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Effect of Neem Products on The Survival and Reproduction of Egyptian *Heterorhabditis bacteriophora* Used against Lepidopterous Insect Pests

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ABSTRACT

The effect of neem products on the survival rate of an Egyptian entomopathogenic nematode (EPN), *Heterorhabditis bacteriophora* (EBN34) was tested. Two commercial products of NeemAzal T/S (1% Azadirachtin) and neem powder (NP- 30% Azadirachtin) were used at different concentrations. The suspension of both (neem products and nematodes) was tested against last instar larvae of *Galleria mellonella* and second instar larvae of the Egyptian cotton leafworm, *Spodoptera littoralis*. The infective juveniles (IJs) stage of EBN34 were not affected when added to low concentrations of neem products. Meanwhile, high concentrations of neem products significantly affected the survival of nematode infective juveniles. whereas, the survived IJs didn't cause significant mortality of *G. mellonella* larvae but nematode reproduction was affected significantly. The neem liquid commercial product (NeemAzal T/S) resulted in 22.0 % mortality in EBN34 juvenils at 100 IJs/ml. when contacted directly by 1.0 % of NTS for 24 hours. When nematodes exposed to NTS for longer periods up to 48 and 96 hours, the mortality percentage increased directely to 40 and 56 rescpectively. Toxicity has increased gradually according to the increase in product concentration and exposure period at different of nematodes concentrations. Treated nematodes with NTS caused more than 80 % mortality in last instar larvae of *G. mellonella*, meanwhile treatment of the juveniles with NP resulted in ca 50% mortalitry. Reproduction of nematodes was highly affected as a result of treatment with neem products in comparison with the control. Using higher concentrations of neem products with nematodes (500 IJs/larva) resulted in 40-50% mortality of the juveniles and flactuated mortality percentage of *Galleria* larvae. Minimum larval mortality (14 %) was recorded after two days of feeding period on treated plant leaves with 0.125 % NTS at both nematode concentrations, while the maximum (96 % mortality) was found when 1.0 % NP was added at the same feeding period and juvenile concentrations. Mean number of emerged juveniles from dead larvae was low when high concentrations of both neem products was used than low concentrations. Increasing feeding period of larvae on treated leaves to 4 and 6 days decreased the propability of juvenile emergence due to the increase in larval mortality at both juvenile concentrations and the increasing levels of the tested neem products, especially in case of neem powder.

Key Words: Entomopathogenic nematodes, *Galleria mellonella*, *Heterorhabditis bacteriophora*, lepidopterous pests, NeemAzal T/S (NTS), reproduction, *Spodoptera littoralis*, survival.

INTRODUCTION

Entomopathogenic nematodes (EPN) can kill broad spectrum of insects in laboratory bioassays performed under optimal conditions where no ecological and behavioral barriers of infection exist. However, the potential to kill an insect in laboratory assay can not always be transferred to field applications, particularly when high levels of control is required (Kaya, 1990; Gaugler, 1988). Entomopathogenic nematodes have been recognized as excellent biological control agents of soil dwelling insect pests. Recent advances in mass-production and formulation technology have made insecticidal EPN available commercially for large-scale field applications in citrus groves, strawberry plantation, artichokes, mint, mushrooms, ornamentals in nurseries and greenhouses, and turf grass (Grewal and Georgis, 1998; Fife, *et al.*, 2003).

Entomopathogenic nematodes are often used in integration with other pest management tactics and the lack of compatibility information is a major impediment in further expansion of their use. The effects of different formulations of neem and a fungicide commonly used in greenhouses on *Steinernema feltiae* which is used for the control of fungus gnats was evaluated. It was found that, neem as pure oil at the field recommended doses of (5-10 ml./L^{-1}) had no effect on the viability and/or virulence of *S. feltiae* up to 120 hours of incubation. However the neem formulation, Nimbecidine and neem oil when mixed with a bactericidal soap (commonly used as a surfactant with neem oil) caused 13-25% mortality of *S. feltiae*. This toxic effect was entirely due to the soap that alone caused about 24% nematode mortality. Neither neem oil,

Nimbecidine or soap had any effect on nematode virulence. The fungicide cinnamaldehyde (Cinnamate) was highly toxic, resulting in 100% nematode mortality after 4 hours of incubation, followed by hydrogen dioxide/peroxyacetic acid mixture (ZeroTolerance) that caused 100% mortality after 120 hours of incubation (Krishnayya and Grewal. 2002).

Infective Juveniles (IJs) of *Heterorhabditis bacteriophora* (1H 145 strain), *Steinernema carpocapsae* (195 strain), and *S. glaseri* were exposed to different concentrations (2% w/v at 1/2, 1/4, 1/8, 1/16, and 1/32) of neem kernel extract. Nematodes were affected at the higher concentrations with *H. bacteriophora* more adversely affected than the *Steinernema* spp. It is suggested that entomopathogenic nematodes and neem should not be applied together in control programs (Rovesti and Deseö, 1989). In the present study, we are going to examine the effect of two different products of neem on the infectivity and reproduction of an Egyptian *Heterorhabditid* nematode.

MATERIALS AND METHODS

Nematode used:

H. bacteriophora (EBN34) was isolated from the soil (Abbas El-Akkad village, El-Nubaraya, Behera governorate). This local isolate was cultured on the last instar larvae of *G. mellonella* according to the method by Dutky *et al.* (1964) and infective juveniles (IJs) were harvested from nematode traps as described by White (1927) at 25 ± 2°C. A stock suspension of the IJs in sterilized distilled water was stored at 10°C until used.

Insect hosts:

The greater wax moth, *G. mellonella*, and the Egyptian cotton leafworm, *S. littoralis* were cultured at the Applied Center for Entomonematodes (ACE), Faculty of Agriculture, Cairo University. The last instar larvae of *G. mellonella and* 2nd instar larvae of *S. littoralis*, were used in this experiment.

Experimental techniques:

Five concentrations of two formulation of Azadirachtin (the active ingredient of neem extracts) were used to obtain the direct effect on survival, infectivity and reproduction of the tested entomopathogenic nematode.Tested neem formulation and nematode concentrations were as follows: NeemAzal T/S (NTS), was tested at 4, 2, 1, 0.5 and 0.25%.Neem poder (NP), was tested at 2, 1, 0.5, 0.25, and 0.125%. The concentrations of the tested nematode were 100 and 500 IJs/ml.

Effect of the neem products was evaluated using direct contact between the nematode suspension and each concentration of the neem products in 100 ml. conical flask under laboratory conditions for 24, 48, and 96 hours. Number of dead juveniles was counted and percentages of mortality was calculated to determine the direct toxic effect of these neem products on the nematode IJs. Effect of neem products on nematode infectivity and reproduction was measured by infecting *G. mellonella* last instar larvae with nematode IJs exposed to different concentrations of the neem products. The following concentrations of different neem products was used; NTS at 1.0, 0.5, & 0.25 % and NP at 0.5, 0.25 and 0.125 % with 100 nematode IJs and 500 nematode IJs/ml. Percentages of larvae mortality and numbers of emerging IJs per cadaver were recorded at infection time of 48 and 96 hours.

To determine the indirect effect of neem products on the reproduction rate of the entomopathogenic nematode *H. bacteriophora* (EBN34), *S. littoralis* second instar larvae were used as a host. The cotton leafworm larvae, *S. littoralis*, was fed for 2, 4, and 6 days on custor leaves treated with the neem products at the following concentration of NTS at 2.0, 1.0, 0.5, 0.25, and 0.125 %, meanwhile, the Neem Powder (NP) was used at concentrations of 1.0, 0.5, 0.25, 0.125, and 0.0625 %. The living insect larvae were treated with nematode infective juveniles (IJs) at concentrations of 100 and 500 IJs/ml./larva. Mortality of insect larvae and number of emerging IJs were recorded in the treatments and control. Five replicates were used in all the aforementioned experiments.

Staistical analysis of collected data:

The data presented in percentage values and were normalized using arcsine transformation. The significance of the main effects was determined by the analysis of variance test (ANOVA). The significance of various treatments was evaluated by Dunc an's multiple rang test ($p < 0.05$) (Duncan 1955). All the

statistical analysis were made using a software package called "Costat", a product of Cohort Software Inc., Bekelely, California.

RESULTS AND DISCUSSION

Data presented in table (1) show that, the neem liquid commercial product (NeemAzal T/S) resulted in 22.0% mortality of *H. bacteriophora* (EBN34) juvenils at a concentration of 100/ml, when contacted directly with 1.0 % of the product for 24 hours. With longer exposure periods to 48 and 96 hours nematode mortality percentage will increase to 40 and 56% respectively. Mortality percentages will increase gradually according to the product concentrations of 1.0, 2.0 and 4.0 % and exposure periods of 24, 48, and 96 hours. At nematode concentration of 100 IJs/ml, the nematode mortality percentage has reached 92% when they exposed to 2.0 % of NTS for 96 hours, and 98% when the nematodes exposed to 4.0 % of NTS for only 48 hours and 100% when the nematodes exposed to 4.0 % of NTS for 96 hours. when the used nematode concentration was 500 IJs/ml., the mortality percentage has dropped to 84%. This was recorded at 2.0% of NTS concentration for 96 hours. In contrast, the powder phase of neem product which contain higher amount of the active ingredient was very toxic to the nematode juveniles especially at the low concentration of the tested nematodes (100 IJs/ml.), mortality was 100% at all used concentrations except for the concentraation 0.5% at 24 hours which resulted in 88% mortality as shown in the same table. At a nematode concentration of 500 IJs/ml, 50% less juveniles died than in the concentration of 100 IJs /ml in all examined neem powder treatments after 24 hours of exposure time, the maximum mortality in the control was 4.0% which recorded after 96 hours at a nematode concentration of 100 IJs/ml., while it was 1.2 % with the other juvenile concentrations at the same exposure periods (table 1).

Table (1): Effect of different concentrations of neem products on the survival of *Heterorhabdities bacteriophora* (EBN34) infective juveniles at different exposure times.

Neem Products	Conc. of neem products	Time (hours)	Concentration of IJs/ml. and % mortality			
			100 IJS		500 IJS	
			Mean ±S.E	% mortality	Mean±S.E	% mortality
NeemAzal T/S (1% Azadirachtin)	1.0 %	24	22.0 ± 3.74	22	290.0 ± 9.88	58
		48	40.0 ± 3.16	40	330.0 ± 12.23	66
		96	56.0 ± 2.45	56	400.0 ± 0.0	80
	2.0 %	24	64.0 ± 2.45	64	300.0 ± 15.78	60
		48	74.0 ± 2.45	74	340.0 ± 2.98	68
		96	92.0 ± 1.99	92	420.0 ± 12.23	84
	4.0 %	24	84.0 ± 2.45	84	280.0 ± 19.97	56
		48	98.0 ± 1.99	98	320.0 ± 12.23	64
		96	100.0 ± 0.0	100	350.0 ± 0.0	70
Neem powder (30% Azadirachtin)	0.5 %	24	88.0 ± 3.74	88	200.0 ± 31.57	40
		48	100.0 ± 0.0	100	300.0 ± 31.57	60
		96	100.0 ± 0.0	100	340.0 ± 24.45	68
	1.0 %	24	100.0 ± 0.0	100	200.0 ± 31.57	40
		48	100.0 ± 0.0	100	260.0 ± 24.45	52
		96	100.0 ± 0.0	100	310.0 ± 24.46	62
	2.0 %	24	100.0 ± 0.0	100	180.0 ± 19.96	36
		48	100.0 ± 0.0	100	280.0 ± 19.96	56
		96	100.0 ± 0.0	100	300.0 ± 31.57	60
Control		24	0.0	0	0.0	0
		48	0.0	0	5.0 ± 2.23	1
		96	4.0 ± 0.99	4	6.0 ± 0.99	1.2

The lowest two tested concentrations of the neem products (1.0% of NTS and 0.5 % of NP) were diluted to 50 and 25% and their effects on nematode reproduction were tested. As shown in table (2), mortality of

treated nematodes at 100 IJs /ml. concentration had ranged between 10% (1.0% of NTS for 48 hours and 0.25% NP for 48 hours) and 64% at 0.5% Np for 96 hours. Whereas, the longest exposure period and highst concentration of NTS resulted only in 52% mortality of nematode juveniles.

Treatment of nematode juveniles with NTS has caused more than 80 % mortality in last instar *G. melonella* larvae, while treatment with NP resulted in a maximum 50% mortality of *Galleria* last instar larvae. Reproduction of nematodes was highly affected as a result of treatment with neem products in comparison with the control. The maximum number of emerged juveniles was recorded at 48 hours of exposure to 0.25 % NTS (4530 IJs/cadaver) while it reached 42680 IJs/cadaver in the control at the same concentration of incubated juveniles as cited in table (2).

Table (2): Effect of two different formulations of neem products on the survival, virulence, and reproduction of Heterorhabditis bacteriophora EBN34 infective juveniles (IJs) inside last instar larvae of the greater wax moth.

IJs conc. (IJs/ml.)	Neem products		Exposure time	Effect on survival of IJs		Virulence and reproduction on G. m.*	
	Name	% Conc.		% mortality	Mean ± SE.	% mortality	Emerged IJs/cadaver
100	NTS**	0.25	48	12	12 ± 2.0	80	4530
			96	26	26 ± 2.46	80	3770
		0.5	48	14	14 ± 2.46	100	3240
			96	36	36 ± 2.46	80	3170
		1.0	48	10	10 ± 3.16	100	2440
			96	52	52 ± 3.74	86.7	1780
	NP***	0.125	48	22	22 ± 3.74	46.7	3440
			96	38	38 ± 2.0	46.7	3150
		0.25	48	10	10 ± 0.0	53.3	2590
			96	24	24 ± 2.45	33.3	2370
		0.5	48	44	44 ± 4.0	33.3	1930
			96	64	64 ± 2.45	20	1850
	Control****	0.0	48	2	2 ± 2.0	100	37720
			96	2	2 ± 2.0	100	42680
500	NTS	0.25	48	7.8	39.2 ± 1.2	100	5840
			96	9.1	45.6 ± 2.04	80	4130
		0.5	48	8.4	42 ± 0.89	100	4510
			96	9.9	49.6 ± 2.71	66.7	3380
		1.0	48	8.6	42.8 ± 1.36	100	3300
			96	9.4	47.2 ± 1.02	46.7	2560
	NP	0.125	48	8.5	42.4 ± 2.23	100	5590
			96	10.2	51.2 ± 2.33	20	2330
		0.25	48	8.4	42 ± 1.27	100	3920
			96	9.1	45.6 ± 0.75	20	3420
		0.5	48	7.8	39.2 ± 1.49	60	3030
			96	9.8	48.8 ± 1.49	66.7	4130
	Control****	0.0	48	0	0.0	100	71960
			96	1.2	6 ± 0.99	100	72520

*Last instar of *Galleria mellonella* larvae.
**NeemAzal T/S (1% Azadirachtin).
***Neem powder (30% Azadirachtin).
****The control is the nematode suspension without neem products.

Using higher concentrations of neem products with the nematode concentration of 500 IJs/cadaver resulted in 40-50% juvenile mortality and flactuated percentages of *Galleria* mortality. Maximum number of emerged juveniles from *Galleria* cadaver was found at 0.25 % NTS after exposure for 48 hours (5840 IJs/cadaver), while the minimum was (2330 IJs/cadaver) which recorded at NP 0.125 % for 96 hours post treatment. While in the control experiment, number of emerged IJs/cadaver was 72520 at 96 hours of incubation period as shown in the table (2).

Second instar larvae of the Egyptian cotton leafworm were also used to evaluate the indirect effect of neem products on the nematode juveniles bioactivity. Data in table (3) indicate that, minimum larval toxicity (14%) was recorded after two days of feeding period on plant leaves treated with 0.125% NTS at both nematode concentrations of 100 and 500 IJs/ml., whereas, the maximum 96% mortality was found at 1.0 % NP at the same feeding period and juveniles concentrations (table 3).

Table 3. Mortality percentage and number of emerging infective juveniles (IJs) per cadaver of *Spodoptera littoralis* second instar larvae previously treated with neem products at different concentrations and then infected with infective nematode juveniles (IJs) at both concentrations of 100 and 500 IJs/ml.

Neem products	% Concentrations of neem products	% mortality* (no. of emerging IJs at different periods) infected with 100 IJs/ml.		
		2 days	4 days	6 days
NTS**	0.125	14 (5500)	92 (0.0)	92 (0.0)
	0.25	16 (2420)	96 (0.0)	100 (0.0)
	0.5	24 (3840)	96 (0.0)	100 (0.0)
	1.0	52 (5110)	96 (0.0)	94 (0.0)
	2.0	64 (4280)	96 (0.0)	100 (0.0)
NP***	0.0625	68 (6160)	100 (0.0)	100 (0.0)
	0.125	68 (4000)	100 (0.0)	100 (0.0)
	0.25	72 (4050)	100 (0.0)	100 (0.0)
	0.5	72 (3530)	100 (0.0)	100 (0.0)
	1.0	96 (1250)	100 (0.0)	100 (0.0)
Control****	0.0	0.0 (6550)	0.0 (14200)	0.0 (35000)
Neem products	% Concentrations of neem products	% mortality* (no. of emerging IJs at different periods) infected with 500 IJs/ml.		
NTS**	0.125	14 (2820)	92 (7500)	92 (0.0)
	0.25	16 (2400)	96 (5000)	100 (0.0)
	0.5	24 (2290)	96 (5600)	100 (0.0)
	1.0	52 (3300)	96 (0.0)	94 (0.0)
	2.0	64 (0.0)	96 (0.0)	100 (0.0)
NP***	0.0625	68 (4750)	100 (0.0)	100 (0.0)
	0.125	68 (4280)	100 (0.0)	100 (0.0)
	0.25	72 (4900)	100 (0.0)	100 (0.0)
	0.5	72 (3570)	100 (0.0)	100 (0.0)
	1.0	96 (5250)	100 (0.0)	100 (0.0)
Control****	0.0	0.0 (5980)	0.0 (14200)	0.0 (30000)

*% mortality and IJs emerged from infected insect larvae previously fed on food treated with neem products.

**NeemAzal T/S (1% Azadirachtin).

***Neem powder (30% Azadirachtin).

****The control larvae were fed on the same food but free of neem products.

Mean number of emerged juveniles from the cadavers was lower at high concentrations than low concentrations of both used neem products. When 100/ml. of nematode IJs were used to infect 2nd instar larvae of *S. littoralis*, the lowest number of emerging nematodes was 1250 IJs/cadaver, which recorded at

1.0% neem powder, while the highest used concentration of NTS resulted in the emergence of 4280 IJs/cadaver (table 3). Meanwhile, when a nematode concentration of 500 IJs/ml. was used, the mean number of emerged nematodes was higher when larvae was treated with the higher concentrations of both the neem products. Treated insect larvae with NTS resulted in the emergence of 3300 IJs/cadaver at 1.0 % and 2400 IJs/cadaver at 0.25 % of the product. The same trend was recorded also with NP treated larvae which resulted in 5250 IJs/cadaver and 3570 IJs/cadaver at 1.0 and 0.5 % respectively as shown in table (3).

Increase of larval feeding period on neem treated leaves to 2, 4 and 6 days decreased the propability of juvenile emergence due to larval mortality at both juvenile concentrations and tested neem products especially the neem powder (NP). In the control, when larvae were fed on neem free leaves, the numbers of emerged infective juveniles were 6550, 14200, and 35000 IJs/cadaver at the three incubation periods 2, 4, and 6 days, respectively (table 3).

The present investigation involved an attempt to determine effects of two different commercial neem products on a local isolate of the entomopathogenic nematodes *H. bacteriophora.* In the light of the current results, both of the neem products was toxic to the nematode juveniles. Toxicity was corelated positively with the amount of azadirachtin in the product and negatively with the concentration of nematode juveniles. Exposure period also play a great role in the toxicity of neem products ar direct contact and that agree with the resuls discovered by Pezowicz *et al.,* (1997), they stated that, a high juvenile mortality (>50%) in *S. feltiae* was observed after 5 days of exposure to neem seed shell (*Azadirachta indica*) extract, 5 g./100 ml. of water. They also stated that, the exposure of nematode infective juveniles to neem water extracts did not affect their ability to infect *G. mellonella* larvae but limited their establishment in the insect and the mean number of established nematodes per *G. mellonella* decreased with increasing Neemix concentration (Pezowicz *et al.,* 1997).

Meanwhile, Margosan-O, a commercial neem-based insecticide affected the entomopathogenic nematode species, *S. carpocapsae, S. feltiae,* and *S. glaseri* infectivity after incubation with this insecticide which was toxic to all the 3 nematode species, but only at much higher concentrations than the recommended field rate of 20 mg azadirachtin / liter water.

The entomopathogenic nematodes belong to the genus *Heterorhabditis* were affected at higher concentrations of neem kernel extract more adversely than the Steinernematid species. It is suggested that entomopathogenic nematodes and the neem products should not be applied together in the control programs (Rovesti and Deseö, 1989).

Some studies indicate that entomophagous nematode species may survive being tank- mixed with commercial formulations of neem (Margosan-O). Nematode infectivity of the mix was greatest when the soil was irrigated prior to the application and if applied on the day it was prepared. In contrast, 3 species of *S. carpocapsae, S. feltiae,* and *S. glaseri* survived and were infective when left in a tank for up to 15 days in solution with neem (Margosan-O). However, *S. feltiae* and *S. carpocapsae* were more adversely affected by the neem (Stark, 1996).

Other studies were conducted in India to explore the integration between neem seed kernel extract (NSKE), neem oil (NO) and *S. carpocapsae* (*S. c.*) on root grub *Holotrica serrata.* Soil include field collected 3rd instar grubs was exposed to various concentrations of NSKE, NO and *S. carpocapsae.* Infective juveniles were added 0 to 16 days after application of NSKE and NO, root grub mortality was noted every day. After combining and immediately applying the *S. carpocapsae,* higher mortality was obtained against root grubs. The survival and virulence of *S. c.* in neem were followed for 20 days with or without aeration. Contrary to our results, *S. c.* survived well in all tested neem concentrations and its virulence was not affected by the neem concentrations with and without aeration. It was concluded that, neem enhances the activity of entomopathogenic nematodes and can represent an eco-friendly strategy to control root grubs in agriculture and forest ecosystems (Sivaramakrishnan, personal communication).

In contrast, our results show that, NeemAzal N/S and neem powder were slightly effective on reproduction of the nematode strain EBN34 at low concentrations and short feeding period of 2 days. Meanwhile, reproduction of this nematode was affected mostly at the highest tested concentrations of neem powder (1.0%) and when 100 IJs/ml. were used to infect the insects. Longer feeding periods, more than 2 days, of cotton leafworm larvae halted nematode reproduction completely at all tested concentrations either with 100 IJs/ml. or with higher nematode concentration of 500 IJs/ml. No reproduction was observed when insect larvae were fed for 6 days on both neem products (table 3).

Our results also indicate that, treated larvae of *S. littoralis* were highly affected only when these insect larvae were fed on both neem products for long periods. Meanwhile, nematode reproduction wasn't affected when insect larvae were fed on both neem products for less than 2 days (table 3).

This was also observed when azadirachtin affected the cellular immune responses with a significant reduction in numbers of hemocytes (De Azambuja and Garcia, 1992). The weakness of the cellular immune responses will increase susceptibility of insects to the infection by pathogens especially nematodes which disagree with our results which indicated that, the used nematode strain "EBN34" was more virulent on insect larvae fed on plant leaves free of neem products.

When 2nd instars larvae of *S. littoralis* were fed for 6 days on castor-oil leaves treated with neem products, they lost weight. These data agree with El-Sayed (1982) who found the same results when 3rd instar larvae of *S. Littoralis* were fed for 3 days on castor-oil leaves treated with neem seed suspension. whereas, Ahmed (1995), found that, the methanolic extract of *Melia* has a significant effect on protein quality and quantity. Curently tested neem products play the same role with *S. littoralis*, and the emergence of nematode juveniles is usually low in treated insect larvae (table 3).

Both of the tested neem products were effective on the viability and virulence of nematode juveniles bred on *G. mellonella* larvae. Reproduction of the nematodes was also higly affected specially at high neem concentrations and long exposure periods. These results disagree with those obtained by Pezowicz *et al.*, 1997, who concluded that, exposure of nematode infective juveniles (IJs) to water extracts of the neem didn't affect their ability to infect larvae of *G. mellonella.* The authors want to call attention to the fact that mean numbers of established nematodes per *G. mellonella* larva decreased with increasing Neemix concentration (Pezowicz *et al.*, 1997) which agree with our results.

In conclusion, we recommend that, entomopathogenic nematodes should not be used at the same time or parallel with neem products. The nematodes should be used before or after application of neem products with enough time.

REFERENCES

Ahmed, A. A. I. 1995. Studies on the effect and mode of action of *Melia azedarach* L. extract on some lepidopterous insects. Ph. D. thesis, Dept. of Plant Protection, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt, pp. 137.

De Azambuja, P. and ES. Garcia. 1992. Effect of azadirachtin on *Rhodnius prolixus*: immunity and Trypanosoma interaction. *Mem Inst Oswaldo Cruz.* 87 (5), 69-72.

Duncan, D. B. 1955. Multiple range and multible F-test. *Biometrics* 11, 1-42.

Dutky, S.R., J.V. Thompson, and G.E. Cantwell. 1964. A technique for the mass propagation of the DD-136 and nematode. J. Insect Pathol. 6: 417-422.

El-Sayed, E. I. 1982. Evaluation of the insecticides properties of the common indian Neem, *Azadirachta indica* A. Juss., seeds against the Egyptian cotton leafworm, *Spodoptera littoralis* (Boisd.) Bull. Ent. Soc. Egypt, Econ. Ser., 1982-1983, (39-47.

Fife, J. P., R. C. Derksen, H. E. Ozkan, and P. S. Grewal. 2003. Effect of pressure differentials on the viability and infectivity of entomopathogenic nematodes. *Biological control*, 27, 65 – 72.

Gaugler, R. 1988. Ecological considerations in the biological control of soil-inhabiting insects with entomopathogenic nematodes. Agriculture Ecosystem and Environment 24: 351-360.

Grewal, P. S. and R. Georgis. 1998. Entomopathogenic nematodes. In: Biopesticides: Use and Delivery, (Hall, F. R. and J. J. Menn, eds.), Humana Press, pp. 271-299.

Kaya, H. K. 1990. Soil ecology, In. Entomopathogenic Nematodes in Biological Control (Gaugler, R and Kaya, H. K., eds.), CRC Press, Boca Raton, Florida., pp. 93-115.

Krishnaya, P. V. and P. S. Grewal 2002. Effect of neem and selected fungicides on viability and virulence of the entomopathogenic nematode *Steinernema feltiae.* Biocontrol Science and Technology, 12 (2) 259-266.

Pezowicz E., S. Ignatowicz, and M. Kamionek 1997. Comparative effects of water extracts of seeds of the Indian neem tree, *Azadirachta indica* A. Juss, a plant-derived insecticide, NeemixTM, and a synthetic insecticide, Bancol 50 WP, on the efficacy of the entomogenous nematode, *Steinernema feltiae* (Filipjev). Annals of Warsaw Agricultural Univerisity Animal Science. 18: 31-39.

Rovesti L., and K. V. Deseö, 1989. Effect of neem kernel extract on steinernematid and heterorhabditid nematodes. Nematologica. 35: (4) 493-496.

Stark J. D. 1996. Entomopathogenic nematodes (Rhabditida: Steinernematidae): Toxicity of neem. Journal of Economic Entomology. 89:(1) 68-73.
White, C. F. 1927. A method for obtaining infective larvae from culture. *Science* 66: 302-303.

تأثير مركبات النيم على حيوية وتكاثر النيماتودا المصرية من النوع *Heterorhabditis bacteriophora* والمستخدمة ضد آفات حشرية من حرشفية الأجنحة

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أجريت هذه الدراسة لإختبار فعالية مركبات النيم على معدل حيوية النيماتودا المصرية الممرضة للحشرات من النوع هيتيرورابديدس باكتيريوفورا (إي بي إن ٣٤)· تم إستخدام إثنان من مركبات النيم التجارية النيمازال تي/إس (١% أزادراختين) و بودرة النيم (إن بي ٣٠% أزادرختين) بتركيزات مختلفة· تم إختبار محلول كلاً من مركبات النيم والنيماتودا ضد العمر اليرقي الأخير لدودة الشمع الكبرى (جاليريا ميلونيللا) والعمر اليرقي الثاني لدودة ورق القطن المصرية (سبودوبترا ليتوراليس)· لم تتأثر حيوية الأطوار المعدية للسلالة (إي بي إن ٣٤) عند تعريضها للتركيزات المنخفضة من مركبات النيم ، بينما كان للتركيز العالي من مركبات النيم تأثيراً معنوياً على حيوية الأطوار المعدية ، في حين أن هذه الأطوار المعدية لم تسبب نسبة موت معنوية ليرقات الجاليريا ميلونيللا بينما تأثر تكاثر النيماتودا معنوياً· المحلول التجاري لمركب النيم (نيمازال تي إس) أدى إلى موت ٢٢% من الأطوار المعدية للسلالة (إي بي إن ٣٤) عند تركيز ١٠٠ طور معدي/ملل عند تعرضها مباشرة لتركيز ١% من ال إن تي إس لمدة ٢٤ ساعة· وعندما عرضت النيماتودا لمركب ال إن تي إس لفترة أطول حتي ٤٨ ، ٩٦ ساعة وصلت نسبة الموت حتي ٤٠ و ٥٦% علي التوالي· من هذه الدراسة تبين أن سمية مركبات النيم للنيماتودا تتزايد تدريجياً مع تزايد التركيز ووقت تعريض الأطوار المعدية لهذه التركيزات المختلفة من مركبات النيم مع تركيزات النيماتودا المختلفة· النيماتودا المعرضة لمركب ال إن تي إس سببت أكثر من ٨٠% موت للعمر اليرقي الأخير للجاليريا ميلونيللا ، بينما النيماتودا المعرضة لمركب بودرة النيم أعطت نسبة موت تقدر بنحو ٥٠ %· أظهرت النتائج تأثر إنتاج النيماتودا المعرضة لمركبات النيم عنه في المقارنة ، أعطى التركيز العالي من مركبات النيم موت ٤٠-٥٠% من الأطوار المعدية وسببت موت متذبذب ليرقات الجاليريا· أقل نسبة موت لليرقات (١٤%) تم تسجيلها بعد تغذية يرقات دودة القطن علي أوراق نباتات معاملة بتركيز ٠.١٢٥ % من ال إن تي إس لمدة يومين لكلاً من تركيزي النيماتودا ، بينما كانت أعلى نسبة موت (٩٦%) عند التغذية لمدة يومين علي أوراق نباتات معاملة بتركيز ١٠٠ % من ال إن بي· كان متوسط الأطوار اليرقية الخارجة من اليرقات الميتة نتيجة الإصابة بالنيماتودا منخفضاً عند المعاملة بالتركيزات العالية من كلا مركبات النيم أكثر من التركيزات المنخفضة ، بزيادة فترة التغذية لليرقات على الأوراق المعاملة لمدة ٤ ، ٦ أيام إنخفضت فرصة هجرة الأطوار المعدية من الجثث المعاملة بكلا تركيزى النيماتودا وكذلك بزيادة تركيز مركبات النيم المختبرة و خاصة بودرة النيم·