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EFFICACY OF *BEAUVERIA BASSIANA* AND *METARHIZIUM ANISOPLIAE* TO CONTROL MEDITERRANEAN FRUIT FLY, *CERATITIS CAPITATA*

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A B S T R A C T

The efficacy of *Beauveria bassiana*, *Metarhizium anisopliae* and their mixture to control last instar (prepupae) Mediterranean fruit fly, *Ceratitis capitata* larvae in the soil using different volumes of spore suspension concentrations were investigated under lab condition. *M. anisopliae* revealed higher mortality rate than *B. bassiana* reaching 73.80% at 1×10^9concentration of volume 20ml/100g. However, the mixture of *M. anisopliae* and *B. bassiana* expressed the highest mortality rate among *Ceratitis capitata* larvae reaching 96.60 % at the concentration 1×10^9 and 20ml volume with lowest LC_{50} of 234x 10^7. The mortality rates of *Metarhizium anisopliae* fungus were 40.6 and 61.3% at 1×10^6 and 1×10^9, respectively. According to the study outcome, fungal mixture 1×10^9 concentration can be applied in the field with 200 ml /m^3 soil according to the volume transformation.

Keywords: *Beauveria bassiana*, *Metarhizium anisopliae*, *Ceratitis capitata*, biological control, entomopathogenic fungi.

INTRODUCTION

Mediterranean fruit fly, *Ceratitis capitata* (Diptera: Tephritidae) is considers as one of the most destructive pest on fruit and vegetables worldwide (Vargas et al., 1984). Although Citrus are the main preferable host of this insect, it infests peach, pear, apple, grape, apricot, coffee, pomegranate, date palm, moreover, it infests cucurbits and some flowers with less frequency reaching almost 350 host (Liquido *et al*., 1990, Liquido *et al*., 1991). Mediterranean fruit fly can destroy 100% of susceptible fruits such as apricot and peach and little less than that in pears and apple in the case of none using available controlling programs (Broughton and Francis, 1998). Number of techniques was used to manage *C. capitata* exemplified by applying chemical pesticides alone or with attractive bait traps(Clark *et al.*, 1996). Because of the negative effects of chemical pesticides on humans, animals, and the environment, the demand to find alternative control programs, such as biological control agents, has been increased ever since. The entomopathogenic fungi *Beauveria bassiana* (Ascomycota: Hypocreales) and *Metarhizium anisopliae*(Deuteromycotina: Hyphomycetes) that belong to the Hyphomycetes group were used worldwide to control large number of insect pests (Toledo *et al.*, 2008). These two entomopathogenic fungi shown high mortality rate among fruit fly in Thailand, reducing their density by 50% (Aemprapa, 2007). *M. anisopliae* and *B. bassiana* has been reported as an effective biological control agent against insect pests and from over 200 species belongs to Orthoptera, Hemiptera, Lepidoptera, Diptera, Hymenoptera and Coleoptera (Veen, 1968). The main objective of this study is to measure the efficacy of *B. bassiana* and *M. anisopliae* to control Mediterranean fruit fly, *C. capitata* last larval instar (pre-pupae) in the soil using different spore concentration and volumes.

MATERIAL AND METHODS

Insect rearing: Mediterranean fruit fly, *C. capitata* were collected from infested citrus fruits from several Iraqi states (Baghdad, Wasit, and Diyala). Collected fruits were kept in 1 Liter jars contained a layer of soil (about 5 cm) enabling larvae transformation to the pupal stage and were incubated in the rearing room at 27±2 ˚C and 75±5%Rh. Emerged adults were transferred into

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30×30×30 cm Plexi-glass rearing boxes that have two sides covered with organza cloth to allow air circulation. Eggs were collected after 6 days in rectangle plastic containers that contain water after filtering them through the cloth sides and kept on wet filter papers for three days tell hatching. Young larvae were transferred to sterile petri dishes containing 30 gm of artificial diet (300 gm wheat husks, 100 gm sugar, 100 gm yeast extract powder, 5 ml HCl, 2 gm sodium benzoate) according to (Gazit *et al*., 2004).

Fungal purification: The two fungal isolates *B. bassiana* and *M. anisopliae* were sub-cultured many times on full potato dextrose agar (PDA) media. The inoculum was transferred onto quarter-strength plates which contain 100 µg streptomycin sulphate and 10 µg tetracycline hydrochloride/ml. Plates were incubated at ambient temperature and placed under standard white fluorescent light (35098 F18E/33 General Electric, USA) for 24 hours 5 -7 days. Spore suspension was made by adding 3-4 drops of sterile distilled water on the fungal colony that were grown on the plate using flame-sterilized loop. The spore suspension was streaked onto 2% water agar media by using flame-sterilized metal loop and plates were incubated under laboratory conditions for 24 h. A single germinated spore was transferred onto full-strength PDA media plate and incubated at ambient temperature for one week according to Scott and Chakraborty (2010).

Bioassay test: Spore suspension was prepared by adding 5 ml of sterile distilled water to pure full growth isolate Petri dish; then, by using a sterile metal scraper, fungal mycelia were scraped and the solution was poured into a 50 ml Falcon tube after filtering the solution through sterile miracloth. The spore concentration of *B. bassiana* and *M. anisopliae* and their mixture was determined using haemocytometer and adjusted to 1×10^6, 1×10^7 and 1×10^9 conidia/ml. Mixture treatments were prepared via mixing the same volume of each fungal spore suspension to reach the desire concentration. Spore suspension was added to 100gm sterile soil in 30 ×20 cm jars and mixed very well. Then, 10 newly hatched larvae of medfly were released on plastic petri dish containing 30 gm of the artificial media and placed in the jar upon the soil layer which removed within two days, after checking that all larvae were turned into pupal stage in the soil. Soil was sprayed with sterile distilled water every three days to keep wet. Three volume (10, 15 and 20 ml) suspensions were used for each spore concentration. All experiments were done in three replicates. Sterile distilled water was used for control treatment. Jars were incubated in the rearing room at 27±2 ˚C and 75±5%Rh for 10 days. Number of emerged adults was counted from day 8 to 10 after inoculation. Pupae were failed to emerge checked under dissecting microscope.

Statistical analysis: The experiment was designed using Complete Random design (CRD), mortality percentages were modified according to Abbott (1925). Data were grouped according to volume and concentration. Data were analyzed using SPSS version 20 statistical analysis software.

RESULTS

The result of treating last instar Mediterranean fruit fly larvae with *B. bassiana* spore suspension mixed with soil (Table1) revealed that the highest mortality rates were at the highest concentration used (1×10^9 conidia/ml) irrespective to the volume of the spore suspension. Meanwhile, the highest volume of spore suspension (20 ml) used had the highest mortality rates in all concentration. In general, the highest mortality rate of 65.70% was encountered with volume 20 ml of 1×10^9 conidia/ml.

Table 1. Mortality rate of last instar *Ceratitis capitata* larvae exposed to three volumes of different concentrations of *B. bassiana* spore.

Concentration conidia/ml	Mortality %		
	Spore suspension volume/100 g soil		
	10 ml	15 ml	20 ml
1×10^6	32.83 a *	36.00 a	37.93 ab
1×10^7	38.06 ab	47.66 ab	50.43 ab
1×10^9	55.71 ab	59.40 ab	65.70

* a or ab: The same columns and row are not significantly different.

The results of *M. anisopliae* showed that the highest mortality rate (73.80%) was observed at 1×10^9 concentration of volume 20 ml/100 g soil, which was significantly higher than the other concentrations of the volumes (Table 2). The concentration 1×10^7 revealed high mortality percentage at the volume 20ml without

any significant differences by the other volumes reaching 59.00%. In addition, the lowest mortality rate was observed at the 1×10^6 concentration in comparison to the concentrations of the other volumes.

Table 2. Mortality rate of last instar *Ceratitis capitata* larvae exposed to three volume spore suspension of different concentrations of *M. anisopliae*.

Concentration conidia/ml	Mortality %		
	Spore suspension volume/100 g soil		
	10 ml	15 ml	20 ml
1×10^6	40.60 ab *	36.50 a	48.10 bc
1×10^7	51.10 bc	57.10 bc	59.00 bc
1×10^9	61.30 c	64.03 cd	73.80 d

* a or ab: The same columns and row are not significantly different.

Table 3 illustrates the mortality rate of last instar *Ceratitis capitata* larvae after treating soil with mixture of different concentrations and volumes of spore suspension of *M. anisopliae* and *B. bassiana*. The 1×10^9 concentration recorded significantly the highest mortality rate among all other concentrations and volumes 10, 15, and 20ml/100g soil with 85.80, 90.40, and 96.60% respectively. Both 1×10^6 and 1×10^7 concentrations showed significantly high mortality among larvae at the volume 20 ml/100 g soil with 76.80 and 81.80% respectively. The lowest mortality was at the volume 10 ml with the 1×10^6 concentration with 60.70%.

Table 3. Mortality rate of last instar *Ceratitis capitata* larvae exposed to different concentrations of the mixture of *M. anisopliae* and *B. bassiana* spore suspension.

Concentration conidia/ml	Mortality %		
	Spore suspension volume/100 g soil		
	10 ml	15 ml	20 ml
1×10^6	60.70 a *	61.20 a	76.80 a
1×10^7	71.40 a	77.10 a	81.80 ab
1×10^9	85.80 bc	90.40 cd	96.60 d

* a or ab: The same columns and row are not significantly different.

Table 4 demonstrates the comparison between the three entomopathogenic fungi treatments: *M. anisopliae*, *B. bassiana* and their mixture used at each volume of spore suspension. The results illustrated that using 20 ml of spore suspension/100 g soil scored significantly the highest mortality rate with all concentrations 1×10^6, 1×10^7 and 1×10^9 conidia/mL. The 1×10^9 conidia/ml concentration of the three treatments recorded the highest mortality at all volumes. However, the mixture of *M. anisopliae* and *B. bassiana* treatment showed significantly the highest mortality at all concentrations and the volumes in comparison with using fungi individually. *M. anisopliae* scored higher mortality than *B. bassiana* in almost all volumes and concentrations.

Table 4. Comparison of mortality rate of last instar *Ceratitis capitata* larvae exposed to *M. anisopliae* and *B. bassiana* and their mixture.

Concentration conidia/ml	Spore suspension Volume/100 g soil	Mortality %		
		B.bassiana	*M. anisoplae*	*B.bassiana +M.anisoplae*
10^6	10	32.83 a *	40.60 a	60.70 b
	15	36.00 a	36.50 a	61.20 a
	20	37.93 a	48.10 a	76.80 b
10^7	10	38.06 a	51.10 a	71.40 a
	15	47.66 a	57.10 ab	77.10 b
	20	50.43 a	59.00 ab	81.80 b
10^9	10	55.71 a	61.30 a	85.80 a
	15	59.40 a	64.03 a	90.40 b
	20	65.70 a	73.80 a	96.60 b

* a or ab: The same columns and row are not significantly different.

Table (5) expresses the LC_{50} using 20 ml spore suspension/100 g soil. It can be seen from the table that mixture treatment had has the lowest LC_{50} at (234x 10^7) in comparison with other treatments. Meanwhile, *B. bassiana* showed the highest LC_{50} (1.17x 10^{10}) required followed by *M. anisopliae* that reached (552 x 10^8).

Table 5. LC_{50} of three 1×10^6, 1×10^7 and 1×10^9 (conidia/mL) concentrations at the volume (20 ml/100 g soil) that scored highest mortality rate.

Fungal species	LC_{50}	Ch square(x^2)	df
B.bassiana	1.17 x 10^{10}	2.599	7
M.anisoplae	552 x 10^8	3.381	7
B.bassiana + M.anisoplae	234 x 10^7	1.033	7

DISCUSSION

The study results revealed that *M. anisopliae* alone expressed the higher mortality rate than *B. bassiana* at mostly all spore suspension concentrations and volumes. Ihara *et al.* (2001) found that *M. anisopliae* isolates virulence were significantly higher than *B. bassiana* on controlling brown-winged green bug, *Plautia stali*. Herlinda (2010) also reported that *M. anisopliae* tested isolates produce high spore density on *Aphis gossypii* in comparison with *B. bassiana* isolates which might be an explanation to the higher mortality rate. Moreover, Garrido-Jurado *et al.*(2011) found that *B. bassiana* isolates were less virulent than *M. anisopliae* on fruit fly, *Ceratitis capitata* third instar larvae. Mixture of both entomopathogenic fungal species used revealed significantly higher mortality rate than the individual fungal species in all volumes of concentrations, with mortality rate of over 96% at the concentration 1×10^9 and volume 20ml/100gm soil. Moreover, the lowest LC_{50} of 234x 10^7 was depicted for treatment with the mixture of both fungal species. The increased mortality rates encountered with the mixture might be due to the synergistic effect of both fungal species. Bukhari *et al*.(2011) found that the mixture of *B. bassiana* and *M. anisopliae* increased mortality rate among malaria mosquito larvae in comparison with using them individually. Maranga *et al.* (2005) as well reported that formulating both fungi species scored high mortality rate among of the tick *Amblyomma variegatum*. The study results showed that increasing spore suspension concentration and volume can increase mortality rate. This explains the highly scored mortality rate at 1×10^9 concentration of volume 20ml/100gm soil. As a result, for field treatment : the study recommend applying the mixture 1×10^9 with 200 ml /m^3 according to the volume transformation.

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