COMPATIBILITY BETWEEN ENTOMOPATHOGENIC NEMATODE *STEINERNEMA FELTIAE* AND PLANT EXTRACTS FOR TREATING THE COTTON LEAF WORM, *SPODOPTERA LITTORALIS* (BOISD.) (LEPIDOPTERA: NOCTUIDAE)

By **MANAL M. ADEL; FAYZ. N. ZAKI AND NAGWA. Y. SALEM**

Plant protection Department, National Research Centre, Cairo, Egypt

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INTRODUCTION

Insecticides are usually more harmful to biological control agents than to pest species. Therefore, pest management strategies based on the utilization of other relatively safe alternatives to conventional chemical insecticides are now strongly advocated.

Plants produce a variety of compounds that provide certain protection against insect attack (Felton and Gatehouse, 1996). High saponin content in some cultivars of the Lucerne or alfalfa, *Medicago sativa* L., is correlated with resistance to the pea aphid *Acyrthosiphon pisum* (Petersen *et al.*, 1976). Alfalfa contains an array of saponins derived from triterpenoid aglycones (Lee *et al.*, 1996; Oleszek, 1996; Tava and Odoardi, 1996). These saponins were shown to reduce larval growth and cause mortality in the flower beetle, *Tenebrio molitor* (Pracros, 1982).Extracts of the neem tree *Azadirachta indica* have been traditionally used in India to control insect pests. Azadirachtin proved to be the dominating and most effective among several related limonoids present in the extracts, (Ley *et al.*, 1993, El-Gengaihi *et al.*, 2002, and Opender *et al.*, 2004).

Entomopathogenic nematodes have a considerable potential as biological control agent for various insect pests. Some nematode species belonging to Steinernematidae, in particular, have been successfully used against harmful species from several insect orders, (Weiser& Mracek, 1988 and Eculica *et.al.* 1997, Shamseldeen *et al.*, 1999; Alchanatis *et al.*, 2000; Navon *et al.*, 2002 and Pramila *et al* 2003).

The im of the present work is to study the combined effect between extract Suneem oil of neem tree and saponin mixture from alfalfa (root and aerial parts) on

the susceptibility of *S. littoralis* larvae to infection by the entomopathogenic nematode *S. feltiae* as possible means for use in programs of integrated pest management.

MATERIAL AND METHODS

Fourth instar larvae of the cotton leaf worm *Spodoptera littoralis* (Biosd) obtained from laboratory culture maintained on castor bean leaves *(Ricinus communis* L.) for successive generations at 25+ 2C and 65+5 % R.H. was used in this study.

Materials tested are: (1) Azadirachtin was routinely administered in the commercial neem seed extract called Suneem oil, which was obtained from the Sunida Exports, India. (2) Saponins were prepared from the roots (Oleszek *et al.*, 1990) and foliage (Oleszek *et al.*, 1992) of various varieties of alfalfa (*Medicago sativa* L., Leguminaceae).

Fourth instar larvae were starved for 4 hrs and then fed on a disc of castor leaves (4 cm diameter) previously dipped at the desired concentration (1, 0.5 and 0.25 ul/ml of Suneem oil) in Petri dishes (9 cm diameter) furnished with moistened filter paper to avoid desiccation. Control larvae were fed on fresh castor leaves sprayed with water only. Two replicates, each of 10 larvae, were used for each concentration. After 48 hrs of feeding on the treated leaves, the larvae were kept in clean Petri dishes (9 cm in diameter) bottomed with filter paper, containing the different concentrations of entomopathogenic nematodes *S. feltiae* (500, 250 and 125 ijs/ml). Nematodes isolated from different soil samples each 1 dm^3 in volume; the samples were transported to the laboratory and then place in peteri dishes (25 cm diameter) together with 20 larva of *Galleria mllonella* serving as bait for nematodes (Eculica *et al,* 1997). The nematodes were used in this experiments were obtained from rearing for several generations. Microscopic examination was used for estimating the dose (numbers) of ijs/ml. Petri dishes were incubated at 25°C. After 7 days, numbers of nematodes (males and females) were counted. Control larvae, which fed on untreated leaves, were kept in dishes containing the same concentrations of nematodes (500, 250 and 125 ijs).

Saponin compounds were extracted from alfalfa (roots or aerial parts). Discs of castor leaves were dipped for 3 minutes in the desired concentration of saponin root and aerial parts (at 100 and 10 ppm). The same technique was carried out as mentioned before (in Suneem oil).

Statistical analyses of the results were conducted using f test (ANOVA) (Hayslett, 1970).

RSULTS AND DISCUSSION

Effect of the plant extract (Suneem oil) on the susceptibility of *S. littoralis* to the infection by the entomopathogenic nematode, *S. feltiae* is illustrated in Table (1). Data show that numbers of nematodes in *S. littoralis* cadaver were decreased significantly by the increase in concentration of Suneem oil. At the highest concentration of 1 ul/ml of the plant extract and 500 ijs/ml of nematode adults, nematodes found in one cadaver averaged 87 and 95.5 (♂&♀). These averages decreased to 37.5&42 (♂&♀) nematodes/cadaver at 0.25 ul/ml of the extract, and 250 ijs/ml of nematode comparing to 62 and 66.5, (♂&♀) nematodes in the control. In addition, at the highest concentration of Suneem oil (1 ul/ml) and 125 ijs/ml, the average number of nematodes in each cadaver decreased to 17 and 18.5, (♂&♀) nematodes (Table I). At the lowest concentration of Suneem oil (0.25 ul/ml) and 125 ijs/ml, the average numbers of nematodes/cadaver were, 19.5&40. (♂&♀), compared to 23.3&53.5 (♂&♀), nematodes/cadaver in the control.

The percentage of invading nematodes (♂&♀) was markedly decreased, especially at higher concentrations of the plant extract. It decreased from 43.7 % at 500 ijs and 0.25 ul/ml of Suneem oil to only 28.4 % at 125 ijs and 1 ul/ml of Suneem oil. In control, the percentages of invading nematodes in cadavers were 55.3, 51.4 and 80 % at 500, 250 and 125 ijs, respectively.

Statistical analysis (ANOVA) showed the effect of different concentrations of Suneem oil on the susceptibility of *S. littoralis* to the infection by *S. feltiae*. Data in Table (I) show that at 1 ul /ml of Suneem oil, the decrease in concentration of nematodes from 500 to 125ijs resulted in significant decrease of both males and females numbers of invading nematodes in the body of *S. littoralis*. The same results were obtained in case of using other concentrations of Suneem oil (0.5, 0.25, ul /ml) as well as in the control. Also, at any dose of nematodes (ijs), the number of nematodes /cadaver decreased markedly by the increase in Suneem oil concentration. At the same time, numbers of nematodes /cadaver were markedly higher in control (untreated) larvae of *S. littoralis*, compared with any of the used Suneem oil concentrations. In this experiment, nematode males and females showed nearly the same response to Suneem oil concentration.

TABLE (I)

Effect of Suneem oil on the susceptibility of *S. littoralis* to infection by the nematode *S. feltiae.*

Conc. of nematodes [js/ml		Concentration of Suneem oil ul/ml							
		1		0.5		0.25		control	
		No. of nematodes / cadaver ± S.E.							
		♂	♀	♂	♀	♂	♀	♂	♀
500	Average ±S.E.	87 a C ±5.1	95.5 a D ±15.3	97.5 a B ±0.25	107.5 a C ±1.75	106 a B ±4	118.5 a B ±4.75	136 a A ±1.5	140.5 a A ±10.25
	% total	36.5		41		43.7		55.3	
250	Average ±S.E.	31.5 b B ±0.75	24.5 b D 2.25±	34 b B ±0.5	32 b C ±2	37.5 b B ±0.75	42 b B ±1	62 b A ±2.5	66.5 b A ±2.75
	% total	22.4		26.4		31.8		51.4	
125	Average ±S.E.	17 c A ±1	18.5 c D ±1.25	24.5 c A ±0.75	33.5 b C ±1.75	19.5 c A ±0.75	40.5 b B ±5.75	23.3 c A ±11.7	53.5 c A ±2.75
	% total	28.4		46.4		48		80	

For Male "F=4.47** LSD=13.8 AA=between suneem oil concentrations (n=4)
F=79.26** LSD=15.9 aa=between nematode concentration (n=3)
Fore Female "F=69.3** LSD=4.9 AA= between suneem oil concentrations (n=4)
"F=613.9** LSD=5.7 aa=between nematode concentration (n=3)

Data in Table (2) show the effect of saponin extracted from root and aerial parts of alfalfa on the susceptibility of *S. littoralis* 4th instar larvae to infection by *S. feltiae*.

Using aerial parts extract at 100 ppm, it was found that the average number of nematodes/cadaver decreased from 35 and 88 (♂ & ♀) at 500 ijs to 8 and 17 (♂ & ♀) by decreasing the concentration of nematodes to 125 ijs.At 10 ppm of saponin extract of alfalfa root, these numbers decreased from 31and 90 (♂ & ♀) at 500 ijs to 7 and 11 (♂ & ♀) nematodes/cadaver at 125 ijs, with total percent of (14.4 %). Saponin from alfalfa roots at 100 ppm, decreased the numbers of nematodes/cadaver from 32 and 55.5 ((♂ & ♀) at 500 ijs to 2.5 and 9.5 (♂ & ♀) at 125 ijs (9.6%). The total percentage of invading nematodes was markedly decreased by increasing the concentration of the plant extract, while highest numbers of nematodes/cadaver were obtained in control (4^{th} instar larvae of *S. littoralis* treated with nematodes only at 500, 250 and 125 ijs) comparing to the numbers of nematodes/cadaver obtained when the plant extract was applied, at any concentration.

Differences between saponin mixture (Arial and root parts) showed nearly the same effect within the same concentration of nematodes. In addition, the different concentration of plant extract showed slight effect on the numbers of nematodes found in the host cadaver. It was clearly shown that the numbers of *S. feltiae* females were markedly higher than the numbers of males in *S. littoralis* cadavers. In all cases, numbers of nematodes were significantly higher in control comparing with any treatment by saponin mixture of alfalfa.

The compatibility of entomopathogenic nematodes and various plant extracts has been past studied. The plant extracts tested in this study caused mortality and reduction in nematode infectivity especially at higher concentrations of Suneem oil. This may be due to the slow movement of nematodes in of the oily nature of Suneem oil and to low concentration of oxygen. The results are in agreement with the finding of (Hara and Kaya, 1983; Rovesti and Deseo, 1990 and Hussaini *et al.*, 2001), who studied the compatibility of some commercial pesticides (e.g.: fungicides, acaricides, herbicides, synthetic pyrthroid fenvalerate; insect growth regulators, endosulfan...etc) at different concentrations with entomopathogenic nematodes *Steinernema* spp. The results indicated that the infective juvenile stages (ijs) of this nematode species tolerated most of the tested chemicals. Meanwhile, Stark, (1996), Pezowicz, (1997) and Hussaini *et al*, (2001). used the *Steinernema* species in combination with Margozan O, Neemix and Neem oil as effective insecticides applied at low concentration. Neem product was found

TABLE (II)

Effect of saponin mixture from alfalfa (root and aerial parts) on the susceptibility of *S. littoralis* larvae to infection by the nematode *S. feltiae*.

Conc., of nematodes	Concentration of plant extract								control	
	Saponin compound from aerial parts of alfalfa				Saponin compound from root parts of alfalfa					
	100 ppm		10ppm		100 ppm		10 ppm			
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
500 ijs	35 a B ±2.5	88 a A ±9	43 a B ±3.5	97.5 a A ±1.25	32 a B ±7.5	55.5 a B ±4.25	31 a B ±2.5	90 a A ±3.5	113.5 a A ±7.75	89.5 a A ±3.25
	24.6 %		28.1 %		17.5 %		24.2 %		40.6 %	
250 ijs	7.5 b B ±0.75	29 a A ±3.5	14.5 b B ±2.75	40.5 b A ±1.25	6.5 b B ±0.25	18 b B ±2.5	10.5 b B ±1.25	21.5 b B ±2.75	42.5 b B 2.25	49 b A ±4
	14.6 %		22.0 %		11.8 %		12.8 %		36.6 %	
125 ijs	8 b A ±1.5	17 a B ±1.5	10.5 b A ±1.25	20 b B 2.5	2.5 b C ±0.25	9.5 b B ±1	7 b B ±1	11 b B ±1.5	24 c A ±1	43 b A ±2
	20.0 %		24.4 %		9.6 %		14.4 %		53.6 %	

Fore Male "F=11.6**" LSD=21.8 AA=between suneem oil concentrations (n=5)
F=5.4* " LSD=16.9 aa=between nematode concentration (n=3)
Fore Female"F=2.1 not sig. AA= between suneem oil concentrations (n=5)
F=5.4* LSD=16.9 aa=between nematode concentration (n=3)

to be safe for all nematode populations. The entomopathogenic nematodes may be viable candidates for an integrated approach.

SUMMARY

Laboratory experiments were carried out to determine the combined effect of using entomopathogenic nematodes with plant extracts on the target pest, *Spodoptera littoralis* suneem oil and saponin compounds from alfalfa roots and aerial parts reduced the numbers of *Steinernema feltiae nemato*des in *S. littoralis* cadaver when applied together on 4th instar larvae. Higher concentrations of both plant extracts reduced the number of nematodes developed inside the cadaver of the host larvae.

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