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Laboratory evaluation of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* against adults of *Dacus ciliatus*

BASSIM SHEHAB HAMAD
Phd Biology
Ministry of Science and Technology
Directorate of Agric. Res. / IPM Center, Iraq
MOHAMMED J. HANAWI
Phd Biology
University of Wasit, College of Science, Iraq
MOHAMMED KHALAF ABDULKAREEM
MSc. Agriculture
Ministry of Science and Technology
Directorate of Agric. Res. /IPM Center, Iraq
AKMAM ALI HABBEB
MSc. Biology
University of Wasit, College of Science, Iraq

Abstract:

The susceptibility of adults of Dacus ciliatus to the fungal isolates of Metarhizium anisopliae (MARD106 and MARD10) and Beauveria bassiana (MARD54 and MARD66) were evaluated under laboratory conditions. The results of the effect of different concentrations of these isolates on the survival rate of adults showed the percentage of survival female which treated with 10^5 ,$10^7$and 10^9spore ml^{-1}concentrations of MARD106 were 96 , 80 and 32% , respectively, and the survival of male were 100 , 96 and 28%, respectively. The mortality of females at age 1, 4, and 7 days treated with the isolate MARD66, were 100, 62.5 and 35% respectively (r = -0.95), and the mortality of males were 100, 80, and 5%, respectively (r

= -0.99). The spores transferred between untreated males and treated females during mating causing mortality of males at rate don't differ from females significantly. The highest volume spray on fruit (9 ml/fruit (10^{-7} spore ml^{-1}) of B. bassiana MARD66 producing the highest number holes of female oviposition (22.6 hole/fruit),comparing with 6 and 2 ml /fruit (10^{-7} spore ml^{-1}) that produce 12 and 2 hole/fruit, respectively. One of the most important stages in the life insect aimed by control agents is the last larval instar; in this study treated this stage with the fungi B. bassiana and M. anisopliae were caused 100% mortality.

Key words: entomopathogenic fungi, *Beauveria bassiana*, *Metarhizium ansiopliae*, *Dacus ciliatus*

INTRODUCTION

Dacus ciliates is a major significance agricultural pest of a wide range of Cucurbitaceae in Africa, Asia and the Middle East (Hancock, 2012). The pest was recorded in Iraq in 1988, which represents the main serious tephritid pest that primarily attacks cucurbit crops (Moanas & Abdulrassol, 1989). Punctures on attacked fruit represent the main signs of oviposition. Eggs are laid inside the attacked fruit, then hatch into larvae feed for six days. These two stages of insect life causing reduction in fruit quality and quantity. Synthetic fungicides are primarily used to control the loss that caused by the pest. However, recently, the integrated pest management is considered an effective, profitable and environmental-friendly management of cucurbit flies by many countries (Bano2003)**.**Of various biological approaches, the use of fungal antagonistic microorganisms is most widely accepted mechanism throughout the world. The fungal entomopathogens *Metarhizium ansiopliae*(met.) and *Beauveria bassiana*(Bals.), belonging to the Hyphomycetes group, are natural important enemies of

insect species that spend at least one stage of their life cycle in the soil (Toledo, et al., 2008). Aemprapa (2007) shows that the two fungi caused 50% reduction in population of *D. ciliatus.* Furthermore, studies show that the two fungi cause high rate of mortality up 85% in some species related to orders Diptera, Hymenoptera, Coleoptera and Hemiptera (Veen 1968; Mahmoud 2009; and wriaght *et al* 2000).The successful infection of an insect host is through adhering a sufficient number of conidia to the cuticle layer. Conidia germinate on and penetrate all regions of the cuticle (Pekrul and Grula, 1979). Penetration happens via secreting fungal enzymes and may occur after 16 - 18 hrs.post inoculation by aggressive pathogenic strains (Pekrul and Grula, 1979). The clean hole at the cuticle layer of infected insects is the main sign of penetration. The fungus may also enter the respiratory system via conidial contamination or through the opening or side of the spiracle. Although they can also be ingested and enter the organism through the wounds (Madelin 1963; Sahayaraj et al 2014).

The susceptibility of various developmental stages of *Dacus ciliatus*(larvae, and adults) to the entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana* was evaluated under laboratory conditions. This study investigates the efficacy of fungal infection at adults and efficacy of spore transferring of both fungi among mating adults and the effects of fungal isolates on the last larval instar.

MATERIALS AND METHODS

Insect rearing

Infected cucumber fruit were placed in the cubic glass boxes (40 cm^3) under laboratory condition at 27 ^oc, 60 ±5% humidity and 16 hours photoperiod. After completing insect life cycle the adults were transferred into 20cm^3 glassy cubic boxes equipped

with dried yeasts as a protein source (Morelli et al; 2012), and 15 cm Petri dishes containing 5% sugar solution (5g glucose and 95 ml tap water). Uninfected cucumber fruits were placed for ovipostion, and replaced frequently with new uninfected fruits (kieser, 1972).

Fungal spore suspension preparation

Two isolates of each fungi *Metarhizium anisopliae* (MARD106 and MARD10) and *Beauveria bassiana* (MARD54 and MARD66) 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 haemocytometer. The viability of spore was determined as in Lacey (1997).

Effects of different spore suspension concentrations on the adult insects

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 insect 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 °c allowing fungi to grow.

Fungal pathogenicity at different adult's age

To investigate the effects of all fungal isolates on insects at different age, sixteen adults, 8 females and 8 males (which represents one replicate) at age 1,4, and 7 days, were sprayed with fungal suspension ($1x10^7$) each isolates separately until runoff. The treated insects were monitored and the percentages of morality were determined daily. Each treatment were distributed randomly and repeated five times.

Determination of spore's transmission among mating adults

To study the possibility of the spore movement from infected female to healthy males, five females were treated as above then placed in 20 cm^3 glassy boxes with five healthy males. Each box represents a replicate and all treatments distributed randomly with four replicates.

Influenced oviposotion behavior by volume of spore suspension

Uninfected cucumber fruits were sprayed with 2, 6, 9 ml a fruit at concentration $1x10^7$ spore ml^{-1} of *Beauveria bassian*a (m66 isolate) at 40 cm distance with hand sprayer then left for one hour to dry. Treated fruits were placed in boxes containing five of each sex and supplied with water and food for adults feeding. Holes will be counted due to oviposition. Each box represents a replicate and all treatments distributed randomly with four replicates.

Effects of fungal isolates on last larval instar

Larvae were dipped in the suspension of each isolates $1x10^7$ +1ml of 0.05% Tween80 for five second where the control was by dipping the larvae in the solution of water and 1ml of 0.05% Tween80 . After that all larvae were placed in tubes 46x 42 cm

(diameter x high) containing 40g sterile sand and 5 ml SDW and left to pupate. Average of pupation was measured after eight days. All dead larvae were transfer into 9 cm Petri dishes containing wet filter paper at 22 ^{o}c allowing fungi to grow. The experiment was designed to randomized complete design with four replicates (10 insect/ replicate)

Statistical analysis

All experiments were randomly distributed; ANOVA was used to compare means of three groups according to Duncan's test and T-test for means of two groups. The analysis was made using SPSS software 20 editions.

RESULTS AND DISCUSSION

Effects of different spore suspension concentrations on the adult insects

Understanding of the interaction between the pest and its pathogen is particularly important so that the virulent isolates of *M. anisopliae* and *B. bassiana* can be identified to adults of *D. ciliates* and to investigate the effects of conidial concentrations on adults' mortality. This understanding can lead us to better control methods with very effective and cost-efficient. The results shows that the effect of different concentrations of fungal isolates used in the treatments have varied impact on the survival adults. The variation in the mortality may be associated with amount of the spores that access insect bodies. The 1 x10^{9} spore ml^{-1} concentration achieved the highest ratio of mortality with shortest period time .The percentage of survival female which treated with 10^{5} and 10^{7} 10^{9}spore ml^{-1}concentrationsof *M.anisopliae*(MARD106) after 24 hours were 96 , 80 and 32% , respectively, and of male survives were 100 , 96 and 28%, respectively (Fig. 1). Other isolates also show differences in the survival rate of adult. The

rate of survivals treated with *M. anisopliae* (MARD10) were 96, 92 and 24% for males and 88, 68 and 8 % for females. Figs (2, 3, and 4) illustrate a negative relation between the timeline of total death and three concentrations of all isolates. Similar results were reported from *Bactrocera cucurbitae* infected with *Paecilomyces lilacinus*, Mortality was dose-dependent, with the highest mortality occurring at ($2.4x10^9$) after 5 and 7 days of treatment (Amala.2013). Dose–mortality responses have also been reported on many other arthropod pests, laboratory study for six concentrations of fungal isolates of Beauveria bassiana, *Metarhiziumanisopliae*. *Verticillium lecanii*,*Hirsutella thompsonii* and *Cladosporium oxysporum* on adult aphids *Aphis craccivora*. Showed the highest concentration of (10^8Spore ml^{-1}) resulting in highest death rate (Saranya *et al.* 2010).

Ekesi *et al.* (2000) find out that the highest rates of death were at highest concentrations (10^8Spore ml^{-1}) and the death rate decreased when concentration decreased.

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) evaluated 12 strains of *B. bassiana*, 7 of *M. anisopliae* and one of *Hirsutella citriformis*a gainst *Bactrocera dorsali* was treated with 1×10^8 spore $ml^{-1}$and found one strain of *B. bassiana*was virulent achieving mortalityup to 68%. Similarly, Munoz (2000), testing 16 isolatesof*B.bassiana*for the control of mediterranean fruit fly reported a mortality range between 20-98.7%. Our results are in agreement with other studies that confirm

susceptibility of adults of fruit flies to the entomopathogenic fungi. 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) 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.

Fungal pathogenicity at different adult's age

The effects of four fungus isolates on the mortality of females of the cucurbit fly at different ages(1, 4, and 7 days) were assessed in a laboratory experiment to find an isolate suitable for biological control. The effects of fungus treatments on mortality, of females varied considerably among the isolates. The mortality of females at age 1, 4, and 7 days caused by MARD66, were100, 62.5 and 35% respectively (r= -0.95) (fig 5). For males were 100, 80, and 5%, respectively (r =-0.99), (fig 6). The rest of isolates caused the same mortality levels.

Fig 7 shows the comparison between treated females and males at the same age, none of isolates (except M10 isolate) caused major mortality differences between the sexes at age one day. MARD10 isolates caused death in male 100% and females 90% (p= 0.028). At age 4 day the differences among isolates were significant except M54 which caused 37.5 %females and 42.5% males mortality (p= 0.08). The effects of fungus treatments on mortality of adult flies at age seven day were insignificant (p= 0.082).

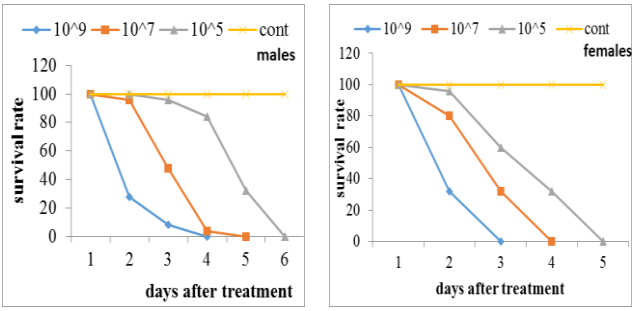
Determination of spore's transmission among mating adults

The spores are also transferred between males and females during mating that may resulting in the death of males at end of the experiment (6 days). The percentage of mortality between

sexes was insignificant except MARD106 which caused 75% males and 100% females. In some studies, it has been that the effects of *M. anisopilae* on the mortality of untreated males of three species of fruit fly C*eratitis capitata, Ceratitis cosyra* and, *Ceratitis fasciventris* were 83, 73, and 72%, respectively, after mating with treated females. (Dimbi, 2013).And mating between untreated females with treated males resulting in the death of the former at percentage 100, 83, and 95, respectively. Indicting that the probability of spore transmission between sexes is likely to happen in the fields (Meadow et al.,2000)

Influenced oviposition behavior by volume of spore suspension

The highest of spray volume9 ml/ fruit (10^{-7} spore ml^{-1}) of *B. bassiana* MARD66 isolate producing the highest number holes (22.6 hole/fruit) due to oviposition comparing with 6 and 2 ml/ fruit (10^{-7} spore ml^{-1}) that have 12 and 2 hole/fruit respectively(fig.9),. Similar studies were reported different results on *Ceratitis capitata* treated with *B. bassiana* the number of holes due to females oviposition were 4, 0.6, and 1.4 of the volumes 1.8, 5.4, and 9 ml/ fruit (Salvatore et al.2009). Rosas-Acevedo et al. (2003) mentioned that the exudates from *Hirsutella thompsonii* might affect oviposition of *Tetranychus urticae* negatively. However, the egg hatchability was not affected. Further studies are needed to estimate egg hatchability of *D. ciliatus treated with the B. bassiana*and *M. anisopliae.* These characteristics make the two entomopathoginic fungi very promising control agents for *D. ciliates* (wekisa et al, 2007).



Fig(1) Survival curve of *Dacusciliates* adults after exposure to fungal isolate *Metarhizium anisopliae* MARD106

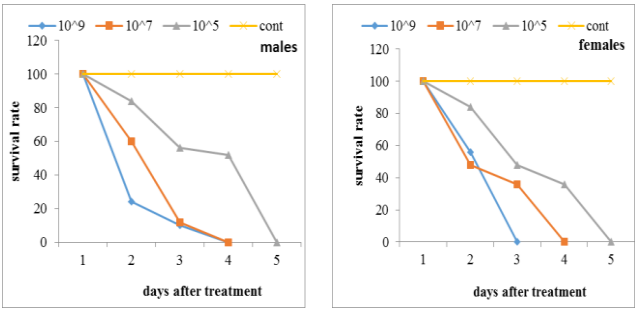


Fig.(2) Survival curve of *Dacus ciliates* adults after exposure to fungal isolate *Beauveria bassiana* MARD66

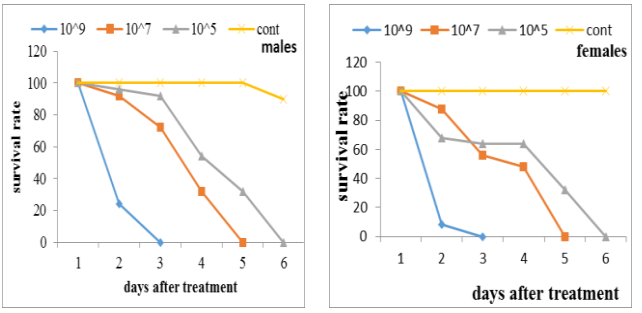


Fig.(3) Survival curve of *Dacus ciliates* adults after exposure to fungal isolate *Metarhizium anisopliae* MARD10

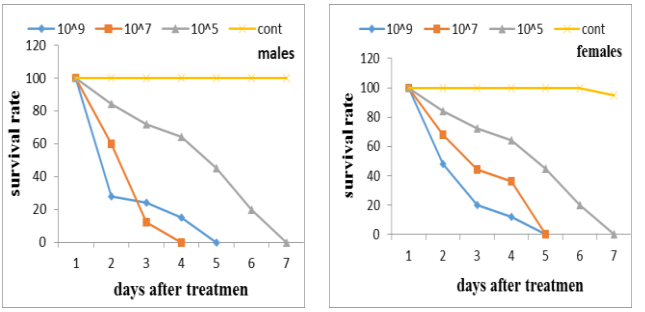
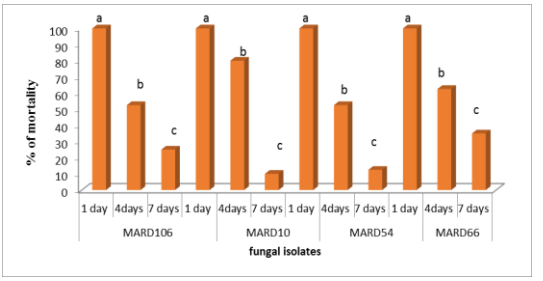
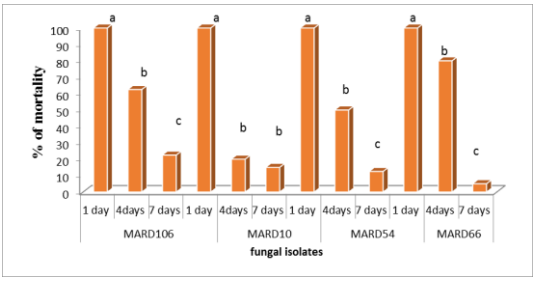


Fig.(4) Survival curve of *Dacus ciliates* adults after exposure to fungal isolate *Beauveria bassiana* MARD54



*Values followed by different letters are significantly different (Duncan's multiple range test,$p<0.05$

Fig. (5) Efficacy of different fungal isolates *Metarhizium anisopliae* (MARD106 and MARD10) and *Beauveria bassiana* (MARD54 and MARD66) on *D.ciliatus* female adults at different ages



*Values followed by different letters are significantly different for each isolate(Duncan's multiple range test,$p<0.05$

Fig.(6) Efficacy of different fungal isolates *Metarhizium anisopliae* (MARD106 and MARD10) and *Beauveria bassiana* (MARD54 and MARD66) on *D.ciliatus* male adults at different age

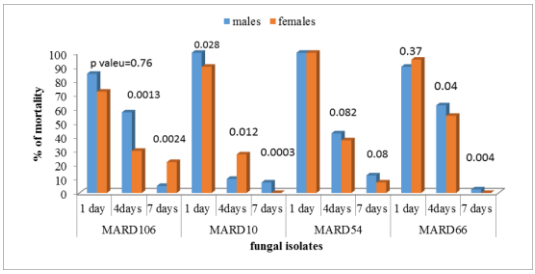
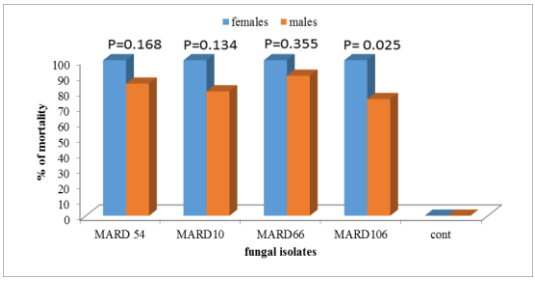
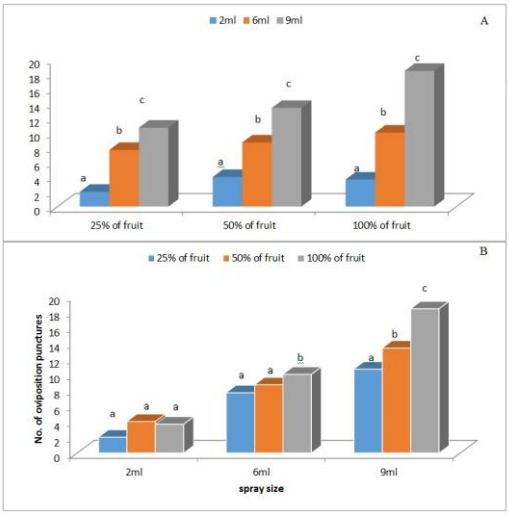


Fig.(7) Effect of fungal isolates *Metarhizium anisopliae* (MARD106 and MARD10) and *Beauveria bassiana* (MARD54 and MARD66) on male and female adults of *Dacus ciliatus* at the same ages



***** *Metarhizium anisopliae* (MARD106 and MARD10) and *Beauveria bassiana* (MARD54 and MARD66)

Fig. (8) Spore's transmission from inoculated females of *Dacus ciliatus* to clean males during the mating.



*Values followed by different letters in same percent of fruit(A) and same spray size(B) are significantly different (Duncan's multiple range test,$p<0.05$

Fig.(9) Effect of percent of fruit surface sprayed and spray size of fungal suspension at concentration $1x10^7$ spore ml $^{-1}$of *Beauveria bassiana* (MARD66) on cucumber fruits on oviposition behavior of *Dacusciliates* females.

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