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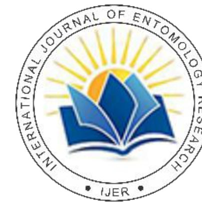

Pathogenicity of *Metarhizium anisopliae* isolates on adults of the Mediterranean fruit fly *Ceratitis capitata* (Diptera: Tephritidae) under laboratory condition

[*1] Hayder Shammer Abbas, [2] Ammar Ahmed Sultan, [3] Bassim Shehab Hamad
[1] M.SC. Biology, University of Diyala, College of Education for Pure Science, Baqubah, Iraq
[2] Ph. D Biology, University of Diyala, College of Education for Pure Science, Baqubah, Iraq
[3] Ph. D Biology, Ministry of Science and Technology Directorate of Agric. Res. / IPM Center, Iraq

Abstract
The effectiveness of some Iraqi isolates of *Metarhizium anisopliae* (Metchnikoff) Sorokin (Ma-h, Ma88, Ma19 and Ma29) was evaluated against adults of the Mediterranean fruit fly *Ceratitis capitata* under laboratory condition. Depending on the LC50 values, the isolate Ma88 was the highest mortality for males (8×10^6 spores\ ml) followed by Ma29 isolate with median lethal concentration of 1.3×10^{7} spores\ ml. Exposing of females to mentioned isolates demonstrated that the LC50 values were very close, it was 2.0×10^6 spores\ml. for Ma88 isolate and 2.2×10^6 spores\ml for Ma29 isolate. The median lethal time (LT50) of tested isolates explained that the Ma88 isolate was the most virulence against males (4.99 days) followed by Ma-h isolate (6.27 days). In treatment of females, the LT50 was 5.17 days for Ma88 isolate and 5.42 days for Ma29 isolate. The result revealed that males were greater susceptible than females when exposed to Ma-h isolate, while the females were higher susceptible to all local isolates. The results also demonstrated that Ma88 isolate was the most capability in analyzing the culture medium to obtain the growth requirements, especially carbon element from chitin medium as only source with significant differences from other fungal isolates, this was compatible with its virulence against adults. All fungal isolates were statistically equivalent in analysis of the protein source in culture medium.

Keywords: *metarhizium anisopliae*, pathogenicity, *ceratitis capitata*

1. Introduction

The Mediterranean fruit fly (Medfly, *Ceratitis capitata*) (Wiedermann) (Diptera: Tephritidae) is one of the most destructive pests of horticultural crops which infects more than 300 species of fruits and vegetables (Aluja and Mangan, 2008). Citrus fruits are the most harmful, peaches, pears, apples and others, where larvae feed on the pulp of the host fruit and eventually turn into an inedible. The predominant method of control has been the use of traditional insecticides. However, the continued use of insecticides has caused enormous problems; environmental pollution, development of insecticide resistance and contamination of products (Roessler 1989; Cabras *et al*. 1997) [33, 5]. As an alternative to chemical control or as a part of Integrated Pest Management (IPM) programs. Fungal agents belong to the most promising group of biological control agents against insect pests, particularly the Deuteromycete fungi (Barson *et al*. 1994 [4]; Watson *et al*. 1996 [35]: Reithinger *et al*. 1997 [32]. The *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) [34]. The conidial phase (spores) of a large number of strains of this 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 [17], Campos 2000 [6], Castillo *et al*. 2000 [8], Lezama-Gutierrez *et al*. 2000 [24], De la Rosa *et al*. 2002 [10], Ekesi *et al*. 2002) [14]. The objective of this study was to evaluate the virulence of *Metarhizium anisopliae* (Met.) (Ma-h, Ma88, Ma19 and Ma29) against adults fly of *Ceratitis capitata*

2. Materials and methods

Fungal culture

The entomopathogen isolates of *Metarhizium anisopliae* (Ma-h, Ma88, Ma19 and Ma29) were cultured on potato dextrose agar medium (PDA) autoclaved at 121○C (15 Psi) for 15-20 minutes and poured into sterilized Petri plates. The Petri plates containing PDA medium were incubated at 27 ± 1○C, 80 ± 5% relative humidity and photoperiod of 12 hours. The conidia were harvested gently by scraping the surface of 15-days old culture with inoculation needle. The conidia were suspended in distilled water containing 0.1% Tween-80. The mixture was stirred on a magnetic shaker for 10 minutes. The hyphal debris was removed by filtering the mixture through fine mesh sieve. The conidial concentration of final suspension was determined by direct count using haemocytometer. Suspension concentrations (1×10^5, 1×10^7 and 1×10^9 conidia ml^{-1}) was prepared and used in bioassay.

Bioassay

Three concentrations of each fungal isolates 1×10^5, 1×10^7 and 1×10^9 (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 ºc allowing fungi to grow.

Enzyme activity of fungal isolates

i) Chitinase enzyme

Assay of chitinase activity was performed with colloidal chitin as a sole carbon source, The method of Baharlouei *et al*. (2010) [3] was used to prepare the colloidal chitin, then chitin culture medium was prepared by adding 5 ml of colloidal chitin, 2 g of NaO3, 1 g of KH2Po4, 0.5 g of MgSO4 and 0.5 g KCl to a liter of distilled water and adjusted the pH to 5 then add 20 g of agar, the culture medium was sterilized with the autoclave and then poured into Petri dishes. 5 mm diameter discs of 7 days culture of each isolates were placed in the center of the Petri dishes with chitin culture medium. Dishes were incubated in the dark at temperatures of 27 ºC. radial growth was recorded (Ouedraogo *et al*. 1997) [25].

ii) Protease enzyme

Assay of general proteolytic activity was performed with peptone as a sole of nitrogen source, protein medium was Prepared by adding 10 g glucose, 2 g peptone, 1 g $KH2PO_4$, 0.5 g $MgSO_4$ and 0.5 g KCl to a liter of distilled water and then pH adjusted to 5 then the same steps of preparation of chitin was continued.

Statistical analysis

Mortality data were analyzed with one-way analysis of variance (ANOVA) of

Arcsine transformed corrected mortality (Abbott, 1925). [1] Means were separated by the Duncan Multiple Range Test. LT50s were calculated by probit analysis (Finney, 1971) [19]. The SPSS 20.0 software was used for statistical analysis.

3. Results and discussion

The mortality rate of *C. capitata* was varied according to the concentrations of fungal suspension of Ma-h, Ma88, Ma19, and Ma29 isolates of *M.anisopliae*. After 5 days of treatment, females mortality rate by exposure to Ma88 isolate was 30 and 50% for the concentrations of 10^5 and 10^9 spores / ml respectively, the highest mortality rate was 83-100% after 15 days. mortality rates ranged between 0 and 20% for the concentrations of 10^5 spores / ml and 10^9 spores / ml respectively by treating with Ma-h isolate and increased to 15 and 40 % after 10 days. Exposing to Ma19 and Ma29 isolates at concentration of 10^5 spores / ml the mortality were 56 and 40% increased to 70 and 90% at concentration of 10^9 spores\ml. after 15 days of treatment. The difference between the concentrations was significant except the concentrations of 10^5 and 10^7 spores\ml of the isolate Ma88 after 5 and 10 days of treatment (Fig. 1 B and A). The Ma88 isolate gave the highest mortality rate for males (Fig. 2 A, B) that ranging between 27 and 56% at concentrations of 10^5 and 10^9 spores / ml, respectively after 5 days of treatment and 60 and 100 after 15 days. The Ma-h isolate gave 30 and 100% mortality after 15 days at mentioned concentrations. Mortality rates of these concentrations for the Ma19 isolate was ranging from 50 to 63% and for Ma 29 isolate were 56 and 73% respectively. The concentrations differed statistically, except the concentrations 10^7 and 10^9 of Ma88 isolate after 5 days. Comparing the fungal isolates depending on the LC50 values demonstrated that the isolate Ma88 was the highest effectiveness as mortality factor for males, the LC50 value was 8×10^6 followed by the isolate Ma29 with LC50 value 1.3×10^7 (Table 1). The mentioned isolates were very close in their effect on females, the LC50 was 2.0×10^6 and 2.2×10^6 for Ma88 isolate and Ma29 isolate respectively (table 2). The shortest LT50 for males treatment was 4.99 days for Ma88 isolate and 6.27 days for Ma-h isolate. It was 5.17 days for isolate of Ma88 and 5.42 days for Ma29 isolate for females treatment. Comparing the susceptibility of males and females to tested fungal isolates (Fig. 4) showed greater susceptibility of males when exposed to the Ma-h, while the susceptibility of females was higher than males for all local isolates. Efficacy of pathogenic fungi against pests under laboratory bioassay is used as initial indicator for the preference and the selection of fungal isolates as successful biocontrol agents is depending on their pathogenicity, specialization and easiness of mass production and adapt with environmental conditions (Reay *et al.* 2008; Ptlamul and Parasertan, 2012) [27, 31]. The differences among isolates of the same species is due to genetic variability, it was recorded in the isolates of *Beauveria bassiana* and *Metarhizium anisopliae* (De La Rosa *et al.* 2002; Garcia *et al.* 1984) [10, 22]. The effectiveness of entomopathogenic fungi such as *M. anisopliae* and *B. bassiana* on *C. capitata* adults and puparia have been reported by several authors (Garcia *et al*., 1989; Ekesi *et al*., 2002; 2003; 2005 [20, 13, 14, 16]; Konstantopoulou and Mazomenos, 2005; Quesada-Moraga *et al*. 2008; Dimbi *et al*., 2009) [23, 29, 12]. 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 log value of LC50 ranged from 3.8-10.5 depending on isolate, mortality by contact was significantly higher than by feeding, and treatment of fruits, especially by P. Bv32 and P. Bv39 that significantly reduced the infection by this pest (Qazzaz *et al.* 2015) [28]. Castillo *et al.* (2000) [8] reported 100% mortality in *C. capitata* treated with 1 x 10^6 con/ml of *M. anisopliae.* Other strains of this species have been found to be virulent to adults and immature of *Anastrepha fraterculus* (Wied.). Espin *et al.* (1989) [17] reported a mortality rate of 69-78% in his study on this pest. Quesada, *et al.* (2006) [30] evaluated effectiveness of *M. anisopliae* against pupae of Mediterranean fruit fly, he found that mortality were 30-100% during 6.5- 8.6 day period time. The susceptibility difference of the males and females to entompathogenic fungi have reported by Dimbi *et al.* (2003) [3] that found the females were more susceptible from males to *M. anisopliae*. Whereas, Carsewell *et al.* (1998) [7], found the mortality of females and males was not considerably different.

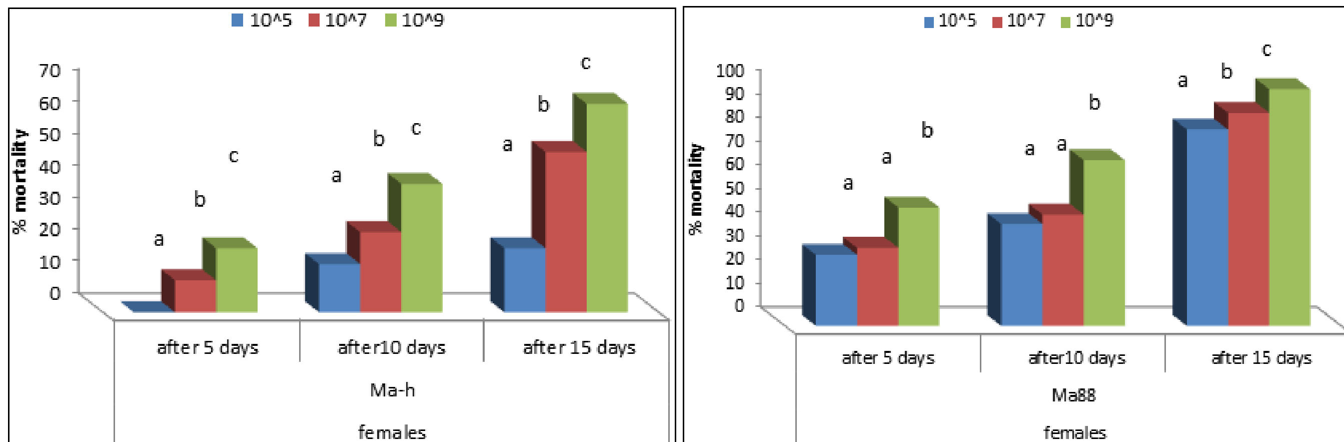

Fig (1-a): Mortality rates of adults (females) of *Ceratitis capitata* treated with different isolates of *Metarhizium anisopliae*

The rates followed by the same letter did not differ significantly according to the Duncan test (0.05)

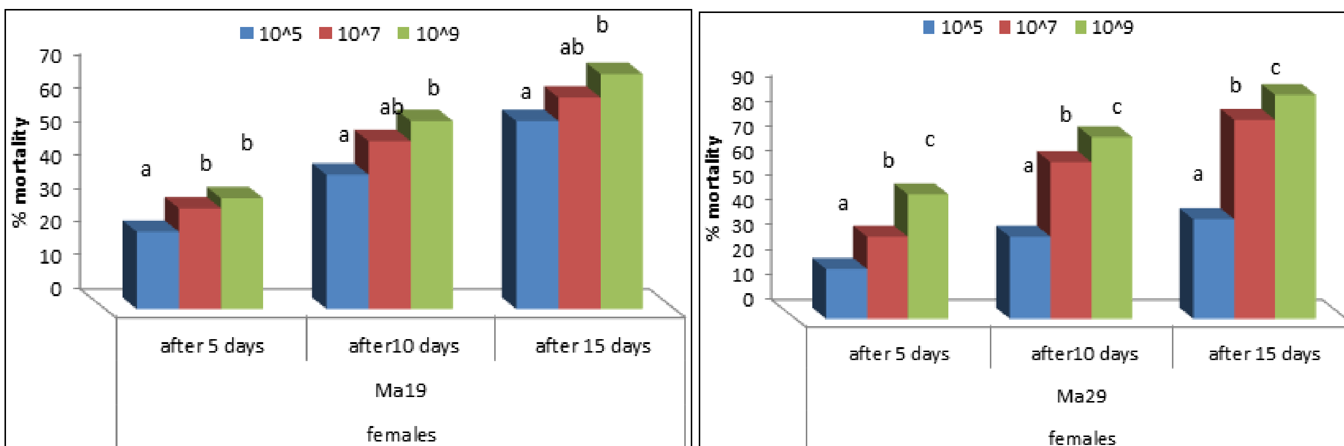

Fig (1-b): Mortality rates of adults (females) of *Ceratitis capitata* treated with different isolates of *Metarhizium anisopliae*

The rates followed by the same letter did not differ significantly according to the Duncan test (0.05)

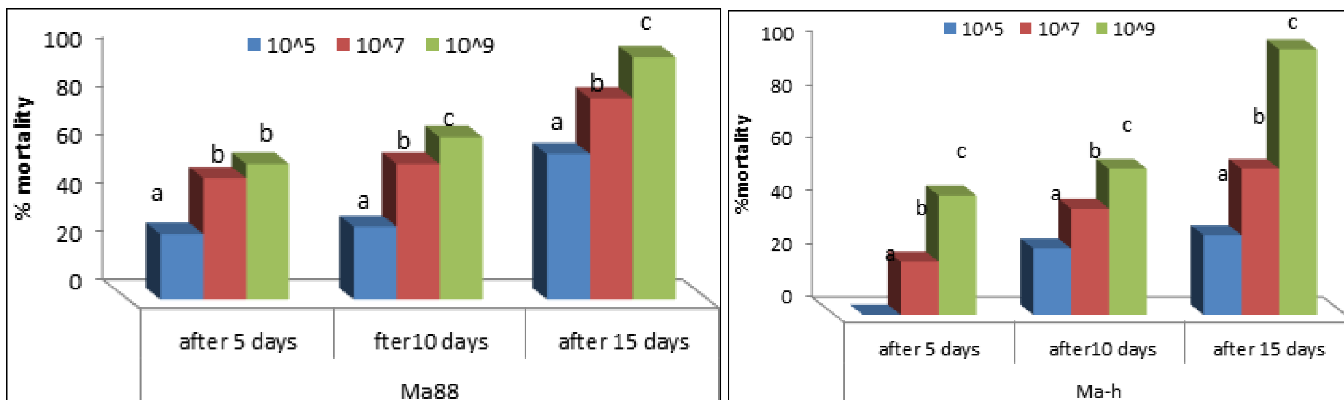

Fig (2-a): Mortality rates of adults (males) of *Ceratitis capitata* treated with different isolates of *Metarhizium anisopliae*

The rates followed by the same letter did not differ significantly according to the Duncan test (0.05)

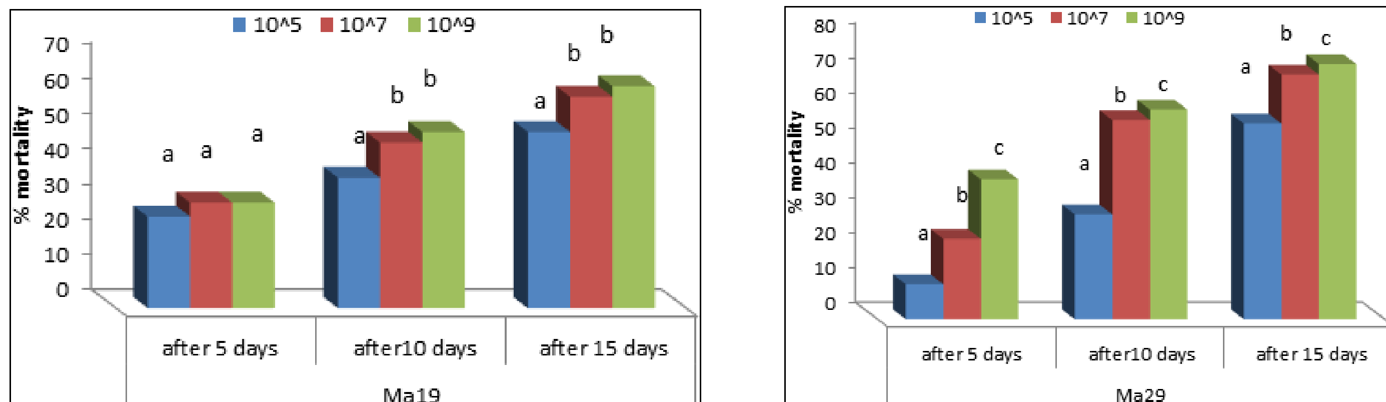

Fig (2-b): Mortality rates of adults (males) of *Ceratitis capitata* treated with different isolates of *Metarhizium anisopliae*

The rates followed by the same letter did not differ significantly according to the Duncan test (0.05).

Table 1: LC $_{50}$ values and LT 50 of *Ceratitis capitata* adults (males) treated with *Metarhizium anisopliae* isolates

Fungal isolates	LC50	X ^{2}	P Values	LT50	X ^{2}	P Values
Ma-h	2×10^{8}	4.7	0.6	6.27	95	0.00.
Ma88	8×10^{6}	12.35	0.08	4.99	67	0.00
Ma19	4.3×10^{8}	9.15	0.24	9.87	3.7	0.82
Ma29	1.3×10^{7}	18.2	0.01	7.0	5.28	0.63

Table 2: LC $_{50}$ values and LT 50 of *Ceratitis capitata* adults (females) treated with *Metarhizium anisopliae* isolates

Fungal isolates	LC50	X^2	P Values	LT50	X^2	P Values
Ma-h	2.1×10^{10}	8.78	0.27	13.1	8.2	0.31
Ma88	2.0×10^{6}	14.7	0.04	5.17	57	0.00
Ma19	2.2×10^{7}	9.7	0.23	8.3	6.15	0.52
Ma29	2.2×10^{6}	9.7	0.2	5.42	5.39	0.6

Enzymatic activity of fungal isolates

Entomopathogenic fungi are recognized biological control agents of insects. Basically, the entomopathogenic fungi pathogen activity depends on the ability of its enzymatic equipment, consisting of lipases, proteases and chitinases, which are in charge of breaking down the insect's integument. (de Carolina Sánchez-Pérez *et al.* 2014) [9]. The radial fungal growth is an indicator of the effectiveness of the isolates, its represent the ability of their enzymatic activity. Ma88 isolate had the most ability of analyzing the culture medium and obtain the growth requirements, especially carbon element from chitinous medium as sole source (fig 3), it was significantly higher than other fungal isolates, this activity was corresponded with their virulent against adults in previous trials. The analysis of protein of culture medium revealed that all fungal isolates weren't differ significantly in their enzymatic activity. Gupta *et al.* (1994) [21] found a relationship between the production of the highest levels of chitinase and protease with the virulence of *B. basiana* against *Galleria mellonela* and *Trichoplusiani*. Fang *et al.* (2009) [18] demonstrated that the gene expression of chitinase (Bbchit1) enhanced the virulence of *B. bassiana* on the *Myzus persicae* Sulzer. Studies have shown different enzymatic expression according to the different type of insects cuticle, Dias *et al.* (2008) [11] and Montesinos-Matías (2008) [26] produce Pr1 after fungal growth in presence of cuticle of *Tenebrio molitor* and *Hypothenemus hampei*.

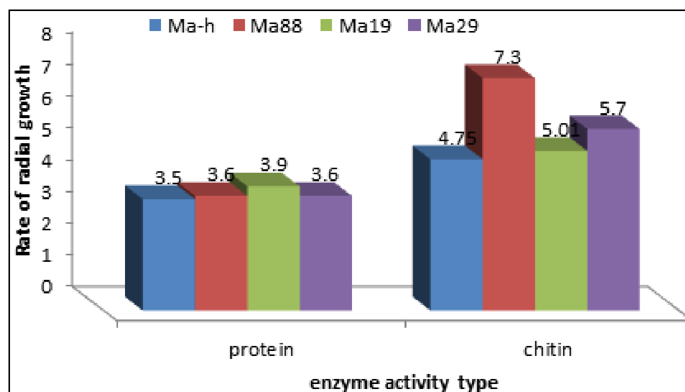

Fig 3: Rate of radial fungal growth on culture medium supplied with chitin as carbon source and peptone as nitrogen source.

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