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Susceptibility of *Spodoptera littoralis* (Boisd.) to treated entomopathogenic rhabdities, *Heterorhabditis bacteriophora* and *Steinernema* sp. by different pesticides

Atwa A. Atwa

ABSTRACT

Spodoptera littoralis (Boisd.) is a major plant pest that causes substantial economic losses worldwide especially in vegetable crops. Entomopathogenic nematodes (EPNs) in the families Steinernematidae and Heterorhabditidae are generally considered beneficial organisms which can serve within integrated pest management (IPM) in agroecosystem. The effect of EPNs *Steinernema* sp. (EBN1e), and *Heterorhabdities bacteriophora* (EBN10k) that exposed to 11 different chemical pesticides on the cotton leafworm larvae was determined under laboratory condition. Generally, *Steinernema* sp. (EBN1e strain) exposed to different tested chemical pesticides was more affected on fifth instar larvae of *S. littoralis* than *H. bacteriophora* (EBN10k strain). More than 90% of fifth instar larvae of *S. littoralis* was killed after exposure to EBN1e strain treated with all used chemical pesticides except EPN1e strain treated with chlorfluazuron, thiocyclam and benomyl which caused 75.45, 80.65 and 81.5 % mortality of cotton leafworm fifth larvae respectively. In otherness, the mortality of fifth instar larvae of *S. littoralis* required to *Heterorahbdities* strain was less than *Steinernema* strain treated with different tested chemicals insecticides. There were significant differences in mortality of *S. littoralis* fifth instar larvae between EBN1e and EBN10k nematodes strains exposed to different chemical insecticides. The EBN1e strain was highly virulent than EBN10k in all treatments. In general, there was significant difference in mortality rates of cotton leafworm larvae required to EPNs concentrations (500IJs and 1000IJs) exposed to different chemicals and between exposure times.

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INTRODUCTION

The cotton leafworm, *Spodoptera littoralis* Boisduval (Lepidoptera: Noctuidae), is similarly one of the most destructive agricultural lepidopterous pests within its subtropical and tropical range. It can attack numerous economically important crops (27 plant species belonging to 16 families) all the year round (Salama, *et al.,* 1971). Field crops and vegetables are infested by *S. littoralis,* e.g. on cotton, may cause considerable damage by feeding on the leaves, fruiting points, flower buds and, occasionally, also on bolls (Salama *et al.,* 1971). In vegetables e.g. on tomatoes, larvae bore into the fruit which is thus rendered unsuitable for consumption. Numerous other crops are attacked, mainly on their leaves (Salama *et al.,* 1971). Most control strategies involve chemical insecticides, but this approach is becoming less attractive (Wang *et al.,* 1995) due to resistance, cost, and the lack of availability of pesticides (Mosallanejad and Smagghe 2009). Therefore, numerous studies have been carried out on possible biological control has the potential to be a useful strategy (Atwa *et al.,* 2013). Among the alternative measures to chemical control of insect pests, in recent years attention has focused on biological control using entomopathogenic nematodes of the families Steinernematidae and Heterorhabditidae (Kaya, 1985; Poinar 1986; Georgis *et al.,* 2006).

Entomopathogenic nematodes (EPNs) in natural environment are exposed to a variety of biotic and abiotic stressors that may drastically reduce their longevity and infectivity. These stressors may be

anthropogenic (e.g. pollutants) as well as natural (e.g. natural enemies and pathogens). The natural habitat for EPNs, the soil is a difficult environment for persistence of many organisms considering its complexity of physical, chemical and biological components. EPNs in the families Steinernematidae and Heterorhabditidae can survive exposure to many chemical pesticides (Atwa *et al.,* 2013). However, infective juveniles are highly susceptible to several nematicides likely to be found in the agroecosystem (Rovesti *et al.,* 1988; Rovesti and Dese, 1991). Gordon *et al.* (1996) and Atwa *et al.* (2013) reported that, no toxic effect of several insecticides, fungicides of a variety of organophosphates and carbamates and mineral oils on nematodes survival, infectivity and reproduction.

Increased efforts in recent years have been focused on biological control using EPNs (*Steinernema* and *Heterorhabditis*) in developing integrated pest management (IPM) strategies with chemical pesticides, it is important to ascertain the degree to which these nematodes may be affects by the chemicals involved. These nematodes are capable of parasitizing many economically important pests including the Egyptian cotton leaf worm *Spodoptera littoralis* (Boisd.) (Atwa, 1999). The EPNs are mobile, highly virulent, capable of being cultured *in vitro*, and have a high reproductive potential. Despite their broad host range and high virulence, these nematodes have shown no mammalian pathogenicity (Gaugler and Boush, 1979), and are safe to vertebrates, plants, earthworms, honey bees, and other non-target organisms (Kaya and Gaugler, 1993). The EPNs strain used in these studies (*Steinernema* sp. (EBN1e), and *Heterorhabditis bacteriophora* (EBN10k)) was isolated in Egypt and identified by Atwa (2003). It has been suggested that combining low-impact insecticides or reduced rates of insecticides with EPNs could achieve adequate control while reducing the adverse effects of insecticides.

The main objective of this study to determine the effect of chemical insecticides and fungicides on virulence and efficacy of the EPNs, *Steinernema* sp. (EBN1E), and *Heterorhabditis bacteriophora* (EBN10K) on susceptibility infection of *S. littoralis* fifth instar larvae.

MATERIALS AND METHODS

Susceptibility of *S. littoralis* to treated EPNs by different pesticides was tested herein under laboratory condition. Two EPNs (EBN-1e and EBN-10k) with two concentrations (500 IJs and 1000 IJs) were mixed with recommended dose of tested chemical insecticides (7 chemical insecticides and 4 chemical fungicides) as cited in Table (1). The mixed suspension of nematodes pesticides complex were done for two exposure times (48 h and 96 h), then the nematodes was washed for three times in distillated water as mentioned below and susceptibility of fifth instar of *S. littoralis* was tested using the treated nematodes.

EPNs

Two Egyptian strains of *Steinernema* and *Heterorhabditis* used in this study are *Steinernema* sp. (EBN1e), and *H. bacteriophora* (EBN10k) isolated and identified by Atwa (2003). Method of Dutky *et al.* (1964) was used for cultured EPNs on last instar larvae of the greater wax moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae). White trap technique as described by White (1927) was used for harvesting nematodes progeny (infective juveniles "IJs") 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.

Rearing of the cotton leafworm

Five instar larvae of *S. littoralis* was obtained from a laboratory culture established at the Department of Entomology, Faculty of Agriculture, Alexandria University. The colony of *S. littoralis* originated from field cultivated with cotton in Alexandria, Egypt. However, feral individuals were added to the colonies twice a year to maintain genetic diversity. Larvae of *S. littoralis* were reared on an artificial diet (Hegazi *et al.*, 1977) at 27 ± 1° C, 60–65% RH, and a 14:10 L:D photoperiod.

Chemical pesticides and bioassay methods

Seven chemical insecticides and four fungicides were used in this experiment with two Egyptian species and/or nematodes strains are mentioned below in Table1. The mixtures of pesticides and nematodes in this experiments were conducted in plastic cups (7 cm high with a diameter of 6 cm) and 20 cups were used for each pesticide and nematodes mixture (Atwa *et al.,* 2013). Each cup contained 10

Table 1. Listed of examined pesticides chemicals and entomopathogenic nematodes

Pesticides group	Commercial name	Field recommended dose in PPM	Manufacturer
Chemicals insecticides			
Insecticides	Penconazole	1000 PPM	Ciba Geigy
	Diafenthiuron	2500 PPM	Ciba Geigy
	Imidacloprid	1000 PPM	Bayer
	Chlorfluazuron	3000 PPM	Zeneca
	Thiocyclam	1000 PPM	Novartis
	Methiocarb	3000 PPM	Bayer
	Methomyl	1000 PPM	Dupont
Fungicides	Trimiltox forte	3000 PPM	Sandoz
	Benomyl	1000 PPM	Dupont
	Mancozeb	5000 PPM	Makhtcheem
	Captan	3000 PPM	Makhtcheem
Entomopathogenic nematodes			
Nematodes species/strains		**Nematode concentrations**	**Original country**
Steinernema sp. (EBN1e)		500IJs/ml	Egypt
		1000IJs/ml	
Heterorhabdities bacteriophora (EBN10k)		500IJs/ml	
		1000IJs/ml	

ml of tested pesticides at recommended dose for field application with two concentrations (500 and 1000 IJs/cup) of each testes nematodes were introduced to the cups; 10 replicate each. Control experiment tested by using IJs with only distilled water. Two exposure (48 h. and 96 h.) times were tested for IJs to different pesticides. Five replicates for each concentration of IJs in each exposure time were used to determine the nematodes viability (Atwa *et al.,* 2013) and then used for bioassay experiment.

A sieve, 500-mesh was used to obtain the IJs suspensions maintained in pesticides nematodes mixture; the nematodes were retained on the top of sieve and washed for three times, then collected to calculate survival IJs and used in bioassay experiment. The final suspensions obtained for each replicate were different; for this reason, in all replicates, one hundred nematodes were counted randomly per count, and three counts were made for each replicate (a life and dead IJs was recorded) (Hegazi *et al.,* 2012). The numbers of live nematodes in a 0.1 mL aliquots were counted to obtain 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 (Atwa *et al.,* 2013). The data obtained were submitted to analysis of variance for comparisons between means for the pesticides effect, and for regression analysis to evaluate the exposure time and nematodes concentration interaction.

The washed IJs collected from the sieve were concentrated in 3 ml of distillated water and used for infected fifth instar larvae of *S. littoralis*. For each replicates of each nematodes species and/or strains concentration, 5 Petri dishes (150mm × 30 mm) lined by the washed IJs on 3 filter papers; 10 larvae were exposed to every Petri dishes. Four days after exposure to IJs, the good or healthy infected larvae depend on death and changing characteristic color and odor smile extract using White trap (White, 1927) at 25±2^{o}C for approximately 12-15 days. The numbers of dead larvae were recorded for data analysis and dead larvae were dissected to examine the present and absence of nematodes infection.

DATA ANALYSIS

The experimental design was completely randomized and balanced (equal numbers of subjects were assigned randomly to each treatment group). Data were subjected to analysis of variance (one-way ANOVA) for determination of differences between means (SigmaStat, 1995). Where significant differences occurred, a least significant differences test was applied for mean separation. The level for significance testing was set at $p < 0.05$ (Winer *et al.*, 1991). Duncan's multiple range test or Student's t-test were applied to significant differences for mean separation.

RESULTS AND DISCUSSION

Based on the results of this research, treated EPNs by insecticides and fungicides can offer better data about the environment stress of chemical insecticides on EPNs in nature, this will be offer better control management of an IPM program. Figure 1 shows the mean effect of different chemicals insecticides on survival of EPNs species/strains required to different IJs concentrations and different exposure times.

Heterorhabdities bacteriophora (EBN10k) in general was highly sensitive to Penconazole, Imidacloprid, Chlorfluazuron, Thiocyclam, Methiocarb, Methomyl, Trimiltox forte and Benomyl (Figure 1A). While as, it was sensitive to Diafenthiuron, Mancozeb and Captan. The data in Figure (1A and B) demonstrated that, there are significant variations in the survival of nematodes IJs required to difference in exposure time (48 and 96 h.). The survival of nematodes IJs are decreasing with the increasing of IJs exposure time to chemical pesticides before infect host (Figure 1A and B). Penconazole, Thiocyclam, Methiocarb, Methomyl and Mancozeb was highly sensitive more than the other tested insecticides by increasing exposure time 96 h. of EBN10k nematodes IJs to tested insecticides (Figure 1A and B).

Mean effect of different chemicals insecticides on survival of *Steinernema* sp. (EBN1e) required to different IJs concentrations and different exposure times was demonstrated in Figure (1-C and D). *Steinernema* sp. (EBN1e) in general was highly sensitive to Chlorfluazuron,

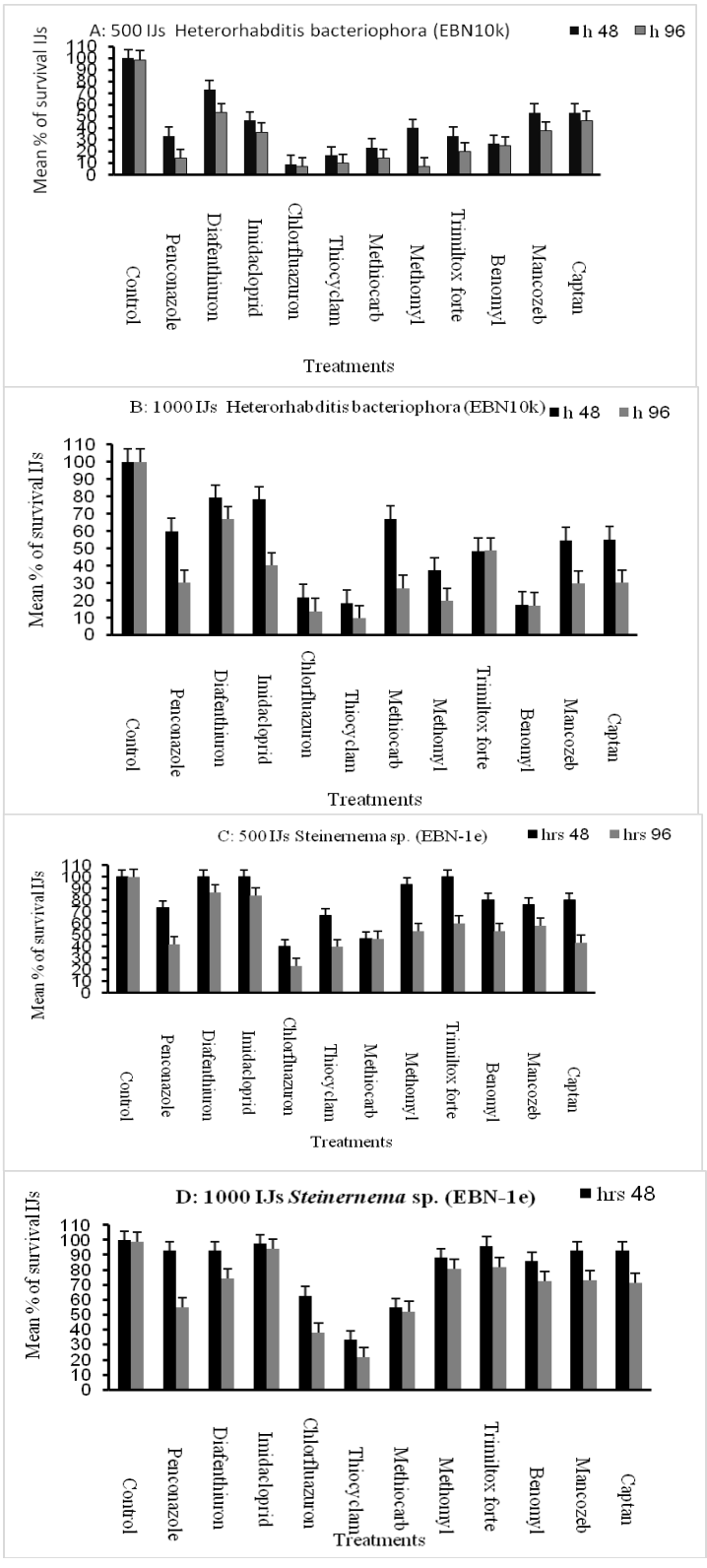

Figure 1. Mean effect of different chemicals insecticides on survival of EPNs species/strains required to different concentration and different exposure time; A: 500 IJs of *H. bacteriophora* (EBN10k), B: 1000 IJs of *H. bacteriophora* (EBN10k), C: 500 IJs of *Steinernema* sp. (EBN1e) and D: 1000 IJs of *Steinernema* sp. (EBN1e).

Thiocyclam and Methiocarb (Figure 1C and D). While as, it was sensitive and moderate sensitive to Penconazole, Diafenthiuron Imidacloprid, Methomyl, Trimiltox forte, Benomyl, Mancozeb and Captan (Figure 1C and D). Significant variations in the survival of nematodes IJs required to difference in exposure times are illustrated in Figure 1A and B. The survival of nematodes strain "EBN10k" IJs are decreasing with the increasing of IJs exposure time to pesticides chemicals before infect host. Penconazole, Thiocyclam, Methiocarb, Methomyl and Mancozeb was highly sensitive more than the other tested insecticides by increasing exposure time 96 h. of EBN10k nematodes IJs to tested insecticides. While as, survival of both tested nematodes (EBN10k and EBN1e) are increasing by increasing the nematodes concentration (1000 IJs) exposure to tested pesticides.

In general steinernematid strains 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 divide to four categories (Atwa *et al.,* 2013) required to percentage of IJs survival (Highly sensitive, sensitive, moderate sensitive and tolerance). Steinernematid strain EBN1e was highly sensitive (mean of survived IJs was less than 50%), to Chlorfluazuron and Thiocyclam (Figure 1C and D). While, Methiocarb and Penconazole were sensitive, (means of survived IJs were from 50 to 70%). Simultaneously, moderate sensitive group (means of survived IJs were from 70 to 90%) include; Captan, Methomyl, Mancozeb and Benomyl, whereas tolerance group include (mean of survival IJs more than 90%), control treatment, Diafenthiuron, Trimiltox forte, and Imidacloprid (figure 1-C and D). On the contrary, heterorhabditid strain was highly sensitive to Penconazole, Diafenthiuron, Chlorfluazuron, Trimiltox forte, Benomyl, Thiocyclam, Methiocarb, Mancozeb, Captan and Methomyl, while as, it was sensitive to Diafenthiuron and Imidacloprid (Figure 1A and B).

Some reports indicated that certain insecticides, particularly organophosphates and carbamates, possess some nematicidal properties. These insecticides induced adverse effects ranging from impaired movement, infectivity and reproduction to death of *Neoaplectana carpcapsae* IJs (Kamionek, 1979; Hara and Kaya, 1983). Prakasa Rao *et al.* (1975) indicated that the organophosphates monocrotophos (50ppm), chlorfenvinophos, fenitrothion (250 ppm), and formothion and phosalon (500ppm) caused 90 to 100 % mortality of IJs of *N. carpocapsae* after 24 h. of exposure. Sub-lethal effects were not reported, but partial paralysis with the curled posture could have occurred at lower chemical concentrations. Zimmerman and Cranshaw (1990) reported that the carbamate carbaryl was significantly more toxic to *H. bacteriophora* (HP88) than to *Neoaplectana* spp. after 24 h. and 48 h. of exposure to 1000ppm. Gaugler and Campbell (1991), Ishibashi and Takii (1993) and Hara and Kaya (1993) indicated that treatment with organophosphate and carbamate pesticides impaired the infectivity of *S. carpocapsae* under laboratory conditions, even though the IJs were more active in the presence of these compounds. Such chemicals may stimulate inactive nematodes and thereby enhance their infectivity against the target insects. This chemical compatibility indicates a potential for combined applications of nematodes with chemicals. Apparently, the type and dose of insecticide, exposure period, nematode species or strain are determining factors in formulating the most profitable combination chosen in integrated programs of insect control.

Regarding the influence of exposure time on nematode infectivity, Fedorko *et al.* (1977a) and Fedorko *et al.* (1977b) showed in laboratory tests that IJs of *S. carpcapsae* were unaffected by short – term exposure to a wide variety 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 *N. carpocapsae* and *N. bibionis* was observed during 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 has high adverse effects on the nematode survival at higher concentrations and longer exposure period while muthrin and nudrin were harmless to the nematodes

especially in short exposure period. Gordon *et al.* (1996) observed no time–related response for *S. carpocapsae* and *S. feltiae* with carbofuran and fenoxycarb insecticides combinations since the effect of insecticides occurred within the initial 24 h. exposure period and did not increase thereafter.

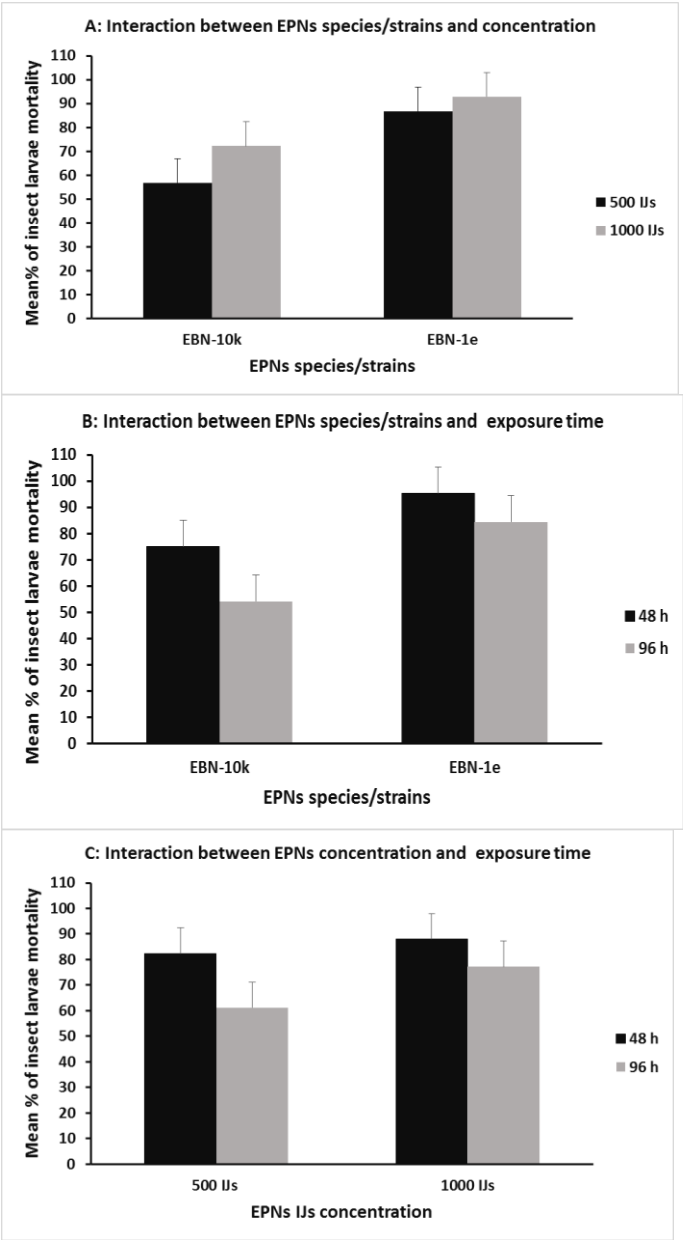

Figure 2. Mortality percentage of fifth instars larvae of *S. littoralis* required to interaction of EPNs species/strains, concentration and exposure time affected by stress of tested chemicals insecticides; A: Interaction between EPNs species/strains and concentration, B:

Interaction between EPNs species/strains and exposure time, C: Interaction between EPNs concentration and exposure time . Atwa *et al.* (2013) demonstrate that the differences in survival percentage of EPNs may be attributed the differences in nematode's acetylcholinesterase concentration, also the higher survival of *Steinernema* group than *Heterorhabdities* group may be attributed to the difference in nematode's acetylcholinesterase in both genera. In contrast, the different effects between insecticides and fungicides on survival of nematode IJs could be related to the different effects on nematodes chemical receptors and the respiratory metabolites as claimed by Atwa *et al.* (2013). This conclusion may suggest that these insecticides and fungicides had some negative lethal effect on nematodes, but it is safer on *Steinernema* genera than *Heterrhabditis* genera (Atwa *et al.*, 2013).

The mean mortality percentage of the pest larvae required to interaction of two tested EPNs concentration and exposure time affect by stress of different chemicals insecticides and fungicides was illustrated in Table 2. The data in Table 2 demonstrated that, there are significant variation in the main percentage mortality of *S. littoralis* fifth instar larvae required to differences of EPNs strains (EBN10k & EBN1e) as pointed in Figure 2. *Steinernema* strain (EBN1e) was high significantly in the mean percentage mortality of *S. littoralis* fifth instar larvae more than *Heterorhabditis* strain (EBN10K). Interaction of EPNs concentration affected on the tested insect larvae mortality was lead to; the high concentration of tested EPNs in general "1000 IJs" was high significantly in the mean percentage mortality of *S. littoralis* larvae more than the low concentration "500 IJs" (Table 2 and Figure 2). On the contrary, date in Table (2) illustrated that, the low exposure time (48 h.) was high significantly in the mean percentage mortality of *S. littoralis* fifth instar larvae more than the high exposure time (96 h.).

The response of *S. littoralis* fifth instar larvae to *H. bacteriophora* (EBN10k) treated by insecticides, with the determinations of insect infestation are indicated in Table (3). Referring to estimates of insect larvae moralities, it was observed that, EBN10k caused mortality average of *S. littoralis* fifth instar larvae was highly infectivity (100 % mortality) in control treatment with all treatments (EPNs concentrations 500 and 1000 IJs and exposure time

Table 2. Mean mortality percentage of *Spodoptera littoralis* required to interaction of two EPNs (EBN10k & EBN1e) species/strains, concentration and exposure time affect by stress of different chemicals insecticides.

Treatment	Mean % mortality of *Spodoptera littoralis* fifth larvae required to:						Mean
	EPNs strains		EPNs concentrations		Exposure time		
	EBN10k	EBN1e	500 IJs	1000 IJs	48 h.	96 h.	
EPNs only							
Control	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Insecticides							
Penconazole	68.7	90.35	65.7	93.35	88.3	70.75	79.53
Diafenthiuron	89.0	97.0	89.2	96.8	97.48	88.53	93.0
Imidacloprid	79.15	96.55	81.4	94.3	96.0	79.7	87.85
Chlorfluazuron	42.75	75.45	43.05	75.15	70.25	47.95	59.1
Thiocyclam	37.3	80.65	57.75	60.2	69.85	48.1	58.98
Methiocarb	68.55	90.5	65.7	92.35	87.8	70.25	79.19
Methomyl	50.65	91.2	67.45	74.4	81.75	60.1	70.93
Fungicides							
Trimiltox forte	48.15	93.4	68.65	72.9	82.0	59.55	70.78
Benomyl	39.75	81.5	64.2	57.05	68.15	53.1	60.63
Mancozeb	72.7	92.2	78.85	86.05	90.75	74.15	82.45
Captan	81.85	90.1	79.6	92.35	93.55	78.4	85.98
Mean	64.88	89.91	71.80	82.91	83.99	69.22	-----
LSD	0.4798 ($p < 0.05$).						

48 h. and 96 h.) as illustrated in Table (3). The efficacy of EBN10K nematode strain concentration (500 IJs) after exposure to Diafenthiuron, Imidacloprid, Mancozeb and Captan for 48 h. was effective in casing 93.0, 86.0, 85.0 and 86.0 % of *S. littoralis* larvae mortality respectively (Table 3). Low of EBN10K nematode strain concentration (500 IJs) after exposure to Penconazole, Methiocarb, Methomyl and Trimiltox forte for 48 h. by causing 66.6, 61.0, 70.0 and 62.0% of *S. littoralis* larvae mortality respectively (Table 3). On the contrary, poor efficacy of EBN10K nematode strain concentration (500 IJs) was after exposure to Chlorfluazuron, Thiocyclam, and Benomyl which affecting 36.0, 46.6 and 56.6 % of *S. littoralis* larvae mortality respectively (table 3). Apparently, all treated of EBN10K nematode strain concentration (500 IJs) for 96 h. to tested insecticides and fungicides was causing poor efficiency of *S. littoralis* larvae mortality except Diafenthiuron and Captan which causing 70.0 and 63.2 % of insect larvae mortality respectively (Table 3).

On the otherwise, highly infectivity rate (causing highly mortality of *S. littoralis* larvae) observed with treated IJs (1000 IJs for 96 h.) by Penconazole, Diafenthiuron, Imidacloprid, Methiocarb, Mancozeb and Captan (Table 3). While as, the efficacy of EBN10K nematode concentration (1000 IJs) after exposure to Chlorfluazuron, Methomyl, Trimiltox forte and Benomyl for 96 h. was effective in casing 73.0, 75.0, 68.0 and 63.2 % of *S. littoralis* larvae mortality respectively (Table 3). On the contrary, poor efficacy of the nematode strain "EBN10K" concentration (1000 IJs) treated by Thiocyclam (causing 56.6 % insect larvae mortality) after 96 h. of exposure time (table 3). Data in Table (3) illustrated that, there are high significantly in the mean percentage mortality of *S. littoralis* fifth instar larvae between treatments with high exposure time at 96 h. Three group of infectivity of the nematode strain EBN-10K concentration (1000 IJs for 96 h.) was observed; highly infectivity group including Penconazole, Diafenthiuron, Imidacloprid Methiocarb and Captan, while as the moderate effective group was affected by Mancozeb only, otherwise the poor infectivity was observed by

Table 3. Mortality percentage of *S.littoralis* fifth instar larvae caused of treated *H. bacteriophora* (EBN10k) IJs to different chemicals insecticides with interaction of between concentration and exposure time.

Treatment	Time required and nematodes concentration interaction				Mean
	48 h. x 500 IJs	96 h. x 500 IJs	48 h. x 1000 IJs	96 h. x 1000 IJs	
EPNs only					
Control	100.0	100.0	100.0	100.0	100
Insecticides					
Penconazole	66.6	26.6	95.0	86.6	68.70
Diafenthiuron	93.0	70.0	98.0	95.0	89.00
Imidacloprid	86.0	46.6	98.0	86.0	79.15
Chlorfluazuron	36.0	20.0	73.0	42.0	42.75
Thiocyclam	46.6	20.0	56.6	26.0	37.30
Methiocarb	61.0	26.6	98.0	86.6	68.05
Methomyl	70.0	22.6	75.0	45.0	53.15
Fungicides					
Trimiltox forte	62.0	26.6	68.0	36.0	48.15
Benomyl	56.6	40.0	63.2	49.2	52.25
Mancozeb	85.0	53.2	86.0	66.6	72.70
Captan	86.0	63.2	95.0	83.2	81.85
Mean	70.73	42.95	83.82	66.85	-----
LSD	0.3393 ($p < 0.05$)				

treated IJs with Chlorfluazuron, Thiocyclam, Methomyl, Trimiltox forte and Benomyl (Table 3).

Data in Table (4) explain the interaction of different variation (exposure time and nematodes concentration) on EPNs infectivity required to the mean percentage mortality of pest larvae by *Steinernema* sp. EBN1e treated with different chemical insecticides. A significant variation was occurred between the mean percentage mortality of insect larvae in control treatment and different treatments with chemical insecticides (Table 4). The highly efficacy of insect mortality required to EPNs treated IJs were observed by Penconazole, Diafenthiuron, Imidacloprid, Methiocarb, Methomyl, Trimiltox forte, Benomyl, Mancozeb and Captan treatments, while as the moderate efficacy was required to Chlorfluazuron and Thiocyclam with the nematodes strains "EBN1e" concentration 500 IJs for 48 h. exposure time (table 4). On the other hand data in Table (4), demonstrate that, there are high effect on mean percentage of insect mortality required to the same nematode concentration (500 IJs) with differences of exposure time (48 h. and 96 h.), the nematodes infectivity was decreasing by increased the exposure time (table 4). On the contrary, there are low significantly differences in the mean percentage mortality of *S. littoralis* fifth larvae by *Steinernema* sp. EBN1e required to *Steinernema* sp. "EBN1e" treated with different insecticides and fungicides with nematodes at 1000 IJs and different exposure time 48 h. and 96 h. for all treatments (Table 4).

Meanwhile, Gordon *et al.* (1996) stated that, carbofuran and fenoxycarb, carbamate insecticides with different mode of action, were toxic, to varying degrees, to steinernematids. Apparently, death of most nematodes is not immediate after exposure to tested pesticides. For example, *N. carpocapsae* and other nematode species remained in partially paralysed with very slow death and, if the partially paralysed nematodes are allowed to recover, carbamate and organophosphate–treated nematode regain their normal activity (Nelmes, 1970; Keetch, 1974; Bunt, 1975; Marban – Mendosa and Viglierchio, 1980; Hara and Kaya, 1983). The species and possibly strain of nematodes appear to be of crucial significance in determining its level of susceptibility to systemic insecticides (Atwa *et al.*, 2013), that is agree with our data in this study. Meanwhile, Hara and Kaya (1983) mentioned that carbamates and organophosphates were found to kill a proportion of the IJs of *S. carpocapsae* (All strain) and cause partial paralysis and reduced infectivity of

Table 4. Mortality percentage of *S.littoralis* caused of treated *Steinernema* sp. (EBN1e) IJs to different chemicals insecticides with interaction of between concentration and exposure time.

Treatment	Time required and nematodes concentration interaction				Mean
	48h x 500 IJs	96h x 500 IJs	48h x 1000 IJs	96h x 1000 IJs	
EPNs only					
Control	100.0	100.0	100.0	100.0	100
Insecticides					
Penconazole	93.0	76.6	98.6	93.2	90.35
Diafenthiuron	97.0	93.8	98.9	95.3	96.25
Imidacloprid	98.0	93.0	99.0	93.2	95.8
Chlorfluazuron	73.0	43.2	99.0	86.6	75.45
Thiocyclam	81.2	65.2	85.0	73.2	76.15
Methiocarb	97.0	78.2	98.2	89.6	90.75
Methomyl	95.0	81.2	96.0	91.6	90.95
Fungicides					
Trimiltox forte	96.5	86.0	98.0	89.6	92.53
Benomyl	87.6	66.6	89.2	76.6	80.0
Mancozeb	92.0	85.2	98.5	91.6	91.83
Captan	93.2	76.0	97.5	91.2	89.48
Mean	91.94	78.75	96.49	89.31	-----
LSD	0.4096 ($p < 0.05$)				

the remainder. Das and Divakar (1987) demonstrated that, circa 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. Koppenhöfer and Fuzy (2008) demonstrate that, EPNs are compatible in tank mixes with many pesticides including numerous chemical and biological insecticides. Our study shows that some insecticides and fungicides had different stress on EPNs infectivity to the list of compatible insecticides as 48 h. and 96 h. exposure to recommended dose of field application of these chemicals. 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 agree with Radová (2011) mentioned that, it is hard 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 result for the interaction effect of chemical insecticides not only makes application of nematodes in agro-ecosystem easier but also facilitates their use in integrated pest management systems (Atwa *et al.,* 2013).

The discussion on role of insecticides in nematode infectivity would evolve physiological points of view. Pristavko (1967) reported that, the addition of small amount of certain insecticides causes physiological weakening of the insect organism and reducing its resistance to EPNs. Another concept was proposed by Forshler *et al.* (1987) in the search of pesticidal power on nematode activity and infectivity. He claimed that, reductions in nematode activity after exposure to chemicals are not accompanied with concomitant reductions in infectivity. According to this author, nematode exposed to these chemicals became quiescent but after being removed from contact with chemicals, became active again and are capable of infecting susceptible insect hosts. The reason for the very slow death state may be due to rates of penetration, metabolism and detoxification of the chemical by the nematode. Other investigators (Saleh and Sammour, 1995) proposed that, the potential joint action of insecticide – nematode was probably due to the easy and fast reaching of insecticides to their target site in the insect. This may be enhanced through pathways induced by the nematodes and

their associated bacteria besides the separate action of both insecticides and nematodes. In these respect, Gordon *et al.*(1996) who reported no toxic effects of several carbamates and minimal effects of a variety of organophosphates on nematode infectivity.

Finally, results of this study increase our knowledge of EPNs, Fungicide and insecticides interaction, in this study we also illustrated that, most of these chemical pesticides used in this study are not toxic to both tested nematode species/strains but there are difference in toxicity rang that is was also agree with the data illustrated by Atwa *et al.* (2013). This study explained effect of used pesticides on survival and efficacy of nematodes are interesting when integrated pest management involves nematodes/pesticides combination in agro-ecosystem. These knowledge about the potential survival and efficacy loses due to used pesticides will be lead to predict the application rate of nematodes in field application.

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Atwa A. Atwa

Plant Protection Research Institute, Agriculture Research Center, Giza, Egypt.

E mail: atwaradwan@yahoo.com