Journal of Basic & Applied Mycology (Egypt) 10 (2019): 1-8 **p-ISSN 2090-7583, e-ISSN 2357-1047**
 http://www.aun.edu.eg/aumc/Journal/index.php

The Potential Effects of Entomopathogenic Fungus, *Beauveria bassiana* (Bals.-Criv.) Vuill., on Certain Genera of Fruit Flies (Diptera: Tephritidae) Under Laboratory Conditions

F.A. Abdel-Galil[1], M.A. Amro[2], Doaa S. Mohamed[3] and Eman M. M. El-Kousy[3]

[1]Plant Protection Department, Faculty of Agriculture, Assiut University, Assiut, Egypt.
[2] Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt.
[3]Zoology Department, Faculty of Science, Assiut University, Assiut, Egypt.

***Corresponding author: e-mail:** faagalil@hotmail.com **Received 13/1/2019, Accepted 10/3/2019**

Abstract: This study evaluated the effect of three different concentrations ($3x10^7$, $3x10^6$ and $3x10^5$ conidia/ml) of the entomopathogenic fungus, *Beauveria bassiana* (Bals.-Criv.) Vuill (AUMC 3864) on the adult stage of the tephritid fruit flies, *Bactrocera zonata* (Saunders), *Ceratitis capitata* (Wiedemann) and *Carpomyia incompleta* (Becker). The highest concentration of the fungus ($3x10^7$ conidia/ml) caused 100% mortality, 72 hours post adult infection for both of *B. zonata* and *C. capitata*, and after 96 hours in case of *C. incompleta*. The concentration ($3x10^6$ conidia/ml) caused 100% mortality after 96, 144 and 120 hours for *B. zonata*, *C. capitata* and *C. incompleta*, respectively. The lowest concentration $3x10^5$ conidia/ml caused 100% mortality of the pest adults after 168, 192, 120 hours for the above metioned species, respectively. Also, the results indicated that, the minimum LT_{50} values for *B. zonata*, *C. capitata*, and *C. incompleta* were 46.06, 49.40 and 41.63 h after infection by the concentration of $3x10^7$, respectively. Consequently, the most susceptible species to *B. bassiana* was *C. incompleta* which presents the least LT_{50} values (41.63, 69.39 and 76.70 h) after infection by the concentrations of $3x10^7$, $3x10^6$ and $3x10^5$ conidia/ml, respectively. So, this biocontrol agent can be used in controlling fruit flies as a promising tool compared with the conventional insecticides.

Keywords:Bioassay, Entomopathogenic fungi, *Beauveria bassiana* AUMC 3864, fruit-flies, *Bactrocera zonata, Ceratitis capitata, Carpomyia incompleta*.

Introduction

Entomopathogenic fungi have attracted many scientists to demonstrate their efficacy on several pests. The entomopathogenic fungus, *Beauveria bassiana* (Bals.-Criv.) Vuill., is a species belongs to the Hyphomycetes that are naturally inhabitants in soil and infects wide range of insect species that spend at least one stage of their life cycle in the soil **(Madelin, 1963** and **Toledo *et al*. 2006**). Importantly, this wide host range has enabled *B. bassiana* to become one of the most widely potentially used fungal biological control agents **(Gillespie 1988).** On the other side, tephritid fruit flies are among the major pests that affect fruit production throughout the world and represent the most economically important group of Phytophagous of order Diptera (**Robinson and Hooper 1989**, **Foote *et al*. 1993**, **De la Rosa *et al*. 2002** and **Abdel-Galil *et al*. 2011**).The current social and environmental problems associated with insecticide use for fruit fly control, either by aerial or ground applications on foliage for adult control or to the soil for larvae or new-emerged adult control, have motivated the search for biological control alternatives, including entomopathogenic bacteria, nematodes and fungi **(Saul *et al*. 1983**, **Penrose 1993** and **Toledo 2002**). Basically, the conidial phase (spores) of a large number of strains of *B. bassiana*, coming from different geographic regions, have been assessed, under laboratory conditions, for controlling different fruit fly species and for different life cycle stages (**Garcia *et al*. 1984**, **Espin *et al*. 1989**, **Campos 2000**, **Castillo *et al*. 2000**, **Lezama-Gutierrez *et al*. 2000**, **De la Rosa *et al*. 2002** and **Ekesi *et al*. 2002**). Several years of laboratory, semi-field and field studies have shown that, tephritid fruit flies, *C. capitata* (Wied.), *Rhagoletis cerasi* (Loew) and *Bactrocera oleae* (Gmelin) are susceptible to the infection by *B. bassiana* strain ATCC 74040 (**Ladurner *et al.* 2007** and **Daniel *et al*. 2008**). **Imoulan and Elmeziane (2014)** studied the pathogenicity of *B. bassiana* isolated from Moroccan Argan forests soil against C.

capitata larvae under laboratory conditions. They reported that the entomopathogenic fungi are an interesting tool for fruit fly control and hold a useful alternative method rather than conventional insecticides. Recently, primary selection of effective pathogens should be taken under laboratory conditions prior to application in the field. Therefor, this study aimed to determine the effect of three different conidial concentrations of *B. bassiana* (Bals.-Criv.) Vuill. strain AUMC 3864, on the mortality of three selected genera of Tephritid flies adults under laboratory conditions.

Materials and Methods

1-*Beauveria bassiana* (Bals.-Criv.) Vuill. strain AUMC 3864:

The strain of *B.bassiana* (Bals. - Criv.) Vuill., obtained from the Assiut University Mycology Center (AUMC), Assiut, Egypt, was used. Propagation of the fungal strain was carried out using the culture medium Sabouraud's Dextrose Agar (SDA) as described by **De la Rosa *et al*. (2002**). The autoclaved culture medium was distributed into test tubes and Petri-dishes that had been previously sterilized. Inoculation was carried out inside a laminar flow Cabinet using a bacteriological loop to deposit small quantities of conidia upon the culture medium, (Figure 1: A and B). Cultures were incubated under controlled conditions (27± 2°C, 80 ±5% RH, and photoperiod of 12:12 h [L: D]). After 15 days, the conidia were harvested by rasping the surface of the culture medium with sterile bacterial loop, and a spore suspension of the strain of *B. bassiana* was prepared, of concentrations of $3x10^7$, $3x10^6$ and $3x10^5$ conidia/ml. The suspension was prepared from 1 g of conidia in 100 ml of sterile water plus 0.025% glycerin as a dispersant their homogenized in a glass flask with the aid of magnetic agitator.

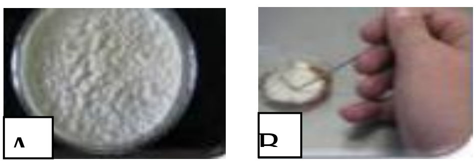

Figure 1: A- *Beauveria bassiana* culture (3 weeks old) and B- Harvest of dry conidia. (Qazaz,2012)

2- Tested flies:

Ripening and newly fallen fruits were randomly collected from host trees in Assiut Governorate. Samples were placed in a plastic tray over sand in a screened box and transferred to the laboratory as described by **Amro and Abdel–Galil (2008) and Abdel-Galil *et al*. (2018).** The emerged larvae or pupae were collected and the sand was renewed, after that the fruit liquids were eliminated. The pupae of *B. zonata*, *C. capitata* and *C. incompleta* as complex were placed in vials on sterile sand until adult emergence under the laboratory conditions, 28+2°C and 60+5% R.H. Newly emerged adult flies were placed in an experimental cages (30x30x45cm) and supplied with diet mixture of sugar and protein hydrolysate as food and water (3:1).

3- Pathogenicity to adult flies:

A total of 25 adult flies (three days old) of *B. zonata*, *C. capitata* and *C. incompleta* were washed in distilled water and divided into 5 replicates, each contains five individuals that had placed in 100 ml glass flasks (25 individual adult per treatment). For each treatment, adult flies were sprayed with 0.5 ml of suspension of the fungal strain, containing $3x10^7$, $3x10^6$ and $3x10^5$conidia/ ml, for 30s.The same number of adult flies was used as control. Furthermore, inoculated flies were placed into glass flasks containing absorbent paper towels to absorb excess humidity and fungal suspension. However, groups of 5 flies were then maintained under controlled conditions (27± 2°C, 80±5% RH and a photoperiod of 12:12 h [L: D] for observation. These flies were provided daily with sterile water and a food source of sugar and hydrolyzed protein (3:1). Mortality was recorded daily after inoculation. Dead flies were placed at the same conditions to ensure the emergence of fungal mycelium followed by sporulation, (Figure 2: A , B and C) (**Qazaz, 2012**).

4- Data processing and statistical analysis:

LT_{50},confidence limits 95% of LT_{50} and LT_{90} slope and Standard Error (SE) values were calculated for each of the three concentrations of *B. bassiana* that tested against the three species of fruit flies (*B. zonata*, *C. capitata* and *C. incompleta*) by using Propan probit analysis program version 1.1 **(Finney, 1971).**

Results and Discussion

The effect of *B. bassiana* strain AUMC 3864 against adults of *B. zonata*, *C. capitata* and *C. incompleta* was estimated by using three different spore concentrations. Data in Table (1) show the mortality percentage of adults of these flies at the concentrations of $3x10^7$, $3x10^6$ and $3x10^5$ conidia /ml. It is noted that the initial kill occurred after 48 hours by using the concentration of $3x10^7$conidia/ml. against *C. incompleta* which recorded the highest mortality percentage (80%) and followed by *B. zonata* then *C. capitata* with (72% and 32%), respectively.

The obtained results revealed that, the mortality percentages caused by the highest concentration of the fungi ($3x10^7$ conidia/ml) recorded 100%, 72 hours post infection for both of *B. zonata* and *C. capitata*, while it reached 100% after 96 hours for *C. incompleta*. However, the mortality caused by $3x10^6$ conidia/ml, reached 100% after 96, 144 and 120 hours for *B.zonata*, *C.capitata* and *C.incompleta*, respectively. On the other hand, the mortality caused by the lowest concentration tested ($3x10^5$ conidia/ml) presented 100% mortality on the pest adults after 168, 192, 120 hours for the above metioned species , respectively. Probit analysis parameters of *B.bassiana*, on the adults of the selected genera were presented in Table (2) and Figures (3,4 and 5).

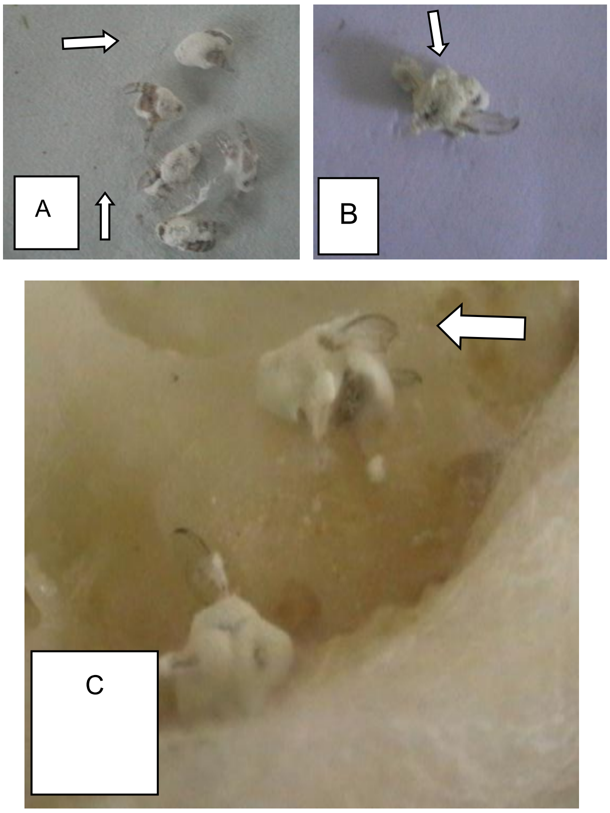

Figure 2: *B. bassiana* AUMC 3864 infection symptoms on *B. zonata* (A), *C. capitata* (B) and ***C. incompleta* (C) adult flies**

The obtained results revealed that, the most susceptible species to *B. bassiana* was *C. incompleta* which presented the least LT_{50} values 41.63, 69.39 and 76.70 h at the concentrations of $3x10^7$, $3x10^6$ and $3x10^5$ conidia/ml, respectively. So, this biocontrol agent can be used for controlling fruit flies as a promising tool compared with the conventional insecticides.

Table: 1- Mortality percentages of the tested tephritid fruit fly adults by using three different spore concentrations of *B. bassiana*.

hours / dose	*B. zonata*			*C. capitata*			*C. incompleta*		
	$3x10^7$	$3x10^6$	$3x10^5$	$3x10^7$	$3x10^6$	$3x10^5$	$3x10^7$	$3x10^6$	$3x10^5$
control	0%	0%	0%	0%	0%	0%	0%	0%	0%
24	0%	0%	0%	0%	0%	0%	0%	0%	0%
48	72%	0%	0%	32%	0%	0%	80%	20%	0%
72	100%	20%	0%	100%	16%	0%	92%	40%	24%
96		100%	20%		56%	12%	100%	84%	76%
120			64%		92%	56%		100%	100%
144			92%		100%	96%			
168			100%			96%%			
192						100%			

Table: 2- Probit analysis of the efficacy of three different doses of *B.bassiana* sprayed on three different genera of tephritid fly adults

Tephritid flies	Dose (Conidia/ml)	LT_{50} (hours)	Confidence 95% limits of LT_{50}		LT_{90} (hours)	Confidence 95% limits of LT_{90}		Slope	SE
			Lower	Upper		Lower	Upper		
B. zonata	$3x10^7$	46.06	NC[a]	NC	50.43	NC	NC	32.55	59.68
	$3x10^6$	82.59	61.80	83.20	84.45	79.49	102.20	30.65	8.80
	$3x10^5$	112.16	105.43	118.56	139.07	130.23	154.20	13.73	2.14
C. capitata	$3x10^7$	49.40	NC	NC	53.45	NC	NC	37.49	31.82
	$3x10^6$	90.49	83.78	96.83	117.49	108.50	133.30	11.30	1.80
	$3x10^5$	116.19	109.45	122.58	143.28	134.52	157.94	14.08	2.13
C. incompleta	$3x10^7$	41.63	35.88	46.87	60.17	52.99	73.32	8.01	1.42
	$3x10^6$	69.39	73.64	76.58	103.38	93.39	118.07	7.07	1.14
	$3x10^5$	76.70	76.56	88.26	105.35	95.71	128.22	13.15	2.39

[a] NC, not calculable

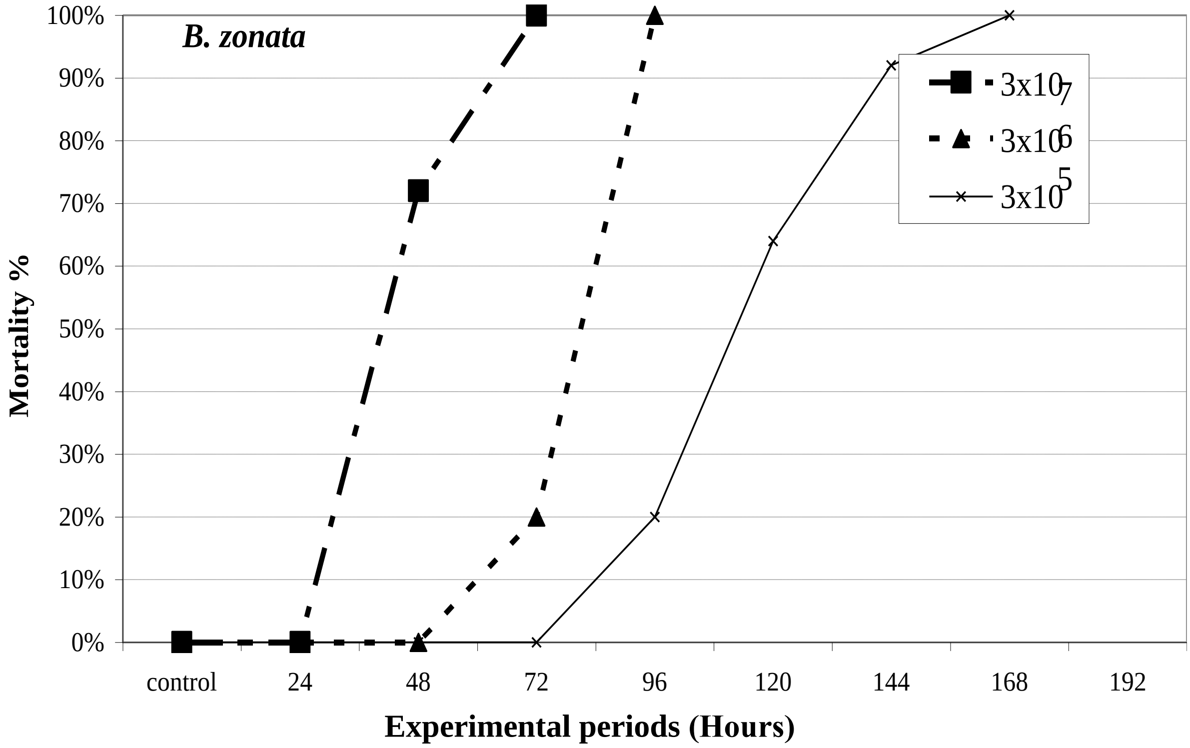

Figure 3: Relationship between experimental periods and rate of mortality of *B. zonata* inoculated with three concentrations of *B. bassiana*

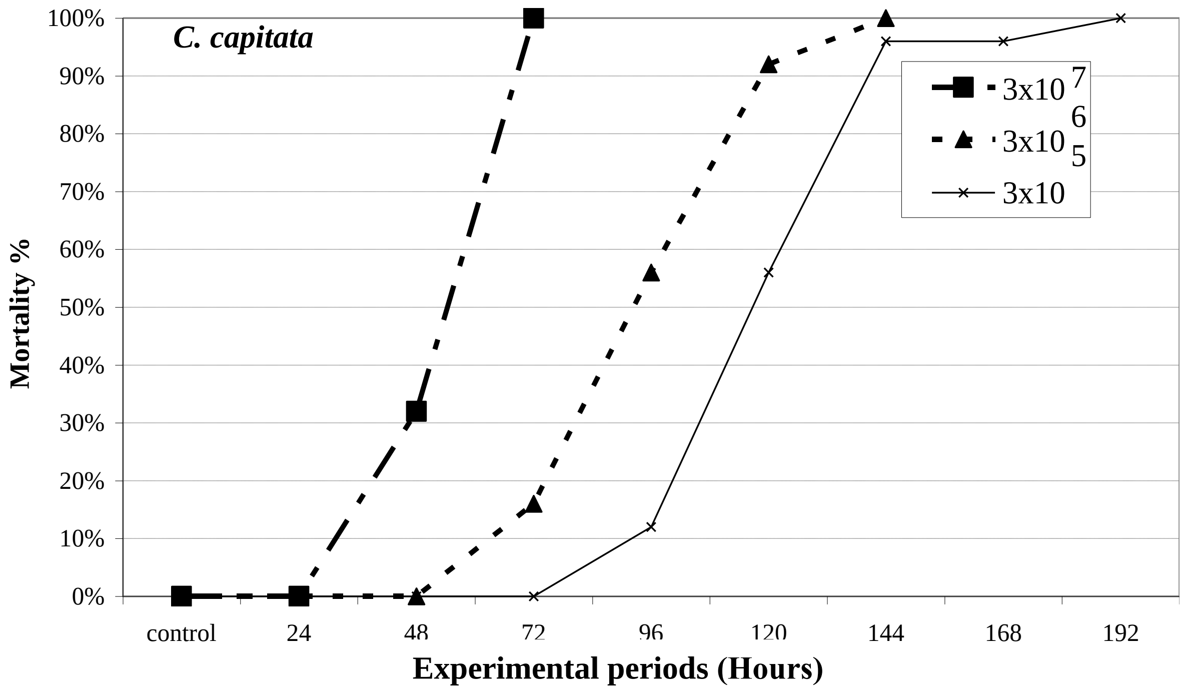

Figure 4: Relationship between experimental periods and rate of mortality of *C. capitata* inoculated with three concentrations of *B. bassiana*

Journal of Basic & Applied Mycology (Egypt) 10 (2019): 1-8 **p-ISSN 2090-7583, e-ISSN 2357-1047**
 http://www.aun.edu.eg/aumc/Journal/index.php

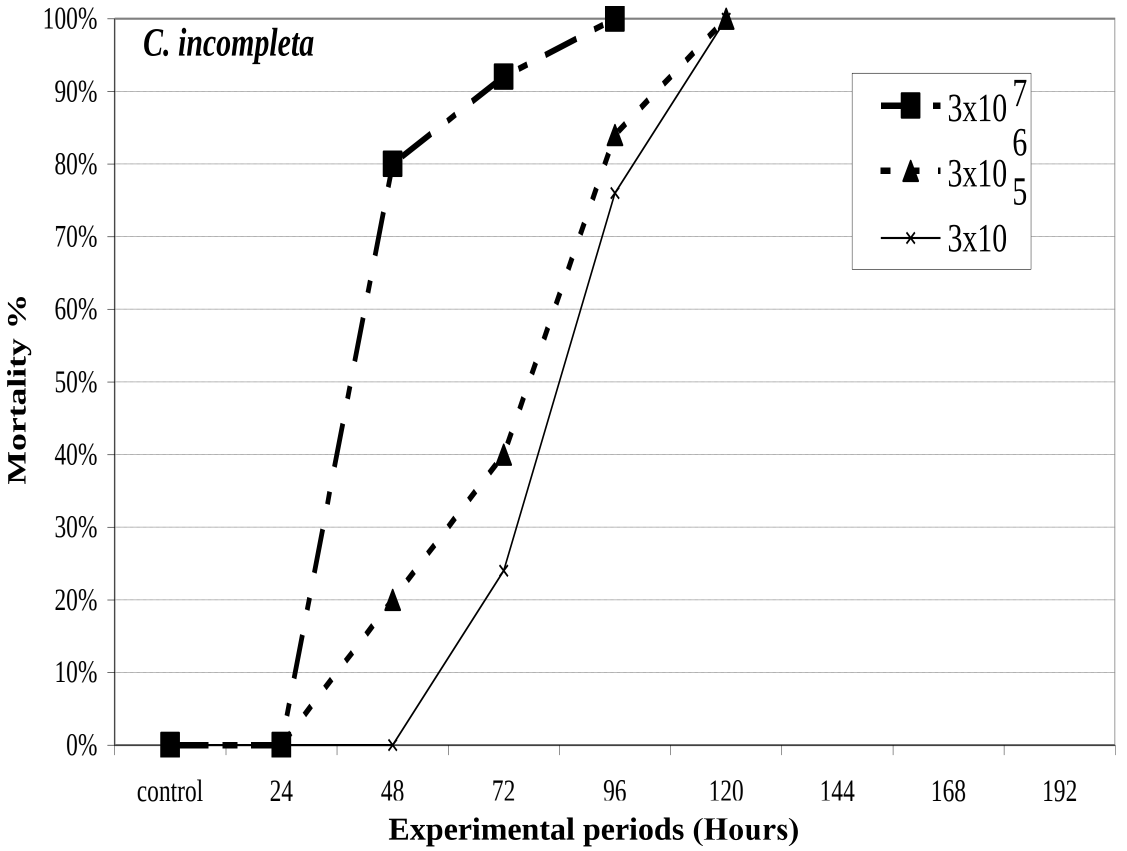

Figure 5: Relationship between experimental periods and rate of mortality of *C. incompleta* inoculated with three concentrations of *B. bassiana*

These results could be due to the unpreviously exposure of *C. incompleta* to this kind of control, while *C. capitata* and *B.zonata* took a lot of attention and exposure to different methods of control especially bio and chemical control for long time. Concequently, the recently established control of *C. incompleta*, led to occupying this ranking order after *C. capitata* and *B. zonata* in the responding to this fungus.

In conclusion, the acceptable values of LT_{50} indicate the possibility of using this entomopathogenic fungus as a component of integrated control programs especially integrated pest management (IPM) against *B. zonata*, *C. capitata* and *C. incompleta*. The slight delay of the lethal time could allow the infected flies to transmit the fungus to other healthy individuals. Morever, the tested strain of *B. bassiana* was more virulent at the concentration of $3x10^7$conidia/ml. It can be suggested that, *B. bassiana* is a suitable and efficient agent for the control of *B. zonata*, *C. capitata*, and *C. incomplete* adults with the possibility for controlling other fruit flies. Once the pathogenicity on adults has been demonstrated, the next step is to develop an application method that can be used under field conditions.

The obtained results in this study, came in agreement with other studies carried out on different insects. **Espin *et al.* (1989)** recorded 69-78% mortality in *C. capitata* adults infected by *Metarhizium anisopliae* (Metsch.), while, **Castillo *et al.* (2000)** found out that mortality of *C. capitata* infected by *B. bassiana* ranged from 8 to 30%. They reported that 100% of *C. capitata* individuals have been killed when infected with $1x10^6$ conidia/ml of *M. anisopliae*. **Munoz (2000)** evaluated 16 strains of *B. bassiana* against *C. capitata* adults and found that mortality values fluctuated between 20 and 98.7%. **Dimbi *et al.* (2003)** reported that mortality in adults of *C. capitata* and *C. rosae* var. *fasciventris* ranged from 7 to 100%, when infected by *B. bassiana*. Similarly,

Sookar *et al* (2008) tested the pathogenicity of seven isolates of *M. anisopliae* and five isolates of *B. bassiana* towards the adults of *B. zonata* by topical application of conidial suspension of 1×10^6 conidia/ml. They found that, all of the tested isolates were pathogenic to this insect pest. Their results revealed that, mortality of *B. zonata* varied between 12 and 98 % at 5 days post-treatment. This finding could lead to utilization of the locally produced entomopathogenic fungi as succesful tool in an integrated pest management programm (IPM) for controlling *B. zonata*. Most workers have not found virulent strains for immature stages. So, future studies could be directed to raise virulence of the present strain to be used as Microbial Biological Control Agent (MBCA) in the future.

Acknowledgements

The authors are grateful to Prof. Dr. Ahmed Moharram, Director of Mycological Center Assiut University, Egypt (AUMC) for technical support, academic advice, and instruction materials. Also, authors thank Dr. Mohamed Ahmed Ibrahim Ahmed, Assistance Prof., Plant Protection Department, Faculty of Agriculture, Assiut University for reading, revising, and editing this manuscript.

References

Abdel-Galil FA, Amro MA, Abdel-Moniem AS and Gameel SM (2011): Comparative study on the potential of Malathion 57% and selected pesticide safe alternatives in reducing fruit flies infestation in the New Valley Orchards. Archives of Phytopathology and Plant Protection 44 (3): 231 — 241.

Abdel-Galil FA, Amro MA, Mohamed DSM, Shafey MH and El-kousy EM.(2018): Factors regulating the population trends of the peach fruit fly *Bactrocera zonata* (Saunders) (Diptera: Tephritidae) attacking guava and mandarin trees in Assiut, Upper Egypt. The 9th International Conference for Development and the Environment. in the Arab world, April, 15-17: 141-149.

Amro MA and Abdel-Galil FA (2008): Infestation predisposition and relative susceptibility of certain edible fruit crops to the native and invading fruit flies (Diptera: Tephritidae) in the new valley oases, Egypt. Assiut University Bulletin for Environtal Researches 11(1): 89-98.

Campos CS (2000): Seleccion de cepas de *Metarhizium anisopliae* (Metsch) Sorokin virulentas a la mosca mexicana de la fruta, *Anastrepha ludens* (Loew) en condiciones de laboratorio. Thesis de Licenciatura. Facultad de Ciencias Agricolas. Univ. Aut. de Chiapas. Huehuetan, Chis., Mexico: 60. Thesis de Doctor en Ciencias. Facultad de Ciencias. Universidad Nacional Autonoma de Mexico. Mexico, 124 p.

Castillo MA, Moya P, Hernandezand E and Primo-Yufera E (2000): Susceptibility of *Ceratitis capitata* Wiedemann (Diptera: Tephritidae) to entomopathogenic fungi and their extracts. Biological Control 19: 274-282.

Daniel C, Keller S and Wyss E (2008): Field applications of entomopathogenic fungi against *Rhagoletis cerasi*. IOBC wprs Bulletin 31: 191-194.

De la Rosa W, López FL and Liedo P (2002): *Beauveria bassiana* as a pathogen of the Mexican fruit fly (Diptera: Tephritidae) under laboratory conditions. Journal of Economic Entomology 95: 36–43.

Dimbi S, Maniania NK, Lux SA, Ekesi S and Mueke JM (2003): Pathogenicity of *Metarhizium anisopliae* (Metsch.) Sorokin and *Beauveria bassiana* (Balsamo) Vuillemin to three adult fruit fly species: *Ceratitis capitata* (Weidemann), *C. rosa* var. *fasciventris* Karsch and *C. cosyra* (Walker) (Diptera: Tephritidae). Mycopathologia 156: 375–382.

Ekesi S, Maniania and NK, Lux SA (2002): Mortality in three African tephritid fruit fly puparia and adults caused by the entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana*. Biocontrol Science and Technology 12: 7-17.

Espin GAT, Laghi de Suza HM, Messias CL and Piedrabuena AE (1989): Patogenicidad de *Metarhizum anisopilae* nas diferentes fases de desenvolvimento de *Ceratitis* *capitata* (Wied.) (Diptera: Tephritidae). Revista Brasileria de Entomologia 33: 17–23.

Finney DJ (1971): Probit analysis.Cambridge University Press, Cambridge : 333 pp.

Foote RH, Blanc FL and Norrbom AL (1993): Handbook of the Fruit Flies (Diptera:Tephritidae) of America North of Mexico. Ithaca, NY/London: Comstock: 571pp.

Garcia AS, Messias CL, Souza HML and Piedrabuena AE (1984): Patogenicidade de *Metarhizium anisopliae* var. *anisopliae* a *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae). Review of Brazilian Entomology 28: 421-424.

Gillespie AT (1988): Use of fungi to control pests of agricultural importance. In "Fungi in biological Control Systems," (M.N. Burge, Ed.), Manchester University Press, London.

Imoulan A and Elmeziane A (2014): Pathogenicity of *Beauveria bassiana* isolated from Moroccan Argan forests soil against larvae of *Ceratitis capitata* (Diptera: Tephritidae) in laboratory conditions. World Journal of Microbiology and Biotechnology 30(3):959-965.

Ladurner E, Benuzzi M and Fiorentini F (2007): Un fungo per combattere i fitofagi che attaccano l'olivo. AZBIO 12: 52-56.

Lezama-Gutierrez R, Trujillo-de la Luz A, Molina- Ochoa J, Rebolledo-Dominguez O, Pescador AR, Lopez-Edwards M, and Aluja M (2000): Virulence of *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes) on *Anastrepha ludens* (Diptera: Tephritidae): Laboratory and field trials. Journal of Economic Entomology 93: 1080-1084.

Madelin MF (1963): Diseases caused by Hyphomycetous fungi, In E. A. Steinhaus [ed.]. Insect Pathology: An Advanced Treatise. Academic Press, New York 2: 233-271.

Munoz RJA (2000): Patogenicidad de *Beauveria bassiana* (Bals.) Bulli. sobre la mosca del mediterraneo, *Ceratitis capitata* (Wied.) en condiciones de laboratorio. Tesis de Licenciatura. Facultad de Ciencias Agricolas. Univ. Aut. de Chiapas. Huehuetan Chis., Mexico: 55 p.

Penrose D (1993): The 1989/1990 Mediterranean fruit fly eradication program in California. In M. Aluja and P. Liedo [eds.]. Fruit flies: Biology and Management. Springer-Verlag, New York: 406-441.

Qazaz FO (2012): Isolation of *Beauveria bassiana* from soil and evaluation of its entomopathogenic and biocontrol efficaccy against the Mediterranean fruit fly, *Ceratitis capitata*. M.Sc.Thesis, Faculty of graduate Studies., Hebron University.

Robinson, AS and Hooper GHS (1989): Fruit Flies: Their Biology, Natural Enemies and Control. World Crop Pests, 3 (A, B.) Amsterdam.

Saul SH, Tsuda D and Wong TTY (1983): Laboratory and field trials soil applications of methoprene and other insecticides for control of the Mediterranean fruit fly (Diptera: Tephritidae). Journal of Economic Entomology 76: 174-177.

Sookar P, Bhagw S and Ouna EA (2008): Isolation of entomopathogenic fungi from the soil and their pathogenicity to two fruit fly species (Diptera: Tephritidae). Journal of Applied Entomology 132: 778-788.

Toledo AJ (2002): Evaluacion de algunos agentes entomopatogenos para el control microbiano de tres species de moscas de la fruta (Diptera: Tephritidae) de importancia economica.

Toledo JP, Liedo S, Campos E, Flores S, Villasenor A and Montoya P (2006): Use of *Beauveria bassiana* and *Metarhizium anisopliae* for fruit fly control: a novel approach Fruit Flies of Economic Importance: From Basic to Applied Knowledge Proceedings of the 7th International Symposium on Fruit Flies of Economic Importance 10-15 September, Salvador, Brazil: 127-132.