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LATENT EFFECT OF *HETERORHABDITIS MARELATUS* AND *STEINERNEMA CARPOCAPSAE* ON SOME BIOLOGICAL ASPECTS OF *SPODOPTERA LITTORALIS* BY

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ABSTRACT

This study was conducted to evaluate the efficacy of two species of parasitic nematodes, *Heterorhabditis marelatus* and *Steinernema carpocapsae* on the 5th instar larvae of *S. littoralis* fed on cabbage leaves *H. marelatus* was comparatively more pathogenic to the tested larvae than *S. carpocapsae* .This was evident in the observed higher mortality rates especially at the lowest dose, where *H. marelatus* caused approximately 50 % mortality. Highest progeny was produced from larvae infected by *H. marelatus* (at concentration 20 IJs/ larva) which gave 4125 IJs/ larva). The combination between the two nematodes had the highest effect on pupation;it gave 40 % pupation as compared to 98 % pupation of the control.

INTRODUCTION

Cabbage, *Brassica oleraceae* var. *capitata* L. is an important vegetable crop in many countries of the world and plants are cultivated in most Egyptian of Governorates. Cabbage plants are liable to infestation by larvae of the cotton leaf worm, *Spodoptera littoralis* (Boisd.) which cause detectable damage affecting quality and quantity of plants,

Among the most suitable biological control agents for controlling the cotton leafworm, *S. littoralis* (Boisd.) are the entomopathogenic nematodes of the families Steinernematidae and Heterorhabditidae, which are considered as good biocontrol agents because they cause rapid death of the insect host without side effects on mammals or plants (Poinar, 1986).

Infective third-stage juveniles of these nematodes, which are capable of long-term survival without feeding, carry symbiotic bacteria, *Xenorhabdus* spp. in their intestine to be released into the host's haemocoel leading to septicemia followed by death of the host insect within a day or two. In most insect species, the nematodes, then, reproduce within the cadaver (Molyneux *et al.*, 1983).

The present work was conducted to evaluate the efficacy of two species of insect parasitic nematodes, *Heterorhabditis marelatus* and *Steinernema carpocapsae* on the 5th instar of *S. littoralis* fed on cabbage plant and its latent effect on some biological aspects of the host.

MATERIALS AND METHODS

1. Pathogenicity of *S. carpocapsae* and *H. marelatus* to the 5th instar larvae of *S. littoralis* and their effect on the infective juvenile production:

Newly hatched larvae of *S. littoralis* were reared in jars (40X20cm.) containing sufficient amount of cabbage leaves.

Freshly moulted fifth instar larvae of *S. littoralis* were exposed for 2days to 2, 5, 10, 20 and 40 infective juveniles (IJs)/ml distilled water (d.w.) distributed on Whatman # 1 filter paper placed in a plastic box (15X10X9 cm). The experiments were incubated at 25°C (Kondo, 1989). Four replicates were used for

each concentration and each replicate consisted of 10 larvae. Control experiment of non-infected larvae fed on cabbage leaves was also performed. Mortality percentages were determined after 48 hr., then, it was corrected against that of control using Abbott's formula (Abbott, 1925). Statistical analysis was performed using probit analysis method as described by Finney (1971) estimate the LC_{50} value. The cadavers were dissected for nematode development and progeny production. Results were also recorded after larval treatment with the two nematode spiecies *S. carpocapsae* and *H. marelatus* at LC_{25} of each in simultaneous application. With all experiments, after 48 hr. each larva was transferred into a clean cell free of nematode juveniles and provided with cabbage leaf pieces until mortality or pupation. The larvae were examined daily to determine the percentage of pupation, pupal weight, emergence and was estimated fecunddity as the total number of eggs laid per female and the percentage of eggs hatching, was determined.

Sterility % was calculated according to Toppozada *et al.* (1966) as follows:

$$\%\text{Sterility} = 100-\left[\frac{aXb}{AXB}\right] X$$

Where:

a = Number of eggs laid/female in treatment.
b = %egg-hatch in treatment.
A = Number of eggs laid/female in control.
B = %egg-hatch in control.

RESULTS

1. Susceptibility of *S. littoralis* larvae to *H. marelatus* and *S. carpocapsae*:

The concentration-mortality lines (Fig. 1) demonstrated pathogenicity of the two entomopathogenic nematode species, *H. marelatus* and *S. carpocapsae* on the fifth larval instar of *S. littoralis* fed on cabbage plants, clearly show a positive correlation between larval mortality and applied number of nematode . .

H. marelatus was comparatively more pathogenic to the tested instar larvae than *S. carpocapsae*. This was evident in the observed higher mortality rates at the lower dose, where *H. marelatus* caused approximately 50% mortality.

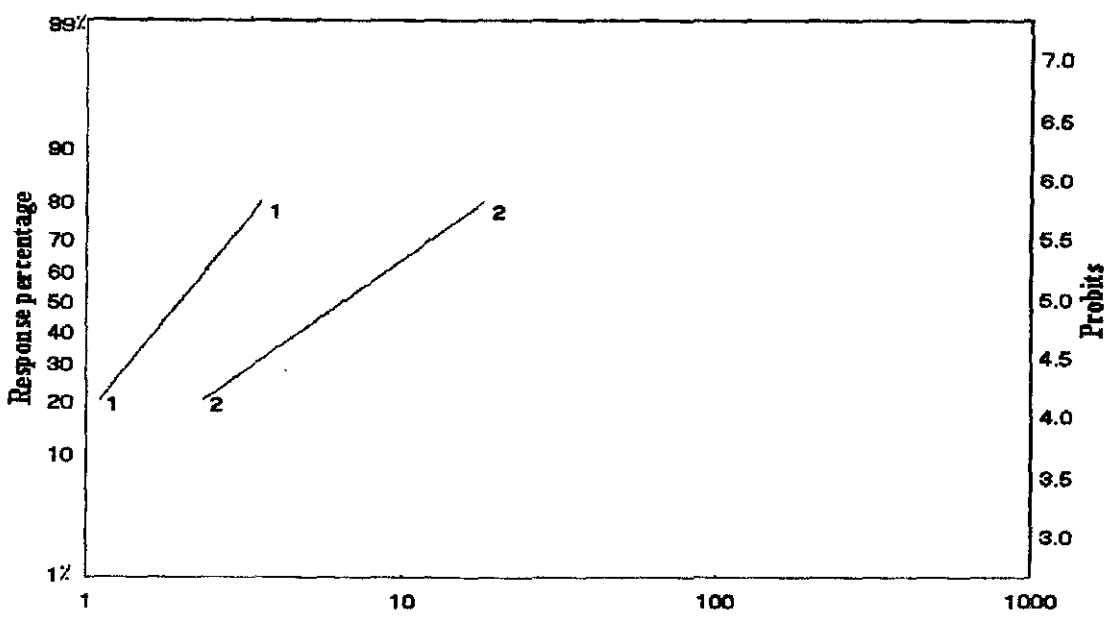

Fig (1): Pathogenicity of *H.marelatus* and *S.carpocapsae* to the 5th instar larvae of *S. littoralis* fed on treated cabbage leaves.

1= *H.marelatus* **2= *S.carpocapsae***

Estimated LC_{50} values were 2.005 and 6.610 IJs/larva for *H. marelatus* and *S. carpocapsae*, respectively.

2. Infective juveniles production:

As shown in Table (1), the total number of juveniles produced / asingle *S. littoralis* larvae varied between the two nematode species.

The highest progeny was produced from larvae infected by *H. marelatus* (at concentration 20 IJs/larva) which gave 4125 IJs/larva) (Table, 1).

2. Latent effect of entomopathogenic nematodes after treatment of the 5 th instar larvae of *S. littoralis*:

2.1. Pupation:

Data in Table (2) demonstrated that *S. littoralis* 5th instar larval infection with either of the two nematode species (*H. marelatus* or *S. carpocapsae*) at LC_{50} values and their applied simultaneously (LC_{25} of *H. marelatus* and *S. carpocapsae*) caused highly significant ($P < 0.01$) reduction in the pupation percent. The combination between the two nematodes had the severest effect on the pupation; it gave 40% pupation as compared to 98% pupation of the control.

Likewise, the infected larvae with the nematodes at the LC_{50} value and the combination between the two nematode species highly significantly ($P < 0.01$) reduced the pupal weight of the resulting pupae as compared to control. The combination between the two nematodes was the most suppressive one on the pupal weight, which recorded 0.257 gm as compared to 0.351 gm of pupal weight produced from untreated larvae (Table, 2).

2.2. Moths, emergence:

Data in Table (2) indicated also that the infection of the 5th instar larvae with the two species of nematodes induced significant ($P < 0.01$) reduction in the moths emergence %. The combination between the two nematode species had the highest effect as it led to 54% adults emergence, as compared to 96% emergence from pupae of control check.

Table (1): Number of juveniles emerged from *S. littoralis* larva infected by *Heterorhabditis marelatus* and *Steinernema carpocapsae*.

Conc. (IJs/larva)	*H. marelatus*	*S. carpocapsae*
2	746.67 ± 53.39^{C}	400 ± 115.6^{C}
5	833.3 ± 96.34^{C}	2350 ± 706.17^{b}
10	1333.33 ± 132.72^{b}	3537.61 ± 113.16^{a}
20	4125 ± 216.7^{a}	2130.7 ± 136.78^{b}
40	1436.66 ± 63.4^{b}	2425.66 ± 58.26^{b}

Means in columns, followed by unlike letters are significantly different ($P < 0.05$).

2.3. Adult fecundity and fertility of eggs:

Data presented in Table (3) demonstrated that the fecundity of *S. littoralis* female moths surviving after infection of the 5th instar larvae by *H. marelatus* and *S. carpocapsae* at LC_{50} values and their combination at LC_{25} values was highly significantly ($P < 0.01$) decreased compared to that of control moth. Likewise, the fertility of eggs (%hatching) demonstrated a highly significant ($P < 0.01$) reduction because of the same treatments.

2.4. Morphogenetic effects:

Data presented in Table (2) demonstrated that the larval infection of the 5th instar larvae by the LC_{50} of entomopathogenic nematodes caused malformations, among the subsequent pupae and adults, while all the obtained pupae & adults from the untreated larvae were normal. Maxima percentages of malformation reached 3.9 % of pupae and 2.5 % of moths from the simultaneous treatment by the twonematode species and treatment by *H. marelatus*, respectively.

Table (2): Effect of *Heterorhabditis marelatus* and *Steinernema carpocapsae* applied at the LC_{50} values to *S. littoralis* 5th instar larvae on pupation %.

Nematode species	%Pupation±SE		Pupal weight (gm)±SE	%Moth emergence±SE	
	Normal	Malformed		Normal	Malformed
H. marelatus	54±4.6**	2.5	0.295±0.09**	66±1.5**	2.5
S. carpocapsae	61±0.7**	2.6	0.304±0.07**	70±3**	1.5
H. marelatus + *S. carpocapsae*	40±3**	3.9	0.257±0.1**	54±0.3**	2.1
Control	98±0.57		0.351±0.02	96±0.77	

**= Highly significant (P <0.01)

Table (3): Effect of *Heterorhabditis marelatus* and *Steinernema carpocapsae* on the adult of *Spodoptera littoralis* infected as 5th instar larvae with LC_{50} values.

Pathogen	Fecundity (No. of eggs/female±SE)	Fertility (Egg-Hatchability %)	Sterility %
H. marelatus	268±6.6**	78	47.4
S. carpocapsae	255±6.6**	65	64
H. marelatus + *S. carpocapsae*	156±4.4**	65	74
Control	408±13	91.8	-

**= Highly significant (P <0.01)

DISCUSSION

The obtained results agree with those of Poinar and Thomas (1996) who indicated that differences in the rate of reproduction of entomopathogenic nematodes were due to the quality of food reserves of the tested insect. Both growth and propagation of *H. marelatus* and *S. carpocapsae* are closely associated with their symbiotic bacterium.

Several authors studied the pathogennicity of nematodes to *S. littoralis* Hatsukada and Grey (1996) and Mogahed (1996) indicated higher infectivity of *S. carpocapsae* to *S. littoralis* larvae. They showed alsothat the efficacy of *H. heliothidis* and *H. bacteriophora* to *S. littoralis* increased with the increase of concentration and period after treatment by different stages. Also, Abbas and Saleh (1998) studied the efficiency of *S. riobrave* against the 4th instar larvae of the same insect and determined the LD_{50} value of 49.6 IJ's/larva.

Several authors studied the interactions between different entomopathogenic nematode species Rosas and Kaya (1990) concluded that the total mortality percentage by *H. bacteriophora* and *S. carpocapsae* in combination was higher than mortality by either species alone to *G. mellonella*. Albrecht *et al.* (1995) exposed the larvae of *G. mellonella* to infective juveniles of *S. carpocapsae* and *H. bacteriophora* both of them produced progeny from the same cadavers. Also, Reyad (2005) revealed synergism to *S. littoralis* larvae after the application (sequential or simultaneous) of *S. riobrave* and *Heterorhalditis* sp.

The entomopathogenic nematodes tested in the present study affected the different biological parameters studied for *S. littoralis* as compared to the control. The reduction in pupation and adult emergence percentage, fecundity and in fertility of *S. littoralis* due to infection by entomopathogenic nematodes was similar to the data obtained by several authors against insect species. Kay and Grieve (1982) demonstrated that adults of *S. exigua* were susceptible to infection by *Neoaplectanta carpocapsae* as they emerged from infected pupae in the soil, among which the most adult mortality (> 60%) occurred within 24 hr of adult

emergence. Kondo (1989), found that the infectivity of *S. bibionis* to last instar larvae of *S. litura* was higher (70%) than that of *S. glaseri;* about 1/3 of the insects used died during their feeding period and the rest died after developing to spinning larvae, pre-pupae or pupae.

The decrease in percentage of pupation and adult emergence of *S. littoralis* may due, in part, to decreased protein biosynthesis needed for growth and development of the organized tissues during the period following larval infection with the entomopathogenic nematodes (Engelman, 1970 and Wigglesworth, 1970).

The suppression of egg production per treated female obtained in this study may be due, in part, to interference of the tested entomopathogenic nematodes with oogenesis and decrease protein contents in the ovaries and the testes of male moths due to damage of sperm bundles (Wigglesworth, 1970).

Reduction in the percentage of egg-hatchability in the present study may be attributed to sterilization of either eggs and/or sperms, or may be due to inability of the sperms to be transferred to females during copulation.

These chromosomal abnormalities in the embryo may cause genetic imbalance in the cleavage nuclei and ultimately lead to embryonic death (Engelman, 1970 and Wigglesworth, 1970).

Smith *et al.* (1996) evaluated *S. carpocapsae, S. feltiae* and *S. glaseri* for suppression of the banded ash clearwing borer, *Podosesia aureocincta* (Lepidoptera : Sesiidae) attacking green ash. Nematodes were applied as bark sprays due to reduced pupation and adult emergence.

Ibrahim (2007) studied the efficacy of *Steinernema riobrave* and *Heterorhabditis* sp. (ISK-2) (Egyptian isolates) on the 4th instar larvae of *S. littoralis*, Data indicated that *S. riobrave* was more effective than *Heterorhabditis* sp. and indicated that the pupae were less susceptible to nematodes infection in soil than pre-pupae and adults. In addition, there was a malformation of adults emerged feom treated pupae. Adults of *S. littoralis* were susceptible to nematodes infection as they emerged from the soil. The majority of nematodes induced mortality within 24 hrs after emergence and many abnormal adults were observed, they could not mate nor lay eggs. Data indicated also that, the appropriate stage for controlling *S. littoralis* by nematodes is the pre-pupae, where 73-93% of the population was killed.

Fetoh and Azazy (2004), found that the larvae and pupae of *Artogia rapae* were highly susceptible to two nematode species, *H. bacteriophora* and *S. carpocapsae,* which gave mortality between 73.3-100 and 53.3-100% among larvae and pupae, which infested cabbage fields.

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دراسة تأثير نوعين من النيماتودا الممرضة للحشرات هما هيتيرورهابتيدي ماريولا تسى وشتينارنيما كربوكابسا علي بعض الصفات البيولوجية لدودة ورق القطن

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في هذه الدراسة تم تقييم تأثير نوعين من النيماتودا الممرضة للحشرات وهما هيتيرورهابتيدي ماريولاس وشتينارنيما كربوكابسا علي العمر اليرقي الخامس لدودة ورق القطن، وتم الوصول الي أن النيماتودا هيتيرورهابتيدي ماريولاس كانت أكثر قدرة علي إحداث الإصابة للحشرة عن النيماتواد شتينارنيما كربوكابسا وخاصة عند التركيزات المنخفضة وأعطت أيضا أعلي إنتاج من الأطوار المعدية الناتجة من حيث الحشرات حيث بلغت ٤١٢٥ طور معدي/يرقة وتم إستكمال الدراسات البيولوجية للتركيز القاتل لـ٥٠% من اليرقات أو خلط نوعي النيماتودا في نفس الوقت علي جيل الحشرة ولقد تبين أن.

تأثر اليرقات التي عوملت بخلط نوعي النيماتودا مع بعضهما مما أعطي نسبة تعذير أقل وكذلك أعلي نسبة خروج الفراشات وحققت نسبة أقل في وضع البيض وفقسه.