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Susceptibility of adults of *Ceratitis capitata* (Diptera: Tephritidae) to the fungal isolates of *Beauveria bassiana* and *Metarhizium anisopliae*

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Abstract:

The pathogenicity of local fungal isolates, Beauveria bassiana (BE17 and BE47) and Metarhizium anisopliae (ME34 and ME87) was evaluated against adults of the Mediterranean fruit fly Ceratitis capitata. Results obtained showed that the mortality rates related with the concentrations, the isolates, and the sex of adults. Percentage of females mortality after five days of treatment were 0.0, 42 and 58% for the concentrations of 10^5, 10^7, 10^9 Spores / ml respectively for the B. bassiana BE17isolate,. Statistical differences among the concentrations were continued after 10 days of treatment, the mortality rates were 58, 75 and 100% respectively. The highest rate of mortality after five days of treatment with the isolate B. bassiana BE47 reached to 92% at the concentration of 10^9 Spores / ml compared with other concentrations (0.0%) that their effect was increased to 8 and 25% respectively after 10 days. The adult's mortality was 17, 50 and 58% for ME34 isolate and 25, 75 and 100 for ME87 isolate for the

mentioned concentrations respectively. High virulence was demonstrated by BE17 isolate according to median lethal concentration (LC_{50}) that was 6.2×10^4, followed by ME87 isolate (6.5×10^5 spore/ml). The shorter median lethal time (LT_{50}) was 2.28 and 2.3 days at the concentration of 10^9 spore/ml for BE47 and ME87 isolates respectively. Results of male treatment with the BE17 isolate showed the mortality rate after 10 days were 42, 67 and 100% for the concentrations of 10^5, 10^7 and 10^9 Spore / ml respectively. The LC_{50} value of this isolate was 6.2×10^4 followed by ME34 isolate (1.6×10^5) and BE17 isolate (4.5×10^5). Lower fecundity (0.0 eggs / female) was due to exposure to the concentration of 10^9 Spore / ml of ME87 isolate without a significant difference with fungal isolates of BE47 and BE17 at the same concentration.

Key words: *Ceratitis capitata* (Diptera: Tephritidae), *Beauveria bassiana*, *Metarhizium anisopliae*

INTRODUCTION

The Mediterranean fruit fly *Ceratitis capitata* (Wiedermann) (Diptera: Tephritidae) is one of the most destructive pests. This is a multivoltine, widespread and highly polyphagous pest which has been recorded in more than 400 host plant species (Liquido *et al*., 1990; Aluja and Mangan, 2008). Females oviposit into the hosts fruits, which are directly damaged by the trophic activity of medfly larvae. In addition to direct damage, severe quarantine policies are imposed by importing countries to avoid importation and establishment of exotic pests. The predominant method to control this pest has been the use of conventional pesticides. However, the continued use of pesticides has caused serious problems such as environmental pollution, development of insecticide resistance, and contamination of products. As an alternative to chemical control, natural enemies, microbial pesticides (bacteria and fungi), have been evaluated as constituents of an IPM strategy

for this pest (Castillo *et al*., 2000; Lacey *et al*., 2001). The *Beauveria bassiana* (Bals.) and *Metarhizium anisopliae* (Met.) are the most important species that used widely in the various countries of the world against a large number of insect pests especially those that spend part of their life cycle in the soil (Toledo *et al*., 2008). The conidial phase (spores) of a large number of strains of both species, coming from different geographic regions, have been assessed, under laboratory conditions, for control of different fruit fly species and on different life history stages (Espin *et al.* 1989, Campos 2000, Castillo *et al.* 2000, Lezama-Gutierrez *et al*. 2000, De la Rosa *et al*. 2002, Ekesi *et al.* 2002).. The objective of this study was to evaluate the virulence of two isolates of *Beauveria bassiana* (Bals.) and *Metarhizium anisopliae* (Met.) Sorokin against adults fly of *Ceratitis capitata*.

FUNGAL SPORE SUSPENSION PREPARATION

Two isolates of each fungi *Metarhizium anisopliae* (ME34 and ME87) and *Beauveria bassiana* (BE17 and BE47) 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 4 °c 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 hypha debris was removed by passing the suspension through fabric cloths. The concentration was determined by the aid of haemocytomer. The viability of spore was determined as in Lacey (1997).

EFFECTS OF DIFFERENT SPORE SUSPENSION CONCENTRATIONS ON THE ADULT INSECTS

Bioassay

Three concentrations of each fungal isolates 1 x 10^5, 1 x 10^7 and 1x 10^9(2 ml of each) were sprayed on 10 adult at age 3 days (5 males and 5 female) in container (3 cm diameter x 12 cm high) with opened ends. The ends were closed with fabric clothes. Control was made by spraying the adults with SDW mixed with 0.05% tween80. The percentage of mortality was measured daily. All dead insects were transfer into 9 cm Petri dishes containing wet filter paper at 22 ^{o}c allowing fungi to grow.

Statistical analysis

Mortality data were analyzed with one-way analysis of variance (ANOVA) of Arcsine transformed corrected mortality (Abbott, 1925). Means were separated by the Duncan Multiple Range Test. LT50s were calculated by probit analysis (Finney, 1971). The SPSS 20.0 software was used for statistical analysis.

RESULTS AND DISCUSSION

The susceptibility of *C. capitata* adults exposing to different concentrations of fungal suspension of the isolates BE17 and BE47 of *B. bassiana* and ME34 and ME87 of *M. anisopliae* were related with the concentrations , fungal isolation, and the sex of the adult, the mortality of females were increased significantly with increasing of concentrations (table 1), *B. bassiana* BE17 isolate gave variance in mortality rates according to the concentrations after 5 days of treatment , the percentage of mortality were 0.0, 42 and 58% for the concentrations of 10^5, 10^7, 10^9 Spore / ml respectively. The statistical differences were continued among the mentioned concentrations after 10 days of treatment, they were 58, 75 and

100% respectively. The highest mortality rate after five days of treatment with the isolate BE47 of *B. bassiana* (92%) was at the concentration of 10^9 Spores / ml compared with the other concentrations (0.0%), that increased to 8 and 25% after 10 days respectively. The isolate ME34 of the fungus *M. anisopliae* achieved 17, 50 and 58% mortality while, ME87 isolate achieved 25, 75 and 100 % for the concentrations of 10^5, 10^7, 10^9 Spores / ml respectively. Comparing among the fungal isolates on the basis of median lethal concentration (LC_{50}), the isolate BE17 showed high virulence according to LC_{50} value that was 6.2×10^4, followed by the isolate ME87 (6.5×10^5). The median lethal time LT_{50} correlated with concentration, at concentration of 10^9 spores/ ml the LT_{50} for isolates of BE47 and ME87 were 2.28 and 2.3 days respectively. Males treatment (table2) showed clear relation between the mortality and the concentrations , after 10 days of treatment with the isolate BE17 the mortality rate were 42, 67 and 100% for the concentrations of 10^5 , 10^7 and 10^9 spores/ml respectively , in the same way the results were by the other fungal isolates. The highest virulence on males was by ME87 isolate according to LC_{50} value that was 6.2 x 10^4 followed by ME34 ($1.6x10^5$) and the isolate BE17 ($4.5x10^5$). The less value of LT_{50} was 2.14 days for the isolate ME34. Males were more susceptibility to the fungal isolates suspension (except of BE17) in comparing with the females as such showed by log of LC_{50} (fig.1).

Survival curves of the adults exposed to the fungal isolates (fig 2-9) showed that the first cases of mortality was related with the isolate and concentration as well as sex (male or female), early mortality of males was recorded after one day of treatment with BE17 isolate, the percentage of survival was 92% at concentration of 10^7 Spores / ml of fungal suspension and 25% at concentration of 10^9 Spores / ml , after 15 and 16 days from treatment the survival rate was 0.0% for these concentrations.

Survival rate of females treated by mentioned isolate was 25% after one day of treatment at concentration of 10^9 Spores / ml, other concentrations the first cases of mortality was after six days of treatment.

The first mortality of males that treated with ME34 isolate at the concentration of 10^9 Spores / ml was in the first day after treatment, while it was in the third day when treated with 10^7 Spores / ml and eighth day by treated with 10^5 spores/ml, the survival percentage was 0.0 % after 14, 12 and 13 days from treatment with concentrations of 10^5, 10^7, 10^9 Spores / ml respectively. Treatment of females with this isolate achieved first cases of mortality after one day from treatment by the concentration of 10^9 Spores / ml and third day for the concentration of 10^7 Spores / ml and the tenth day for the concentration of 10^5 Spores / ml, 100% mortality was at day 15th after treatment for 10^7 and 10^9 spores / ml.

ME87 isolate showed considerable influence in males since the first day after the treatment, the survival rate was 8% and the treatment of female has achieved a survival rate of 17% for the concentration of 10^9 Spores / ml.

Sub lethal effect by the fungal isolates represented by reduction of females fecundity treated with the mentioned fungal isolates (Fig. 6) The results showed that the highest fecundity reduction (100%) was due to exposure to the concentration of 10^9 Spores / ml of ME87 isolate without a significant difference in the effect of the same concentration for the fungal isolates BE47 and BE17 as well as the concentration of 10^7 spores/ml of BE17 isolate (96, 97 and 93%, respectively).
Pathogenicity is the most important indicator of the effectiveness of pathogenic fungi against pests, selection of fungal isolates as successful biocontrol agents relies on their Pathogenicity, specification , ease of production and adapt to the environmental conditions (Reay *et al*., 2008; Ptlamul and Parasertan, 2012)

The results of the present study confirmed the results of several other studies that appeared that the sensitivity of the adults of *C.capitata* to fungal isolates depending on the isolate and concentration. Konstantopoulou and Mazomenos, (2005) has been found that the *B.bassiana* caused 85.6% mortality in the population density of this pest. Munoz',(2000) pointed out in his assessment of 16 isolates of *B.bassiana* against fruit fly of *C.capitata* that the rate of mortality ranging from 20 - 98.7%. Espin *et al.* (1989) recorded 69-78% mortality in his study on this pest. In laboratory experiments to measure the pathogenicity of 10 isolates of *B.bassiana* and 5 isolates of the M.anisopliae against pupae and adults of Mediterranean fruit fly *C.capitata* by inoculation them at ventral surface of the abdomen , Quesada-Moraga *et al.* (2006)pointed out that the rate of death ranged from 30-100% with an average time of survival from 5.6 to 6.8 days and the value of LC_{50} for the most virulent isolates were 4.9×10^5 - 2×10^6 Spore / mL , the LT_{50} value were 4-5.3 days, sub lethal effect of *B.bassiana* on fecundity and fertility, which ranged between 20-71.2% and 28.6-60%, respectively

Castillo *et al.* (2000) measured the effectiveness of seven isolates of entomopathogenic fungi against adults of *Ceratitis capitata* under laboratory condition ,five of them were high pathogenicity , *Metarhizium anisopliae* and of *Paecilomyces fumosoroseus* CG-260 are the most pathogenicity ,the LT50 value were 10-days for the concentration of 5.1 and 6.1 X 10^3 Spore / fly respectively. P. fumosoroseus CECT 2705 isolate was most isolates in reduction of fecundity (65%) at concentration of 1 X 10^6 Spore /adult ,while the isolates of *M. anisopliae* and *Aspergillus ochraceus* has caused 40-50% reduction in fecundity for the most concentration.

Ali *et al.* (2008) tested four concentrations (3x 10^4 and 3 x 10^5 and 3 x 10^6 and 3x 10^7 Spore / ml) of *Beauveria bassiana* and *Paecilomyces fumosoroseus* on adults of *C.capitata* at age

less than 3 days by dipping them for 10 -15 seconds in 10 ml of spore suspension, and the results showed a high susceptibility, in the case of *B. bassiana* the mortality of tested flies were 100% at concentration of 3 x 10^7 Spore/ ml and 82% for the concentration of 3 x 10^6, in the case of *P. fumosoroseus* the mortality rate was70% at the concentration of 3x 10^7 Spore / ml and 62.5% for 3 x 10^6 Spore / ml.

Measuring the effectiveness of the fungal isolates P.Bv52 P. Bv32, P. Bv39, P. Bv41, P. Bv51, of *Beauveria bassiana* against adults of *Ceratitis capitata* appeared that high mortality rates of adults (77% and 65% and 65% and 65 %, and 58%, respectively), in addition to the LT50 value have been ranged from 3.91 - 5.6 days, and the value of LC50 ranged from 3.8-10.5 depending on isolate. Mortality by contact was significantly higher than by nutrition, and treatment of fruits, especially by P. Bv32 and P. Bv39 that significantly reduced the infection by this pest (Qazzaz *et al.*2015) . The variations among the isolates that belong to the same species may be due to genetic variation; this disparity was recorded among the isolates of *Beauveria bassiana* and *Metarhizium anisopliae* in many studies (De La Rosa *et al*., 2002 and Garcia *et al*., 1984)

7 isolates of *Metarhizium* spp. and 12 isolates of *Beauveria* spp. and one isolate of *Hirsutella citriformis* were screened at concentration of 10^8 / ml against the fruit fly of *Bactrocera dorsalis*, the best was *B.basiana* 6241 isolate that achieved 68% mortality(Sirinun, 2007). Sookar (2013) pointed out that the LT_{50} of *Bactrocera cucurbitae* adults treated with the fungi of *Metarhizium anisopliae* and *Beauveria bassiana* has varied between 2.4 and 16.6 days, and the LT_{90} has varied between 5.2 and 23.6 days. The difference of the sensitivity to pathogenic fungi between males and females, which trailed the current study, were noted in other studies, Dimbi *et al.,* (2003) confirmed early mortality of females of *Ceratitis* sp. compared with males when treated with the fungus *Metarhizium*

anisopliae, while other studies (Carsewell *et al.,* 1998) confirmed the absence of this difference in the two species *C. capitata* and *B. tryoni* after treatment with the fungus *Metarhizium anisopliae***.**

Table (1) mortality of *C. capitata* adults (females) at various concentrations of fungal strains of *B. bassiana* and *M. anisopliae*

Fungal isolate	concentration	Mortality after 5 days (%)	Mortality after 10 days (%)	Mortality after 15 days (%)	LC50	X^2	Pvalue	LT50(days)	X^2	P value
BE17	***10^5***	0.0a	58a	100	$6.2x10^4$	***3.8***	***0.8***	***8.5***	***7.55***	***0.037***
	10^7	42b	75b	100				***5.89***	***6.1***	***0.52***
	10^9	58c	100c	--				***4.76***	***4.8***	***0.7***
BE47	***10^5***	0.0 a	8a	100	$1.7x10^7$	***12.19***	***0.09***	***10.93***	***27***	***0.001***
	10^7	0.0a	25b	100				***10.1***	***15.48***	***0.03***
	10^9	92 b	100c	--				***2.28***	***2.24***	***0.93***
ME34	***10^5***	0.0a	17a	100	$6.8x10^7$	***6.62***	***0.47***	***10.49***	***19.25***	***0.007***
	10^7	25b	50b	100				***7.8***	***15.8***	***0.027***
	10^9	50 c	58b	100				***5.75***	***9.7***	***0.2***
ME87	***10^5***	8a	25a	100	$6.5x10^5$	***7.83***	***0.35***	***10***	***15.48***	***0.03***
	10^7	50b	75b	100				***5.16***	***13.25***	***0.07***
	10^9	92c	100c	--				***2.3***	***2.45***	***0.9***

Table (2) mortality of *C. capitata* adults (males) at various concentrations of fungal strains of *B. bassiana* and *M. anisopliae*

Fungal isolate	concentration	Mortality after 5 days (%)	Mortality after 10 days (%)	Mortality after 15 days (%)	LC50	X^2	P value	LT50(days)	X^2	P value
BE17	***10^5***	0.0a	42a	100	$4.5x10^5$	7.95	0.34	***10.58***	***5.05***	***0.65***
	10^7	25b	67b	100				***7.17***	***8.2***	***0.32***
	10^9	92c	100c	--				***5.88***	***6.16***	***0.82***
BE47	***10^5***	0.0 a	50a	100	$4.9x10^6$	6.3	0.5	***8.18***	***4.37***	***0.74***
	10^7	8.3a	50a	100				***7.03***	***2.85***	***0.9***
	10^9	75 b	92b	--				***2.28***	***2.44***	***0.93***
ME34	***10^5***	0.0a	50a	100	$1.6x10^5$	4.9	0.7	***8.9***	***7.95***	***0.34***
	10^7	33b	67b	100				***6.67***	***8.46***	***0.29***
	10^9	83 c	92c	100				***2.14***	***8.55***	***0.29***
ME87	***10^5***	17a	58a	100	$6.2x10^4$	16.6	0.02	***7.99***	***4.03***	***0.25***
	10^7	42b	75b	100				***5.9***	***14.9***	***0.04***
	10^9	92c	100c	--				***2.28***	***2.45***	***0.93***

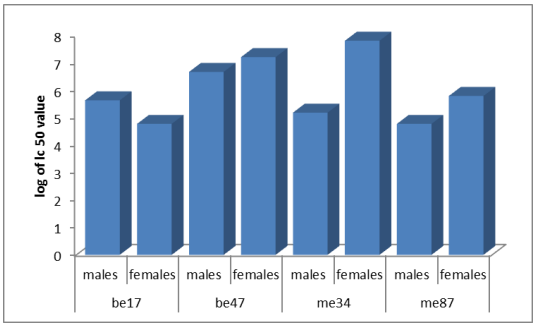

Fig. (1): Susceptibility of males and females of *C. capitata* treated with the isolates of *Beauveria bassiana* and *Metarhizium anisopliae*.

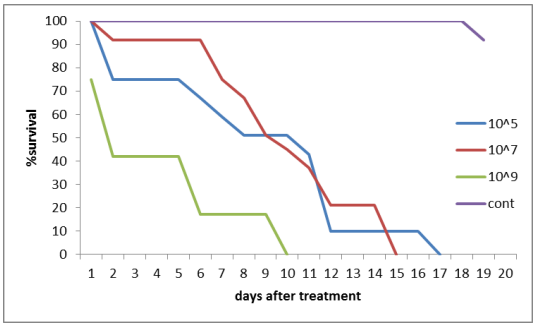

Fig. (2): survival curve of *C. capitata* adults (males) treated with *B. bassiana* Be17 isolate.

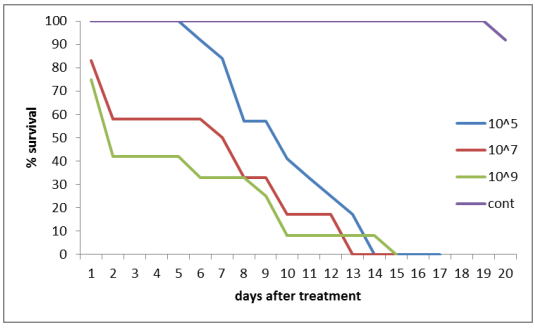

Fig. (3): survival curve of C. capitata adults (females) treated with B. bassiana Be17 isolate.

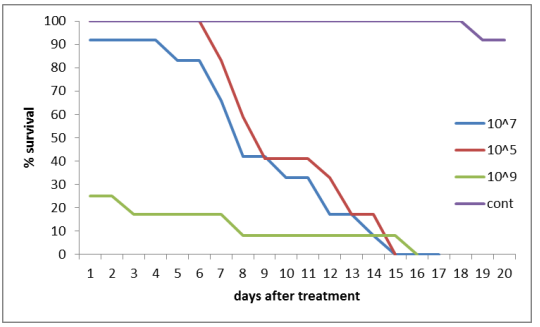

Fig. (4): survival curve of *C. capitata* adults (males) treated with *B. bassiana* Be47 isolate.

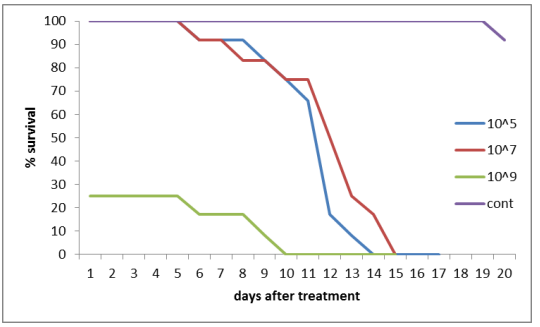

Fig. (5): survival curve of *C. capitata* adults (females) treated with *B. bassiana* Be47 isolate.

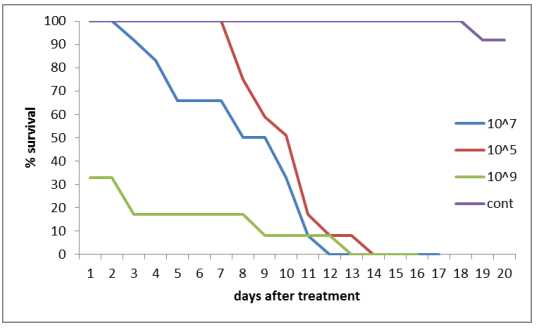

Fig. (6): survival curve of *C. capitata* adults (males) treated with *M. anisopliaeME34* isolate.

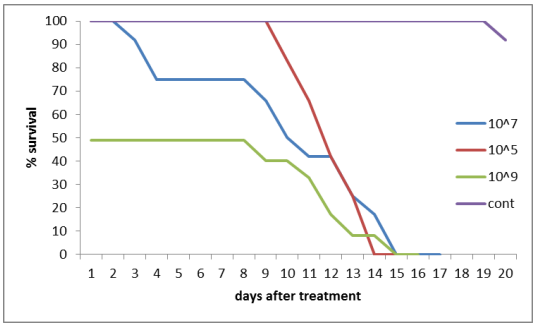

Fig. (7): survival curve of *C. capitata* adults (females) treated with *M. anisopliaeME34*isolate.

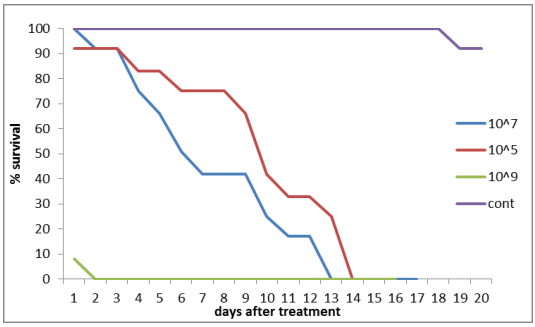

Fig. (8): survival curve of *C. capitata* adults (males) treated with *M. anisopliaeME87* isolate.

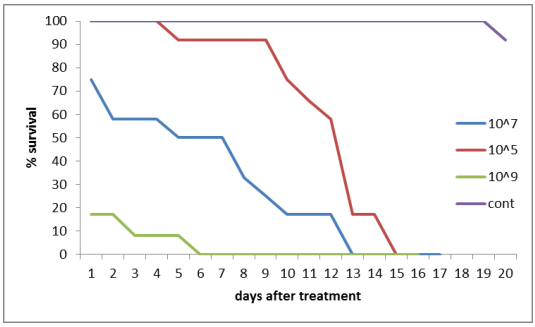

Fig. (9): survival curve of *C. capitata* adults (females) treated with *M. anisopliae ME87* isolate.

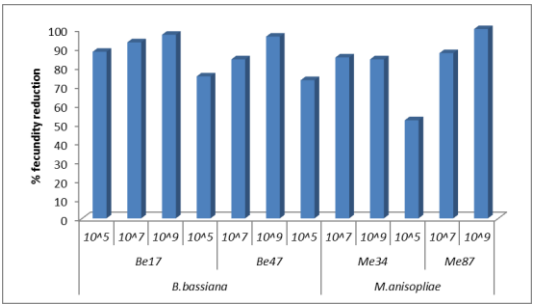

Fig. (10): fecundity reduction of *C. capitata* females treated with the isolates of *Beauveria bassiana* and *Metarhizium anisopliae* .

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