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THE ROLE OF ENTOMOPATHOGENIC NEMATODES, *STEINERNEMA* SPP, IN THE BIOLOGICAL CONTROL OF *AGROTIS IPSILON* (LEPIDOPTERA: NOCTUIDAE)

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ABSTRACT: *The black cutworm, Agrotis ipsilon is one of the major pests with a wide host range; larvae spend most of their life in the soil. In this study, two strains of Entomopathogenic Nematodes (EPNs); Steinernema carpocapsae (All strain) and Steinernema scaptersci (SS) were tested for its virulence against the 4th instar larvae of A.ipsilon at laboratory and semi-field experiments under two types of soils (sandy and clay soil). Four concentrations of each strain were evaluated against 4th larval instar (5, 10, 20 and 40 Infective juveniles (IJs)/larvae) were applied for the laboratory experiments, while the concentrations (50, 100 and 200 IJs/ larvae) for semi-field experiments. Result show that, all treatments of S. carpocapsae induced higher mortality percentages than S. scaptersci, except at 5 IJs/ larvae concentration for sandy soil application. In addition, it could be concluded that, S.carpocapsae in sandy soil treatment was highly effective than clay soil. In most treatments, it was observed that there were insignificant differences between S.carpocapsae and S.scaptersci except at 50 IJs/ml water for sandy soil where S.carpocapsae treatment induced higher mortality percentage at 200 IJs/ml water.*

Key words: *Entomopathogenic Nematodes, EPNs, Steinernema carpocapsae, Steinernema scaptersci, virulence, Black cutworm*

INTRODUCTION

The black cutworm *Agrotis ipsilon* (Hufn.), consider one of the most important seedling pest of several economic plants, have a wide host range, feeding on nearly all vegetables and field crops. It attacks different field crops, such as cotton, soybean, corn, potatoes and tomatoes not only in Egypt but also in several countries. It was recorded to feed on a wide variety of plant (Busching and Turpin, 1977). The heavily use of pesticides to control different pests, caused a serious problems, pollutions, damage the environment and/or pose a threat to public health or ground water (Sharaby and El-Nojiban, 2015). The most usual form of pest control used by growers is the chemical control, where pesticides gradually became less effective and much more costly, in addition the side effect at the environmental pollutions, that is encourage scientists to search for biological alternative more safe method (Goudarzi *et al*., 2015). The pest biocontrol agents are far more environmentally friendly than chemical pesticides and in most cases retain their effect longer. The most chemical pesticides are capable of killing a wide spectrum of arthropods. In contrast, biocontrol agents are generally slower-acting, but cause longer-lasting biotic suppression of a specific pest population than chemical control (Abdel-Alim, 2005). Entomopathogenic nematodes (EPNs) application is one of the most considerable agents in pest management programs. Steinernematidae is the most famous families in entomopathogenic nematodes, it showed a good alternative to chemical insecticides, and it showed highest potential for

controlling insect pests under field condition (Lazink *et al.,* 2010). The infective juveniles (IJs) of these nematodes carry a symbiotic bacterium. When infective juveniles enter to the host body cavity, they release the symbiotic bacterium which kills the host within 24-48 hours through the multiplies rapidly in the blood insect causing septicaemia and killing their hosts Akhurst and Smith (2002). The field of entomopathogenic nematodes as a biological control has experienced exponential over the past decade, it is a one of the famous alternative to chemical insecticides. This study was conducted to evaluate the effect of entomopathogenic nematodes against 4th instar larvae of the black cutworm; *Agrotis ipsilon* in sandy and clay soils.

MATERIALS AND METHODS

Rearing of the black cutworm, *Agrotis ipsilon* :

The adult moth of *A.ipsilon* was collected from light trap at Agricultural Researches Station of Sirs El-lyan, Menofia Governorate. Adults were reared in glass jars with a sex ratio of one male to four females. Maintenance of the culture was carried out according to El-shamy (2001).

Rearing of the grater wax moth, *Galleria mellonella* :

The adult of the greater wax moth, *Galleria mellonella* were collected from infested bee hives at Honey Bees Research Department, Plants Protection Researches Institute, Giza. Rearing technique was carried out under constant conditions according to Metwally *et al*. (2012). Full-grown last instar larvae were removed gently from the culture to use in the experiments and/or maintained the culture.

Rearing of entomopathogenic nematode:

Two strains of entomopathogenic nematodes; *Steinernema carpocapsae* all strain (ALL) and *Steinernema scaptersci* (SS) were supplied by Dr. Mona A. Hussein, pests and plant protection department, International Researches Centre (NRC), Giza. These nematodes were reared in full grown last instar larvae of the grater wax moth, *G. mellonella*.

Evaluation of four concentrations of two strains of nematodes *S. carpocapsae* and *S. scaptersci* against the fourth instar larvae of *A. ipsilon*:

To evaluate the efficiency of the tested nematode strains, the following experiments were carried out using two methods:

Filter paper experiments

This method was performed by using Petri dishes 20 cm in diameter which were furnished with one layers of filter paper on each. Five newly moulted 4th instar larvae of *A.ipsilon* were placed in each one.

Treatments were designed as follows:

1- The 1st experiment was inoculated with nematode suspension at rate of 5 IJs/larva using tap water. The control treatment was inoculated with the same volume of tap water only. The whole experiment was covered with parafilm sheath.

2- The 2nd experiment was prepared as the abovementioned step, with inoculated with 10 IJs/larva.

3- The 3rd experiment was inoculated with 20 IJs/larva.

4- The 4th experiment was inoculated with 40 IJs/larva.

The exposure period was 24 hours, and then larvae were transferred singly to other containers to avoid cannibalism. All containers were covered tidily to prevent larval escaping. Petri dishes were allowed to examine daily for 5 days, the dead larvae (cadavers) were transferred to White's traps to collect the migrated nematodes. The whole experiment was replicated three times.

Sandy soil experiments:

Plastic pots of 7 cm high, 5 cm diameter were filled with 80 g sterilized sieved sandy soil. Each pot was moistened with 20ml tap water in order to reach the final moisture content to 20%. Five newly moulted 4th instar *A.ipsilon* larvae were placed in each one and inoculated as aforementioned experiment using filter paper. The exposure period was 24 hours, and then larvae were transferred singly to other containers to avoid cannibalism. All containers were covered tidily to prevent larval escaping. Containers were allowed to examine daily for 5 days, the dead larvae (cadavers) were transferred to White's traps to collect the migrated nematodes. The whole experiment was replicated three times.

Semi-field experiments:

To evaluate the efficacy of the same tested strains (*S. carpocapsae* and *S. scaptersci* against 4th instar larvae of *A.ipsilon*, three nematode concentrations, 50, 100 and 200 IJs/larva were applied.

Plastic pots of 7 cm high, 5 cm diameter were filled with 120 g sterilized sieved soil. Each pot was moistened with 50ml tap water in order to reach the final moisture content to 20%. Five newly moulted 4th instar *A.ipsilon* larvae were placed in each one and inoculated as aforementioned experiment using filter paper. The exposure period was 24 hours, and then larvae were transferred singly to other containers to avoid cannibalism. All containers were covered tidily to prevent larval escaping. Containers were allowed to examine daily for 5 days; the dead larvae (cadavers) were transferred to White's traps to collect the migrated nematodes. The whole experiment was replicated three times.

To study the role of sandy and clay soil types on the virulence of tested strains of nematodes against the 4th larval instar of the black cutworm, *A. ipsilon,* the following steps were applied:

1- The aforementioned steps were prepared using sandy soil.
2- The 2nd test was using clay soil.
3- While the 3rd test was using Petri dishes embedded with filter paper.

Statistical analysis:

Mortality percentages were corrected using Abbott's formula (Abbott, 1925). LC_{50}'s were calculated using the LDP-Line computer program according to Finney (1971). The variance between treatments was calculated using Analyses of Variance (ANOVA) F-test, and the differences between means were estimated using Duncan's Multiple Range Test (SAS, 2007).

RESULTS AND DSCUSSION

Laboratory experiments:

The obtained data in Table (1) and Figures (1, 2) show the effect of the two tested strains of the entomopathogenic nematode (EPN) against the 4th larval instar of *A. ipsilon* at different substrates. It was observed that the LC_{50} values for *S.carpocapsae* was 8.058 IJs/larva (Fig. 1-a) and was 19.741 IJs/larva for *S.scaptersci* (Fig. 2-a) at filter paper substrate treatment. While the corresponding figure of the sand soil substrate treatment, the LC_{50} values were 6.98 IJs/larva (Fig. 1-b) and 7.07 IJs/larva (Fig. 2-b), respectively.

The LC_{95} values were 41.37 and 30.47 IJs/larva for *S.carpocapsae* treatment at filter paper and sand soil substrate, respectively. As for *S.scaptersci* treatment, the LC_{95} values were 194.25 IJs/larva in filter paper substrate and it was 5547.64 IJs/larva in sand soil substrate (Table 1).

Virulence of tested EPN strains:

Steinernema carpocapsae application:

The obtained results in Table (2) indicated that, the highest mortality percentage of the black cutworm larvae was recorded at the treatment of 40 IJs/larva for *S.carpocapsae* applied on sand soil treated substrate (100%), followed by 40 IJs/larva applied on filter paper treated substrate

(93.11%); being significantly different between each other and with all other treatments . While the 20 IJs/larva concentration, inducing 86.22 and 82.03% mortality, respectively for filter paper and sand soil treatments; being insignificantly different between each other, but both values were significantly different with all other treatments (Table 2).

Table (1): Virulence of the of two tested EPN strains against the 4th instar larvae of *A.ipsilon* applied on two different treated substrates under laboratory conditions

Steinernema spp.	Treated substrate	LC_{50} (IJs/larva)	LC_{95} (IJs/larva)	Slope ± SE
S.carpocapsae All strain	Filter paper	8.058	41.373	2.30±0.23
	Sand soil	6.987	30.475	2.57±0.27
S.scaptersci	Filter paper	19.741	194.257	1.66±0.21
	Sand soil	7.077	5547.642	0.57±0.19

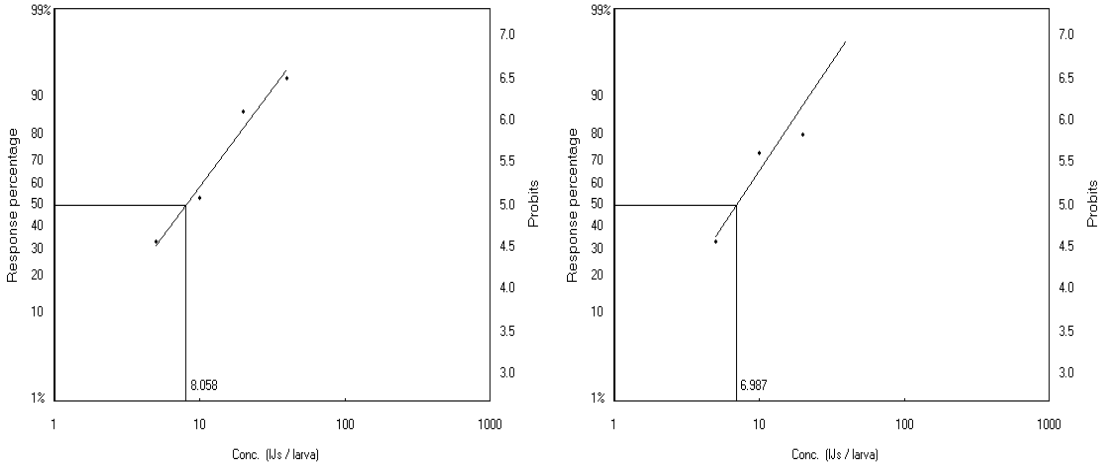

Fig. (1-a&b). Ldp-line of *S.carpocapsae* against the 4th instar larvae of *A. ipsilon* applied by filter paper and sand soil method

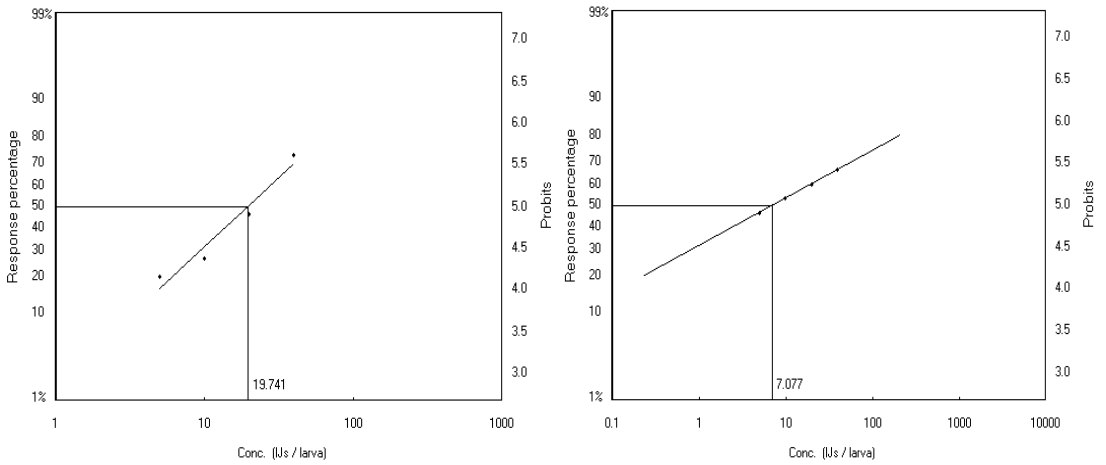

Fig. (2-a&b). Ldp-line of *S.scaptersci* against the 4th instar larvae of *A. ipsilon* applied by filter paper and sand soil method

Table (2): Mortality percentages of 4th instar larvae of *A. ipsilon* by applying different concentration of two EPN strains using two treated substrates under laboratory conditions

Concentration (IJs/larva)	Treated substrate	% Mortality (Mean± SE)		T-value
		S. carpocapsae	*S. scaptersci*	
5	filter paper	33.11±1.80 f A	20.00±1.44 g B	5.628**
	sand soil	33.33±1.45 f B	46.67±1.15 e A	7.186**
10	filter paper	53.10±1.06 e A	26.67±1.15 f B	16.794**
	sand soil	73.11±2.18 d A	53.33±1.73 d B	7.089**
20	filter paper	86.22±1.53 c A	46.67±1.57 e B	26.893**
	sand soil	82.03±1.50 c A	60.66±1.76 c B	9.213**
40	filter paper	93.11±1.82 b A	73.33±1.73 a B	7.854**
	sand soil	100±0.00 aA	66.67±3.46 b B	9.622**
Control		0.00±0.00 g	0.00±0.00 h	
F-value		543.993**	196.064**	

**=Highly significant
Means followed by the same small or capital letter are not significantly different at 5%

The second group was 10 IJs/larva concentration, which induced 53.10 and 73.11% mortality for filter paper and sand soil treatments, respectively; being significantly different between each other and with other concentrations. The least effective concentration was 5 IJs/larva, which induced only about 33% mortality for each of filter paper or sand soil treated substrate; being insignificantly different between each other, but was significantly different with other treatments (Table 2).

Steinernema scaptersci application:

From the corresponding figure of *S.scaptersci* application, it was observed that, the highest mortality percentage was recorded at 40 IJs/larva concentration on filter paper treatment, which gave 73.33% mortality, followed by 66.67% mortality at the same concentration for sand soil application; being significantly different between each other and with other concentrations (Table 2).

While the lowest mortality percentage was recorded at 5 IJs/larva concentration for filter paper application (20.00%), followed by 26.67% at 10 IJs/larva concentration, then 46.67% at 20 IJs/larva concentration (Table 2).

Generally, almost all filter paper treatment gave the low mortality percentages compared with the sand soil treatment of all tested concentrations, except at 40 IJs/larva treatment, where the filter paper treatment induced 77.33% compared to 66.67% in sand soil treatment (Table 2).

A comparison was conducted between the virulence of the two tested strains according to substrate application (filter paper or sand soil) was recorded at Table (2). It was observed that, all treatments, *S.carpocapsae* induced higher mortality percentage than *S.scaptersci*, except at 5 IJs/larva concentration for sand soil application, where *S.scaptersci* induced higher mortality percentage (46.67%) (Table 2).

Semi-field experiments:

Results in Table (3) show the effect of the two tested entomopathogenic nematode against 4th larva instar of *A.ipsilon* in semi-field experiments with different treated substrates.

The LC_{50} value for *S. carpocapsae* in treated filter paper was 30.149 IJs/larva, while in case of *S. scaptersci* application the LC_{50} was 78.881 IJs/larva. In case of sand soil treated substrate the LC_{50} values were 12.317 and 18.628 IJs/larva for *S. carpocapsae* and *S. scaptersci*, respectively. Lastly, the LC_{50} values for *S. carpocapsae* and *S. scaptersci* at clay soil treatment it were 17.104 and 10.222 IJs/larva, respectively.

It could be concluded that, in case of *S. carpocapsae* treatment, sand soil substrate was the most suitable one for EPN activity, followed by clay soil and then filter paper, where the LC_{50} was 12.317, 17.104 and 30.149 IJs/larva (Table 3). While the corresponding figure for *S. scaptersci* treatment was slightly different, where the clay soil was the most suitable substrate, followed by sand soil and then filter paper, where the LC_{50} values were 10.222, 18.628 and 78.881 IJs/larva, respectively (Table 3).

As for the LC_{95} values, it was observed that, in *S. carpocapsae* treatment, clay soil substrate was the most suitable substrate for the EPN activity, giving the least IJs number (57.41IJs/larva), followed by the sand soil (109.961 IJs/larva) then the filter paper substrate (163.961 IJs/larva) (Table 3).

While in case of *S. scaptersci* treatment, the same trend that mentioned above was observed, where the clay soil was the most suitable substrate, followed by sand soil the filter paper, where the LC_{95} values were 59.697, 435.298 and 109.146 IJs/larva, respectively (Table 3).

Virulence of tested nematodes:

***Steinernema carpocapsae* application:**

The obtained results in Table (4) clarify that the larval mortality percentage of the black cutworm larvae showed correlated manner with the concentrations used, *i.e.,* IJs/larva.

The highest mortality percentage was observed at the 200 IJs/larva concentration in all treated substrates, and also at 100 IJs/larva concentration for clay soil substrate.

The percentage of mortality could be arranged as follows with respect to the treated substrate: 93.32 > 93.33 > 86.67 > 79.83 > 73.33% for 100 IJs/larva (sand soil) > 50 IJs/larva (clay soil) > 50 IJs/larva (sand soil) > 100 IJs/larva (filter paper) then 50 IJs/larva (filter paper), respectively (Table 4).

Table (3): Virulence of the two tested EPN strains against the 4th instar larvae of *A.ipsilon* applied by three substrates under semi-field conditions

Steinernema spp	Substrate	LC_{50} (IJs/larva)	LC_{95} (IJs/larva)	Slope ± SE
S.carpocapsae All strain	filter paper	30.149	163.961	2.2365±0.4276
	sand soil	12.317	109.146	1.7360±0.4280
	clay soil	17.104	57.41	3.1279±1.2590
S.scaptersci	filter paper	78.881	1079.387	1.4477±0.3061
	sand soil	18.628	435.298	1.2018±0.3501
	slay soil	10.222	59.697	2.1462±0.8577

Table (4): Virulence of the two tested EPN strains against the 4th instar larvae of *A.ipsilon* applied on three different substrates under semi-field conditions

Concentration (IJs/larva)	Treated substrate	% Mortality (Mean±SE)		T-value
		S.carpocapsae	*S.scaptersci*	
50	filter paper	73.33±1.59 e A	40.00±7.50 d B	4.343*
	sand soil	86.67±2.21 c A	66.67±2.84 c B	5.549**
	clay soil	93.33±1.48 b A	93.33±0.00 a A	0.00 Ns
100	filter paper	79.83±2.45 d A	65.33±15.24 c A	0.939Ns
	sand soil	93.32±1.83 b A	86.67±3.30 a A	1.762Ns
	clay soil	100.00±0.0 a A	100.00±0.0 a A	---
200	filter paper	100.00±0.0 a A	73.33±3.15cb B	8.445**
	sand soil	100.00±0.0 a A	86.67±0.00 a B	11.544**
	clay soil	100.00±0.0 a A	100.00±0.0 a A	---
Control		0.00 f	0.00 e	
F-value		488.766 **	30.535 **	

**=Highly significant *=Significant NS=Not significant

Means followed by the same small letter are not significantly different between concentration and the same capital letters are not significantly different between nematode species at 5%

***Steinernema scaptersci* application:**

The corresponding figure in *S.scaptersci* treatments for the larval mortality percentage in different treated substrates showed that the highest mortality percentage (100%) was observed in clay soil treatment at 100&200 IJs/larva. Tested concentrations could be arranged according to its influence as follows: clay soil at 50 IJs (93.33%)>sand soil at 100 & 200 IJs/larva (86.67%)>filter paper at 200 IJs/larva (73.33%)> sand soil at 50 IJs/larva (66.67%)>filter paper at 100 IJs/larva (65.33%) then filter paper at 50 Ijs/larva (40.00%), respectively (Table 4).

Comparing the virulence of the tested EPN strains:

A comparison was carried out between the virulence of the two tested strains according to substrate application (filter paper, sand soil or clay soil) and was recorded at Table (4).

It was observed that, nearly at all treatments, there was insignificant differences between *S.carpocapsae* and *S.scaptersci* strains, except at 50 IJs/larva concentration for filter paper and sand soil application, on one side and between filter paper treatment at 200 IJs/larva concentration, where *S.carpocapsae* treatments induced higher mortality percentage than *S. scaptersci* treatments (Table 4).

The obtained results are in harmony with those obtained by Hassan *et al.* (2016), who evaluated the virulence of entomopathogenic nematodes, *Steinernema glaseria* and *Heterorhabditis bacteriophora* Poiner (Hp88 strains) against 3th, 4th, 5th and 6th instar larvae of the black cutworm *Agrotis ipsilon*, and found adequate mortality caused by both tested nematodes at different time intervals. They resulted that the two nematode strains; *Steinernema glaseria* and *Heterorhabditis bacteriophora* had a significant effects against different instar larvae of *Agrotis ipsilon*. In addition, Veerle *et al*. (2016) studied the efficacy of

entomopathogenic nematodes against larvae of *Tuta absoluta* in the laboratory, and concluded that the potential of EPNs as a biological control agent against larvae of the tomato leaf miner *Tuta absoluta*. *Steinernema feltiae* and *S. carpocapsae* showed better efficacy than *H. bacteriophora*, where at 18°C and 25°C, *S. feltiae* killed 100% of the third instars, under laboratory conditions.

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دور النيماتودا الممرضة للحشرات *Steinernema* spp فى المكافحة البيولوجية للدودة القارضة

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الملخص العربى

تعتبر حشرة الدودة القارضة *Agrotis ipsilon* من الآفات شديدة الخطورة على الزراعات خاصة فى مرحلة البادرة حيث تقرض النباتات أعلى سطح التربة و تمرالدودة القارضة بستة أعمار يرقية ويعتبر العمر اليرقى الرابع هو أشد وأخطر الأعمار اليرقية ضرراً على الزراعات لذلك اعتمدت هذه الدراسة على مكافحة العمر اليرقى الرابع للدودة القارضة بإستخدام سلالتين من النيماتودا الممرضه للحشرات من عائلة الـ Steinernematidae هما (SS) *Steinernema scapterisci* و(All) *Steinernema carpocapsae* وتم إجراء هذا البحث فى معامل محطة البحوث الزراعية بسرس الليان – محافظة المنوفية وذلك من خلال إجراءالتجارب المعملية والشبه حقلية على عينات من التربة الرملية والتربة الطينية . تم إستخدام أربعة تركيزات من كل سلالة من السلالات محل الدراسة وكانت التركيزات هى (40-20-10-5) طور معدى لكل يرقة فى التجارب المعملية فى حين تم استخدام التركيزات (200-100-50) طور معدى لكل يرقة فى التجارب الشبه حقلية.

وكانت أهم النتائج التى توصل لها هذا البحث أن استخدام النيماتودا الممرضه للحشرات التابعه لعائلة الـ Steinernematidae ضد الدودة القارضة أدى إلى حدوث نسبة موت جيدة ومتفاوتة على يرقات العمر اليرقى الرابع للدودة القارضة وذلك عند تطبيق السلالة *S. Carpocapsae* ، وقد سجل أعلى نسبة موت ليرقات الدودة القارضة مع سلالة الـ *S. carpocapsae* فى جميع التركيزات ما عدا التركيز 5 طور معدى لكل يرقه وذلك تحت ظروف التربة الرملية .

يوصى البحث بإستخدام النيماتودا الممرضة للحشرات *S. carpocapsae* ضمن برامج المكافحة المتكاملة للدودة القارضة وخاصة فى فى الأراضى الرملية.