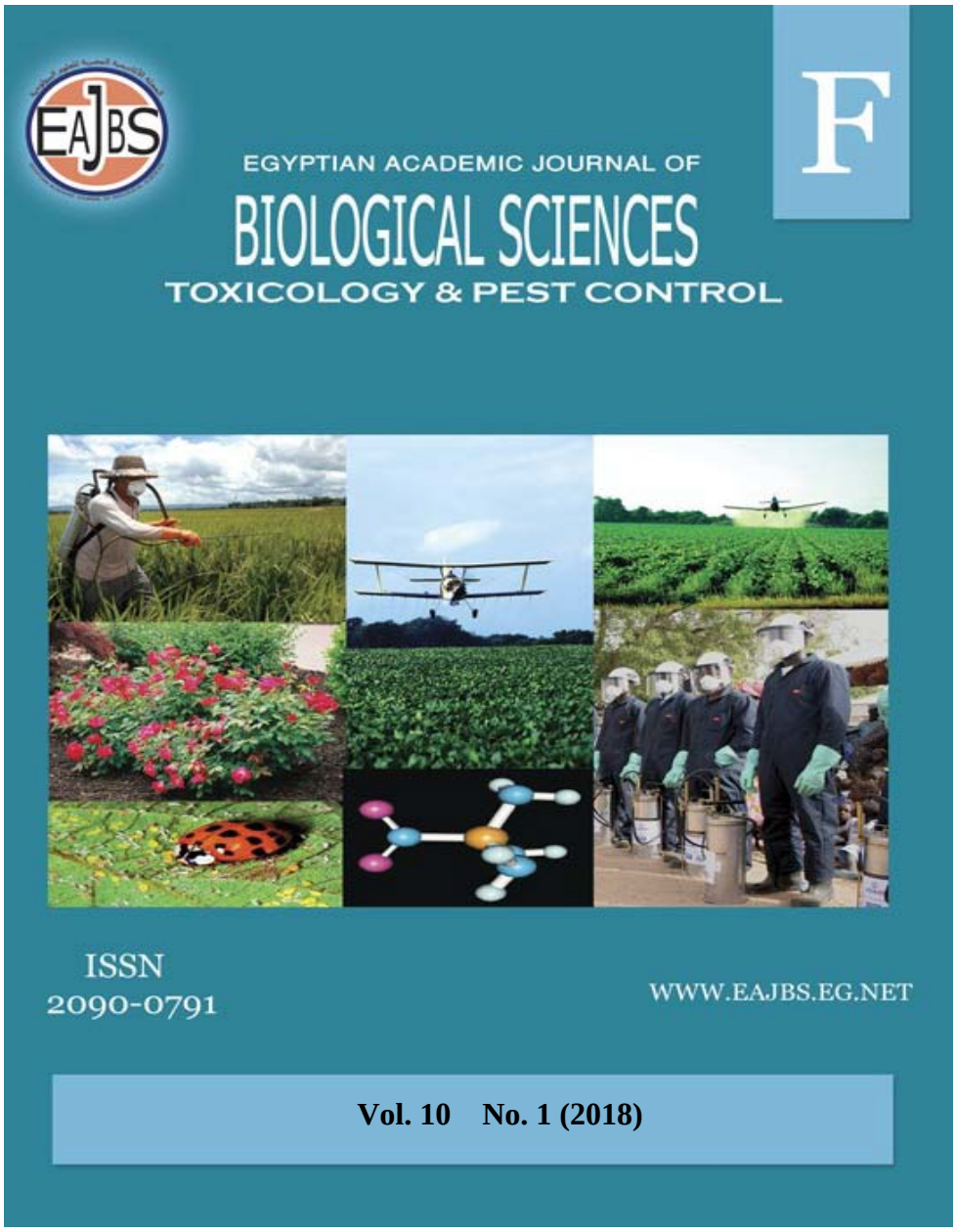

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Evaluation of Entomopathogenic Fungi, *Beauveriabassiana* And *Metarhizium anisopliae* on Peach Fruit Fly, *Bactrocera zonata* (Saunders) (Diptera:Tephritidae)

Mohamed A. hussein[1]; Amany S.Khaled[1]; A.A.Ibrahim[2]; Nehad A. Soliman[2]; Safaa H. Attia[2]

1-Faculty of Science-Ain Shams University, Cairo, Egypt.
2-Plant Protection Research Institute-Agricultural Research Center, Dokki, Giza, Egypt.

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ABSTRACT

This research intended to investigate the pathogenicity of *Beauveria bassiana* (Bals.) and *Metarhizium anisopliae* (Met.) on immature stages and adult flies of the peach fruit fly, *Bactrocera zonata* (Saunders). Percentage mortality was concentration dependent. The LC_{50} and LC_{90} values reflected privilege of *B. bassiana* over *M. anisopliae* on immature stages while *B. bassiana* reflected higher mortality values for young age pupae than those of old age. Both entomopathogenic fungi showed high activity against flies.

INTRODUCTION

The Peach fruit fly, *Bactrocera zonata* (Saunders) (Diptera: Tephritidae) is a destructive polyphagous pest infest most of the horticultural fruits in addition to some vegetables (Allwood *et al*. 1999) causing a great loss to the horticultural sector. Female flies oviposit their eggs inside fruits, which hatch into larvae feeding on the fruit flesh causing its destruction then leave the harmed fruit after passing three larval stages and migrate to the soil burying themselves in soil at shallow depths to pupate and complete its life cycle. Fruits producing countries may also lose potential markets due to stringent quarantine regulations imposed by importing countries to avoid the invasion of undesired pests. Most of the control action methods is the excessive use of insecticides to control this pest causes loss of potential markets. Therefore, it is essential to develop safe and environment- friendly effective alternatives for the ordinarily used chemicals. Fungal agents are among the most promising groups for biological control agents against pest insects as they live naturally in the soil to come in contact easily with full-grown larvae and pupae of *B.zonata*. *Beauveria bassiana* (Bals.) and *Metarhizium anisopliae* (Met.) recognized as some of the most important entomopathogens of dipteran insects (Castillo *et al.* 2000; Destéfano*et al*. 2005; Salvatore *et al*. 2009 and Boudjelida and Soltani 2011).All entomopathogenic fungi have the same basic mode of action when infecting insect host, the conidia or asexual spores, come into direct contact with the host and adhere to the cuticle. Attachment of

the spores to the host- mediated by chemical components in the outer layers of the spore (Parker *et al.,* 2000). The spores are covered by proteins, lipoproteins and polysaccharides which help spores to adhere themselves to the host cuticle. Then the spores germinate producing appressoria (Penetration-peg structure) (Putcheta *et al.,* 2006). The cuticle is penetrated by a combination of mechanism pressure from the appressoria and the action of cuticle-degrading enzymes, such as lipases, proteinases, chitinases (St-Leger *et al.,* 1989), or such as trypsin, metalloproteases, and aminopeptidases (Bidochka and Small 2005). Once the fungus reached hemocoel, it encountered host's immune system by secreting toxins, and then the host died. The fungus grows vegetatively in the host hemocoel and external conidia produced upon the death of the host when fungal hyphae exit through the less sclerotized areas of the cuticle (Inglis *et al.,* 2001).

The aim of this work is to investigate the ability of the entomopathogenic fungi, *B.bassiana* (Bals.) and *M.anisopliae* (Met.) to cause death to the immature stages (full-grown larva and pupae) and flies of *B.zonata* in its natural fauna. In addition, study the susceptibility of these stages to both mentioned entomopathogenic fungi at different concentrations.

MATERIALS AND METHODS

1-The Peach Fruit Fly, *Bactrocera zonata* (Saunders):

Peach fruit fly, *B.zonata* (Saunders) obtained from the laboratory colony reared in the Horticulture Insects Department, Plant Protection Research Institute, Dokki, Giza, Egypt. The insect larvae reared using artificial larval rearing medium according to the technique of Tanaka *et al*., (1969). Flies diet formed from sugar and enzymatic yeast hydrolysate in ratio 3:1, respectively in addition to water source El-Sayed, (1979).

In present work full-grown larvae collected at the same time after they had popped from the artificial larval rearing medium to pupate. Pupation process begins in about eight hours after larval exit from artificial larval rearing medium while pupae need about nine days to complete its duration.

2- Entomopathogenic Fungi *B.bassiana (Bals.)* and *M. anisopliae* (Mets.) as Bioagents against, *Bactrocera Zonata* (Saunders).

2.1-Entomopathogenic Fungi:

Entomopathogenic fungi used in the present study were *Beauveria bassiana* (Bals.) and *Metarhizium anisopliae* (Met.). The first fungus was isolated from the whitefly, *Bemisia tabaci* in Sharkia governorate, while the second from the red palm weevil, *Rhynchophorus ferruginens* in Ismailiyah governorate (Ibrahim, 2006).

2.2- Conidiospores Production:

The Conidia obtained from the fungal culture of *B. bassiana* and *M. anisopliae* were grown at 25 °C, in dark on sabourad dextrose agar (SDA), consisted of peptone 10 g/L, glucose 20 g/L(sterilized alone before mixed) and agar-agar 20 g/L, (constant volume of 15 ml) in standard Petri-dishes (90 mm diameter). Conidia harvested from 15 days old plates by scraping into the sterile solution of 0.01% Tween 80 (polyoxyethylene, sorbitan mono-oleate; 1 ml/L). The suspension was vortexed for 2 min and agitated for 1.5 hours on a flask shaker (Griffen and George Ltd) at room temperature before filtering through four layers of sterile muslin. The conidial concentration of the resulting stock suspension estimated using an improved Neubauer bright line hemocytometer (Reichart) under a Leitz Dialu X 20 EB microscope (40 x magnifications). series of dilutions were made to give range concentrations of 1×10^6 ,

1×10^7 , 1×10^8 , 1×10^9 and 1×10^{10} conidia/ml. Suspensions were held overnight on the ice and then routinely checked for conidial germination to use in bioassays as described by Yeo *et al*., (2003).

3. Bioassay tests of entomopathogenic fungi *B.bassiana* (Bals.) and *M. anisopliae* (Mets.) on peach fruit fly, *B.zonata* (Saunders):

3.1. Bioassay Test of Entomopathogenic Fungi on Full-Grown Larvae:

Full-grown larvae of the peach fruit fly *B. zonata* tested in sterilized glass jars of 7 cm diameter containing 75 ml of fine sand sieved through 2 mm sieve. The glass jars and the sand sterilized in an oven at 200 °C for an hour. Different conidial concentrations (1×10^6, 1×10^7, 1×10^8, 1×10^9 and 1×10^{10} conidia/ml) of *B.bassiana* used and incorporated into the sand using small sprayer then the sand vigorously mixed with the spatula. Ten fresh full-grown larvae of *B. zonata* popped at the same time, placed on the surface of the treated sand in each jar allowing them to burrow themselves naturally into it. The jars closed with pieces of muslin and rubber bands. Each concentration replicated five times and other five replicates without conidia run in parallel as control treatment. The same steps are done for *M.anisopliae*. The preparations maintained under laboratory conditions with the relative humidity of approximately 75% and temperature of 25 ± 2 °C. After ten days, the number of emerged adults in each jar recorded. The infection confirmation by the fungus was done by collecting dead individuals (larvae, pupae, adult flies) and then were transferred to the sterilized Petri-dish with moistened piece of cotton and incubated at 25 °C until fungal sporulation on the cadavers (Ekesi et al., 2002).

3.2. Bioassay Tests of Entomopathogenic Fungi on Pupae:

Susceptibility of one-day and four days old pupae of *B. zonata* tested separately in glass jars of 7 cm diameter containing 75 ml of fine sand. Conidial concentrations, 1×10^6, 1×10^7, 1×10^8, 1×10^9 and 1×10^{10} conidia/ml of *B.bassiana* (Bals.) used and incorporated into the sand using small sprayer then the sand vigorously mixed with spatula. Half of the treated sand placed in the jars. Ten of one-day old pupae of *B. zonata* placed on the sand surface after their immersion in conidial suspension for one minute and then the pupae covered with the rest of the sand **(Ekesi et al., 2002).** Each concentration replicated five times in addition to other five replicates without conidia as control treatment. The same steps are done for *M. anisopliae* (Met.). The preparations kept at approximately 75% RH and 25 ± 2 °C. Emerged flies recorded in each jar. The infection confirmation by the fungus done by collecting dead individuals (pupae) and then were transferred to sterilized petri dish with moistened piece of cotton and incubated at 25± 2°C until fungal sporulation on the cadavers. The same steps repeated for testing pupae (four-days-old) and other treatment without conidia as control.

3.3. Bioassay of Entomopathogenic Fungi on Flies:

Exposure of newly emerged flies (one-day-old) of *B. zonata* to entomopathogenic fungi *B.bassiana* and *M.anisopliae* carried out separately in sterilized jars of 9 cm in diameter and 23 cm height. Five conidial concentrations 1×10^6, 1×10^7, 1×10^8, 1×10^9 and 1×10^{10} conidia/ml of *B.bassiana* used separately in small sprayers and jars' inner surface well sprayed. Excess of conidial suspension at the bottom of the jars withdrawn with a syringe and then left for an hour to dry. Fifty newly emerged flies of *B.zonata* (one-day old) transferred inside each jar using aspirator allowing them to move freely on the jars' surface. Jars supplied with flies' diet (sugar and enzymatic yeast hydrolysate in a ratio 3:1 in addition to the water source); the jars closed with pieces of muslin for ventilation and rubber bands. Each concentration replicated five times and a control treatment without conidia run as

well. The same steps carried out for *M.anisopliae*. All treatments kept at 25 ± 2 °C and 75 % relative humidity. Dead flies counted daily and the infection confirmation by the fungus done by collecting dead flies and then were transferred to sterilized Petri dish with moistened piece of cotton and incubated at 25± 2°C until fungal sporulation on the cadavers appear.

4. Statistical Analysis:

All experiments contained five replicates. Mortality- adjusted using Abbot's Formula (1925).Statistical analysis conducted using SAS Program (1988). Proc ANOVA used to evaluate the significant differences among cultivars, and means separated using Duncan's multiple range test.

RESULTS AND DISCUSSION

Effect of Entomopathogenic Fungi on Peach Fruit Fly, *B.Zonata* (Saunders) Immature Stages and Flies:

Percentage of larval mortality showed variation after treatment using different conidial concentrations of both *B.bassiana* and *M.anisopliae*. The minimal and maximal values of percentages of larval mortality recorded at the concentrations 1×10^6 to 1×10^{10} conidia /ml of *B.bassiana* and *M.anisopliae* (Table 1). There was a significance (P<0.05) in larval mortality values occurred after treatment with *B.bassiana* and *M.anisopliae* at the concentration 1×10^6 (F=160.00**, P<0.000001, df=2, 12) ($t_{(8)}$ =1.90, P<0.0943) while 1×10^8 (F=281.33**, P<0.000001, df=2,12) ($t_{(8)}$=1.41, P<0.1950). The highest used concentration 1×10^{10} of *B.bassiana* and *M.anisopliae* revealed high significance in the resulted percentages of mortality (F=420.19***, P<0.000001, df=2,12) ($t_{(8)}$=3.79, P<0.0053). Entomopathogenic fungi produce toxins to increase pathogenesis and play an insecticidal role. Beauvericins produced by *Beauveria* species while destruxins of *Metarhizium* species are among such fungal metabolites that increase the pathogenicity of fungus. These metabolites also show invertebrate toxicity (Roberts, 1992 and Hajek 1992). The used concentrations of *B.bassiana* and *M.anisopliae* (1×10^6, 1×10^7, 1×10^8, 1×10^9, and 1×10^{10}) reflected a high significance in percentages of one-day old pupae mortality (Table 2). There was no significance between *B. bassiana* and *M.anisopliae* in the percentages of mortality recorded of one- day- old pupae at the lowest concentration 1×10^6 (F=0.167 ns, P<0.000001, df=2,12) ($t_{(8)=}$0.01, P<0.001). The conidial concentrations 1×10^8 of *B.bassiana* and *M. anisopliae* reflected high significance in the percentages of mortality to one-day old pupae (F=352***, P<0.000001, df=2,12) ($t_{(8)}$=7.06, P<o.ooo1) while the concentration 1×10^{10} resulted in very high significance (F=429.13***, P<0.000001, df=2,12) ($t_{(8)}$=10.61, P<0.0001). *B.zonata* pupae (four days old) showed some resistance for both *B.bassiana* and *M.anisopliae* (Table3). Nevertheless, *M. anisopliae* conidial concentrations revealed higher ability to cause death to four days old pupae of *B. zonata* than those of *B. bassiana*. There was a significance at the lowest used concentration 1×10^6 conidia/ml (F=221.00**, p<0.00001, Df=2,12) ($t_{(8)}$=9.00, P<0.0001) and the moderate concentration 1×10^8 conidia/ml (F=206.60**,P<0.00001, Df=2,12) ($t_{(8)}$=3.16, P<0.0133). Full-grown larvae and pupae reflected lesser mortality values than those occurred in flies. This may be due to those larvae bury themselves into the soil (2.5 cm or slightly more) for pupation. The larvae are moving vigorously in soil searching a suitable place to pupate; therefore, the conidia adhered on them may be lesser than pupae. This may explain the resulted mortality at high concentrations than the lower ones. Pupae showed variation in mortality values after exposure to both *B.bassiana* and *M.anisopliae*. Earlier pupae

(one day old) responded to *B.bassiana* more than older ones (four days old). Ekesi *et al*. (2002) evaluated the pathogenicity of *M. anisopliae* against pupae of three Tephritid species, including *C. capitata*, and they found that pupal susceptibility decreased with increasing pupal age. The cuticle is the main obstacle to infection in insects as it is the main path of fungus penetration. Hence, it needs some physical or enzymatic means to pierce the hard cuticle Gul *et al*., (2014). Early age pupae showed higher susceptibility as compared to older pupae. This may be due to that 1-day pupae have as softer cuticle, which facilitates the mission of conidia. The cuticle of older pupae harden with age and therefore become refractory to fungal infection. In addition, Hajek and St-Leger (1994) showed that, low pathogenicity of these fungi against many insect species was due to the nature of the cuticle, in terms of its density and thickness and the degree of sclerotization. The significance in the percentages of mortality caused by both *B.bassiana* and *M. anisopliae* was clear at the conidial concentration 1×10^{10} (F=275.00**, P<0.000001, df=2,12) ($t_{(8)}$=1.26, P<0.2415). Flies of *B.zonata* were the most susceptible stage to infection of both *B.bassiana* and *M.anisopliae* conidial concentrations (Table 4). A significant variation was reflected among the used conidial concentrations of *B.bassiana* (F=150.00**, P<0.00001, df= 5, 24) In addition, *M. anisopliae* raised a significant variation in the resulted percentages of mortality to flies with its used concentrations (F=237.00**, P<0.000001, DF=5, 24). Significance between the resulted percentages of mortality at the lowest used concentration 1×10^{6} conidia/ml for both *B.bassiana* and *M.anisopliae* (F=634**, P<0.00001, DF=2.12) ($t_{(8)}$=2.36, P<0.0462). There was a significance at concentration 1×10^{8} conidia/ml (F= 957.00**, P<0.000001, DF=2, 12) ($t_{(8)}$ =3.09, P<0.0150) and the highest concentration 1×10^{10} conidia/ml (F=2575, P<0.00001, df=2,12) ($t_{(8)}$=1.00, P<0.3466). It seems that percentages of mortalities achieved at all treated stages increased by increasing entomopathogenic fungi conidial concentrations. All treatments showed significant (P<0.05) mortalities as compared to the control treatment. The obtained results of this work are in agreement with Oliveira *et al*., (2010 a) who reported that mortality of larvae and pupae of *C. capitata* increased by increasing concentrations of conidia of *M. anisopliae* and *B. bassiana*. These entomopathogenic fungi produce many toxins and extracellular enzymes such as proteases and chitinases according to the first hosts' facing layer. Toxicity values of the used entomopathogenic fungi based on LC_{50} and LC_{90} revealed the ability of *B.bassiana* to cause death to all *B.zonata* stages more than *M.anisopliae* (Table 5). The obtained data indicate that, *B.zonata* larvae treated with LC_{50} and LC_{90} of *B.bassiana* were lower than obtained data when larvae treated with *M.anisopliae* and equal to 5.15×10^{5} and 1.53×10^{10}, 1.23×10^{7} and 1.81×10^{11} conidia/ml, respectively. The data also showed lower values of *B.bassiana* LC_{50} and LC_{90} when treated pupae than *M.anisopliae* and equal to 1.45×10^{5} and 8.03×10^{9} , 2.5×10^{5} and 1.56×10^{36} conidia/ml , respectively. The data showed that flies of *B.zonata* respond positively when treated with both *B.bassiana* and *M. anisopliae* as LC_{50} and LC_{90} values were equal to 1.23×10^{6} and 5.31×10^{9}, and 1.44×10^{6} and 6.13×10^{9} conidia/ml, respectively. The entomopathogenic fungi *B.bassiana* and *M.anisopliae* showed pathogenic activity against different stages (full-grown larvae, pupae and adult flies) of *B.zonata* but with different levels. Percentages of mortality seemed to be concentration-dependent. The flies of *B. zonata* showed susceptibility to infection with both *B. bassiana* and *M. anisopliae*. These findings are in agreement with Castillo *et al.,* (2000) who reported 100 % mortality in adult flies of *C. capitata*. Many other studies confirmed the susceptibility of other (tephritids) to infection with *M. anisopliae* and *B.bassiana*. DelaRoso*et al.,* (2002), Dimbi*et al.,* (2003), Quesada-Moraga *et al*., (2006**),** Ladurner*et al.,* (2007), Daniel and Wyss, (2008) and Daniel (2009) had

reported the same findings. Flies of *B.zonata* showed highly effective response to infection by both *B.bassiana* and *M.anisopliae*. *M.anisopliae* showed higher pathogenicity against flies of *B. zonata* at the conidial concentrations $1\times10^{8}$and 1×10^{9} more than *B.bassiana* while there was no significance at the concentration 1×10^{10}. These results are in agreement with Mahmoud (2009) who stated that *M. anisopliae* caused higher percentage of mortality to *B. zonata* flies than *B. bassiana*. Pathogenicity of flies reflected concentration dependent pattern. The obtained data may suppose that when the number of adhered conidia increase the toxins produced by them increase finally causing death to the host. According to the morphological features of flies as its body covered with thin layer of cuticle, bearing large number of hairs and spines (White and Elson-Harris, 1994) that may allow conidia to attach easily. This might explain the susceptibility of flies more than larvae and pupae whom have completely different morphological features. In this piece of work, *B.bassiana* worked more actively and effectively than *M.anisopliae* against the tested stages of *B.zonata*. Other studies reported superiority of *M.anisopliae* on tephritids than *B.bassiana,* Ibrahim *et al* (2014), and Soliman *et al*., (2014). These fundamentals may explain the variation in mortality values screened by different stages of *B.zonata* after exposure to different conidial concentrations. At this point of view, there might be a relation between hosts and entomopathogenic fungi, which draw the line of action between them. In other words, observed virulence are due to factors related to the hosts' health, developmental stage, and its particular ability to resist infection (Gillespie *et al*. 2000) who stated that infection in insects stimulates a complex defensive response. Insect fungal pathogens invade their hosts through the external skeleton. A battery of extracellular cuticle degrading proteases and chitinases facilitate passage through the integument and provide nutrition for the fungus (Charnley and St Leger, 1991 and St Leger, 1995). In conclusion, differences in induced mortality rates among *B.zonata* stages may have been related to differences in the conidial attachments onto the insect cuticle, modes of germination, or to the suppression of the host's immune system Chandler et al., (1993).

Table 1: Percentage Mortality of *Bactrocera zonata* (Saunders) full-grown larvae treated with *Beauveria bassiana* (Bals.) and *Metarhizium anisopliae* (Met.) at different concentrations

Mean % mortality of *B.zonata* full-grown larvae treated with					
B.bassiana					
1×10^{6}	1×10^{7}	1×10^{8}	1×10^{9}	1×10^{10}	Control
$53.51^{d}\pm0.09$	$61.22^{c}\pm0.09$	$79.21^{b}\pm0.08$	$79.62^{b}\pm0.03$	$89.86^{a}\pm0.01$	$0.40^{e}\pm0.25$
F= 216.00** P< 0.000001 df =5 , 24					
M.anisopoliae					
$42.63^{e}\pm0.02$	$49.85^{d}\pm0.03$	$55.32^{c}\pm0.91$	$63.83^{b}\pm0.01$	$70.21^{a}\pm0.02$	$0.80^{f}\pm0.31$
F=127.00** P<0.000001 df=5,24					

Means followed by the different letters are not significantly different (P<0.05)

Table 2: Percentage Mortality of *B.zonata* pupae (one day old) to *B.bassiana* and *M.anisopoliae* at different concentrations

Mean % mortality of *B.zonata* pupae treated with					
B.bassiana					
1×10^{6}	1×10^{7}	1×10^{8}	1×10^{9}	1×10^{10}	Control
$57.61^{e}\pm0.81$	$71.43^{d}\pm0.01$	$79.62^{c}\pm0.02$	$81.63^{b}\pm0.73$	$91.34^{a}\pm0.03$	$1.20^{f}\pm0.89$
F=306.00** P< 0.000001 df=5,24					
M.anisopoliae					
$57.14^{d}\pm0.01$	$57.68^{d}\pm0.02$	$59.18^{c}\pm0.02$	$61.23^{b}\pm0.81$	$63.23^{a}\pm0.01$	$1.09^{e}\pm0.13$
F=64.20** P<0.000001 df=5,24					

Means followed by the different letters are not significantly different (P<0.05)

Table 3: Percentage Mortality of *B.zonata* pupae (four days old) to *B.bassiana* and *M.anisopoliae* at different concentrations

Mean % mortality of *B.zonata* pupae treated with					
B.bassiana					
1×10^{6}	1×10^{7}	1×10^{8}	1×10^{9}	1×10^{10}	Control
$29.17^{e}\pm0.21$	$41.67^{d}\pm0.67$	$47.92^{c}\pm0.09$	$56.25^{b}\pm0.45$	$60.42^{a}\pm0.49$	$0.20^{f}\pm0.43$
F=132.00** P-value 0.00001 df=5,24					
M.anisopoliae					
$38.98^{d}\pm0.23$	$35.14^{d}\pm0.31$	$46.08^{c}\pm0.63$	$49.12^{b}\pm0.52$	$51.23^{a}\pm0.55$	$0.40^{e}\pm0.24$
F=88.53** P<0.000001 df=5,24					

Means followed by the different letters are not significantly different (P<0.05)

(Table 4): Percentage Mortality of *B.zonata* flies (one day old) to *B. bassiana* and *M.anisopoliae* atdifferent concentrations

Mean % mortality of *B.zonata* treated with					
B.bassiana					
1×10^{6}	1×10^{7}	1×10^{8}	1×10^{9}	1×10^{10}	Control
$47.97^{e}\pm0.02$	$62.60^{d}\pm0.08$	$76.42^{c}\pm0.07$	$84.90^{b}\pm0.10$	$96.75^{a}\pm0.11$	$0.00^{f}\pm0.00$
F=574.27*** P-value 0.000001 Df=5,24					
M.anisopoliae					
$51.64^{d}\pm0.05$	$58.27^{d}\pm0.03$	$82.87^{c}\pm0.03$	$93.44^{b}\pm0.03$	$96.72^{a}\pm0.06$	$0.00^{e}\pm0.00$
F=802.00*** P-value 0.000001 Df=5,24					

Means followed by the different letters are not significantly different (P<0.05)

Table (5): Toxicity of *B.bassiana* and *M.anisopoliae* on different stages of *B.zonata*

Stage	LC_{50} conidia/ml	LC_{90} Conidia/ml	slope±SE	χ^2	P
B.bassiana					
Full-grown larvae	5.15×10^{5}	1.53×10^{10}	0.286±0.073	1.208	0.7511
Pupae	1.45×10^{5}	8.03×10^{9}	0.271±0.061	0.801	0.8492
Adult flies	1.23×10^{6}	5.31×10^{9}	0.353±0.042	0.225	0.974
M.anisopliae					
Full-grown larvae	1.23×10^{7}	1.81×10^{11}	0.179±0.565	0.041	0.9978
Pupae	2.5×10^{5}	1.56×10^{36}	0.037±0.057	0.166	0.9829
Adult flies	1.44×10^{6}	6.13×10^{9}	0.494±0.056	5.125	0.165

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ARABIC SUMMERY

تقييم قدرة الفطرين بيوڤاريا باسيانا وميتاريزيم أنيسوبلي لإحداث المرض في حشرة ذبابة ثمار الخوخ (رتبة ثنائية الأجنحة :تيفريتيدى)

محمدعادل حسين[١]، أماني سليمان خالد[١]، أحمدعدلي محمدإبراهيم[٢]، نهاد عبد الحميد سليمان[٢]، صفاء حفناوى عطية[٢]

١- كلية العلوم جامعةعين شمس

٢- معهد بحوث وقاية النباتات - مركزالبحوث الزراعية

هدف هذا البحث التحقق من قدرة كل من الفطرين الممرضين للحشرات بيوفيرياباسيانا و ميتاريزيومأنايزوبلى لإحداث المرض للأطوار الغير كاملة و الكاملة من حشرة ذبابة ثمار الخوخ ، باكتروسيرازوناتا (سوندرز) و قد أثبتت الدراسة عن أن النسب المئوية للموت كانت مرتبطة بزيادة تركيزات الفطريات المستخدمة كما أسفرت عن تفوق فطر البيوفيرياباسيانا عن الفطر الأخر ميتاريزيوم أنايزوبلى في تحقيق الموت لطور العذراء في العمر المبكر عنه في العمر الأقدم و كان الذباب الأكثر تأثرا بالإصابة بالفطر بعد خروجه من العذارى بيوم واحد.