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The Combined Effect of Entomopathogenic Nematodes on some Biological Aspects of Cotton Pests *Spodoptera littoralis* (Boisd) and *Agrotis ipsilon* (Huf.).

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ABSTRACT

Two species of the Entomopathogenic nematodes, *Steinernema carpocapsae* and *Steinernema scapterisici* were studied against cotton leaf worm *Spodoptera littoralis* (Boisd) and black cutworm *Agrotis ipsilon* (Hufn.).Under laboratory conditions, by using different nematodes strains at Lc_{50} values were 3, 6, 7 juveniles IJ/ml of *S. scapterisici* , *S.carpocapsae* , combination of both strains and using water for control .The results investigated that effect of all treatments on some biological aspects of target pests such as, larval duration of *S. littoralis* were recorded that, 11.5, 11.5,11 and 11.8 days, after treatments with *S.carpocapsae* , *S. scapterisici*, combination and control, respectively. Furthermore *A.ipsilon* showed that highly tolerance more than cotton leaf worm,also the percentages of larval mortality were 32 % for *S.carpocapsae* ,44 % for *S. scapterisici*, 26% for combination of both strains and no larvae mortality on control treatments. Also there is no significant differences between pupal duration of both target pests while, the results indicated that, significant differences between pupal weight (mg) of *S. littoralis* and *A. ipsilon*. No emergency of *S.littoralis* moths after treatment with strain *S.carpocapsae* and this percentage increased to 66 % with *S. scapterisici*, however the combination of both strains affected the emergency of moths to reach 33% compared with control 84%. As regard the 2nd insect pest *A.ipsilon* showed that highly emergency of moths 92 % for control treatment with water only and 50 % when treated with *S. scapterisici* and combination of both strains while decreased to 33% for *S.carpocapsae*. Subsequently, all the biological aspects of adults of both pests as ovi-position, fecundity (Eggs/ female), fertility and also the intermediated shapes were affected mostly by *S. scapterisici*, these findings might be taken into consideration in evaluating the success of the Entomopathogenic nematodes as a pathogenic insecticides for *S.littoralis* and *A. ipsilon* as an economic pests of cotton plants and opens new approaches to develop highly efficient combined biological products on the basis of entomopathogenic nematodes as a bio-agents.

Keywords: *Spodoptera littoralis* ; *Agrotis ipsilon* ; Entomopathogenic nematodes ; *Steinernema carpocapsae* ; *Steinernema scapterisici*.

INTRODUCTION

The Egyptian cotton leaf worm *Spodoptera littoralis* Bois. (Lepidoptera: Noctuidae), is an important and widespread agricultural pest in the subtropical and tropical ranges, it has been infested cotton, tomato, lettuce, strawberry and other vegetables throughout Africa, Middle East and Mediterranean basin (Pineda *et al*., 2007) and (Shairra & Nouh, 2014). The pest causes serious and considerable economic losses to many crops in both greenhouses and open fields (Abd El-Razik & Mostafa, 2013).

The black cutworm *Agrotis ipsilon* (Huf.) is one of the agricultural pests that infest various crops worldwide, attacking different field crops, such as cotton, soybean, corn, potatoes and tomatoes not only in Egypt but also in several countries of the throughout the year. losses occurred in yield due to *A. ipsilon* infestation especially at seedling stage(Ladhari *et al,* 2013).

It has a wide host range, feeding on nearly all vegetables and many important grains. At the local level, it has long been established in Egypt as a major pest of cotton and vegetable plants (Beheedy, 1982). Many populations are extremely resistant to pesticides and, if they become well established, can be exceptionally difficult to control. In these cases, it is important that a comprehensive treatment program is implemented, incorporating a range of reliable control methods, including entomopathogenic nematodes. They can be used as alternatives to chemical control where they leave no harmful chemical residues in the environment behind and without inducing resistance in insect hosts (Evans, 1999 & Shamseldean *et al.,* 2009). A promising strategy with good potential to control insect pests and, at the same time, to minimize the adverse effects of chemical insecticides is the use of entomopathogenic nematodes as microbial control agents. After the entrance of the infective juveniles (IJs) into the selected host insect, they release their symbiotic bacteria into the insect hemocoel where they grow causing septicemia and/or toxemia leading to host death (Georgis, 1992).The Entomopathogenic nematodes (EPN) belong to both families Steinernematidae and Heterorhabditidae associated with their symbiotic bacteria Xenorhabdus, and Photorhabdus respectively have been used commercially as bio control agents of economic insect pests (Gaugler, 2002). Biological control methods offer alternative choices to use the unsuccessful pollutant chemicals. Among biological control agents, the Entomopathogenic nematodes, seemed to be the most appropriate weapon for controlling this serious pests in soil (Ibrahim & Shairra, 2011). The present work aims to evaluate the infectivity of Entomopathogenic nematodes, *Steinernema carpocapsae* and *Steinernema scapterisici* effect against the cotton pests, *Spodoptera littoralis* and *Agrotis ipsilon.* Also, the present work aims to study the combined effect of entomopathogenic nematodes on some biological aspects for possible successful use against these target pests.

MATERIALS AND METHODS

- **Laboratory studies:**

1.Insect culture

A laboratory colony of *S.littoralis* and *A. ipsilon* was obtained from the Departments of cotton leaf worm Plant Protection Research Institute, Agricultural Research Centre (ARC).Under laboratory conditions insect larvae were kept at 25 ± 1ºC and 65–70 ± 5 RH.

2. Nematodes:

Two strains of nematode, *Steinernema carpocapsae* and *Steinernema scapterisici* were supplied by Dr. El-Sadawy, National Research Centre, Dokki, Giza, Egypt. According to (Shamseldean *et al.,* 2009) the last instar

larvae of the greater wax moth, *Galleria mellonella* were used as hosts and for mass culturing of nematodes.

- ***Susceptibility of the nematode strains, Steinernema carpocapsae and Steinernema scapterisici aganist A. ipsilon and S. littoralis larvae.***

The 3rd instar larvae of *A. ipsilon* and *S. littoralis* were obtained from culture reared on castor oil leaves for several generations under laboratory conditions 25 ± 2° C and 60–65% RH., fifteen instar larvae of both target pests were putted in plastic cups (4 X 5 cm) filled with 50 gm. of sterilized sand and moistened with 20% water (v/w). Experiments were carried different nematodes strains at Lc_{50} values were 3, 6, 7 infective juveniles IJ/ml/cup. of *S.scapterisici* , *S. carpocapsae* , combination of both strains and using water for control on bioassay tests. All cups were covered and kept at 25 ± 2 °C and respectively were conducted in 7cm high and 6.5cm in diameter plastic containers covered with plastic lid. Ten replicates were made for each target insects.

- **The effects of combination of the nematode strains, *S. carpocapsae and S. scapterisici* against *A. ipsilon* and *S. littoralis* on some biological aspects.**

The accumulative mortality percentages of the host insects were calculated and the efficacious of the two Entomopathogenic nematode species were evaluated for study some biological aspects for the target insects, separately or combination with insect diet and were statistically compared. Each treatment was repeated three times.

- **Statistical analysis:** Statistical analysis was carried out using Analysis of variance (ANOVA) was conducted on all data (SAS Institute Inc., 1996).

RESULTS AND DISCUSSION

- **Laboratory studies**

1. Effect of entomopathogenic nematode species on the different biological aspects of the cotton leaf worm and black cutworm.

a. larval stage

Susceptibility of cotton leaf worm *S. littoralis* and black cutworm *A. ipsilon* larvae to entomopathogenic nematode, was bioassayed in laboratory , as shown in Table (1) and Fig.(1)[A], larval mortality *S. littoralis* is higher than *A. ipsilon*, it was recorded that 94% and 32 % after treatment with *S.carpocapsae* While reached to 88% and 44% when treated with *S.scapterisici*, increased to 94 % by combination both of two strain, *S.scapterisici*, *S.carpocapsae* and 10 % and zero mortality of cotton leaf worm *S. littoralis* and black cutworm *A. ipsilon* on control, respectively.

The biological activity (larval and pupal mortality, larval and pupal duration, pupal weight, adult emergence , and morphogenetic effects) of the tested entomopathogenic nematode against the 3rd larval instar of S. *littoralis* and *A. ipsilon* has been studied. The result showed that in Table (1) larval duration of S. *littoralis* and *A. ipsilon* show that 11.50 ± 0.4 , 14.90 ± 0.55, 11.50 ± 0.4, 16.57 ± 0.49, 11.00 ± 0.0, 14.22± 0.36,11.80 ± 0.17 and 13.35 ± 0.57 days when treatment with *S. carpocapsae, S. scapterisici* , combination of them compared with control, respectively. Results revealed that no significant susceptible to the infection by all treatments, while *A. ipsilon* was more significant susceptible of nematodes treatments, results declared that normal 4th instar larvae of *A. ipsilon* and infected with nematodes (Figs (1) [A & B], (Barberchecki & Kaya 1991) mention that, higher mortality LC_{50} of S. *exigua* by application of S. *carpocapsae* caused 63.0, 65.9, and 74.8% in clay, organic, and fine sandy loam, respectively. Previous research work of Salem *et al*., (2007) agrees with our results, *S. carpocapsae* were more virulent and faster in killing *S. littoralis* larvae especially the younger instar larvae. Hussein (2004) found that the Egyptian nematodes were as efficient as the imported ones and in some cases had higher efficacy in controlling *S. littoralis* and *A. ipsilon*. On the other hand, (Baris, *et al.,* 2014) stated that five native Entomopathogenic nematodes species caused 100 % mortality of *S.cilium* larvae.

b. Pre- pupa and Pupal stage

The average duration of the pre- pupa of *S. littoralis* and *A. ipsilon* were 1.4 ± 0.24 days and 2.05 ± 0.10 days for *S. carpocapsae*, 1.4 ± 0.4 and 2.16 ± 0.15 days for *S. scapterisici*, 1.0 ± 0.0 and 2.51 ± 0.15 days for combination and 2.8 ± 0.1 and 2.46 ± 0.11 days for control , respectively. as shown in (Table 1) indicated that, the highly pupation percentage of *A. ipsilon* and *S. littoralis* were 100 % and 90 % for control treatment , 74 % and 6 % for combination, 68 % and 10 % for *S. carpocapsae* and 66 % and 12 % for *S. scapterisici* ,respectively.

Table 1. Effect of different treatment of nematodes on some biological aspects of *S. littoralis* and *A. ipsilon* under laboratory conditions.

Biological aspect	Target insect pests	Strain of Nematodes			Control	F.	L.S.D. (0.05)
		S. carpocapsae	*S. scapterisici*	Combination " *Sc+Ss*"			
% Larval Mortality	S. *littoralis*	90	88	94	10		
	A. ipsilon	32	44	26	0		
Larval duration (days)	S. *littoralis*	11.50 ± 0.4 a	11.50 ± 0.4 a	11.00 ± 0.0 a	11.80 ± 0.17 a	0.65	0.67
	A. ipsilon	14.90 ± 0.55 b	16.57 ± 0.49 a	14.22 ± 0.36 b c	13.35 ± 0.57 c	14.53	1.09
Pre-Pupal duration(days)	S. *littoralis*	1.4 ± 0.24 a	1.4 ± 0.4 a	1.0 ± 0.0 a	2.08 ± 0.1a	2.25	0.74
	A. ipsilon	2.05 ± 0.10 a	2.16 ± 0.15 a	2.51 ± 0.15 a	2.46 ± 0.11a	2.10	0.315
% Pupation	S. *littoralis*	10	12	6	90		
	A. ipsilon	68	66	74	100		
Pupal duration	S. *littoralis*	10.3 ± 0.0 a	11.0 ± 0.58 a	10.7 ± 0.0 a	10.8 ± 0.62 a	0.07	2.32
	A. ipsilon	12.30 ± 0.19 a	12.88 ± 0.29 a	12.86 ± 0.28 a	12.45 ± 0.19 a	1.94	0.56
Pupal Weight (mg)	S. *littoralis*	325.0 ± 22.17 ab	308.8 ± 56.52 b	366.6 ± 48.07 ab	426.1 ± 11.58 a	5.21	97.6
	A. ipsilon	375.83 ± 7.3 b	388.75 ± 9.3 b	373.06 ± 11.3 b	436.46 ± 10.5 a	16.48	22.76

Means followed by different letters in each column are significantly different (P, 0.05).

Also no significant differences between pupal duration of both target insect pests, On the other hand, the treatments of different nematodes affected on Pupal stages induced mortality counts were recorded daily for five days from the initiation of the experiment, a high mortality and malformed that as shown in Fig.1[E], compared with normal pupae on control treatment as shown in Fig.1[D], after infected with *S. scapterisici* the pupal weight (mg) decreased reached 308.83 mg., 325.0 mg. for *S. carpocapsae*, 366.67 mg. with combination and 426.15 mg with control of *S. littoralis*, respectively (Table, 1). However, pupal weight of *A. ipsilon* revealed that significant between nematode, *S. scapterisici* ,388.75 mg. ; *S. carpocapsae,* 375.83 mg. and 373.06 mg. with combination and 436.46 mg with control treatment, Table (1) .Our results are in conformity with Atwa (2009, 2014) recorded that, *Heterorhabditis bacteriophora* was most effective on the fifth instar larvae of *S. littoralis* whereas *S. glaseri* was effective and the rate of mortality of *S. littoralis* was lowest when treated with *S. glaseri* (2-20%).The nematode, *S. carpocapsae* recorded a highly effect on pupal stage of *S. littoralis* was 100 % mortality after infection. Our results confirm these of (Mogahed and Abbas, 1998) who found that 4th instar larvae were more susceptible to *S. carpocapsae.*

C. Adult stage

Data in Table (2) revealed that, no emergency of *S. littoralis* moths after treatments with strain *S. scapterisici* , while this percentage increased to 66 % when larvae treated with *S. scapterisici* , reached to 33% by the combination of two strains and increased to 84% with control. As the results in Table (2) and Fig (1)[F & G], showed that, the highest effect on full-grown larvae followed abnormal pupae and malformed of adult *A. ipsilon* moths, male wings slightly curled and adult seemed to be normal by infected with "*S. scapterisici"*, *S. carpocapsae* induced high percentage of abnormalities in male and Female wings slightly curled and male wings slightly curled and male seemed to be normal as a results of treatment of combination of both strains *Sc+Ss* , as in Table (3) and Fig. (1)[H, I, J, K, L & M].

Data from the current study is consistent with that obtained by Haukeland and Lola-Luz (2010) stated that, *Steinernema carpocapsae* treatment in late summer produced 49.5% reduction of early instar *O. sulcatus* larvae, and late spring application resulted in 65% control of late instar larvae.

Table 2. Effect of different treatment of nematodes on some biological aspects of mature stage of *S.littoralis* and *A. ipsilon* under laboratory conditions.

Biological aspect	Target insect pests	Strain of Nematodes			Control	F.	L.S.D. (0.05)
		S. carpocapsae	*S. scapterisici*	Combination " *Sc+Ss*"			
% Pupal Mortality	S. *littoralis*	100	34	67	16		
	A. ipsilon	27	50	50	8		
% Moth	S. *littoralis*	0	66	33	84		
Emergence	*A. ipsilon*	33	50	50	92		
Sex ratio (Female:	S. *littoralis*	-	1:3	1:1	1:1		
Male)	*A. ipsilon*	43: 57	52:48	37: 5	40.8:59.2		
Pre-oviposition	S. *littoralis*	-	4.0 ± 0.0 a	0 ± 0.0	1.6 ± 0.24	16.0	1.6
(days)	*A. ipsilon*	1.8 ±0.39 a	1.5 ± 0.41 a	3.0 ± 0.57 a	2.4 ± 0.45 a	0.38	3.11
Oviposition (days)	S. *littoralis*	-	4.0 ± 0.0	8.0 ± 0.0	8 ± 1.30	1.57	8.86
	A. ipsilon	5.75 ± 0.48 a	5.5 ± 0.46 a	3.00 ± 0.78 a	5.25 ± 0.37a	0.83	4.52
Post- oviposition	S. *littoralis*	-	1 ± 0.0	0 ± 0.0	2.25 ± 0.22	5.0	1.77
(days)	*A. ipsilon*	3.5 ± 0.21 a	2.0±0.0 b	1.5 ±0.12 b	1.0 ± 00 b	8.7	1.23
Fecundity (Eggs / ♀)	S. *littoralis*	-	35 ± 0.0	0 ± 0.0	476.4 ± 137.08	1.44	1021
	A. ipsilon	177.25 ± 26.31 b	30.50 ± 7.71 b	309.0 ± 61.99 ab	663.50 ± 97.95 a	3.96	440.5
Female longevity	S. *littoralis*	-	8 ± 0.0	0 ± 0.0	11.4 ± 0.47	15.88	2.19
(days)	*A. ipsilon*	11.05 ± 0.64 ab	9.00 ± 0.91a	7.5 ± 1.43 b	8.65 ± 0.95a b	6.04	5.54
Male longevity	S. *littoralis*	-	9 ± 0.58	0 ± 0.0	10.4 ± 0.37	2.69	2.19
(days)	*A. ipsilon*	19.00 ± 1.51 a	17.33 ± 1.31 a	9.00±0.89 b	9.33 ± 0.59 b	4.7	7.4
Hatchability %	S. *littoralis*	-	0	0	98		
	A. ipsilon	-	-	75	100		
Incubation period	S. *littoralis*	-	-	-	4.5 ± 0.24	0.0	0.0
(days)	*A. ipsilon*	-	-	3.50 ±0.12 b	4.9 ±0.0 a	22.7	0.44

Means followed by different letters in each column are significantly different (P, 0.05).

D. Egg stage

Subsequently all of biological aspects affected oviposion , fecundity (Eggs/female) the treatment with two strain of nematode *S. scapterisici* and combination induced of sterility of moths and no eggs on the second generation and no complete life cycle compared with control the average of eggs/female were 476.4 eggs.Table (2), and Fig. (1)[N, O & P] , normal egg are deposited in single or small clusters *"*Control*"* , abnormal egg in groups after treatment with nematodes and a magnified view of abnormal egg. (Capinera , 2015) observed that, the egg is white in color initially, but turns brown with age, the eggs normally are deposited in clusters on foliage. Females may deposit 1200 to 1900 eggs.

As a results , the high percentage of mortality % of larvae and induced malformed adult moths and more morphological changes on egg mass, that as damaged occurred on protein and enzymes, so reduced on immunity of target insect pests , *A. ipsilon* , that agree with (Hassan *et al*. 2016), studies that, phagocytosis process is known to stimulate production of lysosomal enzymes. This may refer to nematodes exudates which contain high amount of this enzyme that the nematodes can easily penetrate and digest host tissues. Which play important roles in defeating the host immune system by its antiphagocytic activity.

In consolation , results revealed that, both nematodes *S.carpocapsae* , *S. carpocapsae* and combination of two strains more affected on biological aspects of two target pests, and we can used entomopathogenic nematodes as a bio-control agent for to control larval populations of the black cutworm, *A. ipsilon* and cotton leafworm *S. littoralis* larvae in the field.

Table 3. Scoring of larval – pupa – adult of *Agrotis ipsilon* after treated with different strains of nematodes under laboratory conditions

Scores	Characteristics	*S. carpocapsae*	*S. scapterisici*	Combination
0	Adults seemed to be normal	+	+	+
1	Adults with wings slightly curled	++	+++	++
2	Adults wingless	+++	+	+
3	Adults severely curled	+++	+++++	++
4	Adults attached with puprium	+	+	+
5	Partial emergency (head and thorax)	+	+	+
6	Partial emergency with head only	+	+	+
7	Posteriorly partial emergency	+	+	+
8	Dead pupa	+	+	+
9	Larval pupal intermediate	+	+	+
10	Dead larvae	+	+	+

(+):Number of malformed adult moths

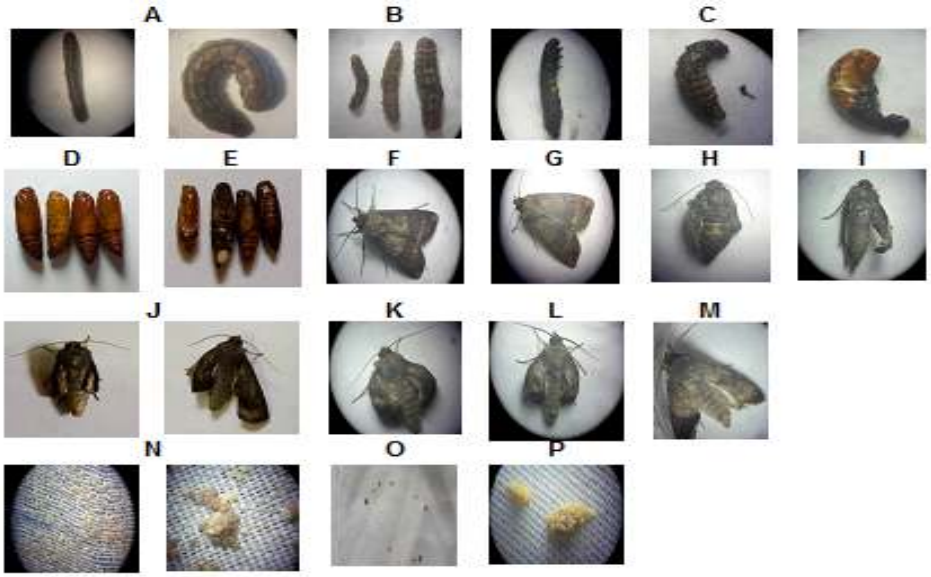

Fig.1. Morphological and physiological changes of immature and mature stages of *A.ipsilon* after infected with nematodes.

- **A. 4th instar larvae on right of photo and 6th instar larvae on left (control).**
- **B. Larvae infected with nematodes (4th, 5th and 6th instar larvae).**
- **C. Intermediate shape.**
- **D. Normal pupae**
- **E. Malformed and dead pupae**
- **F. Normal male**
- **G. Normal Female**
- **H. Male wings slightly curled "*S. scapterisici*"**
- **I. Adult seemed to be normal "*S. scapterisici*"**
- **J. Male wings slightly curled "*S.carpocapsae*"**
- **K. Female wings slightly curled "*S.carpocapsae*"**
- **L. Male wings slightly curled "combination of both strains *Sc+Ss*"**
- ***M.* Male seemed to be normal "combination of both strains *Sc+Ss*"**
- ***N.* Normal egg are deposited in single or small clusters "Control"**
- ***O.* Abnormal egg "treatment with nematodes"**
- **P. A magnified view of abnormal egg.**

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التاثير المشترك للنيماتودا الممرضه للحشرات علي المظاهر البيولوجية لدودة ورق القطن *Spodoptera littoralis* و الدودة القارضة *Agrotis ipsilon*

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تمت الدراسات المعملية لتاثير القدره المرضية للنيماتودا الممرضه للحشرات لكلا من *Steinernema carpocapsae* و *Steinernema scapterisici* علي العمر اليرقى الثالث لدودة ورق القطن و الدودة القارضة . باستخدام تركيزات ٣،٦،٧ / مل من الطور المعدي لكلا السلالاتين من النيماتدوا و معاملة اخري بخلط السلالاتين و المقارنة بالكنترول ماء فقط، وقد اشارت النتائج الى ان كل المعاملات لها تاثير علي المظاهر البيولوجية لكلا الحشرتين .حيث سجلت فترة الطوراليرقي لدودة ورق القطن ١١.٥، ١١.٥ ، ١١، ١١.٨ يوم بعد المعاملة *S.carpocapsa* و *S. scapterisici* و دمج السلالاتين و الكنترول علي التوالي ، بينما أظهرت يرقات الدودة القارضة مقاومة عالية اكثر من دودة ورق القطن ، و سجلت اعلي نسبة موت ٣٢% بعد المعاملة بسلالة *S.carpocapsa* و ٤٤% للسلالة *S. scapterisici* و ٢٦% لدمج السلالتين و لم تسجل اي نسب موت لليرقات في معاملة الكنترول ، أما بالنسبة لفترة طور العذراء فلم تظهر اي فروق معنوية لكلا الحشرتين.بينما ظهرت فروق بين اوزان العذراي للدودة القارضة و دودة ورق القطن . و قد كانت نسبة خروج فراشات دودة ورق القطن ٦٦ % بعد المعاملة *S. scapterisici* و الي انخفاض النسبة الي ٣٣% نتيجة التاثير المشترك لدمج كلا السلالاتين مقارنة بالكنترول ٨٤% ، بينما سجلت نسبة خروج الفراشات الدودة القارضة اعلي نسبة وصلت ٩٢% للكنترول و ٥٠ % للمعاملة بالسلالة *S. scapterisic* وانخفضت النسبة الي ٣٣% عند دمج السلالاتين و من ثما تاثرت باقي المظاهر البيولوجية لطور الحشرة الكاملة خاصة فترة وضع البيض و الخصوبة و المرحل الوسطية للطور اليرقي ، و بالتالي يؤخذ بالاعتبار نجاح استخدام النيماتودا الممرضة كمبيدات حيوية لدودة ورق القطن و الدودة القارضة كآفات هامة علي نباتات القطن مما يلزم تطوير دمج المركبات البيولوجية خاصة النيماتدوا الممرضة كعوامل حيوية.