Infectivity, Production and Host Finding of *Heterorhabditis bacteriophora* HP88 and *Steinernema feltiae* (Filipjev) against *Ceratitis capitata*

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Abstract: The present study aimed to evaluate the influence of temperature on the ability of two entomopathogenic nematodes (EPNs), *Heterorhabditis bacteriophora* HP88 and *Steinernema feltiae* (Filipjev) California, to infect *Ceratitis capitata* (full-grown larvae and one and three days old pupae) under laboratory conditions. Results indicated that the temperature and EPNs concentration had a significant effect on the efficacy of nematode species. Full-grown larvae had the highest sensitivity compared to one and three days old pupae. The mortality percentages in the tested stages of *C. capitata* were increased as the concentration increased. The ability of *H. bacteriophora* was higher than of *S. feltiae* at all tested concentrations and temperatures. There were significant differences in terms of the number of IJs extracted from full-grown larvae and pupae of different treatments. Also, *H. bacteriophora* was the most reproductive, *in vivo* of full-grown larvae and pupae of *C. capitata*, at all tested concentrations. *H. bacteriophora* was superior to *S. feltiae* in reaching and killing the target host of *C. capitata*. Generally, *H. bacteriophora* had more virulent, high production and more ability to host finding than *S. feltiae* at all tested temperatures. Thus, the success of entomopathogenic nematode, *H. bacteriophora* as biological control agent against *Ceratitis capitata* seemes to be suitable for use in integrated pest control strategies.

Keywords: *Heterorhabditis bacteriophora*, *Steinernema feltiae*, *Ceratitis capitata*, temperature, concentrations, mortality

INTRODUCTION

Entomopathogenic nematodes (EPNs) in the families Steinernematidae and Heterorhabditidae are parasites of soil-inhabiting insects and have great potential as biological control agents of many insect pests (Shapiro-Ilan *et al*., 2012). EPNs are found in a variety of soil habitats, and the various species and isolates exhibit considerable variations in terms of host range, reproduction, infectivity and conditions for survival (*i.e.* temperature, soil moisture, etc.) (Bedding *et al*., 1983). EPNs have been used against soil insect pests and attracted attention of many researchers as biological means of insect pest control (Ehlers, 1996). Infective juveniles (IJs) of EPNs enter insects through the mouth, anus, or spiracles, penetrating the hemocoel. The nematodes release symbiotic bacteria into the host where they multiply quickly and kill the host within 24-48 hours (Gaugler and Kaya, 1990). *C. capitata* is very sensitive to a variety of EPNs, usually with larval and adult mortality being higher than that of pupae (Dolinski and Lacey, 2007).

Ceratitis capitata (Diptera: Tephritidae) is a serious economic pest attacking many host plant species of fruits and vegetables throughout the world, most of which are of high commercial value. It was recorded on more than 50 cultivated and wild plant species, mainly those with fleshy fruits including guavas, mangoes, peach, apricots, figs and citrus (Ghanim, 2009). It may cause 20-25% loss of citrus, 91% of peaches, 55% of apricots and 15% of plums in Jordan. Up to 100% of peaches were destroyed in Frankfurt (Fischer and Petersen, 1989).

Hence, the present study was carried out to test the penetration and infectivity of *H. bacteriophora* and *S. feltiae* against *C. capitata* at two different temperatures. The production and host finding of IJs of both EPNs in *C. capitata* larvae were also addressed.

MATERIALS AND METHODS

Rearing of *C. capitata*

C. capitata used in this study were obtained from a laboratory stock culture kept at the Horticultural Insect Department, Plant Protection Research Institute, Agricultural Research Center. Larvae of *C. capitata* were reared on an artificial diet under laboratory conditions at 25±2°C and 55-65% R.H. The artificial diet consisted of (1000 g) wheat bran, (200 g) sugar, (250 g) brewer's yeast, (10 ml) HCl, (12 g) sodium benzoate and (200 ml) water (Sarhan, 1981; Awadallah and El-Hakim, 1987).

Rearing of *Galleria mellonella*

The larvae of greater wax moth, *Galleria mellonella,* were obtained from infested bee hives and reared in plastic jars (2Kg capacity) until adult emergence according to the technique described by Birah *et al.* (2008). Artificial diet consisted of wheat (130g), wheat bran (130g), milk powder (130g), maize flour (97.5g), yeast powder (97.5g), wax (26g), honey (195ml) and glycerol (195 ml).

Entomopathogenic nematodes species (EPNs)

The heterorhabditis nematode, *Heterorhabditis bacteriophora* strain HP88 was obtained from New Brunswick NJ, USA. 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, *G. mellonella.* A Rearing of entomopathogenic nematode using larvae of *G. mellonella* as a host was performed according to the methods of Dutky *et al*. (1964).

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Bioassays

Effect of temperature on the infectivity of EPNs to *C. capitata*

This experiment was conducted to evaluate the effect of two temperature regimes (25 and 30±2°C) on the sensitivity of *C. capitata* larvae and pupae (one-and three-day-old pupae) on the infectivity with EPNs. Fifty full-grown larvae or pupae were placed in five plastic cups (200 cc capacity), 10 larvae/cup/5 replicates for each tested nematode species (*H. bacteriophora* and *S. feltiae*), half-filled with moistened sterilized sandy soil and covered with plastic lids. These cups were treated with one of the two entomopathogenic nematodes. Five concentrations of IJs (25, 50, 100, 200 and 400 IJs/cup) were studied under the two regimes of temperature. These cups were incubated at 25 and 30±1°C. Cups were examined after seven days of treatment and the rate of mortality was calculated.

Production of entomopathogenic nematode *H. bacteriophora* and *S. feltiae*

Ten full-grown larvae and pupae (one-day-old) of *C. capitata* were confined individually 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 of *H. bacteriophora* and *S. feltiae*. After 7 days, the larvae were transferred to Petri-dishes lined with filter paper moistened with distilled water (White-trap). This trap was used for harvesting the emerged infective juveniles. The infective juveniles were harvested daily using White traps according to White (1927). Each treatment had 10 replicates. The whole number of IJs produced/larvae was counted.

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

One ml of the nematode suspension containing 4000 IJs/tube was poured into the bottom of tubes (16 cm in height and 1.5 cm in diameter). After that, sterilized wet sandy soil (10%) was placed to cover the nematode suspension in each tube till reaching the heights of 3, 6, 9 and 12 cm of the soil column. Five full-grown larvae of *C. capitata* was placed inside a net of wire screen and placed on the top of the sand inside each tube. After 7 days, the larvae were then transferred to Petri-dishes lined with filter paper moistened with distilled water (White-trap) to record the numbers of larval mortality and emerged IJs to confirm the infection of larvae with the EPN. Ten replicates were used for each treatment (soil depth) with a total of 40 tubes for each nematode species. Ten tubes treated with distilled water were used as the control treatment for each nematode species.

Statistical analysis

Proportional data were transformed by arcsine square root (ARCsine) before analysis. The obtained data were 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

Infectivtiy of EPNs against *C. capitata* at 25 and 30°C

At 25°C, the mortality rates of full-grown larvae of *C. capitata* exposed to *H. bacteriophora* at 25, 50, 100, 200 and 400 IJs/cm² were 10, 32, 60, 70 and 90%, respectively. The respective mortlity rates for one-day-old pupae were 6, 20, 38, 54 and 70%; 4, 10, 32, 46 and 60% in three-day-old pupae (Table 1). The LC_{50} value for full-grown larvae, one-day-old pupae and three-day-old pupae were estimated at 91.44, 174.91 and 245.25 IJs/cm², respectively.

At 30°C, the respective concenterations of *H. bacteriophora* caused 8, 24, 44, 66 and 86% mortality in full-grown larvae; 4, 16, 34, 48 and 64% in one-day-old pupae; 2, 14, 26, 38 and 50% in three-day-old pupae at 25, 50,100,200 and 400 IJs/cm^2, respectively (Table 1). According to concentration–mortality line, the LC_{50} for larvae, one-day-old pupae and three-day-old pupae were 54.83, 216.28 and 245.06 IJs/cm² of soil surface, respectively.

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

Conc. (IJs/cm^2)	*H. bacteriophora*					
	25°C			30°C		
	Larvae	One-day-old-pupae	Three-day-old-pupae	Larvae	One-day-old-pupae	Three-day-old-pupae
Control	0.00±0 e	0.00±0 e	0.00±0 d	0.00±0 e	0.00±0 e	0.00±0 e
25	10.00±1.64 d	6.00±1.76 d	4.00±1.06 c	8.00±1.89 e	4.00±1.39 e	2.00±2.01 e
50	32.00±2.26 c	20.00±1.45 c	10.00±1.57 c	24.00±4.28 d	16.00±1.56 d	14.00±2.50 d
100	60.00±2.31 b	38.00±3.15 b	32.00±1.40 b	44.00±3.79 c	34.00±1.65 c	26.00±1.70 c
200	70.00±4.43 b	54.00±2.42 ab	46.00±3.99 ab	66.00±2.93 b	48.00±2.47 b	38.00±1.81 b
400	90.00±1.56 a	70.00±2.93 a	60.00±2.88 a	86.00±1.74 a	64.00±2.49 a	50.00±2.88 a
F	120.28	91.81	76.98	44.31	70.66	43.96
P	0.000	0.000	0.000	0.000	0.000	0.000
Lc_{50}	91.44	174.91	245.25	105.21	189.10	262.21

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

At 25°C, the different concentrations of *S. feltiae* had comparable direct effect on the mortality percentage of *C. capitata* full-grown larvae at 8, 30, 50, 68 and 88% for 25, 50, 100, 200 and 400 IJs/cm², respectively. The respective mortality percentages of one-day-old pupae were 6, 18, 36, 52 and 68%. As for precentage of mortality of three-day-old pupae, it was 2, 8, 30, 44 and 58% at 25, 50, 100, 200 and 400 IJs/cm², respectively (Table 2). LC_{50} value of larvae, one and three-day-old pupae of *C. capitata* were 105.21, 189.10 and 262.21 IJs/cm², respectively.

Pertaining to *S. feltiae* at concentration of 25, 50, 100, 200 and 400 IJs/cm² at 30°C, the respective mortality rates were 6, 24, 42, 62 and 80%, in full-grown larvae; 2, 16, 22, 40, 54%, in one-day-old pupae; and 0, 10, 20, 34 and 46%, in three-day-old pupae (Table 2). LC_{50} for *S. feltiae* to full-grown larvae, one-day-old pupae and three-day-old pupae were found to be 135.02, 315.10 and 400.77 IJs/cm² of soil, respectively.

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

Conc. (IJs/cm²)	*S. feltiae*					
	25°C			30°C		
	Larvae	One-day-old-pupae	Three-day-old-pupae	Larvae	One-day-old-pupae	Three-day-old-pupae
Control	0.00±0 e	0.00±0 f	0.00±0 f	0.00±0 e	0.00±0 e	0.00±0 e
25	8.00±1.66 d	6.00±0.54 e	2.00±1.25 e	6.00±1.79 e	2.00±1.25 d	0.00±0 e
50	30.00±3.33 c	18.00±2.06 d	8.00±1.29 d	24.00±1.53 d	16.00±1.41 c	10.00±0.96 d
100	50.00±3.13 b	36.00±1.60 c	30.00±1.11 c	42.00±1.53 c	22.00±1.82 c	20.00±2.41 c
200	68.00±3.34 b	52.00±2.33 b	44.00±1.49 b	62.00±2.30 b	40.00±2.71 b	34.00±1.93 b
400	88.00±2.05 a	68.00±2.21 a	58.00±1.88 a	80.00±2.90 a	54.00±3.77 a	46.00±2.38 a
F	102.40	147.26	226.93	87.43	40.49	84.11
P	0.000	0.000	0.000	0.000	0.000	0.000
Lc_{50}	54.83	216.28	245.06	135.02	315.10	400.77

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

Production of *H. bacteriophora* and *S. feltiae* within *C. capitata* full-grown larvae

H. bacteriophora produced an average of 6869 and 14462 IJs when infected a single full-grown larva of *C. capitata* with a concentration of 2000 and 4000 IJs/cm² of soil surface, respectively. As for infection with *S. feltiae,* the infected full-grown larva produced an average of 3507 and 7082 IJs at concentration 2000 and 4000 IJs/cm² of soil, respectively. In case of infected *C. capitata* pupae with *H. bacteriophora*, an average of 4470 and 10835 IJs was produced at concentration of 2000 and 4000 IJs/cm² of soil surface, respectively. However, *S. feltiae* produced an average of 2190 and 5366 IJs when pupae of *C. capitata* were infected with concentration of 2000 and 4000 IJs/cm² of soil surface, respectively (Fig 1).

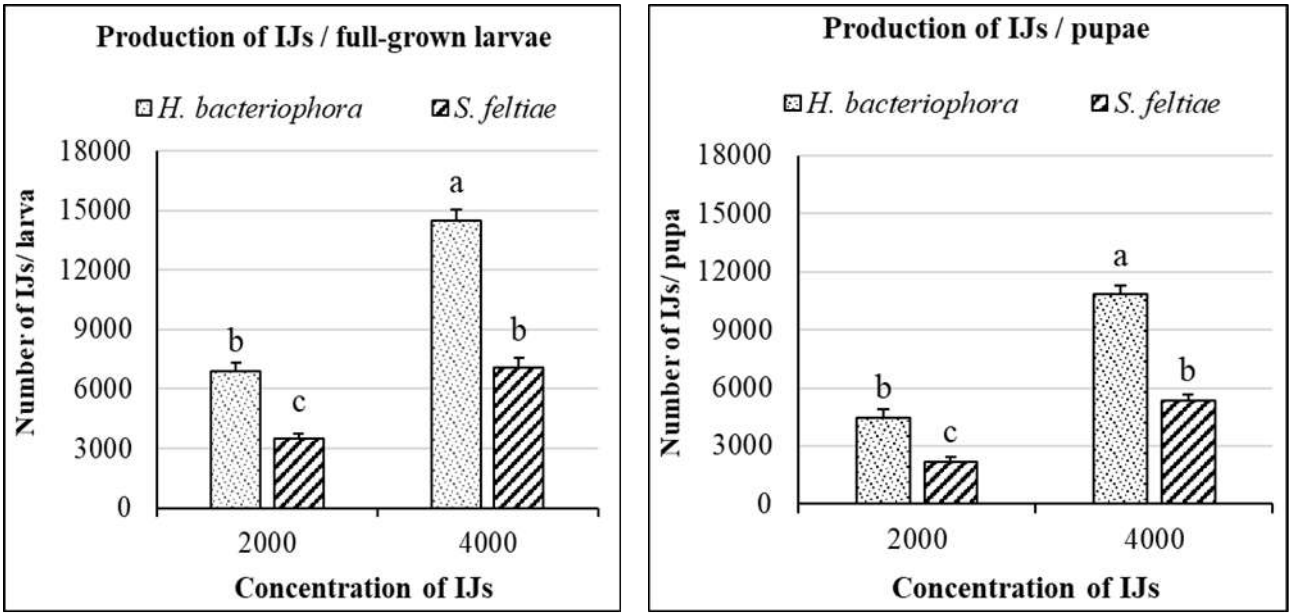

Fig (1): Production of infective juveniles by *H. bacteriophora* and *S. feltiae* from full-grown larvae of *C. capitata* exposed to the tested nematodes at 2000 and 4000 IJs/larva
Bars with different letters indicate significant difference (P<0.05)

Host finding for *H. bacteriophora* and *S. feltiae* IJs to full-grown larvae of *C. capitata*

The two tested EPN species reached their host (full-grown larvae of *C. capitata*) when placed at 3 cm height from the point of release (bottom of the tube). However, at 12 cm height, none of the infective juveniles could reach the exposed host and no mortality was observed in full-grown larvae of *C. capitata*. Generally, *H. bacteriophora* was superior to *S. feltiae* in reaching and killing *C. capitata*, with mortality rate of 100 and 94%, respectively (Fig 2).

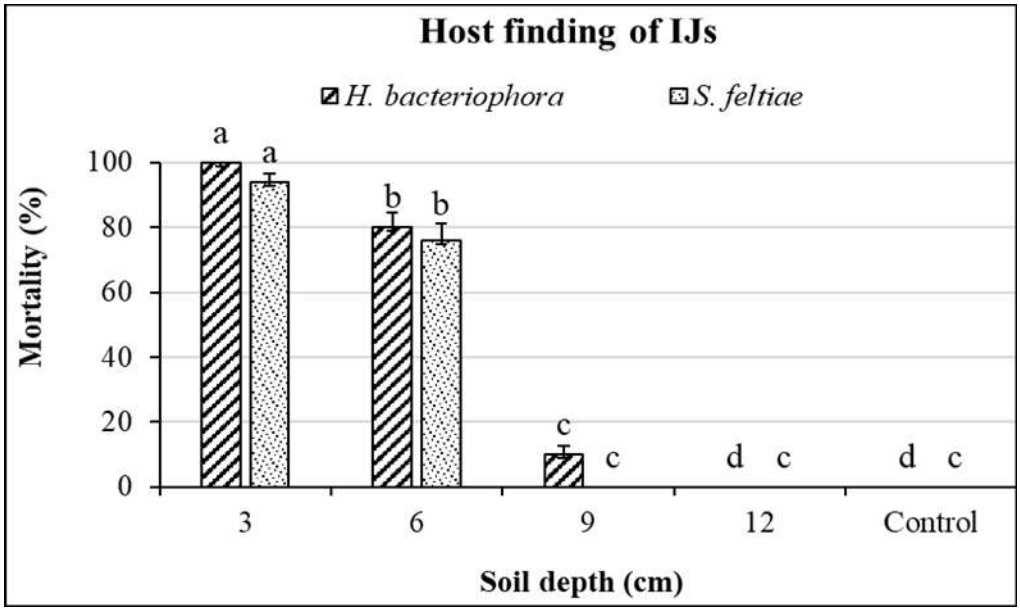

Fig (2): Mortality rate of *C. capitata* full-grown larvae caused by *H. bacteriophora* and *S. feltiae* when placed at different depths of soil surface
Bars with different letters indicate significant difference (P<0.05)

DISCUSSION

The increase in infectivity of the tested ENPs to third instar larvae of *C. capitata* in this study at higher temperature is in agreement with the results recorded for other fruit fly species (Rohde *et al.,* 2010; Kepenekci *et al.,* 2015). It is advantageous that the tested nematodes killed last instar larvae of *C. capitata* effectively at 25°C and 30°C in this study. The temperature range of 25–35°C is approximately the favorite temperature range for *C. capitata* habitats in Egypt (Abed-aall *et al*., 2014). Rohde *et al*. (2010) also found that the infectivity was directly proportional to the increase in temperature, with maximum percent mortality of 86.7% and 80.0% for *S. carpocapsae* and *Heterorhabditis* sp., respectively at 31ºC and the mortality rates at 25ºC were lower than those obtained at 31ºC. It is known that *S. feltiae* is adaptable to lower temperatures (Hazir *et al*., 2001). On the other hand, *Heterorhabditis* sp. is known to be adaptable to warm temperatures (Karagoz *et al*., 2009).

In the current study, *H. bacteriophora* was most reproductive at the two tested concentrations 2000 and 4000 IJs/cm². A single full-grown larva of *C. capitata* infected with *H. bacteriophora* and *S. feltiae* produced an average of 14462 and 7082 IJs when infected at 4000 IJs/cm². These results are close to 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 and *H. bacteriophora* were 14456. (Nouh and Abo Abdalla, 2016) found that production of *H. bacteriophora* and *S. abbasi* from *G. mellonella* larva were 229660 and 177800 IJs/larva at the highest concentration of 200/larvae.

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عدوي وإنتاجيه وإيجاد العائل للنيماتودا *Heterorhabditis bacteriophora* و *Steinernema feltiae* ضد ذبابة فاكهة البحر الأبيض المتوسط

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تهدف هذه الدراسة إلى تقييم تأثير درجة الحرارة على قدرة اثنين من النيماتودا الممرضة للحشرات، *Heterorhabditis bacteriophora* HP88 و *Steinernema feltiae* (Filipjev) California في إحداث العدوى في يرقات وعذارى (عمر يوم وثلاث أيام) ذبابه فاكهه البحر الأبيض المتوسط تحت الظروف المعملية. تمت دراسة تأثير خمسة تراكيزات من النيماتودا (٢٥ و ٥٠ و ١٠٠ و ٢٠٠ و ٤٠٠) يرقه طور معدي/cm^2 تحت نطاقين من درجات الحرارة (٢٥ و ٣٠ ± ٢ درجة مئوية). أظهرت النتائج المتحصل عليها إلى أن درجة الحرارة وتركيز النيماتودا لهما تأثير معنوي على كفاءة أنواع النيماتودا. كانت هناك فروق معنوية من حيث عدد أفراد يرقات الطور المعدي الناتجة من يرقات كاملة النمو وعذارى ذبابه الفاكهة من المعاملات المختلفة. أيضا، كان النوع *H. bacteriophora* هي الأكثر إنتاجيه من يرقات الطور المعدي في يرقات وعذارى ذبابه الفاكهة، بالتركيزات المختبرة. تفوق النوع *H. bacteriophora* على النوع *S. feltiae* في الوصول إلي وقتل عائله ذبابه الفاكهة. بشكل عام، أظهرت نتائج هذه الدراسة أن النيماتودا *H. bacteriophora* قد تفوق على النيماتودا *S. feltiae* في تركيزات المعلقات المختبرة في درجات الحرارة من حيث الإنتاجية والوصول وقتل عوائلها ذبابه فاكهه البحر الأبيض المتوسط. وبالتالي، وبنجاح النيماتودا الممرضة للحشرات، *H. bacteriophora* يمكن استخدامها كعامل تحكم ضد ذبابه فاكهه البحر الأبيض المتوسط، في استراتيجيات برامج المكافحة المتكاملة للآفات.