

EVALUATION OF THREE ENTOMOPATHOGENIC NEMATODES (STEINERNEMATIDAE) FOR THE CONTROL OF THE MEDITERRANEAN CLIMBING CUTWORM, *Spodoptera littoralis* UNDER LABORATORY AND GREENHOUSE CONDITIONS

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ABSTRACT

The virulence and invading efficiency of three *Steinernema* nematode species on the last larval instar of *Spodoptera littoralis* (Boisd.) as well as, average number of damaged leaves and consumed leaf area were investigated in laboratory and greenhouse experiments. The results proved that the indigenous, Mediterranean species, *Steinernema apuliae*, at 500 and 1500 IJs/pot was the most virulent species in laboratory and greenhouse experiments respectively. It also showed significantly higher invading efficiency than did other nematode species, except that when used at a high concentration of 2700 IJs./pot. In green house. The numbers of adult nematodes found in the cadavers of *S. littoralis* larvae were significantly different among nematode species at 500 IJs/dish in the laboratory and 1500 IJs/pot concentration in the greenhouse. The number of adult females found in the cadavers of this insect larvae was always higher than the number of males. Foliage application of *S. apuliae* resulted in a significant reduction in the number of damaged leaves and revealed a lower index of damage as compared with that in the control. As conclusion, *S. apuliae* has significant the capability of controlling potentiality in the management of the Mediterranean climbing cutworm *S.littoralis* in Egyptian and European greenhouses.

Keywords *Steinernema* spp., *Spodoptera littoralis* (Boisd), Biological control

INTRODUCTION

ENTOMOPATHOGENIC NEMATODES (EPNs) in the families Steinernematidae and Heterorhabditidae are of considerable interest because of their use as biological control agents of insect pests (Poinar 1979, Woodring and Kaya 1988), that are an environmentally acceptable alternative to chemical control methods. The infective 3rd – stage juvenile (IJ) of these nematodes, the only soil free-living stage, carries mutualistic bacteria in its intestine (Boemare et al. 1993). Upon encountering a susceptible insect host,

the IJ enters it through a natural body opening (mouth, anus, or spiracles) or, rarely, through the thin intersegmental membrane and releases its mutualistic bacteria into the hemocoel, which then causes a lethal septicaemia (Kaya et al. 1993).

Several species of the genus *Spodoptera* are recognised as quarantinable plant pests by UK and EU legislation. These include Mediterranean climbing cutworm *Spodoptera littoralis* (Boisd.), which occurs in Mediterranean Europe and Africa. Mediterranean Europe and in glasshouses in northwest EU, on, for example, imported

S. littoralis is a destructive insect pest of wide spectrum of forage crops and vegetables in subtropical and tropical zones and has the potential to cause a serious damage to field cultivation as well as in greenhouses (Salama et al. 1970). The larval stages feed on a wide range of plants, including edible ones produce and ornamentals. Many *Spodoptera* populations developed extremely resistant strains to many pesticides and control measures become exceptionally difficult to control. It is important that a comprehensive management program including the utilization of entomopathogens has to be implemented (Glazer et al. 1991).

Despite the ability of EPNs to parasitize many different insect species, including many economic pests (Poinar, 1979), they have been only sporadically used to control *Spodoptera* spp. Larvae of the fall armyworm, *S. frugiperda*, and of the black cutworm, *Agrotis ipsilon* Hufnagel, were found susceptible to infection by *Steinernema carpocapsae* (Weiser) in both laboratory and field trials, as shown by Capinera et al. (1988) and Richter and Fuxa (1990). However, in tests by Epsky and Capinera (1993) using the Mexican strain and the All strain of *S. carpocapsae* only 10-50% of the nematodes infected *S. frugiperda*. The percentage of infective juveniles invading the host (invasion efficiency) was positively related to the increase in the period of host exposure and to the number of hosts per arena. However, it was negatively related to increase in a substrate surface area per host, and or been not affected by the applied number of nematodes

S. littoralis is a major pest in Egyptian cultivation and chemical control commonly fail to decrease this pest. Therefore, we focused our research on testing some entomopathogenic nematodes biological control measure, such as entomopathogenic nematodes. A laboratory and greenhouse experiments were conducted to determine the efficacy of three steinernematid nematode species (including one indigenous species from the Mediterranean region) against *S. littoralis* larvae, in the event that the indigenous species might be more effective than the imported (foreign ones) in the control of *Spodoptera* species than the other two. (Duncan et al. 2003)

MATERIALS AND METHODS

Nematode cultures. The entomopathogenic nematodes *Steinernema oregonense* Liu & Berry (from Oregon, USA), *S. arenarium* Artyukhovsky (from Russia) and *S. apuliae* Triggiani, Mráček & Reid (indigenous species, from Italy) were propagated by passing through the late instar of the (Greater wax moth) *Galleria mellonella* L. adopting of Dutky et al. (1964). Infective

juveniles were harvested from nematode water traps as described by White (1929). The IJs were stored for three weeks in darkness at 15°C in deionized water and then examined microscopically for their viability before being used in the experiments.

Insect culture. The Mediterranean climbing cutworm, *S. littoralis*, was obtained from a laboratory colony reared on an artificial diet (Premix diet [Manduca premix-Heliothis premix] produced by Stonefly Industries, Inc. USA.). This diet consisted of Premix powder (25% w/w), tap water (75% w/w or v/w), 2ml acetic acid and 1 ml formalin (37% formaldehyde) mixed together in blender. The insects were reared in an insect rearing room in 15-cm-diameter petri dishes at 25±2°C, 65±5% RH, and photoperiod of 16:8h(L: D).

Laboratory studies – Bioassay procedure. Groups of newly ecdysed last instars larvae of *S. littoralis* (ten larvae/ groups) were starved for 2-4h. Then exposed each, in each a Petri dish, to one of the three different species of EPNs for 48h. An aqueous suspension of EPNs at a dose of 500 IJs per dish was applied to a 9-cm-diameter water moisted filter paper (Whatman no.1) and placed in each petri dish. Five replicates/dish

Were used (10 larvae/each) per each inoculum concentration.

After exposure to the nematodes, both dead and living insect larvae were rinsed in tap water and transferred to other petri dishes. Final insect mortality was recorded 72h after the initial exposure. Dead larvae were kept in moist conditions for an additional 3-4 days, to obtain the 1st adult nematode generation, then dissected individually in deionized water. The number of male and female nematodes in the larval cadavers was counted using a stereoscopic microscope, Arsenal model SZP 1102 ZOOM.

Invasion efficiency, estimated according to Epsky and Capinera (1993), as the percentage of IJs that successfully invaded the host was calculated from the number of adult nematodes recovered from the dissected cadavers (summed from all hosts in trials with multiple hosts per petri dish arena) divided by the number of IJs applied per dish (nematode dose), multiplied by 100.

Greenhouse tests. The experiments were conducted in the greenhouse at the Institute of Entomology (České Budějovice, CZ) on one-month-old potato, Superior cultivar, plants.. Each 6-litre pot containing three potato plants, each with 6 to 8 leaves, were maintained under conditions resembling the field situation in Egypt during early spring (20-25°C, 70-80% RH, and 14:10 L:D).

The nematode dose, of either 1500 IJs or 2700 IJs per pot in aqueous solution, was sprayed over the top of each plant and onto the soil surface using a hand sprayer. Nematodes were applied after sunset (just before feeding activity of the *Spodoptera* larvae) in an aqueous suspension of 25 ml/plant, and then each pot was infested with 10 newly last instar larvae of *S. littoralis*. Each pot was covered with a white muslin cage to prevent larval escape. Control treatment pots were sprayed with the same volume of uncontaminated water.

Larval mortality was recorded after 3 days and nematode invading efficacies as well as the number of male and female nematodes per larval cadaver determined five days post treatment. Leaf damage caused by insect larvae was evaluated in those plants sprayed with 2700 IJs. Estimation leaf

damage was based on the number of leaves damaged or consumed by feeding insect larvae in each replicate, according to Glazer *et al.* (1992), where four categories represented (1) 0-25%, (2) 26-50%, (3) 51-75%, and (4) 76-100% consumption of the leaf area. Consumed area of all leaves per pot was expressed as an index of damage and calculated as the average of percentages of consumed leaf areas from all damaged leaves per pot.

Statistical analyses. The effect of different concentrations of IJs on the larval mortality, IJ efficiency, and the number of male and female nematodes in larval cadavers were determined using One-way ANOVA in both laboratory and greenhouse experiments. The differences between nematode species were tested using Post-hoc comparisons with Tukey HSD test. The differences in larval mortality, IJ invading efficiency and the number of male and female nematodes in larval cadavers at 1500 IJs/pot and 2700 IJs/pot concentrations were tested using Two-way ANOVA with interactions (concentration* and nematode species). arcsine transformations for proportions was not used as data normality and variance homogeneity did not violate ANOVA assumptions.

The effect of foliage application of three nematode species on the mean number of damaged leaves and total consumed leaf area per replicate was compared with that in the control, using t-tests for independent samples with the associated Bonferroni correction.

RESULTS

Laboratory experiments. At a concentration of 500 IJs/dish, *S. apuliae* caused significantly higher mortality of the insect larvae than did the other nematode species (Table 1). *S. apuliae* had also the highest invading efficiency, which corresponds to the highest mean number of established nematodes in larval cadavers (Table 1, 2).

Table1. The impact of three *Steinernema* nematode species on *Spodoptera littoralis* larval mortality and nematode invading efficiency in laboratory and greenhouse experiments using different concentrations of infective juveniles (IJ) (mean \pm SE).

Effects	<i>S. apuliae</i>	<i>S. oregonense</i>	<i>S. arenarium</i>	N	F-test	P-value
500 IJs/dish in laboratory:						
% of mortality	100.0 \pm 0.0 ^a	66.0 \pm 2.4 ^b	74.0 \pm 5.1 ^b	5	29.6	0.0001
%invading efficiency	60.8 \pm 1.7 ^a	24.5 \pm 1.2 ^b	23.6 \pm 0.5 ^b	5	292.2	0.0001
1500 IJs/pot in greenhouse:						
% of mortality	70.0 \pm 3.2 ^a	44.0 \pm 2.4 ^b	36.0 \pm 2.4 ^b	5	43.1	0.0001
%invading efficiency	15.9 \pm 0.3 ^a	1.6 \pm 0.6 ^b	10.1 \pm 0.6 ^c	5	166.6	0.0001
2700 IJs/pot in greenhouse:						
% of mortality	65.0 \pm 5.4 ^a	40.0 \pm 0.0 ^b	40.0 \pm 3.2 ^b	10	9.32	0.0018
%invading efficiency	5.2 \pm 0.9 ^a	2.3 \pm 0.4 ^a	3.2 \pm 0.3 ^a	10	3.45	0.0551

N indicates the number of petri dish arena replicates. Different letters indicate the significant differences between nematode species at $P < 0.05$ level in Tukey HSD test

Greenhouse experiments. At both concentrations of 1500 IJs/pot and 2700 IJs/pot in greenhouse experiments, *S. apuliae* caused significantly higher mortality of *S. littoralis* larvae than did the other nematode species (Table 1).

While the mortality caused by *S. oregonense* was almost similar to that obtained by *S. arenarium* at 1500 and 2700 IJs/pot concentrations (Two-way ANOVA, factor concentration: $F_{1,29} = 1.21$; $P = 0.28$; interaction concentration*nematode species: $F_{2,29} = 1.59$; $P = 0.22$).

Table 2. The average number of male and female *Steinernema* nematode species found per *Spodoptera littoralis* larval cadaver in laboratory and greenhouse experiments using three different concentrations of infective juveniles (IJ) (mean \pm SE).

Effects	<i>S. apuliae</i>	<i>S. oregonense</i>	<i>S. arenarium</i>	N	F-test	P-value
500 IJs/dish in laboratory:						
No. of nematodes	31.8 \pm 1.6 ^a	22.7 \pm 1.1 ^b	18.4 \pm 1.7 ^b	5	21.1	0.0001
Sex ratio (F/M)	1.4 \pm 0.1 ^a	1.5 \pm 0.1 ^a	1.6 \pm 0.1 ^a	5	1.8	0.2097
1500 IJs/pot in greenhouse:						
No. of nematodes	34.6 \pm 1.3 ^a	6.3 \pm 2.3 ^b	44.9 \pm 1.6 ^c	5	126.3	0.0001
Sex ratio (F/M)	1.4 \pm 0.1 ^a	6.0 \pm 1.9 ^b	1.4 \pm 0.1 ^a	5	5.8	0.0174
2700 IJs/pot in greenhouse:						
No. of nematodes	22.2 \pm 1.9 ^a	22.1 \pm 4.8 ^a	26.1 \pm 2.8 ^a	10	0.5	0.6050
Sex ratio (F/M)	1.1 \pm 0.1 ^a	1.2 \pm 0.2 ^{ab}	1.8 \pm 0.2 ^b	10	5.8	0.0120

N indicates the number of petri dish arena replicates. Different letters indicate significant differences between nematode species at $P < 0.05$ level in Tukey HSD test. F – female, M – male

Similar to the laboratory experiment, *S. apuliae* had significantly higher invading efficiency at 1500 IJs/pot concentration than did the other nematode species. However, no significant difference in invading efficiency was observed at 2700 IJs/pot concentration, even though *S. apuliae* again reached the highest value (Table 1). The invading efficiency was significantly higher at the 1500 IJs/pot concentration than at the 2700 IJs/pot concentration for both *S. apuliae* and *S. arenarium* (Two-way ANOVA, factor concentration: $F_{1,29} = 81.17$, $P = 0.0001$; interaction concentration*nematode species: $F_{2,29} = 29.29$, $P = 0.0001$).

Statistical analysis showed significant differences between the number of established adult nematodes found in the larval cadavers of *Spodoptera* treated with different nematode species at the 500 IJs/dish in the laboratory and at the 1500 IJs/pot treatment in the greenhouse. No significant differences were observed at 2700 IJs/pot concentration (Table 2). Comparing the number of successfully established nematodes in the larval cadavers in both concentrations proved that it was significantly higher at the 1500 IJs/pot than at 2700 IJs/pot concentration in the *S. apuliae* and *S. arenarium* spp. (Two-way ANOVA, factor concentration: $F_{1,29} = 5.40$, $P = 0.027$; interaction concentration*nematode species: $F_{2,29} = 21.62$, $P = 0.0001$) treatments. The total number of nematodes in the cadavers was significantly correlated with the larval mortality in all three dosage treatments (Pearson correlation coefficients: 500 IJs/pot = 0.86, 1500 IJs/pot = 0.61 and 2700 IJs/pot = 0.84). The number of adult females found in the cadavers of *Spodoptera* larvae was always higher than the number of males, but the differences in sex ratio between nematode species were inconsistent across concentrations (Table 2; Two-way ANOVA, factor concentration: $F_{1,29} = 7.27$,

$P = 0.011$; interaction concentration*nematode species: $F_{2,29} = 7.62$, $P = 0.002$).

Foliage application. Foliage application of *S. apuliae* resulted in a significant decrease in the number of damaged leaves and a lower index of damage compared with the control (t-test: 2.98, df = 18, $P = 0.008$; t-test: 2.32, df = 18, $p = 0.032$; respectively). The effect of other nematode showed insignificant differences from the control (t-tests: df = 13, $P > 0.05$). The overall feeding activity of *Spodoptera* larvae was reduced by 43% in *S. apuliae*, 15% in *S. arenarium* and 7.5% in *S. oregonense* as compared with the control (Table 3).

Table 3. The average number of damaged leaves and the percentage of consumed leaf area caused by *Spodoptera littoralis* larvae (mean \pm SE) in control and after foliage application of three *Steinernema* species

Treatment	N	No. of damaged leaves	Consumed leaf area category				Index of damage
			0-25%	26-50%	51-75%	76-100%	
<i>S. apuliae</i>	10	10.8 \pm 1.4 *	3.2 \pm 0.4	2.3 \pm 0.5	0.6 \pm 0.3	0.3 \pm 0.2	190.0 \pm 28.8 *
<i>S. arenarium</i>	5	15.6 \pm 1.2	3.6 \pm 0.3	3.0 \pm 0.4	1.2 \pm 0.5	0.6 \pm 0.3	285.0 \pm 28.3
<i>S. oregonense</i>	5	17.0 \pm 1.4	4.0 \pm 0.4	3.2 \pm 0.4	1.4 \pm 0.4	0.6 \pm 0.3	310.0 \pm 26.3
Control	10	18.0 \pm 2.6	4.0 \pm 0.4	2.7 \pm 0.5	1.4 \pm 0.6	1.1 \pm 0.6	335.0 \pm 55.2

. Index of damage is equivalent to the sum of consumed leaf areas from all damaged leaves per replicate. N indicates the number of pot replicates; * shows significant impact of nematode application versus control at $P < 0.05$

DISCUSSION

The experiments proved that entomopathogenic nematodes infect *S. littoralis*. *Steinernema apuliae* caused 100 % mortality of the target *Spodoptera* larvae followed by *S. arenarium* and *S. oregonense*. The mean number of established adult nematodes in each arena was also higher in *S. apuliae* than in the other two species. Moreover, the invading efficiency of *S. apuliae* reached 61 %, among the tested nematode species, most probably due to the Mediterranean origin of *S. apuliae*. This may be due to thermal adaptation of *S. apuliae* to the warm, and dry Mediterranean climate, whereas *S. oregonense* and *S. arenarium* originate from colder regions in the temperate zones of the north-west of USA and Russia. The pre-assumption that these two non-indigenous nematode species would not be so effective in the control of *Spodoptera* larvae was confirmed by our experimental results. Such natural cold or heat adaptability is a well-known feature among nematodes (Shapiro et al. 1996 and Mráček et al. 1997). Similar results have been reported by Glazer et al. (1991). In their laboratory experiments, the most virulent EPN to *S. littoralis* was an indigenous *Heterorhabditis* sp. originating from Israel. Thus, it is clear that *S. apuliae* has the capacity to effectively invade the target host, proliferate within the host and causing up to 100% of the larval mortality .

A similar pattern of results was obtained from the greenhouse experiment, where *S. apuliae* showed higher invading efficiency and

achieved a higher number of established nematodes in the larval host than did the other two species tested. Thus indicating successful development of the nematodes in the cadaver, as well as a higher mortality of the target pest. However, all these parameters were significantly lower in the greenhouse than those obtained in the laboratory. This is not unusual, as nematodes sprayed on plants in the greenhouse scatter the nematodes in large surface areas (plants and soil) which decrease the chance of such a fortuitous contamination and consequently lowers the mortality of the hosts. However, *S. apuliae* exhibited significantly successful invading efficiency at 1500 IJs/pot, but not at 2700 IJs/pot, since the mean number of nematodes in each cadaver in laboratory and greenhouse study was 60.8 ± 1.7 and 15.9 ± 0.3 , respectively. This is a good indicator that *S. apuliae* has a reasonably high invading efficiency and has the ability to search for the host and to attack it in a situation resembling field conditions.

Upon the application of a higher concentration of nematodes (2700 IJs/pot) in the greenhouse a wide range of mortality between replicates was observed using *S. apuliae* (40 – 100%). The invading efficiency varied between 1.6 – 10.8% ($5.2 \pm 0.9\%$ in average).

The present results at 1500 IJs/pot coincide with Epsky and Capinera (1993) findings, who observed that 10-50% of applied nematodes successfully infected the host. The lower number of *S. apuliae* in a cadaver does not mean that the efficiency of *S. apuliae* is less, because one successfully developed adult or male/female nematode pair makes this cadaver a source for a new generation capable of infecting another pest larva in a soil.

A high positive correlation was observed in the mortality of target insects and the number of nematodes recovered from their cadavers at all tested concentrations of IJs. Hence, the higher the invasive efficiency, the greater the number of nematodes developing in the host ensuring a successful elimination of the target organism. Interestingly, a greater proportion of female than male nematodes were developed in the cadavers. This may have been due, in part, to the overlooking of some much smaller males. *S. littoralis* larvae feed on the foliage of plants, similar to that of many economically important insect pests which destroy the plant canopy. In the past, applying nematodes to foliage faced many desiccation limitations. Adding anti-desiccants (Webster and Bronskill 1968, MacVean *et al.* 1982). However did not entirely showed satisfactory results when applied in the field. Much higher EPN efficiency has been achieved in more limited habitats, such as in greenhouses where the high humidity plus using anti-desiccants can keep infective juveniles viable for several hours or even days (Williams and Walters 2000, Glazer *et al.* 1992, Glazer and Navon 1990).

Foliar applications in greenhouse experiments on indicate that spraying potato plants with an aqueous inoculum of *S. apuliae* reduced pest feeding activity and leaf damage by 43% in due to high mortality of the pest larvae. This is similar to the leaf damage 46% reported by Glazer *et al.* (1992). The high effectiveness of the indigenous species, *S. apuliae*. Against *S. littoralis* has been reported also by Glazer *et al.* (1991).

S. littoralis larvae spend most of their life-cycle at the soil surface around host plants and climb these plants during the night to feed. The application of nematode's at evening, either on the foliage or soil surface extends their survival period and increases the opportunity for these nematodes to find and infect their target hosts. In the past, field applications usually failed to suppress the target hosts even though when applied in more humid temperate zones. Based on the results of our studies we conclude that Mediterranean *S. apuliae* could provide more effective management than other EPN species of the Mediterranean climbing cutworm in Egyptian and European greenhouses and be better also in open field areas.

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تقسيم ثلاث من النييماتودا الممرضة الحشرية من عائلة
Steinernematidae في مكافحة يرقات حوض البحر الأبيض المتوسط
المتسلقة القارضة (دودة ورق القطن) *Spodoptera littoralis* تحت
ظروف كلا من المعمل و الصوب.

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تم دراسة القوة التأثيرية و كفاءة الغزو لثلاث من عائلة نييماتودا *Steinernematidae* ضد العمر اليرقي الأخير ليرقات حوض البحر الأبيض المتوسط المتسلقة القارضة (دودة ورق القطن) و كذلك كمية الضرر الناتج عن تغذية اليرقات على أوراق نبات البطاطس و التي تتغذى عليه تلك اليرقات سواء المعامل بالنييماتودا أو غير المعامل (كنترول). و قد أجريت التجارب و المقارنات بكلا من المعمل و الصوب ذاتية التحكم في الحرارة و الاضاءة . و لقد أثبتت النتائج أن الأنواع من النييماتودا التي تتبع حوض الأبيض المتوسط من جنس *apuliae* عند تركيز ١٥٠٠، ٥٠٠ فرد نشط أعطى أعلى كفاءة في المعمل و كذلك في الصوب و لكن عند تركيز ٢٧٠٠ فرد نشط / أصيص. و لقد وجد أن عدد الأفراد الكاملة التطور من النييماتودا داخل جثث اليرقات من دودة ورق القطن كانت مختلفة المعنوية تبعاً لنوع النييماتودا المستخدمة و أن عدد الأفراد الكاملة التطور الإناث في جثة اليرقة أعلى من عدد الذكور . كذلك اوضحت المعاملة الورقية بالنييماتودا أن نوع *Steinernema apuliae* أحدثت خفض معنوي في حجم الضرر الحادث للمجموع الورقي الناتج عن تغذية اليرقات عند مقارنتها بمثيلاتها الغير معاملة (كنترول). من ذلك نخلص الى أن النييماتودا من نوع *S. apuliae* يمكن أن تكون ليسا قنرة كعامل من عوامل مكافحة المتكاملة لدودة ورق القطن في مصر و الصوب في أوروبا.