A LABORATORY STUDY OF SUSCEPTIBILITY OF HELICOVERPA ARMIGERA (HUBNER) TO THREE SPECIES OF ENTOMOPATHOGENIC NEMATODES

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ABSTRACT: The insecticidal effect of *Heterorhabditis bacteriophora* Poinar (Rhabditida: Nematoda: Heterorhabditidae), *Steinernema carpocapsae* (Weiser) and *Steinernema feltiae* (Filipjev) (Rhabditida: Nematoda: Steinernematidae), was examined against *Helicoverpa armigera* under laboratory conditions. The nematodes were used in the following doses: 0 (control), 1, 10, 50, 100 and 500 infective juveniles (IJs) per insect, and their infectivity was tested at 22° C after 72, 96 and 120 h. of exposure using three methods of filter paper assay, food assay and soil assay. In all trials, *H. bacteriophora* IRA10 had the highest toxicity and *S. carpocapsae IRA18* the lowest. There were no significant differences between strains at the lowest concentration in all exposure times. In filter paper assay, a dose of 500IJ/Larvae of *H. bacteriophora* IRA10 after 120h of exposure time caused 83% mortality, whereas *S. feltiae* IRA24 and *S. carpocapsae* IRA18 caused 71.67%and 30% mortality, respectively. In food and soil assay, similar results were found and *H. bacteriophora* IRA10 was *more pathogenic* against cotton bollworm compared to *S. feltiae* and *S. carpocapsae*.

KEY WORDS: Entomopathogenic nematodes, *Helicoverpa armigera*, *Heterorhabditis bacteriophora*, *Steinernema carpocapsae*, *Steinernema feltiae*.

The American Bollworm *Helicoverpa armigera* Hubber (Lepidoptera: Noctuidae) is extremely polyphagous and one of the major pests of cotton in almost all of the cotton growing areas in Iran, causing quantitative and qualitative losses. It has been reported as a major pest of cotton, sorghum, maize, sunflower and tomato. The infestation by *H. armigera* on these crops is largely restricted to one stage in host-plant development. On cotton, the damage is characterized by feeding activity on flower buds, flowers and cotton bolls.

Entomopathogenic nematodes (EPNs) are used to control several agriculturally important insect pests of different orders. Several species of EPNs are used worldwide against a variety of pests. Some important EPN species of *Steinernema* and *Heterorhabditis* are obligate pathogens and are characterized by their association with symbiotic bacteria present in the digestive tract; *Xenorhabdus* in steinernematids and *Photorhabdus* in heterorhabditids (Boemare et al., 1996). With the aim of determining virulence of different EPNs species, we investigated the ability of EPNs in control of *Helicoverpa armigera* in laboratory conditions.

MATERIALS AND METHODS

Nematode sources

A preliminary test was conducted to compare the pathogenicity of different entomopathogenic nematodes against last instar larvae of cotton bollworm by the same methods used for the filter-paper-substrate Petri dish assay. *Steinernema carpocapsae* (strain IRA18) (Weiser, 1955), *S. feltiae* (strain IRA24) and *Heterorhabditis bacteriophora* (IRA10) recently isolated from north-west of Iran by Eivazian Kary et al. (2009) were used in experiments.

One last instar cotton bollworm larva was placed in each of 30 Petri dishes (35×12 mm) containing 150 µl of 100 IJs suspended in water and evenly distributed on a filter paper (Wattman No. 1) in the dish. The dishes were incubated in the dark at 25ºC in a plastic bag containing a moist paper towel to maintain humidity. After 120 h, the infected cadavers were transferred to White traps (White, 1927) for harvesting IJs. The IJs were collected in Ringer's solution and stored in the dark at 10ºC. Nematode viability was 100%. IJs within two weeks of harvest were used in all experiments.

Insect sources

Early instar larvae of *H. armigera* were collected from cotton field and reared on artificial diets based on cowpea (Shorey & Hall, 1965) in controlled conditions of 26±2ºC, 50±5% relative humidity and a photoperiod of 16:8 (L: D) h. until they reached the life stage to be tested.

Effect of dose and nematode species on infection of cotton bollworm on filter paper

The efficacy of EPNs was tested at five concentrations including 1, 10, 50, 100 and 500 infective juvenile (IJs) against cotton bollworm larvae. Individual last instar larvae were placed in each of 24 wells of plate as the first replicate containing 100µl of IJs suspended in water evenly distributed on a filter paper (Wattman No.1). The plates were incubated in the dark at 22ºC in a plastic bag containing a moist paper towel to maintain humidity. Control insects were treated with water only. Three days after treatment, insect mortality was recorded on a daily basis. Dead larvae were collected every 24 h, held at the same temperature for another 48 h, and then were dissected to verify that mortality occurred as a result of parasitism by nematodes.

Effect of dose rate and nematode species on infection of cotton bollworm via feeding

This experiment was done with the same conditions as above. Last instar larvae were fed with 5 g of arterial diet incorporated with the same concentration of EPNs. Three days after treatment, insect mortality was recorded on a daily basis and dead insects were examined for nematode presence by dissection.

Statistical analysis

Data were submitted to analysis of variance and the means were compared by the Tukey test, using SPSS 14.0 software (SPSS, 2004). The data were transformed into $\sqrt{(x+0.5)}$ before statistical analysis as necessary.

RESULTS

Effect of three different nematode species against larvae of cotton bollworm in filter paper assay

The effect of three species of entomopathogenic nematodes on cotton bollworm larvae in different exposure times is presented in Tables 1 to 3 and Figure 1. The tested nematodes did not show any mortality in two concentrations

(1 and 10 IJ/larvae) during all exposure times, except *H. bacteriophora* that showed little mortality in 10 IJ/larvae concentration after 72 h exposure time. Mortality rate was increased by increasing exposure time and pathogen concentration. There was a positive correlation between concentrations and mortality rates. Three tested nematodes showed the highest mortality at 500 IJ/larvae concentration. Mortality rates were significantly different between three other concentrations (50, 100, 500 IJ/larvae) after all exposure times (P < 0.001). The tested nematodes differed from each other significantly in pathogenicity (Table 1), and *H. bacteriophora* and *S. feltiae* were more pathogenic than *S. carpocapsae* in higher concentrations (P < 0.05). The descending trend of pathogenicity effects of nematodes in higher concentration after 24 and 72 h exposure times was *H. bacteriophora*, *S. feltiae* and *S. carpocapsae*, respectively.

Effect of three nematode species against larva of cotton bollworm in food assay

The mortality effect of mixed food with three entomopathogenic nematodes after three different times is presented in Table 4 to 6 and Figure 1. According to Table 4, there was no mortality in three first concentrations after 24 h exposure time. With increasing exposure time, mortality occurred in lower concentrations. Mortality was observed in 50 IJ/larvae concentration after 48 h and in 10 and 50 IJ/larvae concentrations after 72 h exposure time. Similar to filter paper assay, pathogenicity effect was increased by increasing exposure time and pathogen concentration, and all three used nematodes caused higher mortality at higher concentrations (Tables 4-6) (P < 0.001). However, *S. carpocapsae* showed no significant difference between five concentrations after 24 and 48 h exposures time (P > 0.05). Mortality rates after 72 h exposure time were significantly different between the given five concentrations (P < 0.001). The pathogenicity effect of *S. carpocapsae* especially in the highest nematode dose (500 IJ/larvae) was significantly different from the two other pathogens (Table 1), and *S. carpocapsae* caused lower mortality rate against cotton bollworm larvae (P < 0.01) after all three exposure times. The descending order of pathogenicity effect of three nematodes was similar to filter paper assay; however, no significant differences was observed between *H. bacteriophora* and *S. feltiae* in the two higher concentrations during different exposure times.

Effect of three different nematode species against larva of cotton bollworm through soil assay

All nematode species showed pathogenic effects on cotton bollworm larva in soil application assay (Tables 7 to 9; Fig. 1). No infection or larva mortality occurred by the tested nematodes in the first concentration of (1 IJ/larvae) treatments. Pathogenic effect by *H. bacteriophora* was observed in four higher concentrations after all three exposure times. Moreover, *S. feltiae* and *S. carpocapsae* showed no pathogenic effect in 10 IJ/larvae concentration after 24 and 48 h and 24 h exposure times, respectively. The infection level of cotton bollworm larva was increased as pathogen concentration increased and there was significant difference between concentrations (P < 0.001). Similar to filter paper and food assays, the highest mortality was observed in 500 IJ/larvae concentration after all three exposure times. Three nematode species differed from each other in pathogenicity in some concentrations. *H. bacteriophora* and *S. feltiae* were more pathogenic than *S. carpocapsae* in two higher concentrations (P < 0.001).

DISCUSSION

The efficacy of various nematode species or strains for controlling a particular insect pest may differ significantly (Bedding et al., 1983; Forschler et al., 1988; Kondo et al., 1988). Efficacy is influenced by the rate of IJ penetration into the insect, the time it takes to release the symbiotic bacteria, and the virulence of the latter (Glazer & Navon, 1990).

In the present study, when cotton bollworm was exposed to various dosages of nematodes in the laboratory for 120 h, *H. bacteriophora* IRA10 killed the greatest number of larvae and direct relationships were found between results obtained in dose-response and exposure-time assays. Similar results were obtained in studies of the effect of nematodes on other lepidopteran pests (Glazer, 1992; Ricci et al., 1996). In all treatments, there was a positive correlation between concentrations and mortality rates. A linear relationship between the number of nematodes applied to insects and the number of infecting nematodes has been established for several insect species (Fan & Hominick, 1991).

Application of three species of these biological agents (*S. feltiae*, *S. carpocapsae*, and *H. bacteriophora*) at studied temperatures (22°C) resulted in mortality in different levels. An optimal biological activity of *S. carpocapsae* was determined in the temperature range from 22 to 24°C (Choo HoYul et al., 2002), *H. bacteriophora* from 22 to 26 °C (Doucet et al., 1996), and S. feltiae at 25°C (Belair et al., 2003). Under the conditions of this study, it appears that all species of entomopathogenic nematodes were able to kill last instar larvae of cotton bollworm within six days of application. *Heterorhabditis bacteriophora* had a greater effect on last instar larvae of cotton bollworm than *S. feltiae* and *S. carpocapsae* in all experiments and it appears that under the conditions of this study, the *H. bacteriophora* nematode is most likely to be an effective biocontrol agent for cotton bollworm.

In our experiments, low mortality caused by *S. feltiae* and *S. carpocapsae* could be attributed to the foraging strategies of the nematode species. *Steinernema carpocapsae* displays a "nictation" or ambusher foraging strategy in which it stays on the soil surface waiting for its host, whereas *S. feltiae* displays an intermediate ambusher–cruiser foraging strategy in which it waits for or seeks its host (Lewis, 2002).

Although laboratory screening of entomopathogenic nematodes for infectivity can be an important component of developing a biological control program for a particular pest (Ricci et al., 1996), relative infectivity among nematodes in the laboratory may not be consistent with what is observed in the field (Grewal & Georgis, 1998). Once the biological candidates are defined based on characteristics tested in the laboratory, the ultimate test of efficacy must be conducted under field conditions. For any biocontrol agent to be effective in the field, it must be able to function under realistic climatic conditions.

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Table 1. Mean mortality percentages of *Helicoverpa armigera* larva infected with EPNs at different concentration (mean number of died larva ± SE) after 72 hours exposure time (filter assay).

Nematode species	Concentration				
	1	10	50	100	500
H. bacteriophora	0Ac	0Ac	5.83 ± 1.78Abc	12.50 ± 2.63Ab	30.83 ± 6.09Aa
S. feltiae	0Ac	0Ac	1.25 ± 0.89Bc	16.25 ± 2.81Ab	25.83 ± 2.55Aa
S. carpocapsae	0Ab	0Ab	0.42 ± 0.42Bb	3.75 ± 1.70Bab	4.58 ± 1.15Ba

Table 2. Mean mortality percentages of *Helicoverpa armigera* larva infected with EPNs at different concentration (mean number of died larva ± SE) after 96 hours exposure time (filter assay).

Nematode species	Concentration				
	1	10	50	100	500
H. bacteriophora	0Ac	0Ac	12.92 ± 2.44Abc	26.67 ± 4.36Ab	50.42 ± 8.82Aa
S. feltiae	0Ac	0Ac	7.08 ± 2.98ABc	31.25 ± 4.08Ab	50.42 ± 2.59Aa
S. carpocapsae	0Ab	0Ab	2.08 ± 1.28Bb	12.08 ± 1.81Ba	15.00 ± 3.12Ba

Table 3. Mean mortality percentages of *Helicoverpa armigera* larva infected with EPNs at different concentration (mean number of died larva ± SE) at after 120 hours exposure time (filter assay).

Nematode species	Concentration				
	1	10	50	100	500
H. bacteriophora	0Ad	0.83 ± 0.83Ad	21.67 ± 3.39Ac	48.33 ± 5.28Ab	83.33 ± 5.56Aa
S. feltiae	0Ad	0Ad	15.00 ± 2.79ABc	51.25 ± 4.03Ab	71.67 ± 2.39Aa
S. carpocapsae	0Ac	0Ac	11.25 ± 2.33Bb	23.75 ± 1.97Ba	30.00 ± 1.94Ba

Table 4. Mean mortality percentages of *Helicoverpa armigera* larva infected with EPNs at different concentration (mean number of died larva ± SE) after 72 hours exposure time (food assay).

Nematode species	Concentration				
	1	10	50	100	500
H. bacteriophora	0Ab	0Ab	0Ab	2.50 ± 1.27Ab	9.17 ± 2.31Aa
S. feltiae	0Ab	0Ab	0Ab	1.67 ± 0.68Ab	6.67 ± 1.55Aa
S. carpocapsae	0Aa	0Aa	0Aa	0.42 ± 0.42Aa	0.42 ± 0.42Ba

Table 5. Mean mortality percentages of *Helicoverpa armigera* larva infected with EPNs at different concentration (mean number of died larva ± SE) after 96 hours exposure time (food assay).

Nematode species	Concentration				
	1	10	50	100	500
H. bacteriophora	0Ac	0Ac	1.25 ± 0.64ABc	14.58 ± 3.18Ab	27.08 ± 2.26Aa
S. feltiae	0Ac	0Ac	4.17 ± 1.64Ac	10.42 ± 2.43Ab	22.08 ± 1.65Aa
S. carpocapsae	0Aa	0Aa	0Ba	0.42 ± 0.42Ba	2.92 ± 1.76Ba

Table 6. Mean mortality percentages of *Helicoverpa armigera* larva infected with EPNs at different concentration (mean number of died larva ± SE) after 120 hours exposure time (food assay).

Nematode species	Concentration				
	1	10	50	100	500
H. bacteriophora	0Ad	0.83 ± 0.83Acd	7.50 ± 1.36Ac	23.33 ± 2.86Ab	36.67 ± 2.39Aa
S. feltiae	0Ad	0Ad	8.33 ± 1.86Ac	18.33 ± 2.08Ab	31.25 ± 1.67Aa
S. carpocapsae	0Ac	0Ac	5.42 ± 1.25Abc	10.42 ± 1.42Bb	18.75 ± 2.80Ba

Table 7. Mean mortality percentages of *Helicoverpa armigera* larva infected with EPNs at different concentration (mean number of died larva ± SE) after 120 hours exposure time (soil assay).

Nematode species	Concentration				
	1	10	50	100	500
H. bacteriophora	0Ad	0.42 ± 0.42Ad	9.17 ± 1.73Ac	25.83 ± 3.09Ab	34.58 ± 1.17Aa
S. feltiae	0Ac	0Ac	8.33 ± 1.52ABb	27.08 ± 3.53Aa	30.42 ± 2.16Aa
S. carpocapsae	0Ac	0Ac	4.17 ± 0.88Bbc	7.50 ± 2.22Bb	17.92 ± 2.24Ba

Table 8. Mean mortality percentages of *Helicoverpa armigera* larva infected with EPNs at different concentration (mean number of died larva ± SE) after 120 hours exposure time (soil assay).

Nematode species	Concentration				
	1	10	50	100	500
H. bacteriophora	0Ad	2.92 ± 1.53Ad	23.75 ± 2.41Ac	48.75 ± 5.73Ab	62.08 ± 3.59Aa
S. feltiae	0Ad	0Ad	13.33 ± 1.94Bc	46.67 ± 4.47Ab	62.08 ± 2.44Aa
S. carpocapsae	0Ad	1.67 ± 1.27Ad	9.17 ± 1.36Bc	20.00 ± 2.04Bb	30.83 ± 3.06Ba

Table 9. Mean mortality percentages of *Helicoverpa armigera* larva infected with EPNs at different concentration (mean number of died larva ± SE) after 120 hours exposure time (soil assay).

Nematode species	Concentration				
	1	10	50	100	500
H. bacteriophora	0Ad	5.83 ± 2.08Ad	32.50 ± 2.76Ac	60.83 ± 4.57Ab	89.58 ± 3.30Aa
S. feltiae	0Ad	1.67 ± 1.11Ad	23.33 ± 2.58Bc	61.25 ± 4.35Ab	81.25 ± 3.64Aa
S. carpocapsae	0Ad	5.00 ± 1.94Ad	20.42 ± 2.10Bc	30.83 ± 1.42Bb	41.25 ± 2.81Ba

For all tables, the means followed by the same lowercase letter within the same row or the means followed by the same capital letter within the same column are not significantly different (P>0.05; Tukey).
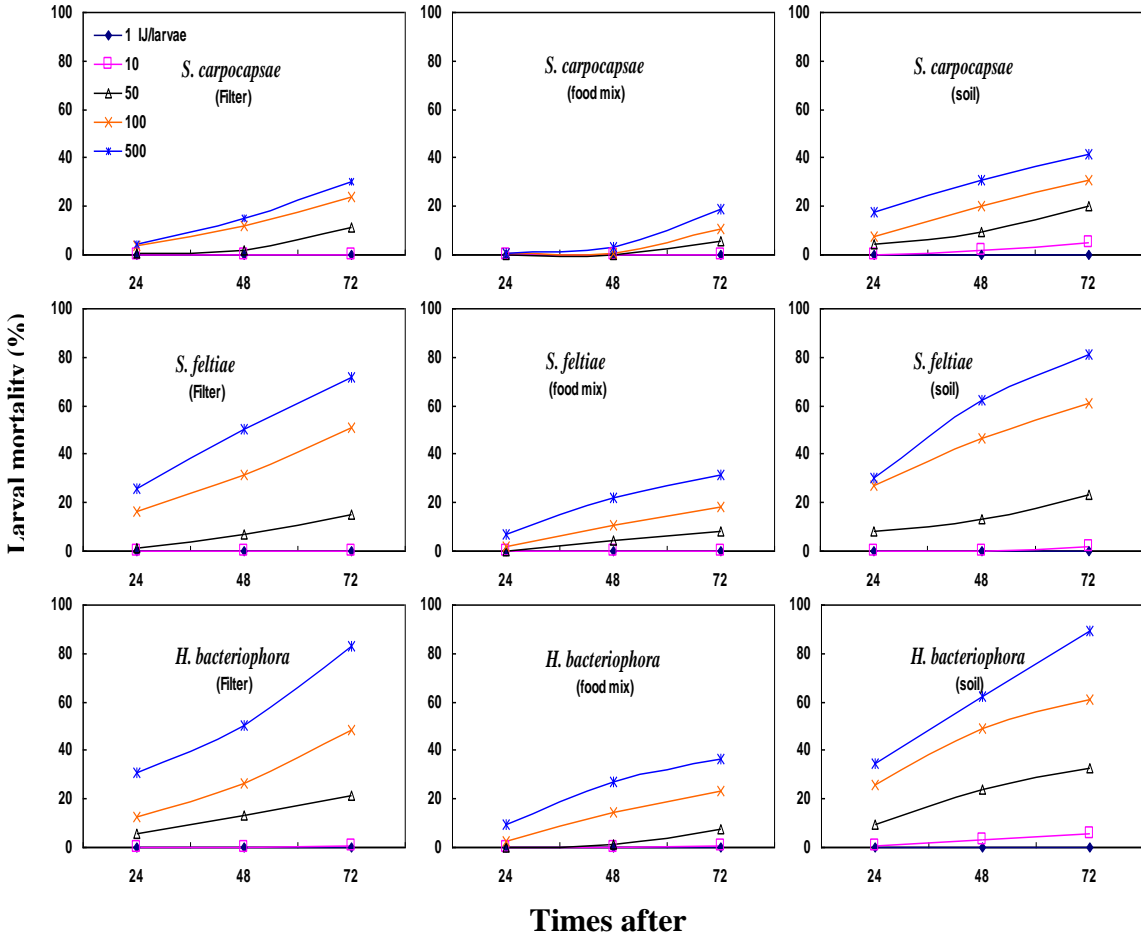
Figure 1. The effects of three species of entomopathogenic nematodes on *Helicoverpa armigera* larva at five concentrations in different treatment way.