	50	66	1.1×10^5	5	0.7	9.77	9.6	0.21
	10^7	30	56	73				8.42	4.9	0.67
	10^9	30	63	83				7.5	3.5	0.83

The rates followed by the same letter in the same column did not differ significantly according to the Duncan test (0.05)

Table 2: Mortality rates, LC 50 value and LT 50 of the adult's cucurbits fruit fly *Dacus ciliatus* (males) treated with different isolates of *Beauveria bassiana*

Fungal isolates	Conc.	% mortality after 5 days of treatment	% mortality after 10 Days of treatment	% mortality after 15 days of treatment	LC50	x^2	P Values	LT50	x^2	P Values
Bb100	10^5	47a	53a	70a	8.3×10^3	3.4	0.84	5.95	2.1	0.96
	10^7	50a	57ab	86b				5.45	3.7	0.82
	10^9	53a	66b	100c				5.2	8.0	0.33
Bb47	10^5	0a	33a	37a	5×10^6	1.6	0.98	17.4	3.91	0.79
	10^7	27b	40a	53b				13.7	0.66	0.99
	10^9	50c	56b	67c				5.5	1.4	0.97
Bb 17	10^5	26a	37a	50a	2.7×10^5	2.4	0.93	16	0.87	0.99
	10^7	30a	47ab	60b				9.9	1.1	0.99
	10^9	30a	50b	63b				9	0.9	0.99

The rates followed by the same letter in the same column did not differ significantly according to the Duncan test (0.05)

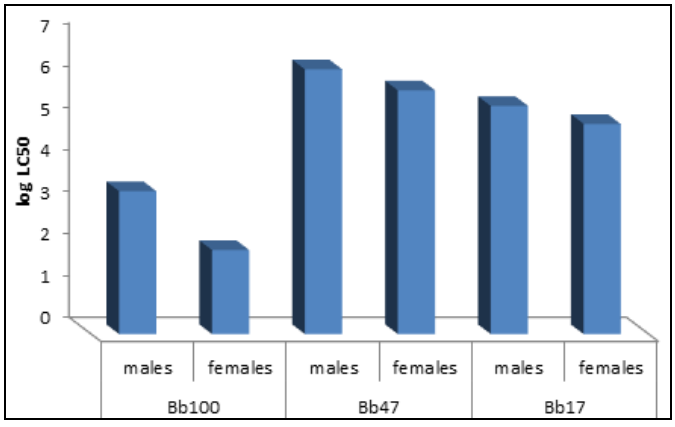

Fig 1: Susceptibly of adults (males and females) of cucurbit fruit fly to isolates of *B.bassiana*

Effect of fungal isolates on larvae

The effect of *B.bassiana* isolates on the cucurbits fruit fly larvae was measured depending on the adult's emergence. The reduction rate of adults emergence was increased with increasing of concentration (fig 2). The highest reduction was at concentration of 10^9 spores\ml. (75%) for the isolate Bb 100 that significantly differed from other isolates, in both of them the reduction rate reached to 66%. In a similar study, the third larvae of fruit fly *C.capitata* were used to investigate the efficacy of 15 isolates of *B.bassiana* species, the results showed that all isolates were able to infect larvae, resulting in a high mortality rate in pupae, ranging between 65-95%, causing significant reduction in adult emergence. The isolates TAM6.2 and ERS 4.16 had an infection ratio of pupae 95% and 90%. Therefore, the highest was the virulence. The LC50 concentration of the isolates was 2.85x 10^3 and 3.16 x 10^3 spores / ml respectively (Imoulan & Elmezian, 2014) [10]. Laboratory and field study studies were conducted by Ekesi *et al.* (2002, 2005) [5] to evaluate the effectiveness of fungal pathogens in the last larval stage of fruit flies. Their results showed the larval stages were generally high susceptible to fungal infection. However, other studies postulated *M. anisopliae*failed to cause infection to *Rhagoletis indifferens larvae* (Yee & Lacey, 2005) [18].

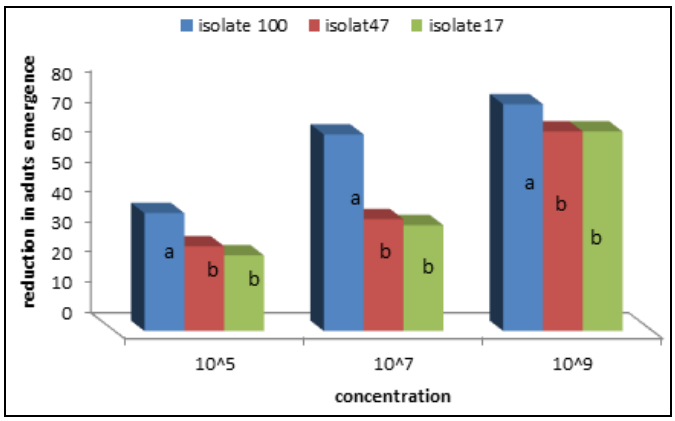

Fig 2: Effect of *Beauveria bassiana* isolates on larvae of cucurbit fruit fly *Dacus ciliatus*

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