Effect of Suneem Oil Extract on the Efficacy of the Entomopathogenic Nematodes (*Steinernema* spp.)

Salem, Nagwa Y.; Manal M. Adel and N. A. Farag
Department of Plant Protection, National Research Center, Dokki, Cairo, Egypt
(Received, May 10, 2005; Accepted, June 13, 2005)

ABSTRACT

The direct and indirect effects of suneem oil extract on the entomopathogenic nematodes, *Steinernema feltiae* and *Steinernema kraussei* were studied using the wax moth larvae; *Galleria mellonella* L. Suneem oil decreased the penetration of both species into the host especially at high concentrations. Direct contact between the plant extract and both species of S. *feltiae* and *S. kraussei* gave more effects against host larvae, than feeding host larvae on diet contains suneem oil extract only.

Key Words: Suneem Oil Extract, Steinernematid Nematodes, Efficacy, *Galleria mellonella.*

INTRODUCTION

Many problems have been encountered because of the extensive use of synthetic insecticides, which more harmful to biological control agents than to the pest species. Therefore, pest management strategies based on the utilization of other safe alternative to conventional chemical insecticides are now strongly advocated.

A compound from the neem tree, *Azadirachta indica*, exhibits high degree of selectivity. The most important bioactive compound, Azadirachtin, contains at least 10 other neem limonoids, which possess insect growth regulating activity (Schmuttrer, 1990, and Ley *et al*, 1993; Gelbic and Nemec 2001; El Gengaihi *et al*, 2002; Viana & Prats, 2003 and Opender *et al*., 2004). Neem seeds and leaves have been shown to possess nematicidal activity against entomopathogenic nematodes (Rovesti and Deseo, 1989 and Grewal, 1989).

The use of entomopathogenic nematodes especially of the family Steinernematidae as biological control agents against pest species has increased in recent years. They already were used against many of insects; kill it by releasing symbiotic bacteria *Xenorhabdus spp.* into the insects homocoel. (Weiser & Mracek, 1988; Hominick, 1990, Georgis, 1992; Eculica *et al* 1997; Navon *et al*, 2000 & 2002 and Pramila *et al*., 2003).

The combination of the two control methods may increase the efficiency of one another against the target pest (Rovesti *et al*, 1989; Rovesti and Deseo, 1990; Stark, 1996; Hussaini *et al*., 2001; Abd- Elkerim and Azazy, 2003 and Adel *et al*, 2005).

The present work deals with assessment of the compatibility of commercially neem product (Suneem oil) with beneficial entompathogenic nematodes *S. Feltiae* and *S. kraussei*, family Steinernematidae as well as the impact of neem on nematodes infectivity and fertility and use of *G. mellonella* as an insect trap to obtain entomopathogenic nematodes serving as a bait (Mracek, 1980).

MATERIALS AND METHODS

Test insect

Galleria mellonella (Lep.: Pyralidae) larvae were obtained from a laboratory culture reared on an artificial diet according to (Dutky *et al*.; 1964).

Bioassay technique

Two different experiments were performed, at both 15°C for 5 days exposure of host larvae to infective juveniles of S. *feltiae* and *S. Kraussei.*

Experimental approach:

1) Direct contact between nematodes and suneem oil extract

Eight concentrations of suneem oil (a commercial neem seed extract, was obtained from the Sunda exports India that contained 1% azadirachtin) were used in different concentrations of 2, 1, 0.5, 0.25, 0.125, 0.0625, .0313, and 0.0156 % nematode infective juveniles (IJs) of both nematode species that reared for several generations on the greater wax moth larvae *G. mellonella*.. Three inoculations of nematode infective juveniles 1000, 500, and 250 were added as suspension to a 1 ml of each suneem oil concentration. In this case, the suneem oil was placed instead of distilled water in the center of each dish (9 cm diameter). Distilled water was used in the dishes to dilute the plant extract to the required concentrations. Three replicates with 10 *Galleria* larvae per Petri dish for each concentration of both nematode species were used. Other three replicates of control insect larvae were kept in dishes containing the same concentration of nematodes without any treatment with suneem oil.

2) Indirect contact between nematodes and suneem oil extract

In this experiment insect larvae were fed on artificial diet mixed with the desired concentration of suneem oil at 2, 1, 0.5, and 0.25% for *S. feltiae* and 0.5, 0.25 and 0.125% for *S. kraussei*, were exposed for 48 hrs, to 3 nematode concentrations of 1000, 500, 250IJs/ml. Larvae infected with nematodes at each concentration of suneem oil and after 5 days, dead insect larvae were dissected and the number of adult nematodes was counted in each cadaver. Three replicates with ten *G. mellonella* larvae per dish were used for each concentration, the same number of replicates were kept in dishes as mentioned before for control.

Statistical analysis of the results were conducted using Student's t- test (Hayslett, 1970).

RESULTS AND DISCUSSION

1) Direct contact between nematodes and suneem oil extract

All *G. mellonella* larvae exposed to different concentrations of suneem oil extract were killed by *S. feltiae* and S. *kuarai*. The suneem oil was toxic to nematodes and its toxicity was observed at higher concentrations of 2, 1, and 0.5% and nematode concentrations of inculcate 1000IJs for both species, only low numbers of nematodes successfully penetrated the wax moth larvae. The percentage of invading nematodes was 4.9, 6.5 and 12.6% for *S. feltiae* and 3.3, 6.3 and 10.4% for *S. kraussei*, respectively. These percentages increased with decreasing the concentration of suneem oil extract (Tables 1&2).

In contrast, parasitism by *S. feltiae* and *S. kraussei* was markedly increased with the highest nematode concentrations of 1000IJs and 0.625% of the suneem oil 432 and 414.3 invading nematodes comparing the two nematode species with the control (254.3 and 255.3 invading nematodes), while, at nematode concentrations of 500 and 250 IJs, the same trend of nematode numbers was recorded (Tables 1 &2).

Statistical analysis indicated that the number of invading nematodes was highly significant at suneem oil concentrations of 2, 1 and 0.5% and not significant at moderate and low concentrations of 0.125 and 0.0313, 0.0156%, respectively except at 0.0625% concentration, the percentage of invading nematodes in treated larvae was significantly higher, 43.2 and 41.4%, than in the control 25.4 and 25.5%, respectively for both species (Tables 1&2).

2) Indirect contact between nematodes and suneem oil

In this experiment, there was no direct contact between the nematodes and suneem oil. Larvae were fed on treated diet with the neem extract and then infected by nematodes. Data obtained in Table (3) show that at the higher nematode concentration of 1000 IJs/ml, the percentage of infected larvae decreased with the increased concentration of suneem oil 19.9, 23.6, and 27.5 at 2,1,0.5%, respectively compared with the control 32.9 % for *S. feltiae* (Table,3), and 9.6 at 0.5% for *S. kraussei*, compared with control 25.1% (Table, 4). In addition, at the concentrations of 0.25 and 0.125% the activity of the nematode (*S. kraussei*) increased and became higher than the control, 28.4 and 33.9% compared to 25.1 % in the control , while at the lower nematode concentration of 250 IJs/ml, for both species decreased below the control at all concentrations of suneem oil extract used. In all cases, the number of infecting nematodes of *S. feltiae* was higher in the control with all nematode concentrations when compared with treatment by suneem oil extract (Table, 3)

Number of nematodes obtained in both experiments was higher in the second experiment than in the first one. Indirect effect of suneem oil on nematodes resulted in higher percentage of invaded nematodes.

Suneem oil extract tested in this study caused mortality and reduction in the nematodes infectivity especially at higher concentrations of the oil, this may be due to the slow movement of the nematodes in the presence of neem oil and to the oily nature of suneem oil at low concentrations of oxygen. No effects were observed at lower concentration, these results are in agreement with Rovesti and Deseo 1989, they evaluated the effect of crude neem extract on five species of entomopathogenic nematodes. *S. feltiae* and *S. carpocapsae*, were susceptible to this neem extract. Same trend was recorded by *Heterohabditis bacteriophora* (Shamseldean *et al.* 2004).

The results indicate that there was no adverse effects of the oil extract on nematodes at lower concentration, specially at the concentration of 0.0625% i.e. the suneem oil at this very low concentration was not toxic or lethal to nematodes. Similar results were obtained by Stark 1996, Pezowicz *et al.*; 1997, Hussaini *et al.*; 2001, Shamseldean *et al* ., 2004 and Adel *et al*, 2005 who used the entomopathogenic *Steinernema* species in combination with Margozan O, Neemix , Neem oil , neem powder, Neem AZAL T/S and suneem oil at different concentrations as effective bioinsecticides. Tolerance was observed at lower concentrations, Neem products was found to be safe for all nematodes populations, may show particular promise for the treatments of some soil pests. Meanwhile, Shamseldean *et al*, 1999 tested the efficacy of some insecticides on entomopathogenic nematodes and they were safer on *Steinernema* species than *Heterorhabditis*, such comparative lethal effect varied with regard to the nematode type , employed insecticides their rates and exposure time.

On the contrary, the present results indicate that there was significantly a negative direct effect of suneem oil on nematode penetration into *G. mellonella* larvae when compared with the indirect effect when insect larvae that were fed on diet contained the suneem oil. Thus, the control of insect pests by Steinernematid nematodes and diluted suneem oil as biological control may enhance their lethal effects on insect pests when applied simultaneously. Plant extracts played as catalytic agent and increased the susceptibility of pests to infection by nematodes specially *S. feltiae*. (Table 3). Plant extracts were safer on *S. feltiae* than *S. kraussei* (Pezowicz *et al.*, 1997) who reported that, Neemix (azadirachtin). 5% had a slight adverse effect on the survival of infective juveniles of *S. feltiae* and didn't affect their ability to infect larvae of *G. mellonella*, the mean number of established nematodes per *G. mellonella* decreased with increasing Neemix concentration.

REFERENCES

Abd-El-Kerim, I.A. and A. M. Azazy. 2003. The relationship between antijuvenile hormone (precocenes) and entomopathogenic,*Steinernema carpocapsae* to nematodes infectivity and energy metabolism supply of *Spodoptera littoralis* (Boisd.). Egyptian-J. Agri. Res., 81 (2): 573-580.

Adel, M.; F.N. Zaki and N.Y. Salem. 2005. Compatibility between entomopathogenic nematodes *Steinenema feltiae* and plant extract on the cotton leaf worm *Spodoptera littoralis* (Boisd). Bull. ent.

Table (1): Direct contact between nematodes (*Steinernema feltiae*) and plant extract of suneem oil.

Concentration of nematodes IJs/ml	Concentrations of Suneem oil% Number of nematodes /cadavar±SE								
	2	1	0.5	0.25	0.125	0.0625	0.0313	0.0156	control
1000	49±1.45^{A}	65±2.7^{A}	125.7±2.7^{B}	289±5.7^{C}	315.3±11.4	432±3.0^{A}	358.3±3.52^{C}	273±3.59	254.3±2.2
%of invasion	4.9	6.5	12.6	28.9	31.5	43.2	35.8	27.3	25.4
500	16.7±1.31^{A}	50.5±1.65^{A}	96.6±1.7^{B}	154.7±3.7	225±6.3	271±2.60^{C}	219±1.41	185.7±2.2	195.6±1.7
%of invasion	3.34	10.1	16.3	30.9	45	54.2	43.8	37.1	39.1
250	14±0.64^{A}	18.7±1.07^{A}	22±0.74^{A}	37.3±1.3^{B}	43±0.98	123.7±1.2^{C}	92.3±1.5	70.7±1.1	8.3±0.9
%of invasion	5.6	7.5	8.8	14.9	17.2	49.5	36.9	28.3	33.2

Table (2): Direct contact between nematodes (*Steinernema kraussei)* and suneem oil concentration.

Concentration of nematodes IJs/ml	Concentration of plant extract % Number of nematodes /cadevar ±SE								
	2	1	0.5	0.25	0.125	0.0625	0.0313	0.0156	control
1000	33± 6.3^{B}	63± 16.9^{B}	104.3±28.3^{C}	246±10.9^{C}	268.7± 2.7	414.3±31.1^{C}	369.3±64.7	260.7±47.8	255.3±32.1
% of invasion	3.3	6.3	10.4	24.6	26.8	41.4	36.9	26.1	25.5
500	22±11.1^{B}	51.3±11.6^{D}	65± 14.5^{D}	193.7±3.8	243.7±48.4	251.3± 43.5	213.7±14.7	182.3±13.9	180.3±27.0
% of invasion	4.4	10.3	13.0	38.7	47.5	50.3	42.7	36.5	63.1
250	8.6±3.7^{B}	32.7±3.8^{B}	21± 1.9^{B}	33± 4.5^{B}	25.3± 3.1^{B}	119.3± 28.5	91±18.6	71. 7±26.4	86.3± 7.9
% of invasion	3.5	13.1	8.4	13.2	10.3	47.7	36.4	28.7	34.5

Table (3): Indirect contact between nematodes (*Steinernema feltiae)* and plant extract of suneem oil.

Concentration of nematodes IJs/ml	Concentration of suneem oil % Number of nematodes /cadvar±SE				
	2	1	0.5	0.25	control
1000	199±5.23^{C}	235 ±6.46^{D}	275 ±5.93	209.5 ±3.88	329.5 ±8.52
%of invasion	19.9	23.6	27.5	20.9	32.9
500	128 ±2.62^{C}	156 ±5.1^{D}	172.5 ±6.8	197.5 ±5.67	217.5 ±4.6
%of invasion	25.6	31.2	34.5	39.5	43.5
250	20.5 ±5.9^{C}	26 ±1.01^{C}	35.5 ±0.8^{C}	21.5 ±3.4^{C}	70.5 ±1.07
% of invasion	8.2	10.4	14.2	8.6	28.2

Table (4): Indirect effect of plant extract of suneem oil on the efficacy of nematodes (*Steinernema kraussei*).

Concentration of Nematodes IJs/ml	Concentration of suneem oil			
	0.5	0.25	0.125	control
	Number of nematodes/ cadavar±SE			
1000	96±20.3^{C}	824.3±32.6	339±98.3	251.3±22.3
% of invasion	9.6	28.4	33.9	25.1
500	75.3±18.0^{C}	151.3±12.3	237.7±48.4	227.7±46.6
% of invasion	15.1	30.3	47.5	45.5
250	17.3±0.66^{A}	30.3±1.77^{B}	31±2.6^{B}	73.7±6.5
% of invasion	6.9	12.1	12.4	29.5

The number with letter means highly significant (^{A}), very significant (^{B}), significant (^{C}) and marginally significant (^{D}). The number without letter means there is no significant different between treated and control larvae.

Significance of differences between the treated and control insects is indicated with letter as follows: ^{A}) $P<0.0001$, ^{B}) $p<0.005$, ^{C}) $p<0.05$ and ^{D}) $p=0.08$

Soc., Egypt, Ser., 31: 19-27.

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

Eculica, J. F.; S. Becvar; Z. Mracek and P. Kindlman. 1997. Laboratory evaluation of control of the European corn borer, *Ostrinia nubilalis* (Hb.) (Lep. : Pyralidae) by nematodes of the genus *Steinernema* (Nematoda: Steinernematidae) at low temperature. J. App. Ent.; 121; 407-409.

El-Gengaihi, S. E.; H. M. Motawe; N. Z. Dimetry and S. M. Mohamed, 2002. Chemical and biological evaluation of neem fruits. Bull. N. R. C., 27(3): 321-328.

Gelbic, I. and V. Nemec. 2001. Developmental changes caused by metyrapone and azadirachtin in *Spodoptera littoralis* (Boisd.) (Lep.: Noctuidae) and *Galleria mellonella* (L.) (Lep.: Pyralidae). J. Appl. Ent., 125(7): 417-422.

Georgis, R., 1992. Present and future prospects for entomopathogenic nematodes product. Biocont. Sci. & Tech., 2: 83-99.

Grewal. P. S., 1989. Nematicidal effect of some plant extract to *Aphelenchoides composticola* (Nematode) infesting mushroom , *Agaricus bisperus* . Revue de.Nematologie 12: 375-377.

Hayslett. H. T., 1970. Statistics, Made simple books W.H. Allen & Co., Itd. London, W. C. pp. 25.

Hominick, W.M.1990. Entomopathogenic Rhabditid nematodes and pest control parasitology. Today 6: 148-152.

Hussaini, S. S; K. J. Staya and M. A. Hussaini. 2001. Tolerance of some indigenous entomopathogenic nematodes isolates to pesticides and their effect on multiplication. Current Nematology. 12 (½): 29-34.

Ley, S. V.; M. M. Denholm and A. Wood.1993. The chemistry of Azadirachtin. Nat. Prod. Reports, pp. 09-157.

Mracek, Z. 1980. The use of "*Galleria* Trap" for obtaining nematode parasites of insects in Czechoslovakia (Nematoda. Steinernematidae). Acta entomologica bohemoslovaca, 77: 378-872.

Navon, A.; V. Alchanatis; I. Glazer; L. Salame; S. Leveski and P. H. Smits. 2000. An image analysis of *Spodoptera littoralis* feeding behavior following ingestion of the insecticidal nematode *Steinernema riobrave* in an alginate gel carrier. Proceeding of the 7th European meeting of TOBC-WPRS working group: Insect pathogens and insect parasitic nematodes, Vienna, Austria, March 22-26, 1999. Bulletin- OILB-SROP. 2000 23(2): 85-90.

Navon, A.; V. K. Nagalakshmi; L. Shlomit ; L. Salama and I. Glazer. 2002. Effectiveness of entomopathogenic nematodes in an alginate gel formulation against lepidopterous pests. Biocont. Sci& Tech., 12(6): 737-746.

Opender, K.; J. S. Multani; S. Goomber; W. M. Daniewski and S. Berlozecki. 2004. Activity of some nonazadirachtin limonoids from *Azadirachta indiac* against lepidopteran larvae. Aust. J. Entom., 43 (2): 189-195.

Pezowicz, E.; S. Ignatowicz and M. Kamionek 1997. Comparative effects of water extract of seeds of the Indian neem tree, *Azadirachta indica* A. Juss, a plant-derived insecticide, Neemix T.M, and a synthetic insecticide, Bancol 50WP, on the efficacy of entomogeous nematodes, *Steinernema feltiae* (Filipjev.). Ann. Warsaw. Agri. uni. SGGW, Horticulture.18:31-39.

Pramila, G.; S. S.; Hussaini, R. J. Rabindra,and M. Nagesh, 2003. Entomopathogenic nematodes work done at Allahabad Agricultural Institute, Allahabad. Entomopathogenic nematodes in India hold on 22 and 23rd January , 2003, 161-165.

Rovesti, L. and K. V. Deseo, 1989. Effect of neem Kernel extract on *Steinernema* and *Heterhabditid* nematodes . Nematologica 34: 493-496.

Rovesti L. and K. V. Deseo.1990. Compatibility of chemical pesticides with the entomopathogenic nematodes *Steinernema carpocapsae* (Weiser) and *Steinernema feltiae* (Filipjev) (Nematoda: Steinernematidae). Nematologica, 36: 237-245.

Rovesti, L.; E. W. Heinzpeter; F. Tagliente; and K. V. Deseo, 1989. Compatibility of pesticides with the entomopathogenic nematoda *Heterorhabditis bacteriophora* Poinar (Nematoda: Heterorhabditidae). Nematologica 34: 462-476.

Schmuttrer, H. 1990. Properties and potential of nature pesticides from the neem tree *Azadirachta indica* . Annu. Rew. Entom. , 35: 271-297.

Shamseldean. M. M.; M. M. Abd-Elgawad and A. A. Atwa. 1999: Factors effecting pathogenicity of Egyptian strain of *Heterorhabditis indicus* infecting cotton leaf worm *Spodoptera littoralis.* J. Nematology, 9: 90-97.

Shamseldean, M. M.; A. A.I. Ahmed and A. A. Atwa. 2004. Effect of neem products on the survival and production of Egyptian *Heterorhabditis bacteriophora* used against lepidopterous insect pests. Egyptian J. Biol. Pest Control, 14(1), 187-194.

Stark, J. D., 1996. Entomopathogenic nematodes (Rhabditidae: Steinernematidae): Toxicity of neem. J. econ, Entomol. 89(1): 69-73.

Viana, P. A. and H. T. Prates, 2003. Larval development and mortality of *Spodoptera frugiperda* fed on corn leaves treated with aqueous extract from *Azadirachta indica* leaves. Bragantia: 62 :(1): 69-74.

Weiser, J. and Z. Mracek, 1988. Parasitic holistic hmyzu (in Czech) in Prague. 258 PP.