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Role of the entomopathogenic fungus *Beauveria bassiana* as a biological control agent for the Mediterranean fruit fly *Ceratitis capitata* (Tephritidae : Diptera) under laboratory conditions

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Abstract:

Fungal pathogens are recognized as biological control agents that are increasingly replacing the chemical compounds for controlling fruit flies. The present study aimed to determine the effects of the entomopathogenic fungus *Beauveria bassiana* (Balsamo) against *Ceratitis capitata* (Wiedemann) (Tephritidae **:** Diptera). In addition, the effects of *B. bassiana* on *in vivo* activity of polyphenol oxidase (PPO) and phenol oxidase (PO) enzymes of *C. capitata* pupae were determined. The toxicity results of *B. bassiana* fungus exhibited mortality of 2.5-12.5% in the pupal stage. Mortality in adult flies is steadily increased, reaching 96.79 and 97.22% on the 35th and 32nd days with 0.88 and 1.75 g/L of fungus treatment. The biochemical analysis of PO and PPO enzymes activity in *C. capitata* pupae showed an increase in enzyme activity with the increase of the fungal concentration and post-treatment duration. This information can aid in pest management in integrated and organic orchards.

Introduction

Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) is a key pest of fruits in the Mediterranean basin that has a worldwide distribution. Globally, this fly attacks more than 250 species of fruits and vegetables (Morales *et al.*, 2004). Its economic importance is due to the direct damage caused by ovipositing of the females to the host fruit and the larvae feeding on the fruit. Control of *C. capitata* can be conducted on the adult fly (Aerial treatment with chemical insecticides, sterile insecticides, chemosterilants, mass trapping, oviposition deterrence) or soil larvae and pupae (Terrestrial treatment with chemical insecticides) (Navarro-Llopis *et al*., 2004; Rendon *et al*., 2006 and Ekesi *et al*., 2007). The heavy application of conventional insecticides against *C. capitata*, may cause serious ecological problems (Magana *et al*., 2008), as the resistance development of the field strain of *C. capitata* (El-Gendy, 2017).

So, several efforts have been made to restrict the use of harmful insecticides by using safer alternative

approaches to control *C. capitata*. Entomopathogenic fungi play a significant role in the natural biological products for many insects (McCoy ***et at***., 1988). The importance of fungi in the control of insects was noticed by ancient Chinees (Roberts and Humber, 1981). Thus, environmentally compatible strategies for managing *C. capitata* populations have been developed, including using entomopathogenic fungi as one of the biological control agents (Castillo *et al*.**,** 2000) as *Beauveria bassiana* (Balsamo) (Ascomycota: Cordycipitaceae) (Beris *et al*., 2013).

B. bassiana is widely spread and considered a natural enemy of a wide variety of insects and arachnids (Roberts and St Leger, 2004)**.** *B. bassiana* is the only insect pathogens infecting their hosts by direct penetration of the cuticle, showing promising perspectives for the *C. capitata* control against pupae in the soil (Soil inoculation) (Garrido-Jurado *et al*., 2011)**.** *B. bassiana* was a safe bio-control organism in an application that has been assessed based on its impacts on non-target insects and mammals, including humans (Zimmermann, 2007).

On the other hand, entomopathogenic fungi were observed to produce several proteases that degrade insect cuticles (Samuels and Paterson, 1995). A broad variety of trypsin, chymotrypsin, elastases, collagenase, and chymoelastase was described (Smith and Grula, 1982) as a liability for insect cuticular deterioration (St Leger, 2008).

The current work aimed to evaluate the efficacy of *B. bassiana* as a soil application against *C. capitata* larvae, as well as the fungus effect on the activity of peroxidase and phenol oxidase enzymes.

Materials and methods

1. Insect rearing:

Adult fly of *C. capitata* was raised in wooden cages (30 x 30 x 30 cm), provided with a food medium (Dry yeast: sugar 1:3), and saturated sponge of water as a source of water in Petri dishes. Eggs were allowed to fall into a water reservoir located beside the base of the cage. The eggs were collected daily from the water reservoir and added to the larval artificial diet which consisted of wheat bran (96.8 g), sugar (64.8 g), dry yeast (9.07 g), citric acid monohydrate (2.4 g) sodium benzoate (2 g) and tap water (200 ml) according to Tanaka ***et al.*** (1969).

2. Virulence assay of the entomopathogenic fungus *Beauveria bassiana* against *Ceratitis capitata* :

The microbial insecticide, *B. bassiana* fungus (WP 2.5%, 2.3 × 108 conidia/gm) was carried out against the 3rd larval instar of *C. capitata*. *B. bassiana* was get from Bioinsecticides Production Unit, Plant Protection Research Institute, Agricultural Research Center, Egypt. The experiments were carried out in plastic cups (250 cm3) closed at the top with a cloth net (1 mm mesh). The cups contained 75 gm of sterilized sand, which had been autoclaved at 105 °C for 24 hours. *B. bassiana* was assayed in six fungal concentrations, 0.75, 1.25, 2.5, 5, 10, and 20 gm/l, and the control treatment was treated with water only. Ten ml of fungal suspension per replicate were added to the soil by the pipette. One hundred of the full-grown larvae of *C. capitata* were used per each concentration (Treatments) in five replicates, 20 larvae each. Popped larvae were transferred to the cups and freely allowed to pupate in the fungus-treated soil. At 2, 4, and 6-days post-treatment, the treatments were inspected, and the dead pupae were recorded. On the 7th day, the pupae were transferred to Petri dishes until emerged flies. Emerged flies were transferred to plastic cups (250 cm3)

covered with a cloth net, provided with a source of food and water as described above. The dead flies were removed daily to Petri dishes containing a wet filter paper. Petri dishes were sealed with Parafilm® and kept in a room at 26˚C to promote the growth of the fungus on the dead bodies to confirm that fungus infection was the cause of death (Ekesi *et al.,* 2002).

3. Determination of lethal concentration (LC50):

The dose-mortality relationship is determined for the concentration that showed the virulence against fruit fly of >0 and <100 % mortality. The concentrations evaluated were between 0.75 -20 gm/L of 2.3×108 conidia/ml. This procedure was repeated with five batches of flies, and the lethal concentrations of each replicate were used for statistical comparisons. LC50 was calculated according to Finney (1971) using the Ldp-Line program (Bakr, 2007).

4. Enzyme preparation:

For biochemical determination of detoxification enzymes, the 3rd larval instar of *C. capitata* was exposed to *B. bassiana* concentrations (As mentioned above) of 1.25, 2.5, 5, and 10 gm/l, in 3 replicates each. About 20 larvae were used for each replicate. After 2-, 4- and 6-days of exposure, pupae were homogenized in 1000 µl chilled phosphate buffer (0.1 M, pH 6.5), containing 0.01% (w/v) of triton X-100. The crude homogenate was centrifuged at 14,000 rpm for 5 minutes at 4°C. The supernatant was collected and used further as an enzyme source for the biochemical estimation of peroxidase (PO) and polyphenol oxidase (PPO) enzymes. Untreated flies were used as control.

5. Assay of phenol oxidase (PO) activity :

The activity of PO was assessed by mixing 500 µl of a sample with 2 ml of Tris-HCl solution (pH 6.5) and then incubated at 30 °C for 3 min. PO activity was measured with a spectrophotometer (PG instrument T80 UV/VIS spectrometer) at 420 nm (Ishaaya, 1971).

6. Assay of polyphenol oxidase (PPO) activity:

PPO activity was assessed by mixing 200 µl of the sample with 1.5 ml of Tris-HCl solution (pH 6.5) and then incubated at 30 °C for 2 min. PPO activity was measured with a spectrophotometer (PG instrument T80 UV/VIS spectrophotometer) at 495 nm (Ishaaya, 1971).

7. Statistical analysis:

The corrected mortality percentages were corrected through Abbott's formula **(Abbott, 1925),** after which LC50 is estimated by Probit analysis. All experiments were completely randomized designs; mortality data were subjected to one-way analysis of variance (ANOVA), and enzyme data were subjected to two-way analysis of variance using CoStat Software (2008) Version 6.4. Means were compared by a Tukey-Kramer test at a 5% probability level where significant differences existed between them.

Results and discussion

1. Virulence assay of the entomopathogenic fungus *Beauveria bassiana* against *Ceratitis capitata* :

Six fungal concentrations of *B. bassiana*, 0.75, 1.25, 2.5, 5, 10, and 20 gm/L, were assayed on the full-grown larvae of *C. capitata* by soil treatment application. Results in Table (1) show no infectious symptom in the pupal stage of *C. capitata* until the 5-days of treatment. On the 6th day of pupal age, *B. bassiana* exhibited little mortality effects ranging from 2.5 to 12.5%, with the tested fungal concentrations (F=2.86, *p*=0.04).

Adults' mortality was observed from the outset. With increasing the

flies' age and fungal concentrations, the flies' mortality rates were steadily increased, which statistically were significant among the different fungal concentrations within each time of post-treatment. On the 8th day, flies' mortality reached < 50% with treatment by 20gm/L of *B. bassiana* ($F=18.92$, $p=0.0001$). The fly mortality increased with elapsed time to reach < 90% on the 23rd day with fungal concentrations of 10 and 20 gm/L ($F=6.09$, $p=0.0018$), to achieve elimination for the fly at the 28th day of fly age with respective fungal concentrations of 5, 10 and 20 gm/L ($F=14.67$, $p=0.0001$). While the concentrations of 2.5 and 1.25 gm/L eliminated the fly at the 32nd and 35th day of the adult age.

The results in Table (2) indicated that LC50 values of the fungus were not registered at the 2nd and 4th-day post-treatment of *C. capitata* pupae, it was recorded on the 6th day with 1115.78 mg/L. LC50 value in the pupal stage was higher than that in the adult flies (589.82-0.007 mg/L). On the 2nd day of adult age, LC50 was 589.82 mg/L, which decreased by increasing time of treatment; On the 5th day of the adult fly age, LC50 decreased by about 7.11 times than those on the 2nd day. A Similar trend was achieved with LC50 reduction of 52.213, 399.592, 2027.822, 40701.286, and 71227.25 times at 8, 13, 18, 23, and 28 days of treatment of that on the 2nd day of the adult stage, respectively.

2. Biochemical assay of the entomopathogenic fungus *Beauveria bassiana* against *Ceratitis capitata* :

2.1. Specific activity of phenol oxidase enzyme:

Results presented in Table (3) indicate that biochemical assay for a specific activity (SA) of PO enzyme in *C. capitata* pupae increased with increasing the tested fungal concentrations and time post-treatment. The highest SA of PO value (General mean ±SE) in pupae was 1.74 µm/mg protein/min when the *C. capitata* larvae are processed at 10 gm/L of fungus. PO activity declined by decreasing the fungal concentration to 1.44, 1.20 and 0.92 µm/gm protein/min at 5, 2.5 and 1.25 gm/L fungal treatments, respectively, compared to 0.34 µm/mg protein/min for control treatment ($F=10884.86$, $df=4$, $p=0.0000$). For PO activity at different days post-treatment, PO was the lowest in the SA (1.094 µm/gm protein/min) at the 6th day of the pupal age, which was significantly differed from those at the 4th (the highest activity; 1.148 µm/gm protein/min) and the 2nd day (1.144 µm/mg protein/min) ($F= 58.76$, $df=2$, $p= 0.0000$). The SA of the PO enzyme ratio was increased by treating *C. capitata* larvae with increasing concentrations of *B. bassiana* fungus by 2.71, 3.54, 4.25, and 5.13-fold at the fungal concentrations of 1.25, 2.5, 5, and 10 g/L of that of the control treatment, respectively. Significant differences were observed between SA values of PO recorded at both treatments and at times post-treatment, followed by high interaction between fungi treatments and elapsed times of treatment ($F= 9.54$, $df=8$, $p= 0.0000$).

2.2. Specific activity of polyphenol oxidase enzyme:

The SA of the PPO enzyme of *C. capitata* pupae was found to be directly related to the fungal concentrations (Table 4). Results show an increase in the SA of the enzyme (general mean ±SE) at the tested fungal concentrations (1.25, 2.5, 5, and 10 g/L) along inspected dates (2, 4, and 6 days). The lowest specific activity was 0.44 µm/gm protein/min at 1.25gm/l of fungus which increased significantly with the increase of the fungal concentrations of 2.5, 5 and 10 gm/l that significantly recorded 0.54, 0.73 and

0.93 µm/gm protein/min, respectively, compared to 0.353 µm/gm for the control one (F= 14277.69, df=4, p= 0.0000). The SA of PPO increased by 1.25, 1.54, 2.07, and 2.65-folds of the untreated one at the tested concentrations, respectively. The general mean of SA of the enzyme for all fungal concentrations at the tested times showed that at the 2nd day (0.58 µm/gm protein/min) of treatment was lower than the other tested days; the 4th day (0.60 µm/gm protein/min) and 6th day (0.62 µm/gm protein/min) with significant differences between the treatments (F= 122.3, df=2, p= 0.0000), followed by a remarkable interaction effect between treatments and inspected time on SA of PPO enzyme (F= 9.77, df=8, p= 0.0000).

The present work was planned to assess the efficacy of the fungus *B. bassiana* on the pupal stage of *C. capitata* and their extended effect on the adult stage. The bioassay of *B. bassiana* treatments of fungus against the *C. capitata* was carried out under laboratory conditions of 25˚C±2.0 and 70%±5.0 R.H. Results of Qazzaz *et al.* (2015) showed that temperature dramatically influenced *B. bassiana* growth and development, with differences observed between the isolates. All the isolates failed to grow and develop at temperatures below 10˚C and above 30˚C. Furthermore, several investigators have reported that the optimum temperatures for *B. bassiana* mycelial growth, conidial germination, sporulation, and virulence are in the range 20-30˚C (Dimbi *et al*., 2004 and Tefera and Pringle, 2003).

The present results didn't show any impacts on the pupal stage of *C. capitata* up to the age of five days, On the 6th day of the pupal stage, *B. bassiana* exhibited little mortality effects 2.5-12.5% in parallel with the fungal concentration., the results of Imoulan and Elmeziane (2014) showed that all isolates of *B. bassiana* were able to infect the larval stage of *C. capitata* and caused a significant reduction in adult emergence. However, *B. bassiana* caused a large mortality rate in puparia ranging from 65 to 95 %. The mortality of adult fly, *C. capitata* from the outset, increases with the increase of the fungal concentration of *B. bassiana* and increase of the flies' age. The higher fungal concentration of 5, 10, and 20 gm/L, had clear mortality effects that began on the 13th day of the fly's age reached 79.17% to eliminate the fly at the 28th day of the adult stage with fungal concentrations of 5, a0, and 20 gm/L. Also, there were responsive mortality percentages at the lower fungal concentrations of 0.75, 2.5, and 1.25 gm/L, to exterminate the fly with respective concentrations at the 37th, 35th, and 32nd days of treatments.

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 Konstantopoulou and Mazomenos (2005) who found that *B. bassiana* induced 85.6% mortality in a *C. capitata* population but was less effective against *Bactrocera oleae*. Similarly, Munoz (2000) 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. Also, Atia (2018) reported 47.08-70.98% mortality in *Bactrocera zonata* (Saunders) (Diptera: Tephritidae) treated with 1-4gm/L of *B. bassiana.* However, the results showed 37.75-55.02 mortality in the pupal stage treated with 1-4gm/L.

Results showed that the pupal stage was more tolerant to the fungal

insecticide than adults fly. Furthermore, LC50 values of *B. bassiana* for *C. capitata* decreased by increasing the time of treatment. LC50 value in the *C. capitata* pupae was more 1.89 times higher than that in the adult stage at 2 days old. The LC50 values of adult fly ranged from 569.8 to 0.008 gm/l (As a log concentration) with obvious differences at the tested times; 2, 5, 8, 13, 18, 23, and 28 days post-treatment, respectively. Such LC50 value was strongly decreased with a reduction ratio of 71227.25- fold. Similar results were obtained by Quesada-Moraga ***et al***. (2006)**,** who found that lethal concentrations (LC50) for *C. capitata* of the four most virulent isolates fungus ranged from 4.9×105 to 2.0×106 CFU/mL, with LT50 ranging from 4.6 to 5.3 days. In addition, Mwamburi ***et al.*** (2010) reported that the LC50 of 34 *B. bassiana* isolates against adult house flies (*Muscadomestica* L.; Diptera, Muscidae) ranged between 103 - 105 conidia/mL.

The findings of the biochemical assay of PO enzyme activity in *C. capitata* pupae exhibited an increase by increasing the fungal concentrations of *B. bassiana*, also as increasing the times tested with high activity of 551.11 µm/gm protein/min in treating flies with 0.88 gm/L. PO enzyme maybe play an essential role in the immunity of the insects to infections. Active PO catalyzes quinones formation that submits to additional melanin-forming reactions (Nappi and Christenson, 2005) that helps the larvae integument hardness of epidermis epithelial cells as a protective mechanism for fungal invasion. These results are supported by the results of Atia (2018), who mentioned that the PO activity in full-grown larvae of *Bactrocera zonata* treated with *B. bassiana* increased to 1.5 folds of control, which increased to 12 folds after 7 days of treatment. The same trend of PPO enzyme activity in *C. capitata* pupae was obtained, where the activity of the PPO enzyme was directly related to the fungal concentrations as well as time post-treatment.

This study concluded the high effects of *B. bassiana* in *C. capitata* control under laboratory conditions. This information could be helpful in pest management in integrated and organic orchards.

Table (1): Cumulative mortality percentages of medfly, *Ceratitis capitata* treated with fungus, *Beauveria bassiana* in the larval stage under laboratory conditions.

Fungus concentration (gm/l)	Corrected mortality percentages (mean ± SE)												
	Post-processing inspection (days)												
	Pupae			Adults									
	2	4	6	2	5	8	13	18	23	28	32	35	37
0.75	00±00	00±00	02.50±1.48 c	02.50±1.90 c	15.00±0.90 d	20.51±0.58d	44.72±1.16 c	62.55±8.46 c	78.89±1.43c	86.38±0.82 c	91.32±0.84a	96.97±0.58 a	100±0.00
1.25	00±00	00±00	02.50+1.50 c	07.50±1.43 bc	12.50±0.43 d	17.93±0.58d	52.50±1.73 bc	71.67± .32bc	81.39±0.58 bc	89.16±0.91 bc	97.22±0.19a	100±0.00a	-
2.5	00±00	00±00	05.00+1.61 bc	10.00±3.58 abc	17.50±0.58 c d	23.08±1.29d	57.78±1.16 b	74.90±7.18 bc	84.16±0.29 bc	91.94±0.01 b	100±0.00a	-	-
5.0	00±00	00±00	05.00+1.59 bc	12.50±2.02 ab	22.50±1.00 bc	38.46±1.16c	60.28±1.16 b	78.37± 2.53a	89.17±0.58 ab	100±0.00a	-	-	-
10.0	00±00	00±00	10.00+0.00 ab	15.00±2.59 ab	25.00±0.59 b	48.72±0.79b	73.33±1.16 a	84.62± .32ab	94.72±0.58 a	100±0.00a	-	-	-
20.0	00±00	00±00	12.50+2.20 a	17.50±2.50 a	40.00±1.01 a	61.54±2.36a	79.17±1.27 a	90.53± 3.22a	97.50±0.44 a	100±0.00a	-	-	-
$LSD_{0.05}$	-	-	7.21	8.57	6.78	9.94	12.87	15.17	8.97	4.8	9.85	7.34	-

Means followed by the same letter (s) are not significantly different at 0.05 of probability.

$LSD_{0.05}$: Least significant difference at 5%.

Table (2): LC_{50} and statistical analysis parameters of the chronic toxicity of *Beauveria bassiana* fungus against the medfly *Ceratitis capitata* under laboratory conditions.

Parameter	Acute toxicity post-treatment (day)							
	Pupae	Adults						
	6	2	5	8	13	18	23	28
LC_{50} (mg/L)	1115.78	589.818	80.126	10.913	1.426	0.281	0.014	0.008
Ratio	0	1.0	7.11	52.21	399.59	2027.82	40701.286	71227.25
χ^2	0.337	0.848	1.39	1.695	0.385	0.25	0.032	2.95
$\chi2$ tabulated	9.5	9.5	9.5	9.5	9.5	9.5	9.5	3.8
Slope ± SE	0.65±0.29	0.59±0.244	0.61±0.20	0.93±0.19	0.68±0.18	0.67±0.21	0.61±0.30	0.40±0.24

LC_{50}: concentration producing 50% mortality; χ^2: Chi square; Slope ± SE; Standard Error of the concentration-mortality regression line.

Table (3): Specific activity of peroxidase (PO) in pupae of medfly, *Ceratitis capitata* treated with fungus, *Beauveria bassiana*.

Fungus concentration (gm/l)	SA (µm/mg protein/min) Time post-treatment (day)			General mean ± SE	Ratio
	2	4	6		
1.25	0.936±0.003	0.936±0.007	0.889±0.006	0.92 ±0.31[d]	2.71
2.50	1.230±0.007	1.240±0.003	1.140±0.006	1.20±0.41[c]	3.54
5.00	1.476±0.004	1.447±0.023	1.411±0.002	1.44±0.59[b]	4.25
10.00	1.757±0.007	1.777±0.008	1.683±0.015	1.74±0.49[a]	5.13
Control	0.327±4.60	0.340±0.26	0.351±0.04	0.34 ±0.12[e]	1.00
General mean ± SE	1.144± 0.45[a]	1.148± 0.46[a]	1.094±0.44[b]	-	-

Means followed by the same letter(s) are not significantly different according to L.S.D.; L.S.D. for con.= 0.015. L.S.D. for date=0.011; SA: specific activity; µM/mg protein/min

Table (4): Specific activity of polyphenol oxidase (PPO) in pupae of medfly, *Ceratitis capitate* treated with fungus, *Beauveria bassiana*

Fungal concentration (gm/l)	SA (µm/mg protein/min) Time post-treatment (day)			General mean ± SE	Ratio
	2	4	6		
1.25	0.425±0.001	0.433±0.002	0.467±0.001	0.442±0.007[d]	1.25
2.50	0.516±0.003	0.542±0.003	0.573±0.004	0.543±0.009[c]	1.54
5.00	0.723±0.001	0.734±1.330	0.733±25.39	0.730±0.006[b]	2.07
10.00	0.914±0.001	0.939±0.006	0.952±0.006	0.934±0.002[a]	2.65
Control	0.344±0.001	0.352±0.002	0.363±0.004	0.353±0.003[e]	1.00
General mean ± SE	0.584±0.06[c]	0.600±0.06[b]	0.618±0.05[a]	-	-

Means followed by the same letter(s) are not significantly different according to L.S.D.; L.S.D. for con= 0.006., L.S.D. for time=0.004; SA: specific activity; µM/mg protein/min

References

Abbott, W.S. (1925): A method of computing the effectiveness of an insecticide. Journal of Economic Entomology, 18: 265-267.

Atia, H.M.S. (2018): Enhancement of the two entopathogenic fungi, as a control measure against the peach fruit fly, *Bactrocera zonata* (Saunders) (Diptera:Tephritidae) in Egypt. Ph.D., Zagazig University.

Bakr, E. (2007): LdP line. http://embakr.tripod.com/ldpline/index.htm.

Beris, E.I.; Papachristos, D.P.; Fytrou, A.; Antonnatos, S.A. and Kontodimas, D.C. (2013): Pathogenicity of Three Entomopathogenic Fungi on Pupae and Adults of the Mediterranean Fruit Fly, *Ceratitis capitata* (Diptera: Tephritidae). Journal of Pest Science, 86: 275-284.

Castillo, M.A.; Moya, P.; Hernandez, E. and Primo-Yufera, E. (2000): Susceptibility of *Ceratitis capitata* (Wiedemann) (Diptera:Tephritidae) to entomopathogenic fungi and their extracts. Biological Control, 19:274-282.

Costat Software (2008): Version 6.4, CoHort software, 798 Lighthouse Ave, PMB 20, Monetery, CA 93940, USA.

Dimbi, S.; Maniania, N.K.; Lux, S.A. and Mueke, J.M. (2004): Effect of constant temperatures on germination, radial growth and virulence of *Metarhizium anisopliae* to three species of African tephritid fruit flies. Biocontrol, 49: 83-94.

Ekesi, S.; Dimbi, S. and Maniania, N. K. (2007): The role of ento-mopathogenic fungi in the integrated management of fruit flies (Diptera: Tephritidae) with emphasis on species occur-ring in Africa, pp. 239-274. In: Use of entomopathogenic fungi in biological pest management (Ekesi S., Maniania N. K. Eds). Research SingPost, Kerala, India.

Ekesi, S.; Maniania, K. N. and Lux, A. S. (2002): Mortality in three African tephritid fruit fly puparia and adults caused by the entomopathogenic fungi, *Metarhizium anisopliae* and *Beauveria bassiana*. Biocontrol Science and Technology, 12:7-17.

El-Gendy, I. R. (2017): Host Preference of the peach fruit fly, *Bactrocera zonata* (Saunders) (Diptera: Tephritidae), under laboratory conditions. Journal of Entomology, 14: 160-167.

Finney, D. J. (1971): Probit Analysis, 3rd ed. Cambridge University Press, Cambridge, UK. p 333.

Garrido-Jurado, I.; Torrent, J.; Barrón, V. and Corpas, A. (2011): Soil properties affect the availability, movement, and virulence of entomopathogenic fungi conidia against puparia of *Ceratitis capitata* (Diptera; Tephritidae). Biological Control, 58: 277-285.

Imoulan, A. and Elmeziane, A. (2014): Pathogenicity of *Beauveria bassiana* isolated from Moroccan Argan forests soil against larvae of *Ceratitis capitata* (Diptera: Tephritidae) in laboratory conditions. World Journal of Microbiology and Biotechnology, 30: 959-965.

Ishaaya, I. (1971): Observation on the phenolixidase system in the armored scales *Aonidiella aurantii* and *Chrysomphalus aonidium*. Comparative

Biochemistry and Physiology, 39: 935-943.

Konstantopoulou, M.A. and Mazomenos, B.E. (2005): Evaluation of *Beauveria bassiana* and *B. brongniartii* strains and four wild-type fungal species against adults of *Bactrocera oleae* and *Ceratitis capitata*. Bio Control, 50: 293-305.

Magana, S.M.; Quintant, P.; Aguilar, D.H.; Toledo, J.A.; Angeles-Chavez, C.; Cortes, M.A.; Leon, L. and Lopez,T. (2008): Antibacteria activity of montmorillonites modifild with silver. Journal of Molecular Catalysis A: Chemical, 281: 192-199.

McCoy, C.W.; Samson, R.A. and Boucias, D.G. (1988): Entomogenous fungi, in: C.M. Ignoffo, N.B. Mandava (Eds.), Handbook of Natural Pesticides, Microbial Insecticides, Part A, Entomogenous Protozoa and Fungi, vol. 5, Mr ic Press, Boca, Raton, Fla.

Morales, P.; Cermeli, M.; Godoy, F. and Salas, B. (2004): Lista De Hospederos De La Mosca Del Mediterráneo ***Ceratitis capitata*** Wiedemann (Diptera: Tephritidae) Basada En Los Registros Del Museo De Insectos De Interés Agricola Del Inia - Ceniap Entomotropica, 19: 51-54.

Munoz, R.J.A. (2000): Patogenicidad de *Beauveria bassiana* (Bals.) Bulli. sobre la mosca del mediterraneo, *Ceratitis capitata* (Wied.) en condiciones de laboratorio. Tesis de Licenciatura. Facultad de Ciencias Agricolas. Univ. Aut. de Chiapas. Huehuetan Chis., Mexico: p. 55.

Mwamburi, L.A.; Laing, M.D. and Miller, R.M. (2010): Laboratory screening of insecticidal activities of *Beauveria bassiana* and *Paecilomyces lilacinus* against larval and adult house fly (*Musca domestica* L.). African Entomology, 18: 38-46.

Nappi, A.J. and Christenson, B.M. (2005): Melanogensis and associated cytotpoxic reaction: application to insect innate immunity. Insect Biochemistry and Molecular Biology, 35:443-459.

Navarro-Llopis, V.; Sanchis-Cabanes, J.; Ayala, I. , Casana-Giner, V. and Primo-Yufera, E. (2004): Efficacy of lufenuron as chemosterilant against *Ceratitis capitata* in field trials. Pest Management Science, 60: 914-920.

Qazzaz, F.O.; Al-Masri, M. I. and Barakat, R. M. (2015): Effectiveness of *Beauveria bassiana* native isolates in the biological control of the Mediterranean fruit fly (*Ceratitis capitata*). Advances in Entomology, 3: 44-55.

Quesada-Moraga, E.; Maranhao, E.A.A.; Valverde-García, P. and Santiago-Álvarez, C. (2006): Selection of *Beauveria bassiana* isolates for control of the whiteflies *Bemisia tabaci* and *Trialeurodes vaporariorum* on the basis of their virulence, thermal requirements, and toxicogenic activity. Biological Control, 36: 274-282.

Rendon, P.; Sivinski, J.; Holler, T.; Bloem, K.; Lopez, M.; Martinez, A. and Aluja, M. (2006): The effects of sterile males and two braconid parasitoids, *Fopius arisanus* (Sonan) and *Diachasmimorpha*

krausii (Fullaway) (Hymenoptera), on caged populations of Mediterranean fruit flies, *Ceratitis capitata* (Wied.) (Diptera: Tephritidae) at various sites in Guatemala. Biological Control, 36: 224-231.

Roberts, D.W. and Humber, R.A. (1981): Entomogenous fungi, in: G.T. Cole, B. Kendrick (Eds.), biology of conidial fungi, Academic Press, New York, pp. 201-236.

Roberts, D.W. and St Leger, R.J. (2004): *Metarhizium* spp., cosmopolitan insect-pathogenic fungi: Mycological aspects. Advances in Applied Microbiology, 54: 1-70.

Samuels, R.I. and Paterson, I.C. (1995): Cuticle-degrading proteinase from insect moulting fluid and culture filtrates of entomopathogenic fungi. Comparative Biochemistry and Physiology. Part B, Biochemistry and Molecular Biology, 110: 661-669.

Smith, R. and Grula, E.A. (1982): Toxic components on the larvae surface of the cornearworm (*Heliothis zea*) and their effects on germination and growth of *Beauveria bassiana*. Journal of Invertebrate Pathology, 36: 15-22.

St Leger, R. J. (2008): Studies on adaptation of *Metarhizium anisopliae* to life in the soil, Journal of Invertebrate Pathology, 98: 271-276.

Tanaka, N.; Steiner, L. F.; Ohinata, K. and Okamoto, R., (1969): Low cost larval rearing medium for mass rearing production of oriental and Mediterranean fruit flies. Journal of Economic Entomology, 62: 967-968.

Tefera, T. and Pringle, K. (2003): Germination, radial growth, and sporulation of *Beauveria bassiana* and *Metarhizium anisopliae* isolates and their virulence to *Chilo partellus* (Lepidoptera: Pyralidae) at different temperatures. Biocontrol Science and Technology, 13: 699-704.

Zimmermann, G. (2007): Review on safety of the entomopathogenic fungi *Beauveria bassiana* and *Beauveria brongniartii.* Biocontrol Science and Technology, 17: 553-596.