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Orijinal araştırma (Original article)

The effect of different pesticides on reproduction of entomopathogenic nematodes

Farklı pestisitlerin entomopatojenik nematodların üremesi üzerine etkisi

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Summary

Entomopathogenic nematodes (EPNs) in the families Steinernematidae and Heterorhabditidae are generally considered beneficial nematodes. These beneficial nematodes can serve in integrated pest management (IPM) in agro-ecosystems. The effect of chemical insecticides (11 different pesticides) on *Steinernema* sp. (EBN-1e), and *Heterorhabditis bacteriophora* (EBN-10k) was determined under laboratory conditions. Generally, EBN-1e *Steinernema* strain was more tolerant to different tested insecticides than *Heterorhabditis* strain. The survival of IJs was more than 90% after treatement with Captan, Methomyl, Mancozeb, Benomyl, Trimiltox forte and Diafenthiuron, for EBN-1e nematode strain, while Chlorfluazuron decreased its survival to less than 5%. In contrast, the survival of *Heterorahbditis* strain was less than the *Steinernema* strain. There were significant differences in reproduction rates between EBN-1e and EBN-10k exposed to different chemical insecticides. The EBN-1e strain had higher reproduction rate than EBN-10k in all treatments. In general, there was significant difference in reproductive rates between the species concentrations (500 IJs and 1000 IJs) exposed to different chemicals or between exposure times (48 h. and 96 h.).

Key words: Entomopathogenic nematode survival, chemical pesticides, *Steinernema* and *Heterorhabdities*.

Özet

Steinernematidae ve Heterorhabditidae familyasına ait entomopatojenik nematodlar (EPN) genellikle yararlı nematodlar olarak kabul edilir. Bu faydalı nematodlar agro-ekosistemlerde entegre zararlı yönetimi (IPM) içinde hizmet edebilirler. Kimyasal insektisitlerin (11 farklı pestisit) *Steinernema* sp. (EBN-1e), ve *Heterorhabditis bacteriophora* (EBN-10k) üzerindeki etkileri laboratuvar koşullarında belirlenmiştir. Genellikle, EBN-1e *Steinernema* ırkı *Heterorhabditis* ırkına göre farklı insektsitlerde yapılan testlerde daha dayanıklı olmuştur. EBN-1e nematod ırkı infektif larvaları Captan, Methomyl, Mancozeb, Benomyl, Trimiltox forte ve Diafenthiuron ile uygulama yapıldığında canlılığı %90'dan fazla olmuş, Chlorfluazuron uygulamasında ise %5'in altına düşmüştür. Bunun aksine, *Heterorahbditis* ırkının canlı kalma oranı *Steinernema* ırkına göre daha az olmuştur. EBN-1e ve EBN.-10k üreme oranları arasında farklı kimyasal insektisit uygulamalarında önemli farklılıklar vardır. EBN-1e ırkının tüm uygulamalarında EBN.-10k ırkından daha yüksek üreme olmuştur. Genel olarak, türler arasındaki konsantrasyonlarda (500 IJs ve 1000 IJs), farklı kimyasallara maruz kalması veya maruz bırakma süreleri (48 ve 96 saat) ile üreme oranları açısından anlamlı bir fark yoktur.

Anahtar sözcükler: Entomopathogenic nematod, canlı kalma, kimyasal pestisitler, *Steinernema* ve *Heterorhabdities*

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Introduction

The entomopathogenic nematodes (EPNs) (Steinernematidae and Heterorhabditidae) are parasites of insects and kill their hosts with the aid of bacteria carried in the nematode's alimentary canal; steinernematids carry *Xenorhabdus* spp., whereas heterorhabditids carry *Photorhabdus* spp. (Poinar, 1990; Adams & Nguyen, 2002). These nematodes can be used as biological control agents to suppress a variety of economically important insect pests (Shapiro-Ilan, 2004; Grewal et al., 2005).

The general life cycle of heterorhabditid and steinernematid nematodes involves a free-living infective third-stage juvenile (IJ or dauer stage) that carries species-specific bacterial symbionts, *Xenorhabdus* or *Photorhabdus*, along its gut (Akhurst, 1986). The IJs of both nematodes commonly seek out and enter a suitable insect host through natural openings such as the spiracles, mouth and anus, or in the case of heterorhabditids, additional means of penetration of the cuticle by use of a tooth. Once the IJs penetrate into the host's hemocoel, the nematode releases the bacteria that propagate and cause a rapid and fatal septicemia. The bacteria digest the contents of the cadaver and the nematode feeds on the bacterial culture. The nematodes pass through 2 or 3 dioecious generations before they produce new infective juveniles (IJs) that emerge from the depleted host cadaver into the soil within two to three weeks, depending on involved conditions (Stuart & Gaugler, 1994; Hominick et al., 1995; Downes & Griffen, 1996). The natural habitat in the soil, chemical stress in agro-ecosystems or chemical persistence can be affect nematode survival and reproduction (Atwa, 1999; Shamseldean et al., 2005).

EPNs in the families Steinernematidae and Heterorhabditidae can survive exposure to many chemical pesticides (Hara & Kaya, 1983; Rovesti et al., 1988; Rovesti & Deseő, 1991; Atwa, 1999). However, IJs are highly susceptible to several nematicides likely to be found in the agro-ecosystem (Rovesti and Deseő, 1991). Zang et al., (1994) and Gordon et al. (1996) reported no toxic effects of several carbamates and minimal effects of a variety of organophosphates on nematode survival, infectivity and reproduction. In addition, the length of exposure to the insecticides had little discernible effect on nematode survival and reproduction which depended on insecticidal concentration (Atwa, 1999).

The objective of this study was to determine the effects of chemical insecticides on reproduction rates of the EPNs, *Steinernema* sp. (EBN-1E), and *Heterorhabditis bacteriophora* (EBN-10K).

Materials and Methods

Differences in reproduction rate of two entomopathogenic nematodes (*Steinernema* sp. "EBN-1e", and *Heterorhabditis bacteriophora* "EBN-10k") due to effects of chemical insecticides (11 different pesticides) was determined under laboratory conditions. Two concentrations (500 IJs and 1000 IJs) of the two tested nematodes were introduced to the recommended dose of the tested chemical insecticides suspension (Table 1) for two exposure times (48 h. and 96 h.).

Entomopathogenic Nematodes used in the experiment

Species and/or strains of *Steinernema* and *Heterorhabditis* used in this study were *Steinernema* sp. (EBN-1e), and *Heterorhabditis bacteriophora* (EBN-10k) that had been isolated and identified by Atwa (2003) from El-Sharawy village, El-Nubaria, Behera Governorate. The nematodes were cultured on last instar larvae of the greater wax moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae), according to the method of Dutky et al. (1964). The IJs were harvested using White traps as described by White (1927) at 25±2^{o}C. A stock suspension of the IJs in sterilized water was stored at 10^{o}C for 2 weeks until used.

Table 1. The name, structure, application use and field recommended dose of tested chemical pesticides

Chemical name	Commercial name	Application Use	Recommended dose	Manufacturer
1-{2-(2,4 dichloropheny) pentyl}-1H-11,2,4 triazol.	Penconazole	Insecticides	1000 PPM	Ciba Geigy
-3- (2,6-disopropyl-4-phenoxy phenyl) –1 tert. Butyl-thiourea.	Diafenthiuron	Insecticides & acaricides	2500 PPM	Ciba Geigy
1-{6 chloro –3- pyridyl (methyl)-N-nitroimidazolidin-2y lideneamine.	Imidacloprid	Insecticides & has some effect as nematicides	1000 PPM	Bayer
1-{3,5-dichloro-4(3-chloro-5)}-trifluoromethyl-2-pyridyloxy-phenyl-3 (2,6 difluoro benzoyl) urea.	Chlorfluazuron	Insecticides	3000 PPM	Zeneca
Mancozeb + Copper carbonate basic + feric ferrous cyanide.	Trimiltox forte	Fungicides	3000 PPM	Sandoz
Methyl 1-(butylcarbamyl)-2-benzimidazole carbamate	Benomyl	Fungicides	1000 PPM	Dupont
Thiocyclam – hydrogene oxalate n,n dimethyl-1,2,3-trithian-5-ylamine hydrogene oxalate.	Thiocyclam	Insecticides	1000 PPM	Novartis
3,5 dimethyl (4-methyl-mercaptophrnyl-N methyl carbamat mercaptodimethur, methiocarb)	Methiocarb	Insecticides	3000 PPM	Bayer
Ethylene bisdithio carbamate	Mancozeb	Fungicides	5000 PPM	Makhtcheem
1,2,3,6 tetrahydro – N – (trichloromethyl thio) phthalimide.	Captan	Fungicides	3000 PPM	Makhtcheem
Methomyl - S – methyl - N – {(methylcarbamoyl) oxy} thioacetimidate.	Methomyl	Insecticides	1000 PPM	Dupont

Chemical pesticides source and Bioassay methods

The chemical insecticides and fungicides were used in this experiment are mentioned in Table1. The experiments were conducted in plastic cups (7 cm high with a diameter of 6 cm) and 20 cups were used for each pesticide. Each cup contained 10 ml of the tested pesticides at the recommended dose for field application. The IJs were introduced to the cups at 2 concentrations (500 and 1000 IJs/cup); with 10 replicates of each. The control experiment used IJs with distilled water. The exposure times of IJs to the pesticides were 48 h. (5 replicates) and 96 h. (5 replicates) for each concentration of IJs. Percentage survival of IJs in each pesticide, each concentration of IJs and each exposure time were estimated in 1 ml of the tested pesticides in each cup. The contents of the 5 cups were washed three times using a sieve of 500-mesh to obtain the IJs from the mixture of nematodes–pesticide; the IJs were retained on the sieve and then collected to calculate survival. Nematode survival was examined by using stereomicroscopy after 48 h. and 96 h. (5 cups each time). Survival was determined by movement of the IJs. The final suspension obtained for each replicate was different; for this reason, in all replicates, one hundred IJs were counted randomly per count, and three counts were made for each replicate (live and dead IJs were recorded). The numbers of live IJs in a 0.1 ml aliquot was counted to obtain the percentage survival, using plastic dishes for serological tests (12.5 × 8 cm, 96 wells, each one with 0.4 cm diameter), under the stereoscopic microscope. The washed IJs collected from the sieve were used to infect larvae of *G. mellonella*. For each nematode species and/or strains, 5 Petri dishes (150mm × 30 mm) lined by 5 filter papers were used; 10 larvae of *G. mellonella* were exposed in every Petri dish. Three days after exposure to IJs, the healthy and infected larvae were separated with a White trap (White, 1927) at 25±2^{o}C for approximately 12-15 days. The concentration of IJs was determined by using a 1 ml sample (5 times) from the final solution and counting IJs using plastic dishes for serological tests (12.5 × 8 cm, 96 wells, each one with 0.4 cm diameter), under the stereoscopic microscope. The total number of IJs that emerged from each larva was used to determine the reproduction rate (Atwa, 1999). By using data of the total number of IJs that emerged (total production), the effect of different pesticides on the EPNs reproduction rates was determined by the equation.

$$\text{Reproduction rate} = \frac{\text{Nematode production / larva}}{\text{Application rate}}$$

Data analysis

Percentage of IJ survival for each nematode species/strain for concentration, exposure time and number of infective juvenile nematodes produced were transformed (arcsine square root) and subjected to analysis of variance (ANOVA) (SigmaStat, 1995). Significant means were separated with the Tukey Test (P≤, 0.05).

Results and Discussion

Figure (1) shows the average of IJ survival of both nematodes after treatment with the tested pesticides (including the two concentrations of IJs; 500 and 1000, and the two exposure times, 48 h. and 96 h.). The EBN-1e steinernematid strain in general was more tolerant to different tested insecticides than the heterorhabditid strain. In addition, there were significant differences between the tested insecticides and fungicides. These significant differences can be divided into four categories according to percentage of IJ survival (highly sensitive, sensitive, moderately sensitive and tolerant). The EBN-1e steinernematid strain was highly sensitive (mean survival of IJs was less than 50%) to Thiocyclam and Chlorfluazuron (Figure 1) and sensitive to Methiocarb and Penconazole (means of survived IJs were from 50 to 70%). The moderately sensitive group (means of survived IJs were from 70 to 90%) included Captan, Methomyl, Mancozeb, Benomyl, Trimiltox forte and Diafenthiuron, whereas the tolerant group included (mean survival of IJs was more than 90%) the control treatment and Imidacloprid (Figure 1). The EBN-10k heterorhabditid strain was highly sensitive to Penconazole, Diafenthiuron, Chlorfluazuron, Trimiltox forte, Benomyl, Thiocyclam, Methiocarb, Mancozeb, Captan and Methomyl, while it was sensitive to Diafenthiuron and Imidacloprid.

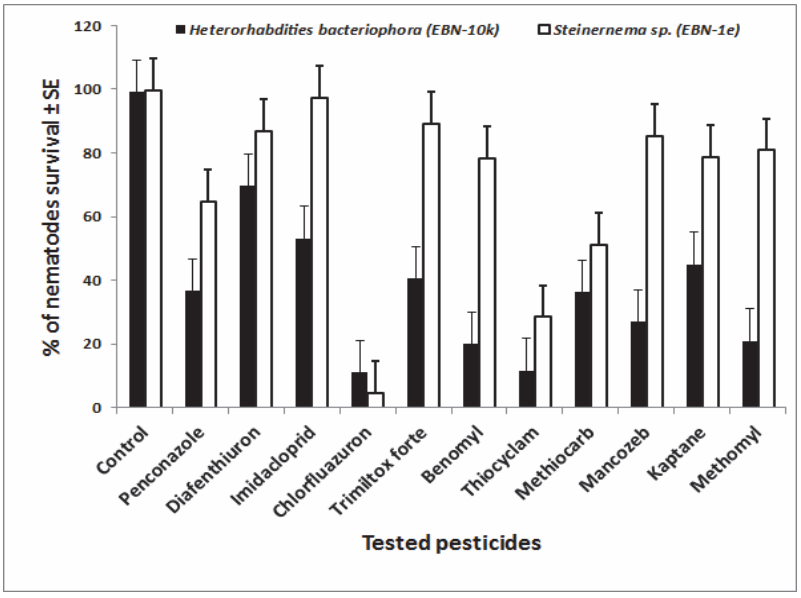

Figure 1. Effect of different insecticides on survival of *Steinernema* sp. "EBN-1e" compared to *Heterorhabditis bacteriophora* "EBN-10k" (Mean ± SE).

The differences in survival percentage of EPNs may be attributable to the differences in nematode's acetylcholinesterase concentration, as reported by (Atwa, 1999). Also, the higher survival of *Steinernema* sp. (EBN-1e) than *Heterorhabdities bacteriophora* (EBN-10k) may be attributed to the difference in acetylcholinesterase levels in both genera, as claimed by Shamseldean et al. (2005). On the other hand, the different effects between insecticides and fungicides on survival of IJs could be related to the different effects on nematodes' chemical receptors and the respiratory metabolites, as claimed by Atwa (1999). This conclusion may suggest that these insecticides and fungicides had some negative or lethal effects on nematodes, but less so on *Steinernema* than *Heterrhabditis*. Regarding the influence of

exposure time on nematode infectivity, Fedorko et al. (1977a,b) showed in laboratory tests that IJs of *S. carpcapsae* were unaffected by short-term exposure to a wide range of insecticides that were toxic to other soil–dwelling nematode species. However, when exposure time to the insecticides was increased beyond 24 h., nematode mortality increased. Zimmerman and Cranshaw (1990) reported that no mortality of *Neoaplectana carpocapsae* and *N. bibionis* was observed after 48 h. of exposure to the organophosphate insecticide diazinon, while *Heterorhabditis* sp. was significantly affected at the end of 48 h. exposure to 400 ppm diazinon. Saleh and Sammour (1995) found that selecron had highly adverse effects on nematode survival at higher concentrations and longer exposure periods, while muthrin and nudrin were harmless to the nematodes, especially with a short exposure period.

Figure 2 shows the significant difference between the nematode strains and concentrations for reproduction rates after exposure to different insecticides. Rate of reproduction of the EBN-1e steinernematid strain was higher than the rate of reproduction of the EBN-10k heterorhabditid strain (Figure 2). The reproduction rate of EBN-1e steinernematid strain was highly significant more than the EBN-10k heterorhabditid strain for both nematode concentrations (500 and 1000 IJs) (fgure 2). Otherwise the over all effect of tested insecticides on reproductive rate of infected larvae with 1000 IJs (in both tested EPN species/strains) had significantly differences more than the reproduction rates of infected larvae with 500 IJs exposure to different insecticides in both tested EPN species/strains (Figure 2).

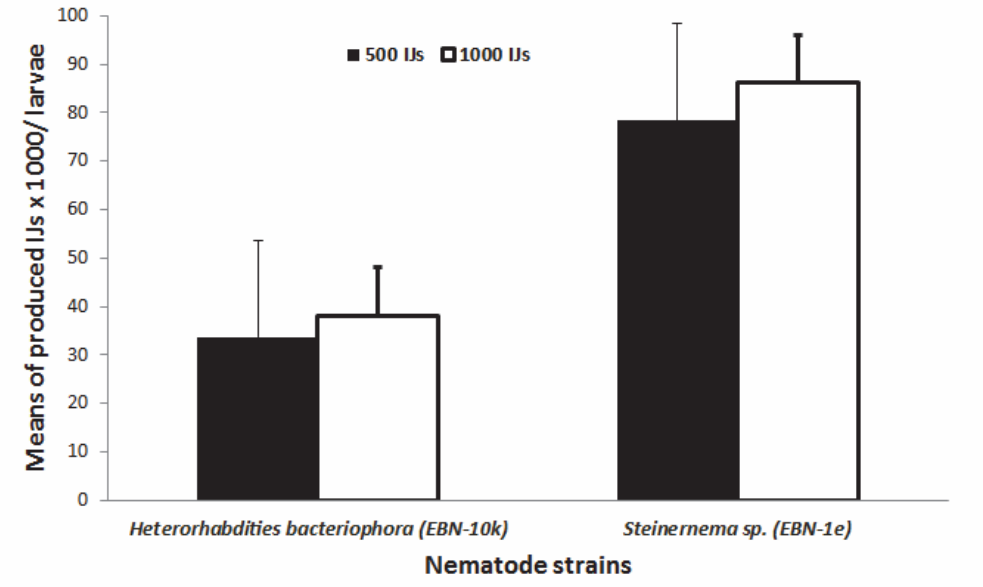

Figure 2. Overall effect of tested chemical insecticides on reproduction rate/larvae (mean± SE) of *Steinernema* sp. (EBN-1e) and *Heterorhabditis bacteriophora* (EBN-10k) at different inoculation rates.

The yield of IJs for differences in exposure time and EPN strains and concentration with the tested pesticides isillustrated in Figure 3. The data in Figure 3 demonstrate significant variation in the yield of IJs for different exposure times (48 h. and 96 h.). The yield of IJs decreased with increasing exposure time to pesticides before infection of the host. The yield of IJs of the EBN-10k heterorhabditid strain was significantly lower than that of the EBN-1e steinernematid strain at 48 h. and 96 h., in both nematode species/strains.

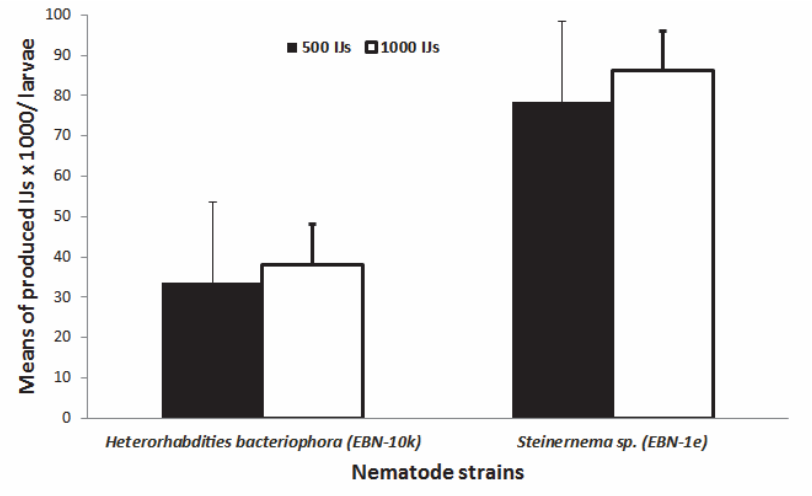

Figure 3. Overall effects of the insecticides on reproduction rate/larvae (mean ± SE) of *Steinernema* sp. (EBN-1e) and *Heterorhabditis bacteriophora* (EBN-10k) for two exposure periods.

Reproduction of *H. bacteriophora* in infected *G. mellonella* was not affected by different pesticides before host infection, but there weredifferences in reproduction rates. The different insecticides had different effects on IJ production for the different nematode strains, concentrations and exposure times of IJs to these chemicals before host infection. No differences in mortality of *G. mellonella* were recorded so the variations in reproduction rate are illustrated. Data in Table 2 shows that Thiocyclam had a negative effect on nematode reproduction in *G. mellonella*; there was no reproduction of IJs in infected *G. mellonella* afterexposure of IJs of the EBN-10k strain to Thiocyclam. On the other hand, a low reproduction rate was observed in IJs treated with Benomyl, Captan and Methomyl, with 75.7, 80.7 and 80% reductions, respectively (Table 2). The reproduction rate was 85.3 x 1,000 IJs/larvae in the control experiment, while the rate was very low (from 16.4 to 20.7 x 1000 IJs/larvae) for Benomyl, Captan and Methomyl (Table 2). Amoderate rate of reproduction is illustrated in Table 2 for the EBN-10k *Heterorhabditis* strain after Penconazole, Diafenthiuron, Imidacloprid, Chlorfluazuron, Trimiltox forte, Methiocarb and Mancozeb exposure, with the range of reproduction rates being 34.0 to 51.5 x 1000 IJs/larvae. The data in Table 2 show that there is a highly significant difference in reproduction rates between the control experiment (85.3 x 1000 IJs/larvae) and the different treatments (range from 0.0 to 51.5 x 1000 IJs/larvae) for interaction of all factors of theexperiment (exposure time and nematode concentrations).

Table 2. Effects of different concentrations of *Steinernema* sp. (EBN-1e), *Heterorhabditis bacteriophora* (EBN-10k), exposure times and different pesticides on reproduction rates of infective juveniles (IJs)

Pesticides	Reproduction of nematodes strains and concentration interaction with exposure time						Means of IJs
	Strains		Concentration		Time		
	EBN-10k	EBN-1e	500 IJs	1000 IJs	48 h.	96 h.	
Control	85.3^{e}	130.9^{a}	102.3^{a}	113.8^{a}	106.8^{a}	109.3^{a}	108.1^{a}
Penconazole	42.2^{g}	98.6^{bc}	69.54^{bc}	71.2^{bc}	66.9^{cde}	73.8b^{c}	70.4^{b}
Diafenthiuron	51.5^{f}	92.1^{cde}	63.8^{cde}	79.8^{b}	65.3^{de}	78.3^{b}	71.8^{b}
Imidacloprid	39.8^{gh}	104.4^{b}	69.7^{cde}	74.5^{bc}	65.9^{de}	78.3^{b}	72.1^{b}
Chlorfluazuron	40.8^{gh}	93.1^{cd}	63.2^{cde}	70.7^{cd}	70.4^{cd}	63.5^{de}	67.0^{bc}
Trimiltox forte	38.8^{gh}	95.2^{cd}	67.3^{cde}	66.7^{cde}	64.8^{de}	69.2^{cd}	67.0^{bc}
Benomyl	20.7^{i}	44.8^{fg}	35.0^{h}	30.4^{h}	65.4^{de}	0.0^{j}	32.7^{e}
Thiocyclam	0.0^{j}	15.0^{i}	0.0^{j}	15.0^{i}	15.0^{h}	0.0^{j}	7.5^{f}
Methiocarb	34.0^{h}	93.1^{cd}	63.5^{cde}	63.7^{cde}	65.6^{de}	61.5^{ef}	63.55^{c}
Mancozeb	44.0^{g}	92.4^{cde}	68.6^{cde}	67.8^{cde}	69.6^{cd}	66.8^{cd}e	68.2^{bc}
Captan	16.4^{i}	89.0^{de}	43.9^{g}	61.5^{cde}	50.6^{ig}	54.8^{ef}	52.7^{d}
Methomyl	17.1^{i}	38.0^{gh}	25.2^{h}	29.8^{i}	55.1^{fg}	0.0^{j}	27.5^{e}
Means	31.39	77.79	51.8	57.37	59.51	49.66	--
LSD				7.417			

* Values followed by different letters within rows or columns are significantly different using LSD test (p <0.05).

The data in Table (3) show the effects of different variables (exposure time and nematode concentration) on *Steinernema* sp. "EBN-1e" reproduction rate required for different insecticides. There was a highly significant difference between the rate of reproduction in the control and insecticide treatments (Table 3). A moderate rate of reproduction was observed for Penconazole, Diafenthiuron, Imidacloprid, Chlorfluazuron, Trimiltox forte, Methiocarb, Captan and Mancozeb, with a decrease in reproduction between 20.3 % to 32.0 %, as illustrated in Table 3. On the other hand, the data in Table 3 demonstrate high sensitivity to Benomyl, Thiocyclam and Methomyl, with a decrease in reproduction rate between 65.8% and 85.5%.

Table 3. Effects of different concentrations of *Steinernema* sp. (EBN-1e) and exposure times with different pesticides on reproduction infective juveniles (IJs) rates

Pesticides	Reproduction rate for time and nematode concentration interaction				Means of IJs/larva	% of decreased in reproduction rate**
	48h x 500 IJs	96h x 500 IJs	48h x 1000 IJs	96h x 1000 IJs		
Control	110.7	122.7	145.3	144.7	130.9[a]	--
Penconazole,	77.3	115.7	101.9	99.5	98.6[bc]	24.7 %
Diafenthiuron	49.0	111.3	103.0	105.2	92.1[cde]	29.6 %
Imidacloprid	85.3	116.0	100.0	116.3	104.4[b]	20.3 %
Chlorfluazuron	85.7	98.0	107.3	81.3	93.1[cd]	28.9 %
Trimiltox forte	94.7	94.7	82.2	109.0	95.2[cd]	27.3 %
Benomyl,	95.7	0.0	83.4	0.0	44.8[fg]	65.8 %
Thiocyclam	0.0	0.0	60.0	0.0	15.0[i]	88.5 %
Methiocarb	88.0	100.7	103.5	80.0	93.1[cd]	28.9 %
Mancozeb	94.0	93.6	95.7	86.2	92.4[cde]	29.4 %
Captan	90.3	85.2	78.0	102.3	89.0[de]	32.0 %
Methomyl	69.7	0.0	82.2	0.0	38.0[gh]	71.0 %
Means	75.43	74.11	90.66	70.89	--	--
LSD			5.246			

* Values followed by different letters within columns are significantly different, using the LSD test ($p < 0.05$).
** % of decreased in reproduction rate = 1- (Means of IJs/larva in treatment/ Means of IJs/larva in control) x 100.

There were significant differences in numbers of nematodes produced between *Steinernema* sp. "EBN-1e" and *H. bacteriophora* "EBN-10k" exposed to different insecticides (Table 4). *Steinernema* sp. "EBN-1e" produced significantly more IJs than *H. bacteriophora* "EBN-10k" in all treatments and the control treatment (Table 4). In general data in table 4 illustrated that, there was significant difference in the number of nematodes produced at different concentrations (500 IJs and 1000 IJs) exposed to different chemical insecticides, except that Thiocyclam had a highly negative effect on the *H. bacteriophora* (EBN-10k) nematode concentration (500 IJs and 1000 IJS). On the other hand, different exposure time (48 h. and 96 h.) showed no significant differences in numbers of nematodes produced fordifferent chemical insecticides, except Benomy and Thiocyclam, which had a highly negative effect when IJs were exposed for 96 h. (Table 4).

It is known that the number of nematode generations inside the host can change according to the nematode species/strain, number of IJs penetrating the host, and the environmental conditions e.g. exposure to chemicals stress (Griffin et al., 2005). The multiplication of nematodes in *G. mellonella* larvae was examined by comparing the number of nematodes produced in *Steinernema* sp. "EBN-1e", and *Heterorhabditis bacteriophora* "EBN-10k" exposed to different pesticides. Some reports demonstrated that certain insecticides, particularly organophosphates and carbamates, possess nematicidal properties (Atwa, 1999). These insecticides induced adverse effects ranging from impaired movement, infectivity and reproduction to death of *Neoaplectana carpcapsae* IJs (Kamionek, 1979; Hara & Kaya, 1983). In all cases, the highest number of nematodes harvested was for *Steinernema* sp. "EBN-1e", compared to *H.*

bacteriophora "EBN-10k". These results suggest that in *G. mellonella* larvae, *S. carpocapsae* was more multistage than *H. bacteriophora* "EBN-10k".

Table 4. Effects of different concentrations of *Heterorhabditis bacteriophora* (EBN-10k), exposure time and different pesticides on infective juveniles (IJs) reproduction rates.

Pesticides	Reproduction rate required to time and nematodes concentration interaction				Means of IJs/larva	% of decreased in reproduction rate**
	48h x 500 IJs	96h x 500 IJs	48h x 1000 IJs	96h x 1000 IJs		
Control	88.7	87.0	82.3	83.0	85.3^{e}	--
Penconazole	44.8	40.3	43.7	39.8	42.2^{g}	50.5 %
Diafenthiuron	50.9	44.0	58.3	52.7	51.5^{f}	39.6 %
Imidacloprid	35.0	42.5	43.3	38.3	39.8^{gh}	53.3 %
Chlorfluazuron	35.6	33.4	53.1	41.2	40.8^{gh}	52.8 %
Trimiltox forte	42.3	37.5	40.0	35.5	38.8^{gh}	54.5 %
Benomyl	44.3	0.0	38.3	0.0	20.7^{i}	75.7 %
Thiocyclam	0.0	0.0	0.0	0.0	0.0^{j}	100 %
Methiocarb	35.0	30.3	35.8	35.0	34.0^{h}	60.1 %
Mancozeb	47.2	39.6	41.5	47.8	44.0^{g}	48.4 %
Captan	0.0	0.0	34.0	31.7	16.4^{i}	80.8 %
Methomyl	31.2	0.0	37.3	0.0	17.1^{i}	80.0 %
Means	33.3	24.33	38.66	29.27	--	--
LSD			5.246			

* Values followed by different letters within columns are significantly different, using the LSD test ($p < 0.05$).
** % of decreased in reproduction rate = 1- (means of IJs/larva in treatment/ means of IJs/larva in control) x 100.

The species, and possibly strain of nematode, appears to be crucial in determining its level of susceptibility to systemic insecticides, according to the data in this study. Hara and Kaya (1983) found that several carbamates and organophosphates adversely affected the *in vitro* development and reproduction of *S. carpocapsae* (all strains), whereas this strain (*S. carpocapsae* "all strains") was unaffected by the chlorinated hydrocarbon methoxychlor or the synthetic pyrethroid fenvalerate. Hara and Kaya (1983) also mentioned that carbamates and organophosphates kill a proportion of the IJs of *S. carpocapsae* (all strains) and cause partial paralysis and reduced infectivity of the remainder. Das and Divakar (1987) demonstrated that 15 insecticides had low toxicity to *S. carpocapsae (*DD-136) strain and concluded that most insecticides can be used with this strain at practical concentrations. Zimmerman and Cranshaw (1990) reported that carbaryl was significantly more toxic to *H. bacteriophora* (HP88 strain) than the *Neoaplectana* spp. after 24 h. and 48 h. of exposure to 1000 ppm, while *N. carpocapsae* and *N. bibionis* were not significantly affected by any of the concentrations tested.

Koppenhöfer and Fuzy (2008) demonstrated that EPNs are compatible in tank mixes with many pesticides, including numerous chemical and biological insecticides. Our study shows that some organophosphate chemicals can be added to the list of compatible insecticides as 48 h. and 96 h. exposure to the recommended dose of field application of these chemicals had little effect. This compatibility level is similar to that of imidacloprid with *H. bacteriophora* and several other nematode species. Because compatibility levels to the same insecticide may differ among nematode species (Koppenhöfer & Grewal, 2005), compatibility of chlorantraniliprole with other nematodes species needs to be determined. That is in agreement with Radová (2011) who reported that it is difficult to explain the differential reaction of EPNs with different pesticides, but these findings show that different nematodes species/strain can react to the same chemicals differently. The observed results for the interaction effects of insecticides on nematodes not only makes application of nematodes in agro-ecosystems easier, but also facilitates their use in integrated pest management systems.

The results of this study increase our knowledge of EPN, fungicide and insecticide interactions. bydemonstrating that most of thechemical pesticides used in this study are not toxic to both the tested nematode species/strains. This study suggests thatnematodes can be successfully included in integrated pest management involving pesticides in agro-ecosystems. Knowledge of the potential reproduction loses attributable to the used pesticides will be help predict the required application rate of nematodes in the field.

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