

Bioefficacy of two entomopathogenic nematodes against *Spodoptera littoralis* Boisduval (Lepidoptera) and *Temnorhynchus baal* Reiche (Coleoptera) larvae

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ABSTRACT

Efficacy of the two entomopathogenic nematode species, *Heterorhabditis bacteriophora* Poinar (HP88 strain) and *Steirnerma glaseri* Steiner (NJ strain), was tested on the fifth and third instar larvae of *Spodoptera littoralis* (Boisd.) and *Temnorhynchus baal* (Reiche) under laboratory conditions. Experiments were conducted on filter paper and sandy soil substrates using nematode at 50, 100, 200 and 400 infective juveniles/5ml of water. *Heterorhabditis bacteriophora* was most effective on the fifth instar larvae of *S. littoralis* whereas *S. glaseri* was effective against third instar larvae of *T. baal* third. Insect mortality was high (60-90%) and low (<45%) at higher and lower nematode concentrations respectively. *Heterorhabditis bacteriophora* treated larvae of *S. littoralis* succumbed to the infection at the higher rates (80-100%) as compared to those treated with *S. glaseri*. The rate of mortality of *S. littoralis* was lowest when treated with *S. glaseri* (2-20%). The differences in the rate of nematode infection and insect mortality under various experimental conditions are attributed to the difference in the behavior, virulence, rate of penetration and host searching abilities of nematodes and the abilities of insect pest larvae to resist nematode penetration. Present study suggests that entomopathogenic nematodes are important and effective biological control agents of most soil dwelling insect pests.

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Key words: Efficacy, entomopathogenic nematodes, *Steirnerma glaseri*, *Heterorhabditis bacteriophora*, *Temnorhynchus baal*, pests, biopesticides, *Bt*, flavonoids

INTRODUCTION

Entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) and their symbiotic bacteria kill broad spectrum of insects under laboratory conditions. Laboratory bioassays showing efficacy against insects cannot be applied under field conditions where high level of control is required (Kaya, 1990). However, laboratory experiments provide directions to better use of biological control agents and understanding of their biology and behavior. Better understanding of nematode biology, host range, epizootiology, their commercial production, storage and formulation have encouraged the use of nematodes as biological control agents and boosted commercialization of biopesticide industry (Friedman, 1990; Ehlers, 1996). Entomopathogenic nematodes (EPNs) are excellent biological control agents of soil-dwelling insect pests, including lepidopteron insects, and

white grubs (Scarabaeidae) in vegetables, sugarcane and turf grass (Atwa, 2014). Despite of the success of EPNs as biological control agents of many soil-dwelling insect pests, the rate of their success against foliar insect pests is minimal (Menn, 2000). *Spodoptera littoralis* Boisduval (Lepidoptera: Noctuidae), the Egyptian cotton leaf worm, is an important and widespread agricultural pest in the subtropical and tropical ranges. *Spodoptera littoralis* is polyphagous in nature, which as a result causes significant crop loss (Carter, 1984). It reduces yield of several economically important crops including 27 plant species belonging to 16 families around the year (Salama, *et al.*, 1971; Anderson *et al.*, 2001). Significant damages have been reported to cotton, tomato, lettuce, strawberry and other vegetables by *S. littoralis* infection throughout Africa, Middle East and Mediterranean basin (Pineda *et al.*, 2007).

The larval stages of *Temnorhynchus baal* Reiche (Coleoptera: Scarabaeidae) (scarab beetle) damage strawberry roots in Egypt, causing significant loss in high cash crops. In order to complete its metamorphic life cycle, larvae feed on the host as much as possible (Atwa, 2003, 2014). Recent advances made on the biological control of scarab beetles in the United States of America, South Africa, Australia, and Egypt (Atwa, 2014) showed susceptibility of scarab beetle to fungi, bacteria, and nematodes (Koppenhöfer, *et al.*, 2000).

Heterorhabditis bacteriophora and *S. glaseri*, the two important insect parasitic nematodes, have many advantages over others. Both species have a broad host range, high rate of efficacy and virulence on many insect pests. Both species are cruiser, a host search technique that increases the chance of host-parasite contacts unlike ambushers. They can be mass produced easily and applied to the field by using conventional application tools. As compared to other nematodes, the field treatments of entomopathogenic nematodes are environmentally safe and their products do not required registration. In the present study, the efficacy of the two species of entomopathogenic nematodes, *S. glaseri* Steiner (NJ strain) and *H. bacteriophora* Poinar, (Hp88 strain) was tested against *T. baal* and *S. littoralis* under laboratory conditions.

MATERIALS AND METHODS

The efficacy of the two species of entomopathogenic nematodes, *Steinernema glaseri* Steiner (NJ strain) and *Heterorhabditis bacteriophora* Poinar, (Hp88 strain) was tested against fifth instar larvae of *Spodoptera littoralis* (Boisd.) and third instar larvae of *Temnorhynchus baal* (Reiche) at the four nematode concentrations i.e., 50, 100, 200, and 400 infective juveniles/5ml of water. Two substrates, the filter paper and the sandy soil were chosen to test nematode's efficacy against the third instar larvae of *T. baal* and fifth instar larvae of *S. littoralis*.

Nematode cultures

Steinernema glaseri (NJ strain) and *H. bacteriophora* (Hp88 strain) were reared on the fifth instar larvae of greater wax moth, *Galleria mellonella* (L) under laboratory conditions (Woodring and Kaya (1988). *Galleria mellonella*, obtained from the infested beehives, was reared on

the artificial diet at 27±2°C and 65±5% RH (Singh, 1994). The infective juveniles of nematodes were harvested by using White Traps as described by White (1927) at 25±1°C. A stock suspension of infective juveniles was made in sterile distilled water and stored at 10°C for two weeks.

Insects

The original population of *S. littoralis*, collected from cotton field in Alexandria (Latitude & Longitude: 31.21°, 29.93°), Egypt, was cultured in the laboratory at 27 ± 1° C. The fifth instar larvae were obtained from laboratory culture for various experiments. In order to prevent inbreeding and maintain genetic diversity, several field collected individuals of *S. littoralis* were added to the culture two times/year (Hegazi *et al.*, 1977). *Spodoptera littoralis* was cultured on artificial diet (Hegazi *et al.*, 1977) at 27 ± 1° C, 60–65% RH, and 14:10 L:D photoperiod. The third instar larvae of *T. baal* was collected from the infested field at Ismailia Governorate (Latitude & Longitude: 30.6°, 32.2°), Egypt and cultured in the laboratory. Prior to the experiment, the insect larvae were washed three times with distilled water to remove soil and organic particles.

Bioassay

The experiments with the third and fifth instar larvae of *T. baal* and *S. littoralis* respectively were conducted in 7cm high and 6.5cm in diameter plastic containers covered with plastic lid. Each container contained 50 gm of autoclaved sandy soil or shredded filter paper. Five milliliter of sterile distilled water was added to all experimental units. Nematode inoculums were prepared in 5ml suspension at concentrations of 50, 100, 200 and 400 infective juveniles. Three larvae from each insect group were inoculated in each container followed by nematodes. Ten replicates were made per infective juvenile concentrations and insect larvae. The percentage mortality was determined 72-96 h after nematode inoculation.

Data analysis

Percentage of larval mortality was analyzed by one-way analysis of variance (ANOVA) and means were compared by least significant difference (LSD). SPSS 18.0 software (SPSS Inc., Chicago, IL, USA)

Table 1. Rate of mortality (%) of *T. baal* and *S. littoralis* at different concentrations of *S. glaseri* and *H. bacteriophora*

Substrate	Insect	Nematodes species	Percentage of insect larvae mortality (Mean \pm SD)			
			50 IJs	100 IJs	200 IJs	400 IJs
Filter paper	<i>T. baal</i>	<i>S. glaseri</i>	0.00 \pm 0.00 ^a	51.50 \pm 6.69 ^b	82.00 \pm 3.50 ^{ab}	89.00 \pm 3.94 ^{ab}
		<i>H. bacteriophora</i>	0.00 \pm 0.00 ^a	0.00 \pm 0.00 ^a	15.00 \pm 5.78 ^b	21.50 \pm 4.74 ^b
	<i>S. littoralis</i>	<i>S. glaseri</i>	13.50 \pm 3.38 ^b	34.00 \pm 7.38 ^b	69.50 \pm 5.50 ^c	90.00 \pm 5.27 ^{ab}
		<i>H. bacteriophora</i>	79.50 \pm 4.97 ^c	98.50 \pm 2.41 ^{ab}	100.0 \pm 0.00 ^{ab}	100.0 \pm 0.00 ^{ab}
Sandy soils	<i>T. baal</i>	<i>S. glaseri</i>	1.50 \pm 2.42 ^a	51.50 \pm 6.69 ^b	85.00 \pm 2.36 ^{ab}	93.00 \pm 2.58 ^{ab}
		<i>H. bacteriophora</i>	1.50 \pm 2.42 ^a	4.00 \pm 3.16 ^a	17.00 \pm 5.87 ^b	21.50 \pm 5.30 ^b
	<i>S. littoralis</i>	<i>S. glaseri</i>	16.50 \pm 2.42 ^b	38.50 \pm 5.80 ^b	81.00 \pm 5.16 ^c	92.00 \pm 2.58 ^{ab}
		<i>H. bacteriophora</i>	81.00 \pm 4.60 ^c	100.0 \pm 0.00 ^{ab}	100.0 \pm 0.00 ^{ab}	100.0 \pm 0.00 ^{ab}

Mean values followed by the same letter in the same row or column are not significantly different at $P < 0.05$; ANOVA

was used for statistical analyses. Percent mortality was subjected to arcsine square root transformation to increase the homogeneity of variance and normality. The transformed data were normally distributed.

RESULTS AND DISCUSSION

Experiments with *S. littoralis* and *T. baal* showed that the insects were significantly susceptible to the infection by *S. glaseri* ($F = 8412.88$, $df = 1$, $p < 0.05$) and *H. bacteriophora* ($F = 9382.58$, $df = 1$, $p < 0.05$). Results showed that EPN were more virulent and effective against insect pests. Nematode dosage at higher concentrations yielded highest insect mortality as compared to the lower dosages when filter paper or sandy soil was used as the substrate ($F = 1.66$, $df = 3$, $p < 0.05$) (Table 1). *Temnorhynchus baal* was more susceptible to *S. glaseri* ($F = 1007.13$, $df = 1$, $p < 0.05$) as compared to *H. bacteriophora* ($F = 2684.02$, $df = 1$, $p < 0.05$) when filter paper or sandy soil substrates were used (Table 1). *Spodoptera littoralis* showed highest mortality when infected with all concentrations of *H. bacteriophora* on both substrates (Table 1).

Results reveal that mortality of fifth and third instar larvae of *S. littoralis* and *T. baal* respectively due to nematode infection on different substrates at various concentrations. The efficacy of *H. bacteriophora* was high on fifth instar larvae of *S. littoralis* whereas, *S. glaseri* was most effective against third instar larvae of *T. baal* (Table 2).

The mortality of *T. baal* is higher than *S. littoralis* when these insects were treated with *S. glaseri* on filter paper or sandy soil substrates (Fig. 1). *Heterorhabditis bacteriophora* was most effective against *S. littoralis* larvae when tested on either

substrate ($F = 1.24$, $df = 1$, $p < 0.05$). There was, however, no significant difference between the mortality of *T. baal* and *S. littoralis* larvae when they were treated with *S. glaseri* on filter paper or sandy soil substrates. Infection with higher concentrations of nematode inoculums yielded higher insect mortality ($F = 39.17$, $df = 3$, $p < 0.05$) (Table 2).

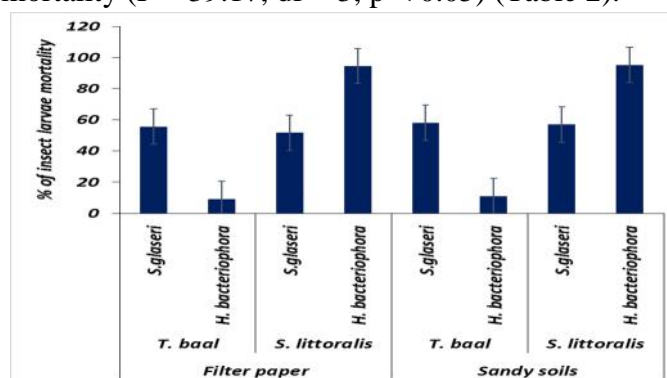


Fig. 1. Impact of *S. glaseri* and *H. bacteriophora* on insect mortality (%)

At lower dosages, the mortality of both insect species was significant but less than 45%. Infection with *S. glaseri* has resulted in the higher mortality as compared to *H. bacteriophora*. Except, when the insects were treated with *H. bacteriophora* at 400 infective juveniles, the rate of mortality of *S. littoralis* was significantly higher with *S. glaseri* infection. Higher nematode concentrations resulted in high insect mortality, nevertheless, *S. littoralis* larvae were most susceptible to *H. bacteriophora* as compared to *T. baal*. Present study reveals significant susceptibility of insect pests to EPNs. The third instar larvae of *T. baal* were more susceptible to *S. glaseri*, whereas fifth instar larvae of *S. littoralis* to *H. bacteriophora*. Rate of mortality of both insect species was high at higher concentrations, whereas lower concentrations

Table 2. Rate of insect mortality at different nematode concentrations and substrates by ANOVA analysis

Source	Sum of Squares	df	Mean Square	F	Significance level
Substrate	500.00	1	500.00	30.90	0.00
Insect	136125.00	1	136125.00	8412.88	0.00
Nematode species	750.31	1	750.31	46.37	0.00
Nematode concentration	130286.88	3	43428.96	2684.02	0.00
Substrate × Insect	20.00	1	20.00	1.24	0.27
Substrate × Nematode species	112.81	1	112.81	6.972	0.01
Substrate × Nematode concentration	80.63	3	26.88	1.66	0.18
Insect × Nematode species	151815.33	1	151815.31	9382.58	0.00
Insect × Nematode concentration	941.88	3	313.96	19.40	0.00
Nematode species × Nematode concentration	48887.83	3	16295.94	1007.13	0.00
Substrate × Insect × Nematode species	90.31	1	90.31	5.58	0.02
Substrate × Insect × Nematode concentration	45.63	3	15.21	0.94	0.42
Substrate × Nematode species × Nematode concentration	131.56	3	43.85	2.71	0.05
Insect × Nematode species × Nematode concentration	6115.31	3	2038.44	125.98	0.00
Substrate × Insect × Nematode species × Nematode concentration	116.56	3	38.85	2.40	0.07
Error	4660.00	288	16.18		
Total	1413800.00	320			
Corrected Total	480680.00	319			

* Significant differences at $P < 0.05$; ANOVA.

reduced insect population at the lower rate. Our results are in agreement with the studies earlier made by Atwa and Shamseldean (2008). Such differences in the insect mortality by the two nematode species may be attributed, first to the host preference by nematodes, second to the nematode's compatibility with its host, and third to the vulnerability of insect pests to specific nematode infection (Shapiro-Ilan and Cottrell, 2005).

Nematode-bacteria complex, as it plays significant role in insect mortality, may also be attributed to the differences in the rates of mortality between the two insect species (Berry *et al.*, 1997). During present study, different efficacy rates of *S. glaseri* and *H. bacteriophora* on *T. ball* and *S. littoralis* suggests that the two nematode species have wider host range. Due to different virulence of the two nematode species, their associated bacteria *Photorhabdus luminescens* and *Xenorhabdus poinarii* also reacted differently when in contact with insect hosts (Rosa *et al.*, 2002).

In the present study, EPNs showed differences in efficacy against insect pests, as was also observed in other studies made earlier by Glazer *et al.* (1991). As a result, the selection of appropriate EPNs to manage insect pests becomes essential (Mannion and Jansson, 1992). Bioassay, nematode virulence, nematode fecundity, rate of mortality and host search behaviour etc., are therefore important determinant in the selection of most effective nematode-insect combinations for successful and effective biological control. Our results are in conformity with Atwa and Shamseldean (2008) and Jansson *et al.* (1993). In contrary to the observations made by Atwa (2003, 2009), *S. glaseri* and *H. bacteriophora* showed differential efficacy on the larvae of *S. littoralis* and *T. ball* despite having similar host-finding behaviour.

In the present study, the low infectivity and insect mortality by *H. bacteriophora* may have been resulted due to the failure of its symbiotic bacteria to establish in the host. Use of different substrates might have also made the differences in nematode virulence and insect mortality. However, our results

with *T. ball* are in conformity with Solter *et al.* (2001) who reported that *S. carpocapsae* isolates have wide host range (Finney and Walker, 1979). Gouge *et al.* (1999) and Menti *et al.* (2000) observed different virulence in *S. glaseri* and *H. bacteriophora*, whereas studies made by Saunders and Webster (1999) and Shapiro-Ilan *et al.* (2002) showed identical rate of infection by *H. bacteriophora* and *S. glaseri*. Later authors suggested that the rate of nematode infection and insect mortality varies due to the differences in the rate and time of nematode penetration. Our studies, where rates of nematode infection and insect mortality varied with conditions conform to the observations of the above authors.

It may be concluded that EPNs are effective biological control agents of numerous soil-dwelling insect pests including coleopteran and lepidopteron. Rates of nematode infection and insect mortality is dose dependent and varies depending on the type of substrate, insect and experimental conditions.

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