Utilization of the Entomopathogenic Nematodes *Heterorhabditis bacteriophora* HP88 and *Steinernema feltiae* (Filipjev) as Biological Control Agents against the Peach Fruit Fly *Bactrocera zonata* Saunders (Tephritidae: Diptera)

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Abstract: Entomopathogenic nematodes (EPNs) of the genera *Steinernema* and *Heterorhabditis* are associated with bacterial symbionts of the genus *Xenorhabdus* and *Photorhabdus*, respectively. They are produced and used as biological control agents against many soil insect pests. The present study was carried out to investigate the effectiveness of entomopathogenic nematode species, *Heterorhabditis bacteriophora* and *Steinernema feltiae* in producing the infective juveniles and host finding effects against the full-grown larvae of *Bactrocera zonata*. Results indicated that the mortality percentage increased as the concentration of IJs increased. Also, *H. bacteriophora* was more effective at 25 than 30°C. The production rate increased as the concentration of IJs increased. *H. bacteriophora* was the highest reproductive strain than *S. feltiae* at the two tested IJs concentrations. Generally, *H. bacteriophora* was more effective than *S. feltiae*.

Keywords: Entomopathogenic nematodes, *Bactrocera zonata*, *Heterorhabditis bacteriophora*, *Steinernema feltiae*, production, host finding

INTRODUCTION

Fruit flies (Tephritidae: Diptera) attack a wide range of commercially produced fruits and vegetables. Infested fruits often drop prematurely. Such infestation resulted in a significant reduction in fruit production, increasing control cost due to insecticide use, and causing problems with international trade (Radonjić *et al.,* 2019). These pests cause direct damage to important export crops leading to losses of 40%-80%, depending on locality, crop variety and season (Kibira *et al.*, 2010).

The peach fruit fly, *Bactrocera zonata* (Saunders), is recognized as a very devastating tephritid insect pest and serious pests attacking tropical and subtropical fruits (Hosni, 2011). It is established in South and South-East Asia (White and Harris, 1994). In Egypt, it has been established as a new pest of guava and mango in 1998 in the northern region, owing to the suitability of climate and the extension in planting favorable host fruits such as peach, guava, mango and apricot (El-Minshawy *et al.*, 1999). It is now a serious pest of fruits and some vegetables replacing *Ceratitis capitata* in most of the Egyptian Governorates (El-Heneidy *et al*., 2016).

Entomopathogenic nematodes (EPNs) in the genera *Steinernema* and *Heterorhabditis* are commercially available for controlling of soil-inhabiting insects (Grewal *et al*., 2005). They can kill their hosts within 48h, be easily produced in *vivo* and in *vitro*, are safe for humans and other non-target organisms, and have negative effects on the environment. They life in mutualistic symbioses with bacteria from the genera *Xenorhabdus* and *Photorhabdus,* respectively. They are the only nematodes that have evolved the ability to carry and introduce symbiotic bacteria into the body cavity of insects and the only entomopathogens with a host range including the majority of insect orders and families (Chaston *et. al*., 2011). Infective stage enters the host's body via the natural opening (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 juveniles can survive in moist soil without a host in this respect, the infective juveniles (IJs) actively seek for several months (Harlan *et al*., 1971).

Therefore, this work aims to assess the susceptibility of larvae and pupae of the peach fruit fly, *B. zonata* to the entomopathogenic nematodes, *Heterorhabditis bacteriophora* and *Steinernema feltiae* as a step for the application of such nematodes as biological control agents against fruit flies.

MATERIALS AND METHODS

Rearing of *B. zonata*

B. zonata used in this study were obtained from a laboratory stock culture at the Biological Control Research Department, Plant Protection Research Institute, Agricultural Research Center. The rearing technique of *B. zonata* was closly similar to that of *C. capitata*. The rearing was carried out under laboratory conditions of 25±2°C and 55-65% R.H. Eggs were collected daily and placed on an artificial diet and kept in an incubator at 25±1°C and left for hatching and larval development. Third larval instars were transferred to trays with a small layer of sand for pupation. Pupae were sieved and transferred to adult cages till emergence then adult flies were reared in cages (Hosni, 2011).

Rearing of *Galleria mellonella*

The greater wax moth, *G. mellonella* larvae were obtained from infested hives and reared in jars (2 Kg capacity) until emergence of moths according to the technique described by Birah *et al.* (2008) using (wheat (130g), wheat bran (130 g), milk powder (130 g),

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maize flour (97.5 g), yeast powder (97.5 g), wax (26 g), honey (195 ml) and glycerol (195 ml).

Entomopathogenic Nematodes Species:

The EPNs used in this study were obtained from a laboratory stock culture at the Biological Control Research Department, Plant Protection Research Institute, Agricultural Research Center. The heterorhabditid nematode, *Heterorhabditis bacteriophora* strain HP88 was orginally obtained from Randy Gaugler, Rutgers University, New Brunswick NJ, USA. Also, the steinernematid nematode, *Steinernema feltiae* (Filipjev) California was received from California, USA. Both species were reared *in vivo* on the full-grown larvae of the greater wax moth, *Galleria mellonella.* Rearing of the EPNs in larvae of *G. mellonella* was performed as described by Dutky *et al*. (1964).

Laboratory Experiments:

Effect of temperature on the infectivity of EPNs

Nematode ability of (*H. bacteriophora* and *S. feltiae*) to control *B. zonata* full-grown larvae and pupae (one and three days old insects) was studied under two temperatures (25 and 30°C±2). Ten full-grown larvae or pupae were placed in plastic cups 200 cc capacity, half-filled with moistened sterilized sandy soil, and covered with plastic lids. The cups were treated with two nematode species using five concentrations (25, 50, 100, 200, and 400 IJs/cup). Ten larvae were used/cup with 5 replicates for each concentration. These cups were inspected after one week of treatments and daily to record the numbers of mortality larvae and pupae and dissected for the confirmation of insect infestation with EPNs. Control treatment was performed using five cups, treated with distilled water only.

Production of entomopathogenic nematode

Ten full-grown larvae and pupae (one-day-old) of *B. zonata* were placed in plastic cups (100 cc capacity) lined with filter paper and covered with plastic lids. The treatment took place using two concentrations of 2000 and 4000 IJs /cup with 10 replicates for each tested nematode species (*H. bacteriophora* and *S. feltiae)*. The infective juveniles were harvested daily using White traps according to White (1927). The total number of IJs was estimated as IJs produced/larva.

Host finding ability of *H. bacteriophora* and *S. feltiae*

One ml of the nematode concentration (4000 IJs/tube) was poured into the bottom of tubes (16 cm in height and 1.5 cm in diameter). After that sterilized and wet sandy soil, 10% was placed to cover the nematode concentration in each tube till reaching the heights of 3, 6, 9 and 12 cm of the soil column. Five full-grown larvae of *B. zonata* were placed inside a net of wire screen and placed on the top of the sand layer inside each tube. Ten replicates were used for each soil depth with a total number of 40 tubes/each nematode species. Ten tubes treated with distilled water were also used as control. After 7 days, the larvae were transferred into (White-trap) to record the numbers of mortality larvae and the emerged IJs to make sure that larvae were infected with the nematode.

Statistical analysis

Proportional data were transformed by arcsine square root (ARCsine) before analysis. The obtained data statistically analyzed through ANOVA (SAS Institute 2003). When F-test was significant, means were separated using Tukey's HSD Test at the 0.05 level of significance. Obtained laboratory mortality results were fitted to the Log-probit model according to (Finney, 1971).

RESULTS

Effect of temperature on the infectivity of EPNs at 25 and 30°C

Mortality increased as the concentration of EPNs increased. The highest mortality in *B. zonata* full-grown larvae and (one and three-day-old) pupae exposed to *H. bacteriophora* (74, 66 and 56%) were achieved at a concentration of 400 IJs /cm^2 of the soil surface, respectively at 25°C. LC_{50} were 166.20, 211.28 and 276.91 IJs/cm² of soil surface at *B. zonata* full-grown larvae and (one and three-day-old) pupae, respectively (Table 1).

Table (1): Mean (%±SE) mortality of full-grown larvae and pupae (one-and three-day-old pupae) of *Bactrocera zonata* exposed to different concentrations of *H. bacteriophora* at 25 and 30°C

Conc. (Ijs/cm^2)	*H. bacteriophora*					
	25°C			30°C		
	Larva	One-day-old-pupa	Three-day-old-pupae	Larva	One-day-old-pupa	Three-day-old-pupae
Control	0±0 f	0±0 f	0±0 d	0±0 f	0±0 f	0±0 e
25	6.00±1.56 e	4.00±1.72 e	0±0 d	4.00±1.60 e	2.00±1.06 e	0±0 e
50	18.00±3.13 d	16.00±1.92 d	14.00±2.02 c	16.00±2.37 d	14.00±1.57 d	12.00±1.90 d
100	34.00±1.56 c	30.00±1.24 c	28.00±1.41 b	30.00±2.06 c	28.00±2.08 c	24.00±1.28 c
200	58.00±1.88 b	50.00±2.93 b	42.00±2.54 a	50.00±2.68 b	48.00±2.44 b	34.00±2.22 b
400	74.00±2.30 a	66.00±2.13 a	56.00±3.01 a	68.00±1.64 a	64.00±1.87 a	48.00±1.31 a
F	72.17	68.73	81.41	68.72	91.36	118.43
P	0.000	0.000	0.000	0.000	0.000	0.000
Lc_{50}	166.20	211.28	276.91	204.97	227.37	376.74

Means followed with the different letters in the same column are significantly different (P>0.05).
Obtained values of % mortality, transformed to ARC sine before conducting ANOVA

Mortality percentage of *B. zonata* full-grown larvae and (one and three-day-old) pupae at concentrations of 0, 25, 50, 100, 200 and 400 IJs/cm² of *S. feltiae* were 0, 4, 16, 32, 54 and 70% for larvae, 0, 2, 14, 30, 44 and 60% for one-day-old pupae and 0, 0, 12, 26, 38 and 50% for 3 day-old pupae at 25°C. LC_{50} for *S. feltiae* to full-grown larvae and (one and three-day-old) pupae of *B. zonata* were 188.25, 250.54 and 334.12 IJs/cm² of soil surface, respectively (Table 2).

The use of concentrations of *H. bacteriophora* 0, 25, 50, 100, 200, and 40 IJs/ cm² caused respective mortality percentages of 0, 4, 16, 30, 50, and 68% for full grown larvae, 0, 2, 14, 28, 48 and 64% one-day-old pupae and 0, 0, 12, 24, 34 and 48% for 3 day-old pupae of *B. zonata* at 30°C (Table 1). LC_{50} for *H. bacteriophora* to the respective stages of *B. zonata* were 204.97, 227.37 and 367.74 IJs /cm² of soil surface (Table 1).

One week after treatment full-grown larvae and (one and three-day-old) pupae of *B. zonata* by different concentrations of *S. feltiae* 30°C, mortality rate were 0, 2, 14, 24, 38 and 52% for full grown larvae, 0, 0, 10, 20, 32 and 44% for one-day-old pupae and 0, 0, 6, 18, 28 and 40% for 3 day-old pupae due to application of *S. feltiae* at concentration of 0, 25, 50, 100, 200 and 400 IJs/cm², respectively. LC_{50} for *S. feltiae* to the respective stages of *B. zonata* were 333.85, 436.40 and 504.63 IJs/cm² of soil surface (Table 2).

Table (2): Mean (%±SE) mortality of full-grown larvae and pupae (one-and three-day-old pupae) of *Bactrocera zonata* exposed to different concentrations of *S. feltiae* at 25 and 30°C

Conc. (Ijs/cm²)	*S. feltiae*					
	25°C			30°C		
	Larva	One-day-old-pupa	Three-day-old-pupae	Larva	One-day-old-pupa	Three-day-old-pupae
Control	0±0 e	0±0 d	0±0 d	0±0 f	0±0 e	0±0 d
25	4.00±1.06 d	2.00±1.39 d	0 ±0 d	2.00±0.94 e	0±0 e	0±0 d
50	16.00±2.60 c	14.00±1.57 c	12.00±2.99 c	14.00±1.13 d	10.00±1.27 d	6.00±1.20 c
100	32.00±2.77 b	30.00±2.44 b	26.00±2.05 b	24.00±1.52 c	20.00±1.64 c	18.00±2.93 b
200	54.00±3.27 a	44.00±2.63 b	38.00±3.26 ab	38.00±1.32 b	32.00±1.96 b	28.00±1.74 ab
400	70.00±2.47 a	60.00±2.83 a	50.00±3.70 a	52.00±2.46 a	44.00±1.95 a	40.00±2.67 a
F	51.29	56.12	41.18	87.82	107.10	85.30
P	0.000	0.000	0.000	0.000	0.000	0.000
Lc_{50}	188.25	250.54	334.12	333.85	436.40	504.63

Means followed with the different letters in the same column are significantly different (P>0.05).
Obtained values of % mortality, transformed to ARC sine before conducting ANOVA

Production of *H. bacteriophora* and *S. feltiae* within *B. zonata* full-grown larvae and pupae

B. zonata full-grown larvae hosted a large number of IJs. Infected full-grown larva of *B. zonata* with *H. bacteriophora* produced an average of 8659 and 16943 (IJs) at concentrations of 2000 and 4000 IJs/cm² of the soil surface, respectively. The full-grown larvae of *B. zonata* produced an average of 4972 and 7565 (IJs) when exposed to *S. feltiae* at concentrations of 2000 and 4000 IJs/cm² of the soil surface (Fig 1).

B. zonata pupae infected with *H. bacteriophora* produced an average of 5940 and 11070 (IJs) at concentrations of 2000 and 4000 IJs/cm² of the soil surface, respectively. As for *S. feltiae* when infected pupae of *B. zonata* with concentrations of 2000 and 4000 IJs/cm² produced an average of 3110 and 6460 (IJs) at 4000 IJs/cm² of the soil surface (Fig 1).

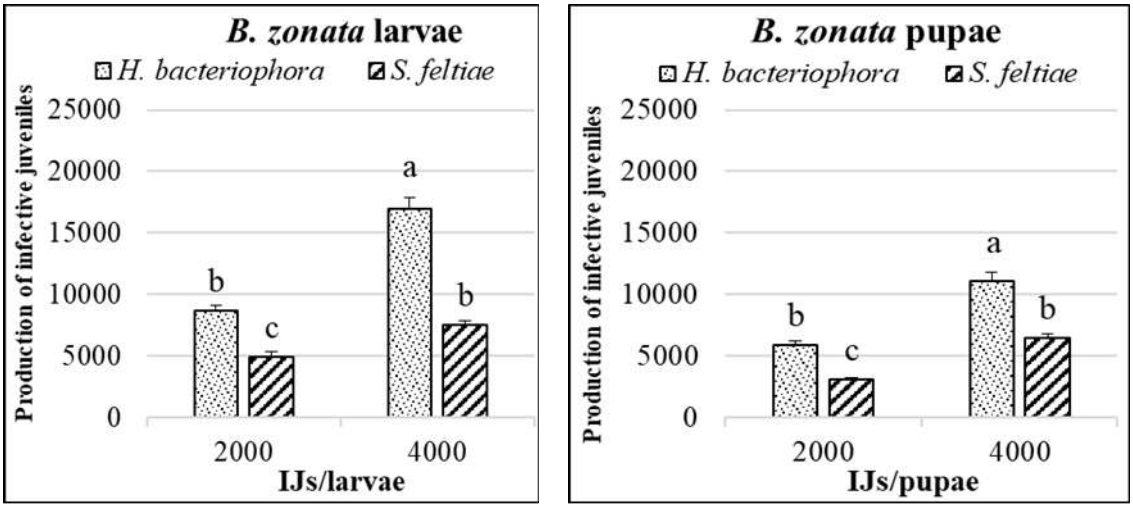

Fig (1): Production of infective juveniles of *H. bacteriophora* and *S. feltiae in vivo* of full-grown larvae and pupae of *B. zonata* exposed to nematodes concentrations of 2000 and 4000 IJs/ larva or pupa

Host finding of IJs *H. bacteriophora* and *S. feltiae* to full-grown larvae of *B. zonata*:

The two tested nematode species reached successfully full-grown larvae of *B. zonata* when placed at 3 cm height from the bottom of the tube. However, at 12 cm height, IJs could not reach the host, and no mortality was observed in the full-grown larvae of *B. zonata*. *H. bacteriophora* caused the highest mortality rate and reached the host causing mortality of 92%, whereas *S. feltiae* caused mortality rate of 86%.(Fig. 2)

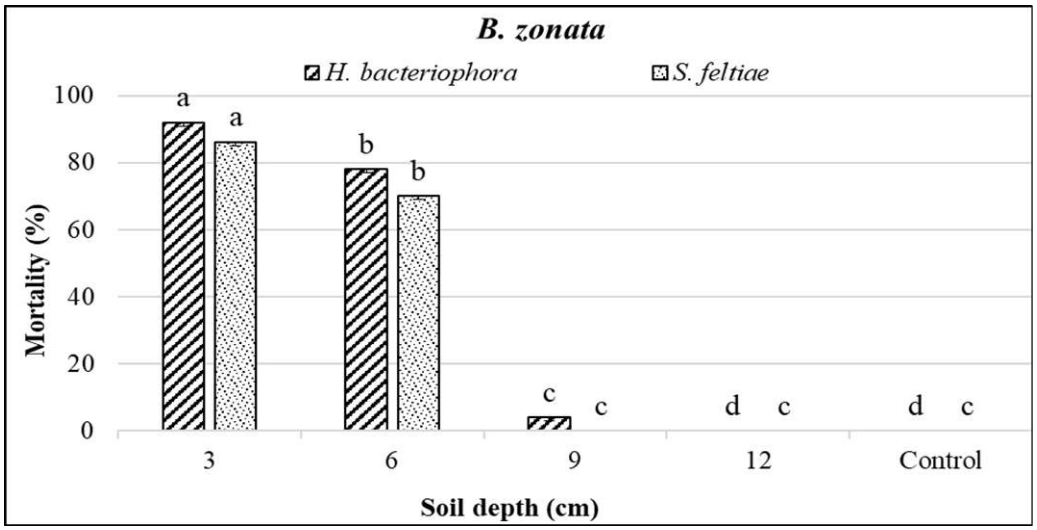

Fig (2): The mortality rate of *Bactrocera zonata* full-grown larvae exposed to *H. bacteriophora* and *S. feltiae* when placed at different depths of the soil surface.
Bars with the same letters indicate no significant difference

DISCUSSION

Effect of temperatures on nematode performance varies with nematode species and strains (Choo *et al*., 2002; Chen *et al.,* 2003). Kaya (1977) reported that *S. carpocapsae* did not develop at 10°C and above 33°C, whereas between 15°C and 27°C the IJs developed and reproduced, with the optimum at 25°C. The optimum range for *H. bacteriophora* was 22-26°C (Doucet *et al.,* 1996) and 25°C for *S. feltiae* (Belair *et al.,* 2003).

Nouh and Hussein (2014) found that *B. zonata* treated with *S. carpocapsae* at 125000, 250000 and 500000 IJs/cup showed mortality percentages of 18%, 46% and 72%), respectively. The efficacy of ENPs (*H. bacteriophora* strain HP88) on full-grown larvae and pupae led to higher efficacy than application of *S. carpocapsae* All strains. Also, treatments of *B. zonata* by either of the two strains at 25°C resulted in higher mortality rates than among the treated at 20°C. The tested of full-grown larvae were the highest mortality rates compared to the pupae. The mortality percentage increased as the concentration increased. These results are in harmony with those of Unlu and Ozer (2003) who found that the average number of nematodes developing in *G. mellonella* larvae treated with *S. feltiae* was 13829. The average number of nematodes per *G. mellonella* larvae treated with *H. bacteriophora* were 144562. Nouh (2016) also found that the production of *H. bacteriophora* from *G. mellonella* larvae was 229660 IJs/larva at the highest concentration of 200/larvae. *G. mellonella* larvae treated with *S. abbasi* produced 177800 IJs/larva at the highest concentration of 200 IJs/larva. Nouh and Hussein (2014) revealed that not only the nematode, *S. feltiae* not only could be used to control the tephritid flies but also could be successful. Culturing and application of *S. feltiae* requirements are in need of special conditions which could be not available in warm countries such as Egypt.

S. feltiae Cross N 33 showed high virulence toward third instar larvae of *B. zonata*; however its pathogenicity toward pupal stage were less pronounced particularly in old pupae (Mahmoud and Osman, 2007). Although, pupae appear to be less susceptible to EPNs infection than larval instars, *H. bacteriophora* IJs were able to cause moderate pupal mortality rate 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 enable IJs to direct penetration into host body through cuticle (Bedding and Molyneux, 1982). *S. feltiae*, the only portal of entry to intact pupae of *B. zonata* is via the spiracles, but the presence of spiracular slits within these openings may prevent penetration Mahmoud and Pomazkov (2004). Such results come in agreement with those obtained by found that 3rd instar larvae and 1 day old pupae of *B. zonata* were significantly more susceptible to nematode infection than second instar larvae and 4, 6 days old pupae at all concentrations of *S. feltiae* tested.

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استخدام النيماتودا الممرضة للحشرات *Heterorhabditis bacteriophora* and *Steinernema feltiae* كأداة مكافحه حيوية ذبابة الخوخ *Bactrocera zonata* Saunders (Tephritidae: Diptera)

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ترتبط النيماتودا الممرضة للحشرات من جنس *Heterorhabditis* و*Steinernema* بالتعايش مع البكتريا من جنس *Xenorhabdus* و *Photorhabdus* ، على التوالي. يتم إنتاجها واستخدامها كأحد عوامل المكافحة البيولوجية لمكافحه العديد من الآفات الحشرية في التربة. أجريت الدراسة الحالية لمعرفة مدى فاعلية أنواع النيماتودا الممرضة للحشرات *Heterorhabditis* و *Steinernema* في إنتاج الأطوار المعدية واكتشاف والوصول للعائل ليرقات ذبابه الخوخ كاملة النمو. وجد أن نسبة الموت زادت مع زيادة تركيز الطور المعدي. أيضا، النوع *Heterorhabditis* كان أكثر فعالية عند ٢٥°م عن ٣٠ درجة مئوية. زاد معدل إنتاج الطور المعدي مع زيادة تركيز الطور المعدي و كان النوع *H. bacteriophora* هو الأعلى إنتاجيه مقارنه بالنوع *S. feltiae* في التركيزين المختبرين. بشكل عام، كان النوع *H. bacteriophora* هو الأعلى تأثيرا مقارنه بالنوع *S. feltiae* .