

**On the Pathogenicity of Entomopathogens to the Peach Fruit Fly,
Bactrocera zonata (Saunders) (Diptera: Tephritidae)**

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ABSTRACT

A study to evaluate the pathogenicity of entomopathogenic nematodes and fungi against the peach fruit fly, *Bactrocera zonata* (Saunders) (Diptera: Tephritidae) was carried out under laboratory conditions. The nematodes species tested were; *Heterorhabditis bacteriophora*; *Steinernema carpocapsae* and *S. riobrave*. The fungi were; *Metarhizium anisopliae* and *Beauveria bassiana*. Different concentrations were used against immature stages of *B. zonata*, by contact and/or soil inoculation techniques. Results showed that both *Steinernema* species were more effective on full-grown larvae than *H. bacteriophora* in contact treatment, whereas *H. bacteriophora* was more effective in soil, as it induced higher mortality of *B. zonata* pupae at both techniques. Mortality rates of *B. zonata* pupae increased significantly by increasing the concentration of the two tested fungi at the same time of exposure. Adult mortality was affected by soil treatments as *B. bassiana* treatments significantly increased mortality rates compared to *M. anisopliae* and control treatments. Emerged adults from treated pupae with both fungi showed mycosis on the outer surface, additionally some emerged adults showed malformation and mostly failed to emerge. Results suggested that entomopathogenic nematodes and fungi can be used as alternative tools of pesticides for controlling *B. zonata* after validating protocols of field conditions.

Key words: *Bactrocera zonata*; Entomopathogenic nematodes; Entomopathogenic fungi; Biological control, Pathogenicity.

INTRODUCTION

Fruit flies (Diptera: Tephritidae) are major quarantine pests that cause devastating direct losses to many different types of commercial and wild fruits, and vegetables throughout tropical and sub-tropical countries and thus they have a great impact on international marketing and world trade in agricultural production (FAO/IAEA, 2000). The peach fruit fly (PFF), *Bactrocera zonata* (Saunders), is an invasive species, native to Southeast Asia. It was first recognized as a new pest of guava and mango in the northern region of Egypt in 1998 (El-Minshawy *et al.*, 1999). Now it is a serious pest of fruits replacing the Mediterranean fruit fly (Medfly), *Ceratitis capitata* (Wied.) in most of the Egyptian governorates. PFF attacks a wide range of host plants including; peach, guava, mango, date palm, apples, bitter gourd, okra, pomegranate, papaya, common fig, quince, sweet and bitter orange and possibly melons and water melons, in addition to numerous ornamentals (FAO/IAEA, 2000). Use of insecticides as the only way to control pests in fruits and vegetables has caused environmental pollution and hygienic problems that represent a risk for people and animals (Gallo, 2007).

Biological control methods offer alternative choices to the use of unsuccessful pollutant chemicals; among the biological control agents are the entomopathogenic nematodes (families: Steinernematidae and Heterorhabditidae) and the entomopathogenic fungi (*i.e.* *Metarhizium anisopliae* and *Beauveria bassiana*) (Singkaravanit *et al.*, 2010). These pathogens seem to be the most appropriate weapons for controlling this serious pest.

In this context, the objective of the present study was to evaluate the pathogenicity of selected entomopathogenic nematodes and fungi species against *B. zonata* immature stages and the selected fungi against the adults under laboratory conditions.

MATERIALS AND METHODS

Insect

B. zonata used in the present study was obtained from the laboratory culture at Biological Control Department (DBC), Plant Protection Research Institute (PPRI), Agricultural Research Center (ARC) in Giza, Egypt. Rearing of *B. zonata* was carried out under the laboratory conditions of 25±2°C, 54-65% R.H. and 14:10 L: D photoperiod on artificial diet according to Marwa *et al.* (2011).

Pathogens

Entomopathogenic nematodes

Three nematode isolates, *Steinernema carpocapsae*; *S. riobrave* and *H. bacteriophora* Poinar [HP₈₈ strain] were supplied by Dr. S. Shairra, DBC, PPRI, ARC, Giza, Egypt. Stock cultures of the nematode isolates were maintained on 6th instar larvae of *Galleria melonella* L., according to Dutky *et al.* (1964) and modified by Shamseldean (1994). Infective juveniles (IJs) were harvested using the modified White traps (White, 1927). The IJs were maintained at sterile conditions at 20±5°C ready for tests against the last instar larvae (3rd instar) and pupae of *B. zonata*.

Pathogenicity of entomopathogenic nematodes against full grown larvae and pupae of *B. zonata*

1- Contact method

Appropriate amounts of nematodes' concentrations of (100, 200, 400, 800 and 1600 IJs/ ml) were counted under a microscope and added to a filter paper with 1ml distilled water in a Petri dish (10cm) (Mahmoud and Mahmoud, 2007). All concentrations were tested on filter papers against full grown larvae and pupae of *B. zonata* in three replicates. Eighteen individuals of each were exposed for 72 h to each concentration of *S. carpocapsae*; *S. riobrave* and *H. bacteriophora* Poinar (HP₈₈) strains suspensions. Control was treated by 1ml distilled water. Mortality percentages were recorded at 24, 48 and 72 h for larvae and only at 72 h for pupae post treatments.

2- Soil method

About 120g of dried autoclaved sand were placed into plastic cups (5x6x6 cm) and moistened with 1ml of nematodes concentrations (100, 200, 400, 800 and 1600 IJs/ 6 pupae/ cup). Pupal mortality rates were estimated and corrected according to (Abbott, 1925). LC₅₀ for each nematode's species was calculated according to (Finney, 1952). Control experiment was conducted without nematodes. Number of dead pupae in each cup was recorded 24, 48 and 72 h. post infection. Each treatment was replicated 3 times.

B- Entomopathogenic fungi

Two entomopathogenic fungi isolates, *M. anisopliae* and *B. bassiana*, were obtained from BCD, PPRI, ARC, Giza, Egypt. The fungi were incubated at 4°C as stock cultures. Conidial development at 25°C from each stock culture was used for bioassays.

Pathogenicity of *M. anisopliae* and *B. bassiana* against larvae and pupae of *B. zonata*

Pathogenicity of the two tested fungi; *M. anisopliae* and *B. bassiana* was estimated under laboratory conditions. A spore concentration of (1x10⁸ spores/ ml) was used as a stock concentration for the bioassay of *B. zonata*. Sterile distilled water with Tween 80 (0.1%) was used as control. Number of infected pupae was recorded daily after treatments for 2 weeks and then adults emerged from infected pupae were checked daily until their death.

1- Contact method

Full grown larvae were infected by the fungi spore concentrations of (1x10⁴, 1x10⁶ and 1x10⁸ conidia/ml) with 0.1 % Tween 80, larvae were sprayed carefully for 30 seconds, using a hand atomizer. The test was carried out using 20 larvae, placed in Petri dish (10 cm). The same number of full grown larvae was used as control, which were sprayed only by sterile distilled water with 0.1% Tween 80. Dead full grown larvae were counted daily. Each treatment was replicated 3 times. The experimental design was the Factorial Completely Randomized Design (Factorial in CRD) with isolate, as factor (A) and with concentration level as factor (B). LC₅₀ was calculated by COSTAT program, using probit analysis of (Finney, 1952). Virulence levels were performed by calculating log LC₅₀ and then leveling by Duncan's multiple range tests (DMRT) and normal distribution.

2- Soil method

A pathogenicity test of the two selected fungal strains was carried out using plastic pots (7×5×5 cm) and autoclaved soil. Twenty pupae were placed about 5 cm below the soil surface. Conidial suspensions were applied by two ways; drenching and pre-mixing. Conidial suspension was prepared to the final volume of 10⁸ conidia/ ml in both experiments. The pots were kept under a shade net at 29.1±2°C and 80% R.H. Trials were carried out in three replicates according to (Mar and Lumyong, 2012).

Pathogenicity of *M. anisopliae* and *B. bassiana* against adults of *B. zonata*

1- Spray method

Twenty newly emerged adults were placed in experimental cages (35x30x30 cm). Adults were sprayed by the fungal concentration (1x10⁸ conidia/ml) for 30 seconds, using a hand atomizer. Adults' diet and water were supplied and kept under room rearing conditions. Same number of adults was used as control, which was

sprayed only by sterile distilled water. Dead adults were counted at 5, 10, 15 and 20 days post treatment. The experiment was replicated 3 times.

2- Oral method

One ml of each fungi suspension (1×10^8 conidia/ml) was prepared and mixed with 1 ml of adult diet. The control was provided by uncontaminated diet. Each treatment was replicated three times and mortality rate was recorded daily.

Statistical analysis:

Probit analysis of obtained data was calculated according to (Finney, 1952) using “Ldp-line” software (<http://www.Ehabsoft.com>).

RESULTS AND DISCUSSION

Infectivity of entomopathogenic nematodes to *B. zonata* immature stages

The selected nematodes; *H. bacteriophora*, *S. carpocapsae* and *S. riobrave* were tested against immature stages of *B. zonata* under laboratory conditions. Infectivity was significantly different among the three tested nematode strains. Pupae of *B. zonata* showed less susceptibility to the nematode's infection than full grown larvae.

1. Contact method

A- Full grown larvae

All concentrations of the three nematodes showed effective impact on full grown larvae of *B. zonata* but mortality rates differed according to the nematode's strain and/ or concentration used (Table 1).

The lowest concentration (100 IJs / ml) of each of the nematodes' strains caused least mortality percentages among the treated larvae, showing 40.91, 65.81 and 70.81% for *H. bacteriophora*, *S. carpocapsae* and *S. riobrave*, respectively. In this context, mortality rates increased with increasing the concentration to 200, 400, 800 and 1600 IJs/ ml. Respective mortality rates reached 88.1, 96.4 and 93.2% at the concentration of (1600 IJs/ ml). Most of larval cadaver had melanized black spots as a result of nematodes' infection.

Obtained results showed a similarity with those of (Rohde *et al.*, 2012) who reported that *S. carpocapsae* ALL was highly virulent to *C. capitata* larvae and had probably little efficacy against the pupae for being an ambusher strategist, making encounters more difficult between pupae (sedentary) and infective juveniles. Furthermore, (Almeida *et al.*, 2007) reported that efficiency of *Heterorhabditis* sp. in reducing *C. capitata* emergence rate attained 80 and 40% in laboratory and greenhouse, respectively.

Steinernematid strains were more effective than heterorhabditid ones. This may lie in the fact that members of these species are known to adopt a sit and wait strategy for infection hence, they termed “ambushers” and they are more effective against soil dwelling insects (Gaugler, 1997 and Attalla *et al.*, 2002). Other factors may also be responsible for such differences in isolates infectivity, such as their ability to penetrate the host as in *S. carpocapsae* isolate which had a small size of its infective juveniles (ranged between 438-650µm) (Adams, 2002 and Rohde *et al.*, 2012) thus, facilitating the penetration mode of the steinernematids, which takes place through the natural openings of the host.

B- Pupae

1- Contact method

Generally, all concentrations of the three nematode strains showed effective impact on the pupae of *B. zonata* under laboratory conditions but mortality rates differed according to nematode's strain and /or concentration used (Table 1). The lowest concentration (100 IJs/ ml) of each of nematode's strain caused the least mortality percentages in the treated pupae. They were 33.48, 5.98 and 16.61% in case of *H. bacteriophora*, *S. carpocapsae* and *S. riobrave*, respectively. Mortality percentages increased by increasing the concentrations to 200, 400, 800 and 1600 IJs/ ml, as respective mortality rates recorded were 55.41, 43.71 and 49.71% at the concentration of (1600 IJs/ ml) (Table. 1).

Obtained data showed that *H. bacteriophora* was much effective, with a mortality range of 1.1-5.5% on the treated stage, compared with the two species of *Steinernema*; *S. carpocapsae* and *S. riobrave*. The difference in pathogenicity of *H. bacteriophora* to the pupae was higher (5.5%) than *S. carpocapsae* and (2.1%) than *S. riobrave* at the concentration of 100 IJs, while it was less at the concentration of 1600 IJs, as it ranged between 1.1 and 1.2%. Generally, *H. bacteriophora* was more infective to pupae than the two strains of *Steinernema*. This phenomenon can be attributed to the ability and capability of heterorhabditid nematodes to

Table (1): Mortality % of *B. zonata* larvae and pupae after exposure to three entomopathogenic nematode strains

Nematode strain		Mortality %					
		100 IJs	200 IJs	400 IJs	800 IJs	1600 IJs	LC ₅₀
Full-grown larvae	<i>H. bacteriophora</i>	40.91	60.9	78.61	83.78	88.06	23.16
	<i>S. carpocapsae</i>	65.81	72.57	80.33	90.15	96.35	20.08
	<i>S. riobrave</i>	70.8	78.35	84.62	89.55	93.22	13.69
Pupae	<i>H. bacteriophora</i>	33.48	38.74	44.22	49.82	55.41	818.39
	<i>S. carpocapsae</i>	5.98	11.37	19.56	30.57	43.71	2191.86
	<i>S. riobrave</i>	16.61	23.29	31.26	40.21	49.71	1634.36

Table (2): Mortality % values of three entomopathogenic nematode strains tested against pupae of *B. zonata* in soil under laboratory conditions

Nematode strain		Mortality %					
		100 IJs	200 IJs	400 IJs	800 IJs	1600 IJs	LC ₅₀
Pupae	<i>H. bacteriophora</i>	18.37	26.76	36.76	47.74	58.89	920.11
	<i>S. carpocapsae</i>	14.51	21.23	29.51	39.03	49.26	1681.41
	<i>S. riobrave</i>	20.82	28.11	36.45	45.49	54.76	1120.61

Table (3): % Mortality, LC₅₀ and Slope response of *B. zonata* pupae treated with *B. bassiana* and *M. anisopliae* under laboratory conditions (contact method)

Entomopathogenic Fungi	Concentration (conidia / ml)	% Mortality	LC ₅₀	Slope
<i>B. bassiana</i>	1×10^4	50	9241.29	0.2788
	1×10^6	72.22		
	1×10^8	86.66		
	Control	0.8	-	-
<i>M. anisopliae</i>	1×10^4	37.77	283697.3	0.2168
	1×10^6	54.44		
	1×10^8	71.12		
	Control	0.6	-	-

2. Soil method

The lowest concentration (100 IJs / ml) of each of the nematode's strain caused the least mortality percentages among treated pupae. They were 18.37, 14.51 and 20.82% in case of *H. bacteriophora*, *S. carpocapsae* and *S. riobrave*, respectively. Mortality percentages increased by increasing the concentrations to 200, 400, 800 and 1600 IJs/ ml. Respective mortality rates reached 58.89, 49.26 and 54.76% at the concentration of (1600 IJs/ ml) (Table 2). Dead pupae infected with nematodes showed melanized black spots on their outer surface. Highest percentage of infected pupae treated in the soil was (58.89%), recorded in case of *H. bacteriophora* at the concentration 1600 IJs, while the lowest one (49.26%) was recorded in case of *S. carpocapsae* at the same concentration.

Infectivity of entomopathogenic fungi to *B. zonata*

A - Immature stages

A.1. Contact method

All the treated larvae pupated normally without mortality. Pupae were placed on moist peat eight days after pupation to stimulate fungus growth. *M. anisopliae* showed least mycosis incidence on the outer surface of treated pupae compared with *B. bassiana*. Dead pupae infected with *B. bassiana* showed mycosis on their outer surface.

Data presented in table (3) show mortality percentages, LC₅₀ and slope values among *B. zonata* pupae when infected with different concentrations of the two entomopathogenic fungi under laboratory conditions. Generally, adult mortality increased; emergence rate decreased and many flies failed to emerge accordingly. Mortality rates of *B. zonata* pupae increased significantly by increasing the concentration of the two tested fungi and the time of exposure (Mar and Lumyong, 2012). Alves *et al.* (2004) concluded that a higher conidial concentration (1×10^8 conidia/ml) of *B. bassiana* and *M. anisopliae* was needed to prevent *C. capitata* emergence. Mortality percentages of *B. zonata* pupae increased by increasing the fungal concentrations. These results are consistent with those of (Mar and Lumyong, 2012) who reported that the adults' emergence of *Musca domestica* larvae was reduced as the fungal concentration increased. Emerged adults from treated pupae showed mycosis on the surface and malformation. Low amount of mycosis was observed in case of *M. anisopliae*, even at 10^8 conidia/ ml and its appearance was delayed when compared with *B. bassiana*. These results are in agreement with those of Mar and Lumyong, (2012).

penetrate host's cuticle using their teeth found in the labial region (Attalla *et al.*, 2002). Numbers of genus *Heterohabditid* are known to adopt a cruising behavior for infecting a potential host and they are much effective against sedentary insect stage (Gaugler *et al.*, 1989 and Attalla *et al.*, 2002).

A.2. Soil treatment

Data presented in table (4) show significant difference between the two tested entomopathogenic fungi in the soil.

Adult mortality was affected by soil treatments. *B. bassiana* treatments significantly increased mortality compared to *M. anisopliae* and control treatments (Table 4). LC₅₀ values were high at the highest concentrations used in this study and further use of increased conidial concentrations was impractical and unaffordable when translated to field required inoculums levels. This is consented with the findings of Ekesi *et al.* (2002) and Goble (2009) who reported that pathogenicity of several isolates of EP fungi was demonstrated against *Bactrocera invadens* final instar larvae, when exposed to soil treated with 1×10^9 conidia/ml, reduced adult emergence to below 8 and 10%. Other typical LC₅₀ values of the most virulent fungal isolates used against adult fruit flies included 4.9×10^5 to 2.0×10^6 conidia/ml. However, the authors sprayed fruit flies with various conidial suspensions instead of applying the fungal inoculum to soil (Quesada-Moraga *et al.*, 2006). Results showed also that *B. bassiana* was able to induce mycosis in adults emerged from treated soil, the proportion of adult cadavers that produced visible signs of mycosis ranged from 30.4 to 60.2%, with an exception of those exposed to *M. anisopliae*. In that case, none of the insects that died after exposure to the conidia of *M. anisopliae* developed visible mycelium on their surfaces. In this context, Mochi *et al.* (2006) verified that pathogenicity of the fungus *M. anisopliae* to *C. capitata* larvae, prepupae, and pupae under laboratory conditions, caused a survival decrease of up to 95% in adults emergence from the soil with the fungus applied in the form of a conidial suspension.

2. Adult stage

Spray method

Virulence of *B. bassiana* and *M. anisopliae* against adults of *B. zonata* was estimated at four treatments by direct spraying of the adults (Table 5). Significant differences were found between the virulence of the two tested entomopathogenic fungi. The virulence of *B. bassiana* and *M. anisopliae* against adults of *B. zonata* was estimated after 5, 10, 15 and 20 days, with spraying the adults (Table 5). Significant differences were found between the two tested fungi. Virulence % increased as the time of exposure increased. LT₅₀ of *B. bassiana* recorded 15,048% virulence/ days comparing with *M. anisopliae* 22.117% virulence/ days. The results obtained in this study agree with other studies carried out on different fruit fly species; for example, Espin *et al.* (1989) observed 69–78% mortality in *C. capitata* adults infected with *M. anisopliae* and Castillo *et al.* (2000) who reported a virulence of 8–30% of *B. bassiana* against *C. capitata*.

Table (4): Mortality %, LC₅₀ and Slope response of *B. zonata* pupae treated with *B. bassiana* and *M. anisopliae* (soil treatment)

Entomopathogenic Fungi	Concentration (conidia / ml)	% Mortality	LC ₅₀	Slope
<i>B. bassiana</i>	1×10^4	33.33	1×10^6	0.2463
	1×10^6	44.44		
	1×10^8	71.11		
	Control	0.4	-	-
<i>M. anisopliae</i>	1×10^4	16.16	3×10^5	0.2168
	1×10^6	22.22		
	1×10^8	38.88		
	Control	0	-	-

Table (5): % Mortality and time mortality response of *B. zonata* adults sprayed with the entomopathogenic fungi; *B. bassiana* and *M. anisopliae*

Entomopathogenic Fungus	Concentration (conidia / ml)	% Mortality / days				LT ₅₀
		5	10	15	20	
<i>B. bassiana</i>	1×10^8	11.011	32.46	49.85	62.41	15,048
<i>M. anisopliae</i>	1×10^8	14.25	17.87	32.63	45.35	22.117

F = 10.08; P ≤ 0.005 for 5 days, F = 37.79; P ≤ 0.001 for 10 days, F = 41.03; P ≤ 0.0000 for 15 days and F = 42.9; P ≤ 0.0000 for 20 days.

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