Efficacy of the Entomopathogenic Nematodes; *Steinernema riobravis* Cabanillas and *Heterorhabditis bacteriophora* (Native strain) Against the Peach Fruit Fly, *Bactrocera zonata* (Saunders) and the Mediterranean Fruit fly, *Ceratitis capitata* (Wiedemann)

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ABSTRACT

Susceptibility of different stages of the peach fruit fly, *Bactrocera zonata* (Saunders) and the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) to the entomopathogenic nematodes; *Steinernema riobravis* Cabanillas and *Heterorhabditis bacteriophora* (native strain) was measured under laboratory conditions. Both insects were more susceptible to *H. bacteriophora* than to *S. riobravis* at all tested stages. Effect of mixing some natural nutrients with the infective juveniles on their pathogenicity when introduced to adult flies was investigated.

Key Words: Susceptibility, Entomopathogenic nematodes, *Steinernema riobravis*, *Heterorhabditis bacteriophora*, *Bactrocera zonata*, *Ceratitis capitata*

INTRODUCTION

The peach fruit fly, *Bactrocera zonata* (Saunders) and the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) are important and destructive fruit pests in Egypt. The female insect oviposits its eggs inside the fruits and hatched larva feeds on the fruit flesh causing its destruction, rotten and finally falls to the ground. Full grown larvae pop from infested fruit and pupate usually in the soil. Most fruit growers use chemical insecticides to decrease the economic loss resulted from the infestation of these fruit flies. Chemical insecticides applied to control pests are efficient for only short periods, can lead to pest resistance and mostly have a toxic effect on non-target organisms. Entomopathogenic nematodes, whose natural habitat is the soil, do not have these undesirable effects; so, they may serve as alternative measures for chemical control of many insects specially, soil - associated pests (Gaugler, 1981; Kaya, 1985; Poinar, 1986 and Klein, 1990). Entomopathogenic nematodes are environmentally friendly natural insect killing agents as they have mutualistic bacteria in their intestine. Soil is a natural reservoir for steinernematid and heterorhabditid nematodes introducing an excellent site for insect – nematode interaction. The infective stages of such nematodes (juveniles) are non-feeding, and have host finding ability (Schmidt & All, 1979 a, b). This infective stage enters the host's body via the natural openings (mouth, anus and spiracles), bores through the midgut or spiracles, releases the bacteria that multiply in the host's hemocoel and kill it within 24 to 48 hours. The bacteria serve as food for the nematodes, which complete their development for more than one generation after which infective juveniles emerge from the cadavers searching for new hosts. The juveniles can survive in moist soil without a host for several months (Harlan *et al.* 1971 and Lewis & Raun, 1978). Previous studies on the susceptibility of various species of fruit flies to some steinernematid and heterorhabditid nematodes under laboratory or natural conditions suggest that they may have potential as biological control agents for tephritid fruit flies (Lindegren & Vail 1986; Lindegren *et al.* 1990; Lezama – Gutierrez *et al.* 1996; Gazit *et al.* 2000; Soliman 2002 and Toledo *et al.* 2001, 2005, 2006).

Aim of this work is to assess the susceptibility of different stages of the peach fruit fly, *B. zonata* and the Mediterranean fruit fly, *C. capitata* to the entomopathogenic nematodes, *Steinernema riobravis* and *Heterorhabditis bacteriophora* as a step for application of such nematodes as biological control agents against fruit flies.

MATERIALS AND METHODS

Fruit flies

B. zonata and *C. capitata* were reared on an artificial diet (Tanaka *et al.* 1969) at 25 ± 2°C and 65% RH in the Insect Horticulture Department, Plant Protection Research institute (PPRI), Giza, Egypt.

Entomopathogenic nematodes

S. riobravis and *H. bacteriophora* (isolated from El-Wahat region, Egypt) were used in the present work. Nematode stocks were obtained from the Department of Insect Physiology; PPRI and maintained under the laboratory conditions on the greater wax moth, *Galleria mellonella* larvae (Kaya

& Stock, 1997) in PPRI.

Susceptibility of the fruit flies stages to entomopathogenic nematodes

Susceptibility of full grown larvae

Different concentrations $5x10^3$, $7.5 x10^3$, $1.2 x10^4$, $2.5 x10^4$, $5 x10^4$ and $1 x10^5$ of *S. riobravis* and *H. bacteriophora* were applied in plastic cups (25 x 20 x15 cm) contained 50 cm^3 of sandy soil. Moisture in all cups was adjusted to 10% after adding nematode suspensions. All cups were kept at 25 ± 2°C. After 24 hours larvae of the tested insect species were transferred to the cups for nematodes infection and kept at 25 ± 2°C. Each concentration was replicated 5 times and each cup contained 100 larvae. A parallel control treatment contained distilled water only without nematodes. Examination of all treatments was done 14 days later. Emerged adults were counted and all dead larvae were dissected to confirm presence of nematodes as the release of IJs from the cadavers lasted 12 – 14 days for *B. zonata* and *C. capitata*, respectively. Treatments of each tested insect species were carried out separately.

Susceptibility of pupae

Full grown larvae of each tested species were collected after popping from the artificial diet and placed in Petri - dish (9 cm) contained sand for pupation. Pupation process started after approximately 8 hours. Different pupal ages 1, 3,6,8,12,24 hours and 8 days old pupae were tested for their susceptibility to the used nematodes. A hundred pupae from each age was placed at the bottom of a plastic cup (25 x 20 x 15 cm) and covered with 7% moistened sand (3 cm high). A concentration of $1 x10^5$ IJs was added on the sand surface. Moisture reached 10% after adding the nematode suspension. Each treatment was replicated 5 times. A control treatment was carried out without nematodes. All cups were kept at 25 ± 2°C. After 14 days, dead pupae were dissected to confirm penetration of nematodes and emerged adults were counted. Treatments of each insect species were carried out separately.

Susceptibility of adults emerged from treated soil

A hundred of 9-day old pupae (a day before adult emergence) was placed on the bottom of a plastic cup (25 x 20 x 15 cm) and covered with 7 % moistened sand (3 cm high). A concentration of $1 x10^5$ IJs of used nematodes was added on the sand surface. The moisture of sand was 10% after adding nematode suspensions. A cylindrical screen covered with muslin cloth with rubber band at its top was placed on the cover of the plastic cup to allow emerged flies to escape away from soil (mimicking of nature). All cups were kept at 25 ± 2°C and 65% RH. A day later, escaped flies were collected using a plastic aspirator and transferred to nematode – free cups. Escaped flies were dissected and examined for the presence of nematodes after 72 hours using fine needles. Treatments of both insects with nematodes were carried out separately and each treatment was replicated 5 times. A parallel control treatment for each insect species was run out without nematodes.

Susceptibility of adult flies to nematodes mixed with natural nutrients

Sugar solutions 1% and 4%, mango, guava, orange juices, agar suspension and a nematode-water suspension contained $5 x 10^3$ IJs were mixed with $5 x 10^3$ IJs separately and placed in plastic cups (2 cm diameter and 1cm high) lined with a thin layer of sponge. The nematode cups were placed in screen cages (20x20x20 cm) and twenty five adult flies from each tested insect species (3-day old) were exposed to the nematodes. Dead flies were removed daily and washed with tap water to get rid of any attached nematodes and kept in nematode free cups. Head, thorax and abdomen of all dead flies were dissected after 72 hours and examined separately for the presence of adult nematodes. Adult nematodes found in each insect body section were counted. The nematode cups were moistened daily to prevent dryness. Each treatment was replicated 5 times for each insect species separately. Control treatments contained no nematodes.

Statistical analysis

All mortality values were corrected using Abbot (1925). One-way ANOVA was used to analyze the mortality of larvae, pupae, emerged adults and susceptibility of adult flies to nematode suspensions. Probit analysis used to calculate LC50.

RESULTS AND DISCUSSION

Full grown larvae of both *B. zonata* and *C. capitata* were susceptible to the infection with *S. riobravis* and *H. bacteriophora* (Table 1). Nematode concentration and fruit fly stage had statistically significant interaction ($P < 0.05$). Number of nematodes infected the host depends on the stage of the host. Nematode concentrations and host stage were significant factors in predicting pathogenicity of nematodes against fruit fly stage. The lowest LC50's was found in *H. bacteriophora* against, *B. zonata* and *C. capitata* while LC50's of *S. riobravis* showed higher values against both insects (Table 2). Dissection of dead full grown larvae revealed that *S. riobravis* and *H. bacteriophora* reproduced new infective juveniles

Table (1): Pathogenicity of different concentrations of *Steinernema riobravis* Cabanillas and *Heterorhabditis bacteriophora* (Native strain) to full grown larvae of *Bactrocera zonata* and *Ceratitis capitata* under laboratory conditions

Nematode concentrations	% of mortality ± SE *			
	S. riobravis		*H. bacteriophora*	
	B. zonata	*C. capitata*	*B. zonata*	*C. capitata*
5 x 10^3	26.3 ± 1.13 ^{f}	24.2± 0.71 ^{d}	48.2 ± 1.03 ^{f}	43.1 ± 1.75 ^{f}
7.5 x 10^3	39.7 ±0.19 ^{e}	28.3 ± 1.12 ^{d}	56.1± 0.93 ^{f}	51.3± 1.13 ^{f}
1.2 x 10^4	56.8± 1.23 ^{d}	67.1 ± 1.93 ^{c}	73.2 ± 0.81 ^{d}	69.2 ± 2.04 ^{d}
2.5 x 10^4	76.1± 1.81 ^{c}	67.01± 1.19 ^{c}	79.2± 1.13 ^{c}	88.0 ± 1.93 ^{c}
5 x 10^4	81.3± 2.01 ^{b}	79.3 ± 1.31 ^{b}	88.7± 1.81 ^{b}	93.2± 1.86 ^{b}
1 x 10^5	86.9± 1.13 ^{a}	89.2 ± 2.13 ^{a}	98.8 ± 1.53 ^{a}	98.3 ± 1.02 ^{a}

* Mortality percentages followed by different letters are significantly different

Table (2): Estimation of LC50 values of the entomopathogenic nematodes, *Steinernema riobravis* Cabanillas and *Heterorhabditis bacteriophora* (Native strain) against full grown larvae of *Bactrocera zonata* and *Ceratitis capitata* under laboratory conditions

Nematode species	Insect	LC_{50}	Fiducial limits		χ^2	Slope ± SE
			Lower	Upper		
S. riobravis	*B. zonata*	11272.85	9099.22	13582.22	6.59	1.49±0.13
	C. capitata	12509.71	5849.97	21367.86	6.57	1.58± 0.33
H.bacteriophora	*B. zonata*	5445.43	3912.93	6942.01	16 92	1.43±0.29
	C. capitata	6510.62	5204.86	7779.50	17.97	1.78±0.17

Degrees of freedom = 4

Table (3): Susceptibility of different pupal ages and soil emerged adult flies, *Bactrocera zonata* and *Ceratitis capitata* to *Steinernema riobravis* Cabanillas and *Heterorhabditis bacteriophora* (Native strain) at the concentration of 1x105.

Pupal age	% of mortality ± SE *			
	S. riobravis		*H. bacteriophora*	
	B. zonata	*C. capitata*	*B. zonata*	*C. capitata*
1 hour	9.13 ± 0.01 ^{b}	7.23± 0.51 ^{b}	46.93±0.42 ^{b}	33.18±0.83 ^{b}
3 hours	3.81 ± 0.35 ^{c}	3.01±0.73 ^{c}	12.09±1.13 ^{c}	9.12±0.45 ^{c}
6 hours	1.83±0.08 ^{d}	1.05±0.13 ^{d}	5.13±0.93 ^{d}	2.15±0.13 ^{d}
8 hours	0.00	0.00	3.18 ± 0.72	0.00
12 hours	0.00	0.00	0.00	0.00
24 hours	0.00	0.00	0.00	0.00
9 days	0.00	0.00	0.00	0.00
Emerged adults	13.71± 1.13 ^{a}	9.18±0.67 ^{a}	70.13±1.18 ^{a}	53.01±0.76 ^{a}

*Mortality percentages followed by different letters are significantly different

Table (4): Susceptibility of 3-day old adult flies of *Bactrocera zonata* and *Ceratitis capitata* to *Steinernema riobravis* Cabanillas and *Heterorhabditis bacteriophora* (Native strain) at the concentration of 5x103.

	% of mortality ± SE*			
	S. riobravis		*H. bacteriophora*	
	B. zonata	*C. capitata*	*B. zonata*	*C. capitata*
	29.36 ± 0.64	42.68 ± 0.88	60.03 ± 1.1	72.13 ± 0.71
Insect part	Mean number of adult nematodes inside fly body ± SE			
	S. riobravis		*H. bacteriophora*	
	B. zonata	*C. capitata*	*B. zonata*	*C. capitata*
Head capsule	0.78 ± 0.03 ^{c}	1.01 ± 0.11 ^{c}	1.2 ± 0.64 ^{c}	3.7 ± 1.18 ^{c}
Thorax	1.13 ± 0.84 ^{b}	1.85± 0.29 ^{b}	2.6± 1.58 ^{b}	5.3 ± 1.61 ^{b}
Abdomen	3.14± 1.07 ^{a}	5.15 ± 1.17 ^{a}	7.4 ± 2.26 ^{a}	9.13 ^{a} ± 1.98

*Means followed by different letters are significantly different

at all concentrations used in the test.

Infection of different pupal ages of *B. zonata* and *C. capitata* with *S. riobravis* and *H. bacteriophora* was greatly lesser than that of the full grown larvae. *H. bacteriophora* showed higher ability to infect pupae at early ages than *S. riobravis* for the two insects but late aged pupae showed high resistance against infection by both nematodes. Ability of *H. bacteriophora* to infect the emerged adults of the two insects was higher than *S. riobravis* (Table 3). Susceptibility of 3-day old adults to infection with *H. bacteriophora* and *S. riobravis* is presented in table (4).

Effect of mixing sugar solutions, mango, guava, orange juices and agar with nematodes on their pathogenicity to the two insects is presented in table (5). All sugar solutions and natural juices mixed with the nematodes had a negative effect on their efficacy against adult flies of the two insects. *H. bacteriophora* tolerated sugar solutions and the natural juices more than *S. riobravis*.

Carbon dioxide gas plays a role in entomopathogenic nematodes attraction to its host (Wallace, 1958). Full grown larvae showed high activity after exit from their host plant searching for a suitable site to pupate. It is acceptable that this stage produces carbon dioxide gas therefore, high infection occurred by nematodes.

Although pupae were apparently less susceptible to nematode infection than full grown larvae, *H. bacteriophora* infective juveniles were able to cause moderate pupal mortality rates at early pupal ages than *S. riobravis* in the two insects. This may be due to the terminal tooth which is a discriminative feature of heterorhabditids that enables them to penetrate their host body through cuticle (Bedding and Molyneux, 1982). *H. bacteriophora* infective juveniles were smaller in size than *S. riobravis* which may give them privilege to enter the host's body cavity via spiracles before hard sclerotization at late hours of pupation process (Kaya and Gaugler, 1993). Other studies recorded that *S. riobravis* infective juveniles were found adhering to the spiracles or generally at the posterior end of western cherry fruit fly, *Rhagletis indifference* (Diptera: Tephritidae), while *H. bacteriophora* infective juveniles were found clustering around the anterior and posterior ends of the pupae (Stark and Lacey, 1999). Late aged pupa had hard sclerotized cuticle, so, it was difficult for nematodes to penetrate it.

S. riobravis and *H. bacteriophora* infective juveniles were able to infect the newly emerged adult flies of the two insects. This may be due to the slow motion of the newly emerged adult flies to reach the soil surface. Also, the soft body of the newly emerged adult flies may act as another reason helped nematode infection. Infection of adult fruit flies is considered as an important factor in dispersal of nematodes and establishment of new infection foci. Studies on entomopathogenic nematodes as control agents against adult insects have been done infrequently. In the present work, exposure of *B. zonata* and *C. capitata* adult flies to S. *riobravis* and *H. bacteriophora* showed that *H. bacteriophora* infective juveniles could infect adult fly (3-day old). The most infected part of adult fly was the abdomen which may give a hint that infective juveniles enter the adult fly body via the cloacae. Also, some nematodes were observed in the head capsule and the thoracic cavities. Renn 1998, tested the routes of penetration of *Steinernema feltiae* attacked adult houseflies (*Musca domestica*), reported aggregations of *S. feltiae* infective juveniles on cloacae and proboscis apertures. A special point noted by the author of the present work that adult females were more susceptible to infective juveniles' infection. This may be due to adult males were more active

Table (5): Effect of some natural nutrients on efficacy of *Steinernema riobravis* Cabanillas and *Heterorhabditis bacteriophora* (Native strain) against adult flies of *Bactrocera zonata* and *Ceratitis capitata* at the concentration of $5x\ 10^3$

Nutrient	% of mortality of adult flies ± SE			
	S. riobravis		*H. bacteriophora*	
	B. zonata	*C. capitata*	*B. zonata*	*C. capitata*
Sugar solution 1%	5.6 ± 1.63^b	8.1 ± 0.16^b	9.18 ± 1.13^b	12.3 ± 0.16^b
Sugar solution 4%	1.4 ± 0.38^c	2.3 ± 0.18^c	3.09 ± 1.08^c	3.11 ± 0.31^c
Mango juice	1.6 ± 0.43^c	2.1 ± 0.11^c	0.00	0.00
Guava juice	0.13 ± 0.03^d	0.03 ± 0.01^d	0.00	0.00
Orange juice	0.00	0.00	0.00	0.00
Agar	33.6 ± 1.16^a	36.8 ± 0.18^a	63.18 ± 1.12^a	78.03 ± 2.31^a

*Means followed by different letters are significantly different

and exhibited more walking and grooming activity than females. *C. capitata* adult flies were more susceptible to infection with *H. bacteriophora* followed by *S. riobravis* than *B. zonata* adult flies when exposed to the same nematodes (*B. zonata* adult flies were highly irritated when infective juveniles introduced).

Mixing infective juveniles with sugar solutions and natural fruit juices had a highly negative effect on pathogenicity against adult flies. Agar–nematode mixture showed promising results and moderate nematode death rate. Renn (1994 and 1998) found that adult houseflies were highly susceptible to *S. feltiae* formulated into baits.

Control of *B. zonata and C. capitata* could be successful when formulations of nematodes against adults and soil as well as full grown larvae are developed. The results obtained from the present work showed that *H. bacteriophora* was highly pathogenic to full grown larvae of both pests, moderately pathogenic to adult flies and less pathogenic to pupae. It could be concluded that the native strain (adapted to local environmental conditions) had the highest virulence against the tested fruit flies. Such successful results should lead to further studies under field conditions using these nematodes in an IPM program against fruit flies in Egypt.

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