Evaluation of some Entomopathogenic Nematode Strains against the Cotton Leaf worm *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) under controlled conditions

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ABSTRACT

Laboratory and greenhouse tests were conducted determine the virulence of some entomopathogenic nematodes against 4th instar larvae of the cotton leafworm *Spodoptera littoralis* at. Laboratory experiments were performed at two constant temperatures of 15 and 25°C with three concentrations of 100, 50 and 25 IJs/dish. Greenhouse experiments were performed at the same constant temperatures with three concentrations of 3200, 1600 and 800 IJs/pot. Seven strains of nematodes were tested. They showed that not all of them were pathogenic to *S. littoralis* larvae, inspite of their ability to invade the larvae at the two different temperatures. At the highest concentration of 100 IJs/dish, three species (*S. kraussei* strain-69, *S. carpocapse* strain NCR, and *S. feltiae* strain žehrovice) where they caused higher mortalities of 100, 100 and 93.3% among *S. littoralis* larvae respectively than did *S. feltiae* strain Holovousy (60%), *S. kraussei* strain-D (53%), *S. glaseri* (46.7%), and *S. cubanum* (40%). At 25°C, the mortality never reached 100%, where, *S. cubanum* achieved the highest (86.7%) mortality while other nematode strains caused mortalities ranged between 33.3 and 73.3%. At lower concentrations of 50 and 25 IJs, the host mortality decreased proportionally for each nematode strain. The efficiency of all tested nematode strains was higher in loamy soil than in sandy soil. Temperature showed various effects on the virulence of different nematode strains in the greenhouse as well as in the laboratory experiments.

Key Words: *Spdoptera littoralis*, entomopathogenic nematodes, Steinernematidae, Infective juvenile nematodes.

INTRODUCTION

The cotton leafworm *Sopdoptera littoralis*(Boisd.) (Lep., Noctuidae) is an insect pest of economic importance to many crops and vegetables. Although attempts to control this pest with chemical insecticides brought some success, the development of insecticide resistance and pesticide residuals in nature have limited the application of chemical control. This experience encouraged the search for alternative control measures to replace chemical insecticides. (Poiner 1979).

Entomopathogenic nematodes in the families Steinernematidae and Heterorhabditidae are soil inhabiting insect parasites associated with mutualistic bacteria that kills their insect hosts in 24 to 48 hours. The infective 3rd stage juvenile, the only free-living stage harbors the bacterium in its intestine. Upon encountering a susceptible host, the infective juvenile enters it either through natural openings e.g. mouth, anus, or spiracles or direct penetration of weak spots in the cuticle and penetrates into the hemocoel where it releases the bacterium (Poiner 1979 and Kaya *et.al* 1993). The bacterium multiplies, killing the host within 48hours The nematode feeds on the bacterial cells and simple decomposed insect tissues, , reproduces, goes through 2-3 generations, and eventually produces a final generation of infective juveniles.

Glazer and Navon (1990) tested the pathogenicity of entomopathogenic nematodes belong to the genera *Steinernema* and *Heterorhabditids* against *Heliothis armigera* Hubner in the laboratory. Complete mortality was achieved with 200 infective juveniles (IJs) of *S. feltiae* Filipiev, strain 'All'. The LD_{50} was 54 IJs per insect. Similar results were obtained with other nematode strains. The youngest instars were the most susceptible to nematode infection. Eight hours of insect exposure to the nematode were needed to induce >80% mortality.

West and Vrain 1997 considered four Steinernematid strains, *Steinernema carpocapsae* All-strain, *S. carpocapsae* Umea-strain, *S. feltiae* 1 l-c strain, and *S. feltiae* strain-27 in laboratory and field tests, as potential agents for the control of the black army cutworm, *Actebia fennica* (Tauscher).Furthermore, Fuxa *et al.*, 1988 reported that the fall army worm *Spodoptera frugiperda* and the black cut worm *Agrotis ipsilon* are susceptible to infection by *S. carpocapsae* in both laboratory and field trials. A positive releationship between the concentration of nematodes and percentage of host mortality was mentioned by many authors (Morris, 1985; Morris *et al.*, 1990; Glazer, 1992 and Epsky & Capinera, 1993, 1994). Concerning temperature tolerance

Mraček *et al.* (1997) found that cold active canadian isolates except *Steinernema kraussei* (69) isolat were highly infective at the relatively low tempertatures of 4, 7 and 10°C and also found that these isolates infected the *Galleria* larvae at low tempertaures in a significantely greater number than did warm active *Steinernema cubanum.*

Shameldean *et al.* (*1998*) *tested* effects *of* Soil temperature, exposure time and host introduction on pathogenicity and reproduction of an Egyptian isolate of *H. bacteriophora* (EASD98) in the laboratory. A 100% mortality of *S. littoralis* was achieved whether the host insects were introduced before or after incubation for 5, 18, or 60 hours exposure at 10, 15, and 30 deg C. In another trial, the reproduction rate of *H. bacteriophora* EASD98 at 10, 15, and 30 deg C was greater than another isolate HP88 and of *Steinernema riobravae*, but failed to reproduce at 35 deg C. *H. bacteriophora* EASD88 yielded more infective juveniles (Pless than or equal to 0.01) than *S. riobravae* at 35 deg C. It was concluded that entomopathogenic nematode species and/or strains are governed by the temperature range of their original localities

The study aim to evaluate some forigen strains of entomopathogenic nematode against *S.littoralis* under controlled condations.

MATERIALS AND METHODS

Steinernema species *S. krauassei* strain (69) and strain (D) from Canada, *S. feltiae* strains (holovousy and žehrovice) from Czech Rep., *S. carpocapsae* strain-NCR from Russan, *S. Glaseri* strain (NC) North Carolina, USA and *S. cubanum* strain(Cuban) from Cuba were tested for their potency against *S. littoralis* 4^{th} instar larvae. All strains were maintained on the greeter wax moth larvae *Galleria mellonella* (L.). The infective juvenile nematodes (IJs) were stored in fresh tap water and held in refrigerator at 4-6°C, except the Cuban strain which was held in room temperature (25°C) for one month until used.

Insect colony

The Egyptian cotton leaf worm, *S. littoralis* colony was obtained as pupae. All experiminted larvae were obtained from a laboratory colony reared on an artificial diet (*Premix diet [Manduca premix-Heliothis premix]* produced by Stonefly Industries, Inc. USA.).This diet was prepared with premix powder 25% weight, 75% tap water weight or vol., 2ml acetic acid and 1 ml formalin (37% formaldehyde) and mixed together by a blender .The insects were reared in a conditioned insect rearing room, at 25±2°C and 65±5% RH, and photoperiod of 16:8 (L : D) h.

Laboratory bioassay

Experiments were carried out in Petri dishes (9.0cm diameter). Three concentrations of 25, 50 and 100 IJs/500µl deionised water were added, each, into the centre of a filter paper (Whatman # 9.0cm.d.), placed in a Petri dish (9cm diameter).. Five larvae were added to the centre of each dish. 10 replicates were used for each strain. Experiments were performed at temperatures of 15 and 25°C.

Greenhouse experiments:

Five pots were filled with sandy soil and another fives were also filled with loamy soil were used. Each pot was inoculated with either of the tested concentrations (3200, 1600 and 800IJs) in 2ml water. The inoculum was applied to the soil surface of each pot. Ten 4^{th} instar larvae of *S. littoralis* were transferred to each pot.

The infectivity of nematodes strains to *S. littoralis* larvae was assessed by percentage of mortality and number of IJs penetrating and successfully developed to adults. The number of nematodes invading the host was determined by dissecting of the insect cadavers 5-7 days after initial exposure to the nematodes.

Invasion efficiency, which was estimated as the percentage of the infective nematode juveniles that successfully invade the host and develop, was calculated from the number of nematodes recovered during dissection divided by the number of nematodes used in each treatment, multiplied by 100.

Invasion efficiency %=

$$\frac{\text{No. of nematodes in dead larval body}}{\text{No. of Applied nematodes}} \times 100$$

RESULTS AND DISCUSSION

Effect of temperature

At 15°C and 100 Ijs inoculum, *S. kraussei* strains 69, *S. carpocapsae* strain NCR and *S. feltiae* strain žehrovice caused higher mortalities for *S. littoralis* larvae (100, 100 and 93.3%), respectively, than did by *S. feltiae* strain Holovousy (60%), *S. kraussei* strain D (53 %), *S. Glaseri* (46.7%) and *S. Cubanum* (40 %) Table (1).

Meanehile, at 25°C, the mortality never attained 100%. *S. cubanum* caused a higher mortality (86.7%) than did by the other nematode strains where obtained mortalities ranged between (33.3 and 73.3%) Table (2). The above data revealed that *S. kraussei* strains 69 and *S. cubanum* were more effective at relatively lower and higher temperatures respectively

Nematode infectivity was affected by changing in temperature, generally the majority of larval mortality was faster at higher temperature of 25°C, where the most of larval mortality was achieved after 5 days, while at15°C, the effect of nematode began after almost 7 days (*S. cubanum*) and 5 days with *S. kraussei* .

Our results were similar to those published by Griffin & Downes (1991), who exposed larvae of *G mellonella* to four heterorhabditid isolates at 5, 7, 9, 12 and 20°C. They found significant differences among isolates in the number of infective juveniles that entered the host Also Mraček *et al.* (1997) recorded that cold active canadian isolates except *Steinernema kraussei* (69) isolat and *Steinernema cubanum* were active at lower than 15°C.

Effect of nematode concentrations

Mortality percentages were positively correlated with inoculum concentration in all nematode strains. The percentage of mortality within all strains ranged between 33.0 and 86.7% for 100IJs, 6.7 and 66.7% for 50 IJs and 6.7 and 46.7% for 25 IJs at 25°C. At 15°C, the mortality ranged from 40 to 100% for 100Ijs, 26.7 to 80% for 50 IJs and 13.3 to 64.7 % for 25 IJs. (Tables 1&2)

At 15°C, the mean number of adult nematodes that successfully invaded and developed in *Spodoptera* cadaver was based on the corresponding IJs inoculate (25, 50 and 25IJs/dish), in case of *S. kraussei* strain 69, it was 3.16 3.99 and 8.7 nematoda adult per larva. While with *S. cubanum* it was 3.5, 4.49 and 6.3 per larva at those nematode concentrations, respectively.

Similarly, Epsky & Capinera (1993) assumed that nematodes invasion ability was generally poor for the fall army worm, *S. frugiperda* or the black cut worm, *Agrotis ipsilon* with only 10-50% of applied nematodes caused successfully, the host mortality. The number of infective juveniles invading the host was significantly affected by changing in the bioassay conditions. They reported a direct relationship between the number of nematodes applied and the percentage of host mortality where the mortality increased by increasing concentration of nematodes applied, and vice versa with our results . Also A positive releationship between the concentration of nematodes and percentage of host mortality was mentioned by many authors (Morris *et al.*, 1990; Glazer, 1992 and Epsky & Capinera, 1994).

Effect of nematode strain

Penetration and nematode development affected differently by the type of nematode strain At 15°C, *S. kraussei* strain 69 at 100 IJs.scored the highest penetration rate (43.3% while, the lowest one was for *S. glaseri* strain-NCR (10.9%). Similarly, at 25°C, the highest penetration rate was for *S. kraussei* strain 69 (45.3%) and

Table (1): Effect of some strains of entomopathogenic nematode (Stienernematid) on the 4th larval instar of *Spodoptera littoralis* at 15°C

strain	% of mortality			mean nematodes /larva±S.E			% of ivading efficiency		
	100ijs/dish	50ijs/dish	25ijs/dish	100ijs/dish	50ijs/dish	25ijs/dish	100ijs/dish	50ijs/dish	25ijs/dish
S.K 69	100	80	40	8.7±0.93	3.99±1.6	3.16±1.2	43.3	31.9	25.3
S.c	100	86.7	64.7	4.5±0.09	3.36±0.53	1.4±0.4	22.4	29.3	13.06
Holov.	60	26.7	20	8.4±0.93	1.73±0.81	0.48±0.3	25.2	4.6	1.92
NCR	46.7	26.7	26.7	4.7±0.83	2.16±0.38	0.68±0.42	10.9	1.44	2.77
D.	53	46.7	13.3	6.6±1.3	5.99±1.7	2.62±0.50	17.5	27.9	6.98
Cuban	40	40	13.3	6.3±2.1	4.4±1.1	3.5±1.1	17.6	17.9	18.66
žehrov.	93.3	80	26.7	8.64±1.4	6.8±1.2	2.55±0.80	31.7	67.2	30.6
Control	0	13.3	6.7	0.0	0.0	0.0	0	0	0

Table (2): Effect of some strains of entomopathogenic nematode (Stienernematid) on the 4th larval instar of *Spodoptera littoralis* at 25°C

strain	% of mortality			mean nematodes /larva±S.E			% of ivading efficiency		
	100ijs/dish	50ijs/dish	25ijs/dish	100ijs/dish	50ijs/dish	25ijs/dish	100ijs/dish	50ijs/dish	25ijs/dish
S.K.69	73.3	66.7	46.7	12.4±1.3	8.26±0.94	4.73±1.7	45.3	55	44.1
S.c	73.3	53	40	4.72±1.8	2.51±1.3	1.1±0.41	17.3	13.3	8.8
Holov.	73.3	6.7	6.7	4.14±0.63	0.63±0.83	0.2±0.16	15.2	0.4	0.4
NC	33.3	13.3	6.7	0.6±0.36	0.7±0.63	0.12±0.12	2.7	0.93	0.16
D.	53	40	6.7	6.12±1.5	2.2±0.61	1.0±0.12	16.3	8.8	1.3
Cuban	86.7	66.7	46.7	5.99±1.2	4.82±0.81	3.6±0.6	25.9	32.1	33.6
žehrov.	73.3	40	40	8.8±0.73	4.8±1.1	2.4±0.5	20.5	19.4	19.2
Control	0	0	13.3	0.0	0.0	0.0	0	0	0

Table (3): Effect of different entomopathogenic nematodes against *Spodoptera littoralis* larvae in two types of soil at 15°C

Nematode Strain	Sandy soil						Loamy soil					
	Mortality %			Mean No. of nematodes/larva ± S.E			Mortality %			Mean No. of nematodes/larva ± S.E		
IJs/pot	3200	1600	800	3200	1600	800	3200	1600	800	3200	1600	800
S.K. 69	100	80	48.2	23.1±5.2	20.4±3.2	19.2±2.3	78.1	74.6	69.4	26.3±4.4	22.5±4.1	19.4±3.4
S.c	59.4	48.2	38.2	28.2±2.2	17.1±3.2	11.6±2.2	54.8	45.6	42.6	32.4±2.8	16.4±4.2	12.2±2.8
S.F.Holo .	63.6	68.4	58.4	18.2±4.2	27.6±4.2.	16.4±1.4	62.6	72.4	58.8	20.4±2.8	14.4±6.2	16.6±2.2
S.g. N.C	88.6	86.6	62.2	26.14±4.2	22.3±4.2	20.2±2.2	89.8	86.5	79.1	30.4±4.8	24.2±3.2	22.2±4.8
D.	52.4	54.8	52.2	36.1±5.2	28.02±3.2	24.2±4.2	64.2	58.4	54.1	38.4	29.2±1.2	21.8±4.2
S. Cuban	58.2	50.2	13.4	42.2	10.01±1.8	8.2±1.6	54.4	48.8	44.2	12.2±4.2	12.1±1.2	8.6±2.6
žehrovice	68.2	64.4	58.4	19.2±5.2	18.1±1.6	14.2±2.6	71.1	66.6	62	22±2.2	19.8±1.2	16.1±3.2
Control	0	4.8	0	0	0	0	0	0	0	0	0	0

IJs= infective juveniles nematode

Table (4): Effect of different entomopathogenic nematodes against *Spodoptera littoralis* larvae in two types of soil at 25°C

Nematode strains	Sandy soil						Loamy soil					
	Mortality %			Mean No. of nematodes/larva±S.E			Mortality %			Mean No. of nematodes/larva ± S.E		
IJs/pot	3200	1600	800	3200	1600	800	3200	1600	800	3200	1600	800
S.K. 69	69.4	64.5	61.2	33.1±5.2	26.4±3.2	19.5±4.3	70.1	68.9	62.4	31.3±4.4	24.5±5.1	17.6±3.4
S.c	49.4	44.2	30.2	18.2±2.2	14.1±3.2	8.6±2.2	58.8	49.6	42.6	22.4±4.8	16.6±4.2	12.4±3.8
S.F.Holov.	33.6	38.4	18.4	8.2±4.2	7.6±4.2	6.6±1.4	42.6	32.4	28.8	20.4±2.8	14.4±6.2	11.6±2.2
S.g. N.C	44.4	40.2	36.8	28.4±6.2	24.1±4.2	18.6±2.2	49.2	42.8	42.6	32.4±6.8	26.2±6.2	18.4±2.8
D.	50.6	48.8	42.6	38.1±5.2	30.01±3.2	22.22±4.2	65.2	52.4	40.2	48.1±5.2	34.2±1.2	21.02±6.2
S. Cuban	79.6	77.4	69.2	63.5±4	51.1±4.8	38.2±4.6	80.6	80.2	76.2	66.1±4	54.4±	42.8±6.2
žehrovice	42.2	38.4	28.4	12.2±4.2	10.01±1.8	8.2±1.6	48.8	46.6	32.4	16.2±2.2	12.1±1.2	8.4±2.6
control	0	4.8	0	0	0	0	0	0	0	0	0	0

IJs= infective juveniles nematode

the lowest was for *S. glaseri* strain NCR (2.7%).

The pervious data shown in (Tables1,2) clarified that there was a positive relationship between the concentration of nematodes, the percentage of mortality and the total mean number of nematode adults in each cadaver, (100, 50 and 25IJs/dish). The mean number of nematodes successfully invaded and developed to adults (male & female) within each cadaver with *S. cubanum* were (6.3, 4.4 and 3.5, respectively at 15°C) and 8.8, 4.8 and 2.4 respectively. at 25°C. The same trend of concentrations effects was similarly shown at 15°C.

These results agree with Mraček *et al.* (1997), they found that cold active canadian isolates except *Steinernema kraussei* (69) isolat were highly infective at the relatively low tempertatures of 4, 7 and 10°C and also found that these isolates infected the *Galleria* larvae at low tempertaures in a significantely greater number than did warm active *Steinernema cubanum.* Shameldean *et al.* (*1998*)) found that, pathogenicity and reproduction of an Egyptian isolate of *Heterorhabditis.bacteriophora* (EASD98) were affected at 10, 15, and 30°C, compared with *H. bacteriophora* EASD88 and *Steinernema riobravae*.

Effect of soil texture

Results in Tables (3 and 4) show that all tested steinernematid strains were capable to infect the 4th larval instar of *S. littoralis* at two different soils (sandy and loamy) at (800, 1600 and 3200IJs/pot concentrations). All strains at a lower temperature 15°C achieved more than 50 percent of larval mortality except the Cuban strain, where it was clear that all tested strains were affected by the type of soil and temperature and all strains caused lower effects in sandy soil than in loamy soil. Strain SK-69 give 100% mortality at 3200IJs/pot at loamy soil and 78.1 at sandy soil. The means of nematodes which successfully invaded the larvae of *S. littorals* and developed inside it, in Loamy soil were higher than in the sandy soil at different concentrations and temperatures (15 and 25°C) Tables (3 and 4). The efficiency of all nematode strains were higher in loamy soils than in the sandy soil.

The aforementioned results indicated that the laboratory efficiency of steinernematid nematodes against the 4th larval instar of *S littoralis* was influenced by nematode inoculum levels, different nematode strain, temperatures and texture type of soil..

The present results agree with the work done by Dimetry Nadia *et al.* (2002) who trialed to control of the hairy rose Scarabaeid beetle, *Tropinota squalida,* by using *S. cubanum, S. kraussei* at 25°C and 15°C respectively..Also Mogahed (1997) found that all tested strains of nematodes (*Neoaplictana carpocapsae, Heterorrhabditis heliothidis* and *Heterorrhabditis bacteriophora*) had greater effect against the adult of *T. squalida* hiding in loamy soil than in the sandy soil. These work assumed with Shamseldean *et al.*(1998) they concluded that entomopathogenic nematode species and/or strains are governed by the temperature range of their original localities

Results indicated that the efficiency of steinernematid nematodes on the 4th larval instar of *S. littoralis* was influenced by the concentrations of nematode inoculum, different nematode strains, temperatures and soil textures.

According to the field studies, *S. cubanum* is expected to reach its reasonable activity in mid day where temperatures are almost above 20°C. Meanwhile, *S. kraussei* will be highly effective during the night, when the temperatures are about or below 15°C. Thus, it could be concluded that both the foreign strains (*Steinernema cubanum*) at 25°C and *S. kraussei* at 15°C could be integrated successfully for controlling the Egyptian cotton leaf worm in the field.

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REFERENCES

Dimetry Nadia Z., Hussein H.M., Zidan, Z. H.and Iss-Hak,R.R. 2002. A successful trial for the control of the hairy rose Scarabaeid beetle, *Tropinota squalida* Scop., using Entomopathogenic nematodes as a bioagent. Biopesticides and Pest Management.vol. 2: 309-316.

Epsky, Nancy. D., and Capinera. J. L. 1993. Quantification of invasion of two srains of *Steinernema carpocapsae* (Weiser) into three lepidopteran larvae. Journal of Nematology 25(2): 173-180.

Epsky, Nancy.D and Capinera, J.L. 1994. Invasion efficiency as a measure of efficacy of the entomogenous nematode, *Steinernema carpocapsae* (Rhabditida : Steinernematidae). J. econ. Ent. 87(2):366-370.

Fuxa, J. R., Richter, A. R., and Agudelo-Silva F. 1988. Effect of host age nematode strain on susceptibility of *Spodoptera frugiperda* to *Steinernema feltiae*. Journal of Nematology 20: 91-95.

Glazer I. 1992. Invasion rate as a measure of infectivity of Steinernematid and Heterorhabditid nematodes to insects. J. Invertebr. Pathol. 59: 90-94.

Glazer I. and Navon, A. 1990. Activity and Persistence of Entomoparsitic Nematodes tested against *Heliothis armigera* (Lepidoptera: Noctuidae). J. Econ. Entomol. 83(5): 1795-1800.

Griffin, C. T., and Downes, J. 1991. Low temprature activity in *Heterorhabditis* sp. (Nematoda:Heterorhabditidae). Nematologica 37, 83-91.

Kaya, H. K.; Hara, A. H. and Gaugler, R. 1993. Entomopathogenic nematodes. Annual Review of Entomology 38: 181-206

Mogahed, M.I. 1997. Potential role of different bioagents in the management of the hairy beetle, *Tropinota squalida* Scop. (Fam. Scarabaeidae, Coleoptera). J. Egypt Ger. Soc. Zool. 22 (E); 283-290.

Morris, O. N. 1985. Susceptibility of 31 species of agriculture insect pests to entomogenous nematodes *Steinernema feltiae* and *Heterorhabditis bacteriophora*. Candian Entomologist 117,401-407.

Morris, O. N., Converse, V., and Harding, J. 1990. Virulence of entomopathogenic nematode-bacteria complexes for larvae of noctuids, a geometrid and a pyralid. Candian Entomologist 122, 309-320.

Mraček,Z., Bečvāř, S., Řezāč, P., Kindlmann P. and Webster,J. M. 1997. Candain steinernematid (Nematoda) isolates and their infective, under cold condition, to greater wax moth (*Galleria mellonella*) larvae. .Biological Control. (8): 160-164.

Poiner, G. O. Jr. 1979. Nematodes for biological control of insects. CRC, Boca Raton, FL. 1986. Entomogenous nematodes, pp. 95-121. In B. D. Franz [ed.], Biological plant and health production. Fische, Stuttgart.

Shamseldean M.M., Abd-Elgawad M.M., Atwa A.A. (1998). Effect of soil temperature, exposure time and host introduction on pathogenicity and recycling of Heterorhabditis bacteriophora strain EASD98 and its comparison with H. bacteriophora strain HP88 and Steinernema riobravae from USA. International Journal of Nematology. 8:(1) 71-76. .

West R.J. and Vrain, T. C. 1997. Nematode control of black army cutworm (Lepidoptera: Noctuidae) under laboratory and field condations. Canadain Entomologist, vol.129: 229-239.