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Susceptibility of the Peach Fruit Fly, *Bactrocera zonata* (Saunders), (Diptera: Tephritidae) to Three Entomopathogenic Fungi

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ABSTRACT

The peach fruit fly, *Bactrocera zonata* (Saunders) (Diptera: Tephritidae) is one of the most serious polyphagous insect pests in Egypt. Its control depends upon chemical sprays with organophosphate pesticides, especially Malathion mixed with protein baits. Their environmental impacts and development of resistance have justified the need to find sustainable control alternatives. Therefore, susceptibility of *B. zonata* to three entomopathogenic fungi was studied. Laboratory experiments to determine the virulence of the fungi; *Beauveria bassiana*, *Metarhizium anisopliae* and *Lecanicillium muscarium* (formerly *Verticillium lecanii*) on 2nd and 3rd instars' larvae, 1 and 6-day old pupae, newly emerged males and females of the pest were conducted at the recommended dose 5 g/ liter of water (5×10^8 conidia/l) and when each ml was containing 5×10^5 conidia. Obtained results showed that the susceptibility of males to the fungi was higher than that of females in both bioassay treatments. The virulence of fungi was also higher in oral bioassay than contact one in both sexes. Moreover, *M. anisopliae* gave higher mortality than *B. bassiana* and *L. muscarium*, reaching 94.4% in males and 76.8% in females in contact bioassay test. Meanwhile, *M. anisopliae* gave a mortality level of 100 and 95.2% for males and females, respectively in the oral bioassay. In case of *M. anisopliae*, median lethal times (LT_{50s}) were rather short for males (6.23, 8.93 days) than (7.49, 10.89 days) for females, followed by *B. bassiana*, 10.83, 12.69 d. for males and 10.17, 14.39 d. for females. *L. muscarium* showed much longer LT_{50s}; 15.32, 18.05 d. for males and 17.02, 22.36 d. for females. Data revealed that both 2nd instar larvae and 1-day old pupae were more susceptible than 3rd instar larvae and 6-day old pupae to all the tested fungi. Based on the obtained results, 2nd instar larvae, 1-day old pupae and male adults were used to determine the interactions among the three fungi on mortality response. Results also showed some potential effect when *M. anisopliae* was mixed with either *B. bassiana* or *L. muscarium*.

Key words: *Bactrocera zonata*, *Beauveria bassiana*, *Metarhizium anisopliae*, *Lecanicillium muscarium*, virulence, susceptibility.

INTRODUCTION

The peach fruit fly, *Bactrocera zonata* (Saunders) (Diptera: Tephritidae) is one of the serious polyphagous insect pests in the world, particularly several countries in the Near East and Egypt due to its wide host range, high reproductive potential, high mobility and adaptability to climate. It attacks many fruit species (more than 50 host plants), including mango, peach, guava, citrus, fig, apple and tomato. (White and Elson-Harris, 1992).

In Egypt, the pest control depends largely on insecticidal applications, especially Malathion, mixed with protein baits. Intensity of insecticidal treatments against *B. zonata* has resulted in development of resistant populations (Ortego *et al.*, 2005). Their environmental impacts and development of resistance have justified the need to find sustainable control alternatives. Therefore, entomopathogenic fungi were tested. Several reports focused on the potential of bacteria and nematodes as biological control agents against fruit flies (Toledo *et al.*, 2005, Mahmoud 2007 and Mahmoud and Osman 2007). Several species of fungi have the potential as viable biocontrol agents to insects. The most commonly used Deuteromycetes fungi include the genera *Beauveria*, *Metarhizium* and *Lecanicillium*. Entomopathogenic fungi based-biocides are currently commercialized. The viability of the fungus should be evaluated since it is an important component of the quality of the product. Reports concerning the use of entomopathogenic fungi against fruit flies, especially the peach fruit fly are meager (Quesada *et al.*, 2006 and Aemprapa 2007).

Objective of the present study focused on determination of the susceptibility of *B. zonata* to three entomopathogenic fungi namely; *Beauveria bassiana*, *Metarhizium anisopliae* and *Lecanicillium muscarium* under laboratory conditions.

MATERIALS AND METHODS

Entomopathogenic fungi used

Commercial formulations of the bio-pesticides; Bio-Power (*Beauveria bassiana*), Bio-Magic (*Metarhizium anisopliae*) and Bio-Catch (*Lecanicillium muscarium*) (1.15% WP), talc based biological insecticides containing spores and mycelias fragments of 1×10^8 conidia/g were tested. The biocides were obtained from T. Stanes and Company LTD, Tamil Nadu, India. All formulations were used at the recommended dose of 5 g/liter of water. Viability of conidia was determined for each bio-pesticide by spreading conidial suspension in Petri dishes containing thin layers of yeast extract

agar (YPDA) and incubated 20 h at 25 °C and by counting the germinated spores using an optical microscope (400x).

Test insect

Initial culture of *B. zonata* was obtained from infested mango fruits at Ismailia Governorate, Egypt and maintained under laboratory conditions of 25 ± 2 °C, 65–75% RH and a photoperiod of L: D 12h. Adult diet consisted of 1 part of protein hydrolyzate: 3 parts of sugar weight/ weight, while the larval diet consisted of wheat bran 100g, brewer's yeast 17g, granulated sugar 33g, agar 3.5 g, nipagin 0.5 g, hydrochloric acid 20 ml and water 400 ml (Qureshi *et al.* 1974).

Experiments:

1- Contact bioassay for adults

25 newly emerged male and female adults of *B. zonata* were placed in experimental cages (10× 20×15 cm) in five replicates. Adults' diet and water were provided regularly. The recommended dose (5× 10^5 conidia/ml) of the entomopathogenic fungi were prepared and kept in SIGMA small sprayer 50 ml in volume before spraying of adults carefully for 30 seconds. Control was similarly grouped and flies were treated with distilled water only. Dead adults were counted daily and tabulated four times at 5, 10, 15 and 20 days after treatments.

2- Oral bioassay for adults

Suspensions (1 ml) of *B. bassiana*, *M. anisopliae* and *L. muscarium* were mixed with 1 ml of adults' diet for preparation dose (5×10^5 conidia/2 ml of diet). Flies consumed the treated diet within 2-3 days. Control adults were fed on diet without fungal conidia. 25 adults were placed in cages (10×20 ×15 cm) under laboratory rearing conditions. Each treatment was replicated five times and mortality rate was recorded daily and tabulated four times at 5, 10, 15 and 20 days after treatments (Konstantopoulou and Mazomenos, 2005).

3- Contact bioassay for 2nd and 3rd instar larvae

2nd and 3rd instar larvae were washed in distilled water and individually placed in small glass cups in five replicates (25 individuals). Each replicate was dipped in 1 ml of the recommended dose (5×10^5 conidia/ml) for 30 seconds. Similar amount of larvae was used for the control, which was dipped in only sterile distilled water. Replicates of treated larvae were placed in Petri-dishes with larval diet (20 g/ dish). The Petri -dishes were checked daily to record mortality rate.

4- Contact bioassay for pupae (Soil bioassay)

Assays were performed using field soil (Sandy-loam: 67.3% sand, 15.4% silt and 17.3% clay), collected from the Farm of the Faculty of Agriculture, Suez Canal University, Ismailia. The soil was first sterilized in an autoclave then placed in a dry oven at 70°C for 24 h and stored in bags until needed. Five replicates of pupae of *B. zonata* were dipped in 5×10^5 conidia/ml for 30 seconds and the same amount of pupae was used for the control. The treated pupae were kept in sterile soil (50 g/ small glass cup). Each replicate was 25 pupae. Dead pupae were recorded after adult emergence in all treatments.

5- Interactions among entomopathogenic fungi

In this experiment, each two fungi together (*B. bassiana* + *M. anisopliae*), (*B. bassiana* + *L. muscarium*) and (*M. anisopliae* + *L. muscarium*) was tested. Suspension mixture contained 2.5×10^5 conidia/ ml / fungus. Five replicates (25 individuals each) of males, 2nd instar larvae and 1-day old pupae were treated using dipping method. Males' diet and water were provided regularly. Recommended dose was prepared and kept in small sprayer before spraying the males for 30 seconds. Replicates of treated larvae were placed in Petri-dishes with larval diet (20 g/ dish). The treated pupae were kept in sterile soil (50 g/ small glass cup). Mortality was recorded after 20 days for males as accumulative mortality rate and after adult emergence for larvae and pupae. Control treatments were similarly grouped and treated with distilled water.

Statistical analysis

Obtained data were statistically analyzed using ANOVA (SAS Institute. 2002). When F-test was significant, means were separated using Tukey's Honestly Significant Difference (HSD) Test at the 0.05 level of significance. The median survival (mortality) times, LT_{50} of entomopathogenic fungi was calculated by using Kaplan-Meier Survivorship Test (SPSS, 1990). The co-toxicity coefficient values were calculated by the method of Sun and Johnson (1960). When the co-toxicity coefficient of a mixture is 100, the effect of this mixture indicates probability of additive action. If the mixture gives a coefficient significantly greater than 100, it indicates a synergistic action. On the other hand, when a mixture gives a co-toxicity coefficient less than 100, the effect of mixture indicates an antagonistic action. In those cases, where potential occurred the co-toxicity coefficient of mixture was divided by one hundred to obtain the degree of potential (Gera and Gupta, 1978).

RESULTS AND DISCUSSION

Contact bioassay of adults

Virulence of *M. anisopliae, B. bassiana* and *L. muscarium* against adults of *B. zonata* was

estimated four times after treatments (Table 1). Significant differences were found in virulence at all the entomopathogenic fungi tested against adult males.

Oral bioassay of adults

Similar results were obtained when *B. zonata* adults were fed on diet contained conidia of the tested entomopathogenic fungi (Table 2). Data showed significant differences of the virulence at four different intervals after treatment. Highest virulence occurred in adults fed on diet contained conidia of *M. anisopliae*, followed by those treated with *B. bassiana* and *L. muscarium* in both males and females of *B. zonata*. Mortality rates were significantly different than the control in adult males.

As shown in tables (1 and 2), data clearly showed that males of *B. zonata* were more susceptible to all the tested fungi compared to females in both contact and oral bioassays. But, this susceptibility was higher in the treatment with *M. anisopliae* than that with *B. bassiana* and *L. muscarium* in both bioassays. Probit analysis of the time mortality response revealed that *M. anisopliae* killed adults of *B. zonata* much faster than *B. bassiana* and *L. muscarium*. LT_{50s} were 8.93, 12.69 and 18.05 days for males and 10.89, 14.39 and 22.36 days for females treated by contact bioassay. LT_{50s} were shorter in all the fungi treatments under oral bioassay. In males, LT_{50s} were 6.23, 10.83 and 15.32 days while they were 7.49, 10.17 and 17.02 days in females at *M. anisopliae*, *B. bassiana* and *L. muscarium* treatments, respectively.

Contact bioassay for 2nd and 3rd instar larvae

Data in Table (3) showed that 2nd larval instar of *B. zonata* was more susceptible (F=48.04; P≤ 0.0000) than the 3rd instar (F=64.01; P≤ 0.0000) at all fungi tested. Also, *M. anisopliae* showed high virulence toward both larval instars of *B. zonata*. It was 80.0 and 63.2% for 2nd and 3rd instars. However, the fungus *L. muscarium* gave less virulence 30.4 and 17.6% for 2nd instar and 3rd instars, respectively.

Contact bioassay for pupae (in Soil bioassay)

Results clearly indicated that 1-day old pupae of *B. zonata* was fairly susceptible (F=62.67; P≤ 0.0000) than the 6-day old pupae (F=12.56; P≤ 0.0002) at all the tested fungi (Table 4). Newly pupae was fairly susceptible to *M. anisopliae* (36.0%), followed by *B. bassiana* (24.8%) and then *L. muscarium* (4.0%).

Interactions among entomopathogenic fungi

In figures (1, 2 and 3), results illustrate interactions among the tested fungi on mortality response of *B. zonata* compared with using each fungus alone. In this experiment, males, 2nd instar larvae and 1-day old pupae of *B. zonata* were selected, since males were more susceptible than females, 3rd instar larvae and 6-day old pupae. Data indicated that the fungus *M. anisopliae* caused potential effect to both *B. bassiana* and *L. muscarium*. This would imply that *M. anisopliae*

Table (1): Virulence and time mortality response of *Bactrocera zonata* adults treated as contact bioassay with *Beauveria bassiana*, *Metarhizium anisopliae* and *Lecanicillium muscarium* (at the concentration of 5×10^5).

Fungus	% Mortality									
	Males					Females				
	5*	10*	15*	20*	LT_{50}	5	10	15	20*	LT_{50}
M. anisopliae	20.0[a]	52.8[a]	76.8[a]	94.4[a]	8.93	18.4[a]	44.8[a]	64.8[a]	76.8[a]	10.89
B. bassiana	7.2[b]	35.2[b]	56.8[b]	77.6[b]	12.69	11.2[b]	32.8[b]	51.2[b]	66.4[b]	14.39
L. muscarium	0.0[c]	11.2[c]	40.8[c]	54.4[c]	18.05	0.0[c]	0.0[c]	16.0[c]	35.2[c]	22.36
Control	0.0[c]	0.0[d]	1.6[d]	3.2[d]	-	0.0[c]	0.0[c]	0.0[d]	1.6[d]	-

Table (2): Virulence and time mortality response of *Bactrocera zonata* adults treated as oral bioassay with *Beauveria bassiana*, *Metarhizium anisopliae* and *Lecanicillium muscarium* (at the concentration of 5×10^5).

Fungus	% Mortality									
	Males					Females				
	5*	10*	15*	20*	LT_{50}	5	10	15	20*	LT_{50}
M. anisopliae	34.4[a]	79.2[a]	100[a]	100[a]	6.23	33.6[a]	55.2[a]	83.2[a]	95.2[a]	7.49
B. bassiana	20.8[b]	43.2[b]	62.4[b]	79.2[b]	10.83	24.8[a]	48.0[a]	64.8[b]	75.2[b]	10.17
L. muscarium	8.0[c]	32.8[b]	48.0[c]	61.6[c]	15.32	4.8[b]	24.8[b]	42.4[c]	59.2[c]	17.02
Control	0.0[c]	0.0[c]	0.0[c]	1.6[d]	-	0.0[b]	0.0[c]	0.8[d]	1.6[d]	-

*Days after treatment

Means followed by the same letters in the same column are not significantly different (HSD at P ≤ 0.05).

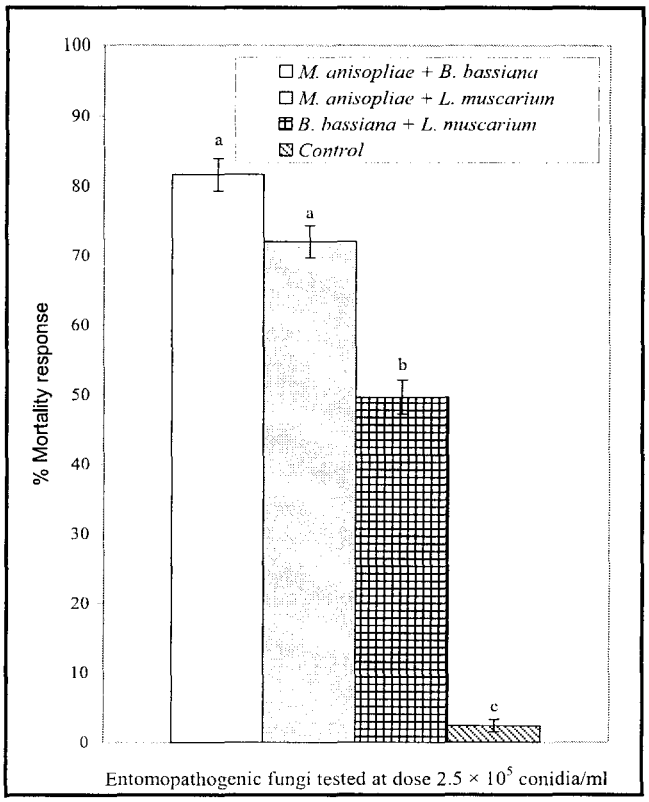

Figure (1): Interactions among *B. bassiana*, *M. anisopliae* and *L. muscarium* on mortality response of *B. zonata* adults (males), treated as contact bioassay.

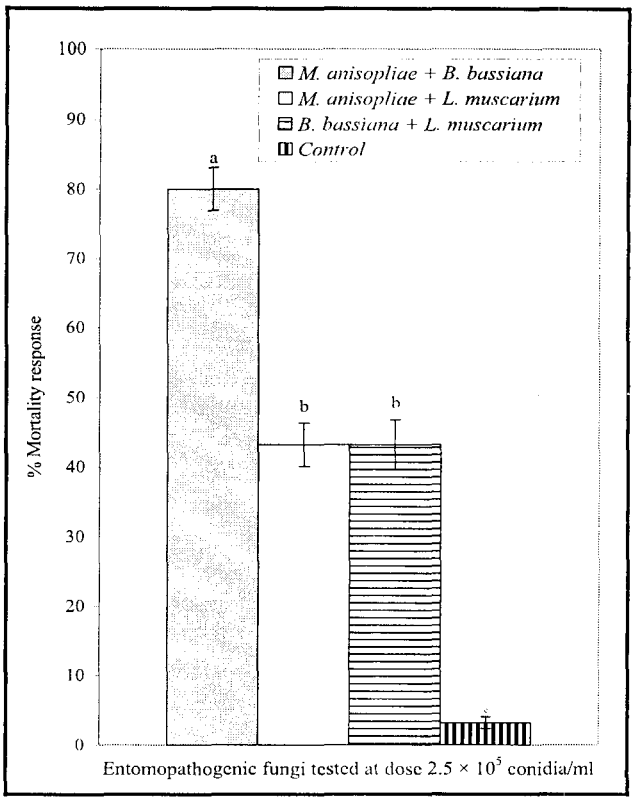

Figure (2): Interaction among *B. bassiana*, *M. anisopliae* and *L. muscarium* on mortality response of *B. zonata* 2nd instar larvae, treated as contact bioassay.

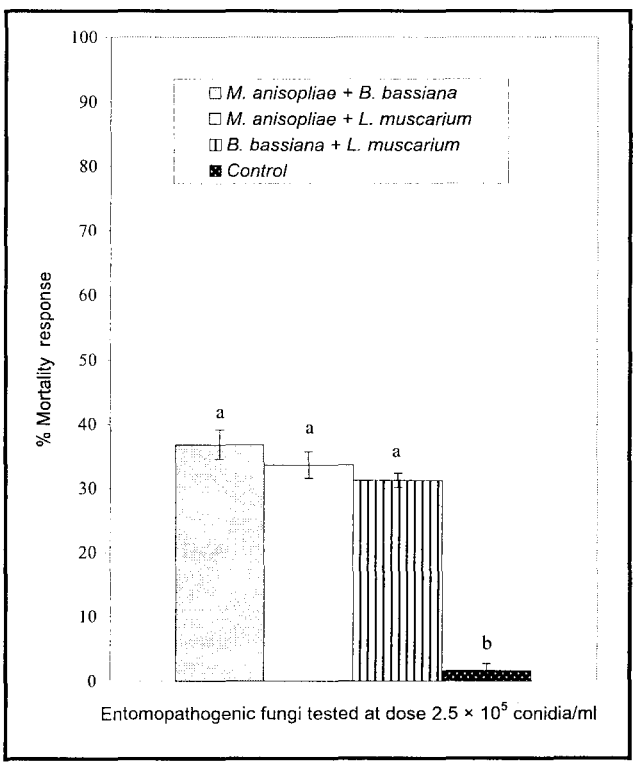

Figure (3): Interaction among *B. bassiana*, *M. anisopliae* and *L. muscarium* on mortality response of *B. zonata* 1-day old pupae, treated as contact bioassay.

Table (3): Susceptibility of *Bactrocera zonata* larvae to contact application with *Beauveria bassiana*, *Metarhizium anisopliae* and *Lecanicillium muscarium* (at the concentration of 5×10^5).

Fungus	% Mortality	
	2nd instar larvae	3rd instar larvae
M. anisopliae	80.0^a	63.2^a
B. bassiana	55.2^b	43.2^b
L. muscarium	30.4^c	17.6^c
Control	2.4^d	3.2^d

Means followed by the same letters in the same column are not significantly different (HSD at $P \leq 0.05$).

Table (4): Susceptibility of *Bactrocera zonata* pupae to contact application with *Beauveria bassiana*, *Metarhizium anisopliae* and *Lecanicillium muscarium* (at the concentration of 5×10^5).

Fungus	% Mortality	
	1-day old pupae	6-day old pupae
M. anisopliae	36.0^a	12.0^a
B. bassiana	24.8^b	3.2^b
L. muscarium	4.0^c	0.0^b
Control	1.6^c	0.0^b

Means followed by the same letters in the same column are not significantly different (HSD at $P \leq 0.05$).

gave synergistic action as co-toxicity coefficient was greater than 100 in all treatments. In adult treatments, the co-toxicity coefficient was 133.3 in the mixture of (*M. anisopliae* + *B. bassiana*) and 222.2 in (*M. anisopliae* + *L. muscarium*). In larval stage, it was 122.5 in (*M. anisopliae* + *B. bassiana*) and 201.6 in (*M. anisopliae* + *L. muscarium*). Also, in pupal stage, it was 102.2 in (*M. anisopliae* + *B. bassiana*) and 115.3 in (*M. anisopliae* + *L. muscarium*).

In this study, susceptibility of *B. zonata* adults to the tested entomopathogenic fungi was high in both *M. anisopliae* and *B. bassiana* than *L. muscarium* and was higher in males than females. These results confirm the susceptibility of some fruit flies to entomopathogenic fungi as reported in the earlier studies of Lezama-Gutierrez *et al.*, (2000) and Ladurner *et al.*, (2007).

Although, *B. zonata* adults were susceptible to the entomopathogenic fungi tested, considerable variations occurred in such susceptibility. The highest was recorded for *M. anisopliae*, reaching up to 100% mortality in adult males. However, females mortality reached 94.4% after 20 days of oral bioassay. The least adult susceptibility was recorded in oral treatment with *L. muscarium* at 61.6 and 59.2% for males and females, respectively. Results revealed that susceptibility of both males and females was higher in all entomopathogenic fungi oral bioassay when compared to contact bioassay. Apparently, most of the earlier studies used the entomopathogenic fungi as pathogens of insect pests via cuticle. The most common route of host invasion is through the external integument, although infection through the digestive tract is possible (Goettel and Inglis, 1997). Ferron (1981) mentioned that method of application of entomopathogenic fungi, life stage and the biochemistry of the insect surfaces are all factors that affect fungi virulence against insect pests. Konstantopoulou and Mazomenos (2005) found that *B. bassiana* had a moderate toxic effect when tested in oral bioassay against *Bactrocera oleae* adults.

Obtained results agree with other studies carried out on different insect pests of Tephritidae. For example, Espin *et al.* (1989) obtained 69-78% mortality in *Ceratitis capitata* adults with *M. anisopliae*. Castillo *et al.* (2000) reported that *Verticillium lecanii* showed low mortalities (>10%) against *C. capitata*. *M. anisopliae* and *B. bassiana* also showed high virulence 92 and 80% mortality rates against *B. oleae*, respectively. Laboratory and semi-field studies showed that Tephritid flies *C. capitata*, *Rhagoletis cerasi* and *B. oleae* were susceptible to infection by *B. bassiana* -based bio-pesticides (Naturalis) (Ladurner *et al.*, 2007; Daniel and Wyss, 2008 and Daniel, 2009).

The value of LT_{50} suggested that *M. anisopliae* was the most virulent followed by *B. bassiana*, but *L. muscarium* was the least virulent one. These results agree with those of Junior *et al.* (2008) who mentioned that *M. anisopliae* caused the highest mortality and the shortest LT_{50} (5 to 6 days) when used against *Oncometopia facialis*. Also, Khashaveh *et al.* (2008) stated that three Iranian isolates of *M. anisopliae* gave LT_{50} values ranged from 5.54 to 7.9 days against *Sitophilus granarius*. However, LT_{50} was 8 days and > 10 days in *Anopheles gambiae* treated with *B. bassiana* (Achonduh and Tondje, 2008).

In the present study, susceptibility of 3rd instar larvae was higher than the 2nd one, this simply due to the longer exposure period of the 2nd instar to the entomopathogenic fungi. No previous reports concerning the virulence of *M. anisopliae*, *B. bassiana* and *L. muscarium* on *B. zonata* were found, but Kaaya and Munyinyi (1995) mentioned that the application of dry spores of *M. anisopliae* and *B. bassiana* produced no mortality in larvae of tsetse flies. Similar results were reported by Dimbi *et al.* (2003) who found no effect of *M. anisopliae* and *B. bassiana* on larvae of *C. capitata*, *C. rosa* and *C. cosyra*, although they were highly virulent against adult stage.

B. zonata pupae showed variations in susceptibility towards tested entomopathogenic fungi. The newly formed pupae (1-day old) were more susceptible to entomopathogenic fungi than 6-day old pupae. Newly formed pupae were susceptible to *M. anisopliae* recording 36.0% and *B. bassiana* 24.8% mortality. However, they showed less susceptibility (4.0%) towards *L. muscarium*. These results disagree with those reported by Ekesi *et al.* (2002) using entomopathogenic fungi against *C. capitata* pupae with mortality reached to 94%. On the contrast, Aluja (1993) found no effect of *B. bassiana* on *Anastrepha ludens* pupae because it possesses a thick and completely sclerotized cuticle and a hard capsule with a scaly surface.

Mortality response of *B. zonata* (2nd instar larvae, 1 day old pupae and adult males) increased by using the mixtures of *M. anisopliae* + *B. bassiana* and *L. muscarium*. Results revealed that *M. anisopliae* caused potential effect to other both fungi in all treatments. Combination among different species of entomopathogenic fungi may enhance the efficacy of their pest control. Entomopathogens have different types of relationships in vivo including independent development, antagonism, synergism and others. There are limited information about results of mixed fungal spores of insects. Several experiments were conducted to establish the effect of two fungal pathogens attacking a single host. *B. bassiana* and *M. flavoviridae* were applied

together against *Melanoplus sanguinipes* (Inglis *et al.*, 1999). Gouli *et al.* (2008) demonstrated that the principal possibility of using the tank mixtures of different species of entomopathogenic fungi for control of *Frankliniella occidentalis* gave a good result for mass production. In addition, formulation of *Trichoderma viride* in combination with *M. anisopliae* could possibly perform for mass production to be used as broad spectrum mycopesticide

In conclusion, this study demonstrated that *M. anisopliae* had the potential to be used for controlling most stages of *B. zonata.* Moreover, it caused a potential action when mixed with *B. bassiana* and *L. muscarium* against different stages of the pest. These experiments based on laboratory trials and need to be tested in field trials under various biotic and abiotic conditions.

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