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Efficacy of some local isolates of *Beauveria bassiana*(Bals.) and *Metarhizium anisopliae* (Met.) in controlling of cucurbit fruit fly *Dacus ciliatus*

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Abstract:

The effects of different isolates of entomopathogenic fungi Metarhizium anisopliae (MARD106 and MARD10) and Beauveria bassiana (MARD54 and MARD66 were evaluated on Dacus ciliatus adults in the lab. The result revealed that the MARD66 isolate reduced survival rate of males adults to (50%) followed by the MARD54 isolate (60%) in the first two days of treatment and 10 and 12% respectively in the third day, While, it was 72%for the MARD10 isolate and 48% for the MARD106 isolate. The treatment of females revealed that the MARD66 and MARD54 isolates reduced the survival rate to 48% and 68% at second day respectively. The result also showed that the MARD66 isolate killed 50% of the population (LC_{50}) at concentration of 1×10^5 spore /ml in comparison with 1×10^6 spore /ml for MARD54 isolate on males and females , the same concentration was pointed out for MARD10 isolate on females and 1×10^8 spore/ml for males. The less LT_{50} value at the concentration of 1×10^9 spore/ml was 1.35 days for the MARD66 isolate on males and 0.94 days on females. According to the obtained results, it can be concluded that the studied fungal isolates of

B. bassiana and M. anisopliae had efficacy to control D. ciliatus adults According to the obtained results, it can be concluded that the studied fungal isolates of B. bassiana and M. anisopliae had efficacy to control D. ciliatus adults with superiority to MARD66 isolate.

Key words: Beauveria bassiana, Metarhizium anisopliae, Dacus ciliatus

INTRODUCTION

The cucurbits fruit fly *Dacus ciliatus* (Loew) is a major pest of cucurbits in some countries (Harris and White Elson-, 1994 and 1992) including Iraq, as recorded for the first time in the fall of 1988 then its harm exacerbate and is threatening many cucurbits crops, (. Moanas, and Abdul - Rassol, 1989) .The infestation of this pest impact on the fruit quality by the holes of the egg-laying followed by deformation and wrinkle of fruits as well as rot after feeding of larvae on the pulp and seeds of those fruits. Its belonging to the Tephritidae family; many of their species cause severe economic losses as a result of yield reduction, this pest causes economic losses up to 100% of the total production (BA-Angood, 1977).

Because of the incompetence of one factor or one way and the difficulty of relying on pesticides in the management of this pest due to the shortage of ripening periods of fruit, harvesting, marketing and consumption. Accordingly, the integrated pest management (IPM) is more acceptance strategy, including entomopathogens, especially fungi that represent the most important factors due to their efficacy and compatibility with the environment and human health. 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). These entomopathogenic fungi showed high virulent to fruit flies in Thailand, their reduction of the infestation was 50% (Aemprapa, 2007). High pathogenicity was registered for *M. anisopliae* fungus against pests that present in the soil, such as termites, locusts, hoppers and roots borers (Aemprapa, 2007), as well as more than 200 insects belonging to the Orders of lepidoptera , hemiptera, diptera, Coleoptera and Hymenoptera (Veen, 1968). High ability of *B. bassiana* fungus in controlling of the olive flies (Mahmoud, 2009). Also, It was reduced the population density of whitefly nymphs at 85% percentage (Wraight and others .2000.)

According to the importance of this pest in all parts of the world, including Iraq and the need to apply the successful control and reduce the use of chemical pesticides as much as possible, this study aimed to measure the effectiveness of local isolates of the two fungi *Beauveria bassiana* (Bals.) And *Metarhizium anisopliae* (Met.) against *Dacus ciliatus*.

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).

Culture of Fungi and preparation of suspension

Two isolates of each fungi *Metarhizium anisopliae* (MARD106 and MARD10) and *Beauveria bassian*a (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 hemocytometer. The viability of spore was determined as in Lacey (1997).

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 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.

STATISTICAL ANALYSIS:

The data were analyzed by standard probit analysis to obtain the Median lethal concentrations (LC50) and LC90 in addition to the time taken to kill 50% (LT50) and LT90 within SPSS system, version 20.

RESULTS AND DISCUSSION:

The results of the survival rate of *D.ciliatus* adults males after exposure to spore suspension of different fungi isolates(Figure 1) revealed that the (MARD66) isolate was more effective as it brought on a rapid decline in the survival rate (50%) after 2 days , followed by (MARD54) isolate (60 % survival). The decreasing of survival rate reached to 10 and 12%, after 3 days for the above isolates respectively, while, it was 72% for the (MARD10) isolate and 48% for the (MARD106) isolate. Similar superiority results (Figure 2) was found on the females, the isolates (MARD66) and (MARD54) gave 48 and 68% of survival rate in the second day after treatment respectively.

Measuring of the median lethal concentrations (LC_{50}) (Table 1) as an expression of isolates efficacy showed that the MARD66 isolate are the most effective, according to the LC_{50} value that was 10^5 Spore / mL on males and females compared with other isolates (10^6 Spore / ml) for MARD54 isolate on males and females and for MARD10 on females and 10^7 Spore / ml for males, finally LC_{50} value was 10^8 Spore / ml for the MARD106 isolate. The lethal concentration of 90 %(LC_{90}) for females treated with MARD66 isolate was 10^8 Spore / ml.

The isolates was differed in the time taken to kill 50% of an insect population (LT_{50}) that was Decreased according to an increase of concentrations (table 2), the less value was 1.35 days at a concentration of 10^9 Spore / ml for MARD66 isolate, to 3.24 days at concentration of 10^5 Spore / ml on males while, it was 0.94 and 3.0 days on females. In general, the results showed females were more susceptibility for the studied isolates compared with the males.

Selection of the fungal isolates as a successful biocontrol agents requires some characteristics such as high pathogenicity, specialty, ease of mass production and adaptation to the environmental conditions (Reay et al. 2008;

Ptlamul and Parasertan 2012) The pathogenicity is the most importance index when measuring the effectiveness of pathogenic fungi against pests and the principle, which relies on at the bioassay (Robert and leger, 2004; Jin, et al.2008). Such effectiveness differences of fungal isolates are pointed out in many studies, the pathogenicity of 10 isolates of *B. bassiana* fungus and 5 isolates of *Metarhizium anisopliae* fungus were measured against pupae and adults of Mediterranean fruit flies *Ceratitis capitata*, the mortality was ranged between 30-100% with survival time ranged from 5.6 to 6.8 days(Quesada *et al* ,2006). At screening of 7 isolates of *Metarhizium* spp. and 12 isolates of *Beauveria* spp. and one of the *Hirsutella citriformis* ,all of them were sprayed at concentration of 10^8 Spore / ml against *Bactrocera dorsalis*, Sirinun, (2007) found the best isolate was *B.basiana* 6241 (68% mortality). The current study compatible with many of the studies that confirm the sensitivity of fruit flies adults to entomopathogenic fungi (Konstantopoulou & Mazomenos, 2005; Yee & Lacey, 2005). The sensitivity difference between males and females to pathogenic fungi which was explained by the current study has pointed in previous studies, Dimbi et al. (2003) confirmed fastest mortality of *Ceratitis* sp. females compared with males when treated with *Metarhizium anisopliae* fungus, while other studies (Carsewell et al. 1998) have confirmed the absence of this difference in the *C. capitata* and *B. tryoni* after treatment with *Metarhizium anisopliae* .

According to the obtained results, it can be concluded that the studied fungal isolates of *B. bassiana* and *M. anisopliae* had efficacy to control *D. ciliatus* adults with superiority to+ MARD66 isolate.

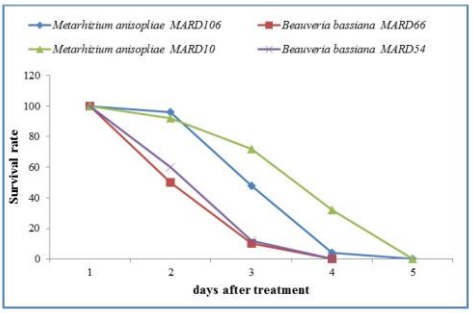

Fig.(1): survival curve for cucurbits fruit fly *Dacus ciliates* (males) treating with differences fungal isolates of *B.bassiana* and *M.anisopliae* at concentration of 10^{7}.

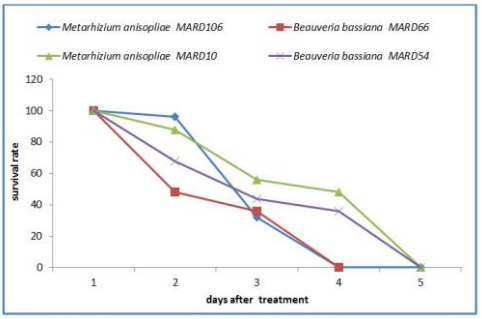

Fig.(2): survival curve for cucurbits fruit fly *Dacus ciliates* (females) treating with differences fungal isolates of *B.bassiana* and *M.anisopliae* at concentration of 10^{7}.

Table (1): Median lethal concentration (LC50) and LC90 of different isolates of *Beauveria bassiana* and *Metarhizium anisopliae* against cucurbits fruit fly *Dacus ciliates* adults

Fungal isolates	Sex	LC_{50}	Confidence limits (95%)	LC_{90}	Confidence limits 95%
M. anisopliae (MARD10)	♂♂	10^{7}	$10^{6} – 10^{8}$	10^{9}	10^{8} -10^{12}
	♀♀	10^{6}	10^{5}-10^{7}	10^{10}	10^{8}-10^{14}
B.bassiana (MARD66)	♂♂	10^{5}	10^{5}-10^{8}	10^{11}	10^{10}-10^{14}
	♀♀	10^{5}	10^{4}-10^{6}	10^{8}	10^{7}-10^{12}
B.bassiana (MARD54)	♂♂	10^{6}	10^{4}—10^{7}	$10^{9.5}$	10^{8} -10^{22}
	♀♀	10^{6}	10^{5}-10^{7}	10^{10}	10^{8}-10^{15}
M. anisopliae (MARD106)	♂♂	10^{8}	10^{7}-10^{9}	10^{11}	10^{9}-10^{22}
	♀♀	10^{7}	10^{6}-10^{7}	10^{8}	10^{8}-10^{9}

Table 2: The time taken to kill 50% of adults' population of cucurbits fruit fly *Dacus ciliates* (LT_{50}) and LT_{90} for different isolates of *Beauveria bassiana* and *Metarhizium anisopliae*

Fungal isolates	Sex	concentration	LT_{50} (days)	LT_{90} (days)
M. anisopliae (MARD10)	♂♂	10^9	1.72	2.26
		10^7	3.32	4.75
		10^5	4.07	5.53
	♀♀	10^9	1.25	2.09
		10^7	3.25	5.19
		10^5	3.3	7.05
B.bassiana (MARD66)	♂♂	10^9	1.35	2.6
		10^7	2.15	2.99
		10^5	3.24	5.46
	♀♀	10^9	0.94	2.86
		10^7	2.06	3.72
		10^5	3	4.83
B.bassiana (MARD54)	♂♂	10^9	1.45	3.79
		10^7	2.15	3.56
		10^5	6.84	20.24
	♀♀	10^9	1.92	3.52
		10^7	2.7	5.1
		10^5	4.82	14.03
M. anisopliae (MARD106)	♂♂	10^9	1.61	2.67
		10^7	3.02	3.89
		10^5	5.67	8.89
	♀♀	10^9	1.7	2.71
		10^7	2.73	3.39
		10^5	5.77	8.76

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