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Effect of Egyptian Entomopathogenic Nematode Isolates on Some Economic Insect Pests

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ABSTRACT

The nematode isolates tested were collected from different localities in Egypt. Some experiments evaluate the infectivity of *Heterorhabditis bacteriophora* and *H.indica* as biocontrol candidates against some economic agricultural insect pests; *Spodoptera littoralis, Agrotis ipsilon, Sesamia cretica, Phthorimaea operculella* and *Spodoptera exigua,*. The tested nematode species grew faster in *S.littoralis* and *Ph.operculella* larvae than in the other host larvae. The survival of *H.bacteriophora* and *H.indica* in distilled water was affected by the two combined factors, exposure time and temperatures. All individuals of *H. bacteriophora* and *H. indica* were viable after exposure for 48 hours to temperatures ranged from 5-30°C. At lower (0°C and -5°C) and higher temperatures (35 and 40°C), the survival decreased especially at higher temperatures. Effect of temperature and exposure time on the infectivity of the nematode *H. indica* (EASD77 isolate) and *H. bacteriophora* (EASD98 isolate) was higher against S. *littoralis*. The highest mortality percentages were recorded at the highest two temperatures tested (30 and 35°C). In contrast, there was no mortality at the lower temperature (10°C). The changes in total haemocyte counts (THCs) and differential haemocyte counts (DHCs) in S. *littoralis* larvae treated with *H. indica* nematode (EASD77 isolate) are important criteria determining cellular immune reactions.

Key Words: Entomopathogenic nematodes, Biological control, Insect pest, Heamocytes.

INTRODUCTION

Steinernematidae and Heterorhabditidae are widely available to be used in biological control (Gaugler and Kaya, 1990). Members of these two families are characterized by their mutualistic bacteria *Xenorhabdus* and *Photorhabdus,* respectively. These bacteria when released from the nematode intestine, multiply rapidly in the host haemolymph, causing septicemia within 24 - 48 hours. The nematodes feed upon the bacterial cells, liquefying host tissues, mature, mate and produce progeny which emerge from the cadaver as infective juveniles (IJs) begin searching for new hosts (Gaugler, 1988).

The present work aims to evaluate the infectivity of different Egyptian isolates of two nematode species belonging to family Heterorhabditidae, *Heterorhabditis bacteriophora* and *H. indica* to some economically important insect pests at different temperatures. The immunity of hosts towards the nematode infection has great significance when applying biological control measures; therefore, additional experiments were carried out to throw light on the possible haemocytic reactions of the cotton leaf worm, *Spodoptera littoralis*.

MATERIALS AND METHODS

1- Nematode isolates and their rearing

Two isolates of *Heterorhabditis bacteriophora* (EASD98 and EAS21) and three isolates of *H. indica* (EASD77, EAS59 and ESF8) were isolated from soil samples representing ecologically diverse habitats in Egypt. The nematode isolates were propagated in laboratory on the last instar larvae of both the greater wax moth, *Galleria mellonella* and of the cotton leafworm, *Spodoptera littoralis* according to Woodering and Kaya (1988).

2- The host insects and their rearing

The last larval instar of the cotton leaf-worm, *S. littaralis* and black cutworm, *A. ipsilon* were reared in the laboratory on castor leaves, according to Ibrahim (1974) and Abdel-Monsef (1984) .The pink borer of sugar-cane, *S.cretica* was reared on green stem of maize plants according to Fayad (1975); the potato tuber moth, *Phthorimaea operculella* was reared on small potato tubers according to Abbas (1981); the beet army worm, *S. exigua* was reared on clover leaves according to Ibrahim (1974).

3- Susceptibility of the host species to different nematode species and isolates

The host larvae were individually placed in plastic cups. Infective juveniles (IJs) were used at doses of 33, 66, 100, 133 and 166 IJs/host larva/cup and incubated at 25±1°C. Each trial was repeated 5 times with

each nematode isolate [EASD98, EAS21, EASD77, EAS59 and ESF8]. Larval mortality was evaluated according to Abbott (1925). Values of LD_{50} were calculated according to Finney (1952). Control experiments were conducted using distilled water. The number of dead larvae in each cup was recorded after 24, 48 and 72 hours.

4- Effect of temperature on the survival of *Heterorhabditis* species and its isolates

500 IJs were placed in glass vials (100 IJs / ml/ vial) of $6cm^3$ capacity. The vials were incubated at 5, 10, 15, 20, 25, 30, 35 and 40°C for 12, 24 and 48 hours. Ather vials were incubated at -5 and 0°C for 15, 60 and 120 minutes to avoid water freezing. After incubation at each specific temperature, the juveniles were transferred to room temperature (25 $\pm$1°C) for 24 hours then the numbers of the alive juveniles were counted using a stereo microscope (Glazer, 1992). Three replicates were used for each experiment.

5- Effects of temperature and exposure time on the infectivity of the nematodes *Heterorhabditis indica* (EASD 77 isolate) and *H. bacteriophora* (EASD 98 isolate)

Last instar larvae of *S. littoralis* were individually placed in plastic cups ($133cm^3$) containing 120 gm. sterilized sand moistened with 3ml suspension of nematodes *H. indica* (EASD 77 isolate) or *H. bacteriophora* (EASD 98 isolate) in distilled water (100 IJs/ml). The cups were held at different temperatures (10, 20, 30 and 35° C) for different times (15, 60 and 120 minutes). Control experiments were carried out simultaneously using larvae placed on sterilized sand moistened with 3 ml, distilled water. Host mortality was determined daily up to 5 days .Thirty larvae were tested for each temperature.

6- Effect of the nematode *Heterorhabditis indica* (EASD 77 isolate) and its symbiotic bacterium *Xenorhabdus nematophilus* on the haemocyte counts of *Spodoptera littoralis* larvae

Sixth instar larvae of *S. littoralis*, were subjected to infection with nematode *H. indica* (EASD 77 isolate). Each larva was placed in a plastic cup with distilled water containing the nematode (100 Ijs/ml). The infected larvae were incubated at 25 + 2°C for 2, 12 or 24 hours. For haemolymph collection, the infected larvae were pre cooled at +4°C for five minutes, surface sterilized with 70% ethyl alcohol, and then bled by cutting a proleg using fine scissors. Drops of haemolymph were collected on microscope slides. Control experiments were conducted simultaneously using non-infected larvae.

a- Determination of the Total Haemocyte Counts (THCs)

To determine the total haemocyte count, haemolymph was allowed to flow onto a clean glass slide, a portion of the blood was quickly drawn to the 0.5 mark of Thoma white blood cell pipette and then diluted with physiological NaCl saline solution (1 : 20). The first 3 or 4 drops of the diluted haemolymph were discarded and the number of haemocytes was counted according to the formula of Jones (1962):

$$THC = \frac{\text{Haemocytes in } x \text{ 1-mm squares X dilution X depth of chamber}}{\text{Number of 1-mm squares counted}}$$

Where: x = mean number of haemocytes/1-mm squares.
The depth factor is usually 10.

b- Determination of the Differential Haemocyte Counts (DHCs).

To detect the differential haemocyte count, blood films were made according to the method of Hunter and Bomford (1959) and then stained with Giemsa.

7- Statistical Analysis.

Data were statistically analyzed using one-way analysis of variance, F-test and T-test using computer soft ware program. Copyright (C) 1989 by H. J. Motulsky, version 1.0, Dr. Leo P. Schouest, UC Riverside Serial # 890168S (SIGMA service).

RESULTS AND DISCUSSION

1. Susceptibility of different host species to the isolates of the nematode *Heterorhabditis bacteriophora* and *H. indica*

Table (1) shows the susceptibility of the different host species to the different nematode isolates in a descending order on the basis of LD_{50} value. *S. littoralis* larvae were found more susceptible to EASD77 nematode isolate with LD_{50} value of 15.1 IJs/ml. Also, *S. cretica* larvae were found more susceptible to the same isolate (EASD77) with LD_{50} value of 25.5 IJs/ml. On the other hand, both *A. ipsilon* and *P. operculella* were found more susceptible to the isolate EASD98 with LD_{50} values of 48.9 and 12 IJs/ml respectively. *S. exigua* larvae were found more susceptible to EAS21 isolate with LD_{50} value of 25.5 IJs/ml.

Table (1): Susceptibility of different host species to the different nematode isolates of the Genus *Heterorhabditis* in a descending order on the basis of the LD_{50} values.

Host species	Descending order of the host susceptibility to different nematode isolates on the basis of the LD_{50} values
S. littoralis	EASD77 (15.1), EFS8 (22), EAS59 (37.5), EAS21 (42), EASD98 (68.2).
A.ipsilon	EASD98 (48.9), EFS8 (56.9), EASD77 (58.2),EAS59 (60.9), EAS21 (66.5).
S. cretica	EASD77 (25.5), EASD98 (29.5), EAS59 (39.2), EAS21 (49), EFS8 (85).
Ph. operculella	EASD98 (12), EFS8 (16.6), EAS59 (20.4), EASD77 (24.8), EAS21 (26.5).
S. exigua	EAS21 (25.5), EASD98 (40.7),EASD77 (44.5), EFS8 (49.5), EAS59 (79.3).

- Values between brackets are the LD_{50} values (IJs / ml) The mortality was recorded after 24,48and72hours

Infectivity of *Heterorhabditis* on insects considerably differs depending on the nematode species and insect host. Comparing the infectivity results of the host larvae such as *S. littoralis, A. ipsilon, S. cretica, Ph.operculella* and *S. exigua,* it was found that they were susceptible to *H.bacteriophora* and *H.indica*, however, the range in number of invading nematodes was varied. These results may imply that variable insect susceptibility to heterorhabditids is not due to numbers of infecting nematodes but it is mainly due to the defense reactions against the nematodes and their associated bacteria. Kondo (1987) and Forschler & Nordin (1988) demonstrated that, in case of *G. mellonella, S.littoralis* or *S. litura*, for example, 100% mortality was achieved with 10, 200 and 1000 *IJs/caterpillar*, respectively, after 48hr exposure to *Steinernema carpocapsae.*

In the present study, the two tested nematode species grew faster in *S. littoralis* and *Ph. operculella* larvae than in the other host larvae. The observed low susceptibility of some insect pests to the nematode isolates may be attributed to physical or behavioral host reaction which prevents the infective juveniles from penetrating the target and this agree with Kondo (1987) and Marcek *et al.*, (1988).

2. Effect of temperature on the survival of the *Heterorhabditis* species and isolates

The used nematode isolates were found in upper and middle Egypt (Aswan and Fayoum), where the temperature in these two Governorates differs variably. Statistical analysis of data in table (2) show that, the percent survival of the nematode isolates decreased significantly at 40°C as compared to the other temperatures tested. This result was only evident at exposure times of 24 and 48 hours. Comparing the nematode isolates, it was found that EAS59 was the most affected isolate especially after exposure to 40° C for 48 hours. This was followed by the isolate EFS8. It is also clear that the nematode isolate EAS21 and EASD77 were not affected by temperature up to 35°C even after increasing the exposure time to 48 hours. At the same time, the isolates EASD98, EAS59 and EFS8 were slightly affected when exposed to 35° C especially for 48 hours. It was also observed that the isolate EFS8 was the only one affected by exposure to 5°C where only 90 and 85% of IJs survived after 24 and 48 hours, respectively.

Data obtained in table (3) show the effect of low temperature (-5°C and 0° C) on the percent survival of the nematode isolates. The nematode isolates were exposed to such temperatures for 15, 60 and 120 minutes to avoid complete freezing of the water. Most of the infective juveniles of all isolates could survive by exposing to -5°C for 15 minutes except those of EFS8 where only 50% remained alive. All juveniles (100%) of isolates EASD98 and EAS59 survived even after increasing the exposure time to 60 minutes at -5° C, in comparison to 80, 65 and 30% for isolates EAS21, EASD77 and EFS8, respectively. By increasing the time of exposure to 120 minutes at -5°C only those of EASD98 survived (100%) followed by EAS59 (85%). A marked reduction in the survival rate was obtained in case of isolate EFS8 (2.6%). Statistical analysis showed a significant difference ($P<0.05$) in percent survival of isolates EASD77 and EFS8 by decreasing the temperature from 0°C to -5°C at an exposure period of 60 minute. Also, significant difference ($P<0.05$) was obtained by increasing the exposure period to 120 minutes for isolates EAS21, EASD77, EAS59 and EFS8. Also, the isolate EASD98 was not affected by low temperatures (-5 and 0°C) where the isolate EFS8 was the most affected one.

It was indicated from the present data that survival of *H.bacteriophora* and *H.indica* in distilled water was affected by the interaction of the two combined factors, exposure time and temperatures. Specifically, temperature was the major factor that affected survival of *Heterorhabditis* isolates. All individuals of *H.bacteriophora* and *H. indica* were viable after exposure for 48 hours to temperatures ranged from 5-30° C. At lower (0°C and -5°C) and higher temperatures (35 and 40°C), the survival decreased but this reduction was more prominent at higher temperatures. These results agree with the findings of Molyneux (1984) who recorded, the poor survival of *Heterorhabditis* species at the higher temperatures, attributed to their relatively

Table (2): Percent survival of infective juveniles of different nematode isolates exposed to temperatures ranging from 5 to 40^0 C.

Nematode		Exposure	% Survival at different temperatures (mean ± S.E.)							
Species	isolate	(time/hours)	5°C	10°C	15°C	20°C	25°C	30°C	35°C	40°C
H.bacteriophora	EAS 21	12	Live ± 0^a	live ± 0^a	live ±0^a	live ± 0^a	live ± 0^a	live ± 0^a	live ± 0^a	live ± 0^a
		24	Live ± 0^a	live ± 0^a	live ± 0^a	live ± 0^a	live ± 0^a	live ± 0^a	live ± 0^a	69 ± 2.6^b
		48	Live ± 0^a	live ± 0^a	live ±0^a	live ± 0^a	live ± 0^a	live ± 0^a	live ± 0^a	33 ± 2.9^b
	EASD98	12	live ± 0^a	live ± 0^a	live ±0^a	livc ±0^a	live ± 0^a	live ± 0^a	live ± 0^a	live ± 0^a
		24	live ± 0^a	live ± 0^a	live ±0^a	live ±0^a	live ± 0^a	live ± 0^a	98 ±1 .2^a	80 ± 2.1^b
		48	live ± 0^a	live ± 0^a	live ±0^a	live ±0^a	live ± 0^a	live ± 0^a	95 ± 1.7^a	49 ± 1.5^b
H. indica	EASD77	12	live ± 0^a	live ± 0^a	live ±0^a	live ±0^a	live ± 0^a	live ± 0^a	live ± 0^a	live ± 0^a
		24	live ± 0^a	live ± 0^a	live ±0^a	live ±0^a	live ± 0^a	live ± 0^a	live ± 0^a	70 ± 2.1^b
		48	live ± 0^a	live ± 0^a	live ±0^a	live ±0^a	live ± 0^a	live ± 0^a	live ± 0^a	40 ± 2.3^b
	EAS 59	12	live ± 0^a	live ± 0^a	live ±0^a	live ± 0^a	live ± 0^a	live ± 0^a	98 ± 1.5^a	99 ± 1.1^a
		24	live ± 0^a	live ± 0^a	live ±0^a	live ± 0^a	live ± 0^a	live ± 0^a	95 ± 2.3^a	40 ± 2.8^b
		48	live ± 0^a	live ± 0^a	live ±0^a	live ± 0^a	live ± 0^a	live ± 0^a	80 ± 2.9^b	20 ± 2.1^c
	EFS8	12	live ± 0^a	live ± 0^a	live ±0^a	live ± 0^a	live ± 0^a	live ± 0^a	live ± 0^a	92 ± 1.1^a
		24	90 ± 2.1^a	live ± 0^b	live ±0^a	live ± 0^a	live ± 0^a	live ± 0^a	87 ± 1.7^{ac}	55 ± 2.9^d
		48	85 ± 2.3^a	95 ± 2.1^b	live ±0^a	live ± 0^a	live ± 0^a	live ± 0^a	68 ± 2.3^c	30 ± 2.8^d

- Means followed by the same letter in the same raw are not significantly differ (P > 0.05).

Table (3): Effect of low temperatures on the survival of the nematode isolates of the *Heterorhabditis spp*.

Nematode		Exposure	% Survival (mean + S.E.)	
Species	Isolate	Time (min)	-5^{0}C	0^{0}C
Heterorhabditis bacteriophora	EAS21	15	95 ± 1.4^a	100 ± 0^a
		60	80 ± 3.2^b	89 ± 1.2^b
		120	65 ± 2.2^c	80 ± 2.1^b
	EASD98	15	100 ± 0^a	100 ± 0^a
		60	100 ± 0^a	100 ± 0^a
		120	100 ± 0^a	100 ± 0^a
Heterorhabditis indica	EASD77	15	100 ± 0^a	100 ± 0^a
		60	65 ± 2.1^c	90 ± 1.3^b
		120	37 ± 1.7^d	73 ± 2.2^c
	EAS59	15	100 ± 0^a	100 ± 0^a
		60	100 ± 0^a	100 ± 0^a
		120	85 ± 1.2^b	100 ± 0^a
	EFS8	15	50 ± 1.5^c	90 ± 1.8^b
		60	30 ± 2.8^d	87 ± 1.5^b
		120	2.6 ± 0.8^e	80 ± 2.1^b

*n = 100 IJs/ml. means followed by the same letter in the same raw are not significantly different (P > 0.05).

high motility. Also, Shamseldean (1994) reported that, the heterorhabditid nematode isolated from Egyptian soils remained alive after 48 hours at 25°C but they eventually died at 37 or 40°C. Kaya (1990) and Kung *et al.* (1991) demonstrated that temperature may be the most important factor restricting efforts to extend the nematode application to many regions because the temperature range of nematode activity is narrow and the infectivity and persistence of most species is significantly reduced above 30°C. According to Molyneux (1984) the native habitats of the nematode species and strains determines their ability to tolerate temperature extremes. Shamseldean (1994) observed the difference in temperature tolerance of two isolates of *Heterorabdeitis* nematode (EAM4 and EBNU 19), which emphasize on the importance of thermo tolerance to entomopathogenic nematodes as biocontrol agents.

3- Effect of temperature and exposure time on the infectivity of the nematode *Heterorhabditis indica* (EASD77 isolate) and *H. bacterlophora* (EASD98 isolate)

Data obtained in table (4) show that, in case of the nematode isolate EASD77 the accumulative host mortality reached 100% after 48 hours at 30 & 35°C compared to 57.1 and 70% in case of EASD98 isolate. At 10°C, no mortality could be detected in both isolates at even after 120 hours from infection. At 20°C, the highest accumulative percent mortality (86.7) was obtained only after 72 hours in case of EASD77 isolate as compared to 10% in case of EASD98 isolate.

Both species of the Egyptian heterorhabditid nematodes proved highly virulent against the Egyptian cotton leaf worm, *S. littoralis*. The isolates (EASD 98 and EASD 77) of *H. bacteriophora* and *H. indica*,

respectively, were highly virulent. The highest mortality percentages were recorded for the two heterorhabditid nematode isolates at the highest two temperature tested 30 and 35°C. In contrast, there was no mortality recorded at the cooler degree of temperature test (10°C). These results agree with Hominick & Briscoe (1990) and Hara *et al.* (1991). On the other hand, Molyneux (1986) suggested that steinernematids were more active at lower temperatures than heterorhabditids, and hence they could be used in temperate regions. The isolation of heterorhabditids from extreme climatic conditions such as Negev desert in Israel (Glazer *et al* . 1991) and sea shores of Hawaii (Hara *et al* . 1991) where steinernematids are rarely encountered, tend to support the view that Heterorhabditidae are endemic to warmer climates while steinernematidae previal in temperate regions (Richardson and Grewal, 1994). In contrast, the distribution of Egyptian isolates of entomopathogenic nematodes varied greatly from one Governorate to another. Meanwhile Shamseldean and Abd-Elgawad (1994) believed that, this difference in distribution may reflect the availability of suitable host insects and the influence of environmental factors such as soil type and moisture. Kondo (1989) also stated that development of invading nematodes in host cadavers differed among the nematode species. Our results also show the influence of temperature on the enhancement of nematode virulence to a certain extent at a maximum temperature of 35° C.

Shamseldean (1994) showed that, temperature affects survival and infectivity of novel heterorhab ditid nematode isolates originally extracted from Egyptian soils. The Egyptian nematode isolates were alive after 48 hours at 25° C but died at 37 or 40° C. Also, differences in nematode tolerance to varying degrees of temperature were evident between the two tested *Heterorhabditis* nematode isolates EAM4 and EBNU19.

4- Effect of the nematode *Heterorhabditis indica* (EASD77 isolate) and its symbiotic bacterium *Xenorhabdus nematophilus* on the haemocyte counts of *Spodoptera littoralis* larvae

The effect of nematode infection on each of THC and DHC was detected before the death of the larvae at 2, 12, 24 hours post- infection. Five different haemocyte types were distinguished in Giemsa stained preparation of blood film of the last instar larvae of *S. littoralis*. These types were identified as Granulocytes (G), Plasmatocytes (P), Prohemocytes (Pr), Oenocytoids (O) and Spherulocytes (S) (fig. 1).

Table (4): Effect of exposure time and temperature on the infectivity of *Heterorhabditis indica* (ESAD77) and *H. bacteriophora* (EASD98) to *Spodoptera littoralis.*

Nematode		Temperature (° C)	*Accumulative (%) host mortality after				
Species	Isolate		24hrs	48hrs	72hrs	96hrs	120hrs
H. indica	EASD77	10	0	0	0	0	0
		20	6.6	56.7	86.7	86.7	86.7
		25	13.3	86.6	100	----	----
		30	36.5	100	----	----	-----
		35	26	100	----	----	-----
H. bacteriophora	EASD98	10	0	0	0	0	0
		20	0	0	10	30	76.7
		25	3.3	70	100	----	----
		30	6.6	57.1	90	96.7	96.7
		35	16.6	70	96.7	96.7	96.7

*Dependent on the original number (30 larvae).

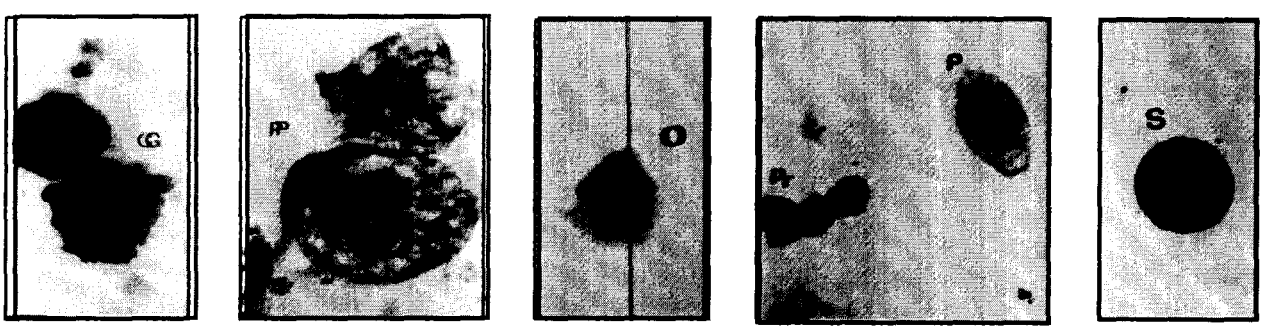


Fig (1). Haemocytes of normal *Spodoptera littoralis* larvae. Granulocyte (G) Oenocytoid (O) Plasmatocyte (P), Prohaemocytes (Pr) and Spherulocyte (S). Giemsa x 1000.

Table (5): Total haemocytes counts in haemolymph of non-infected and infected *Spodoptera littoralis* larvae by *Heterohrabditis indica* (EASD77 isolate).

Post infection time/hours	Total haemocyte count/mm^3* ± S.E.	
	Non-infected larvae	Infected larvae
02	1433.5 ± 220.7^a	1238.2 ± 211.1^a
12	834.9 ± 108.6^b	5140.6 ± 1791.9^c
24	656.5 ± 931^b	1502.7 ± 611.1^a

*Means followed by the same letter, with same column in the same raw of the column are not significantly different (P > 0.05).

Table (6): Percentage of different haemocyte types of *Spodoptera littoralis* larvae at different times post-infection with *Heterorhabditis indica* nematode (EASD77 isolate).

Post infection time/hrs.	% Haemocyte types (mean ± S.E.)*									
	Prohaemocytes		Plasmatocytes		Granulocytes		Spherulocytes		Oenocytoides	
	Non-infected	infected	Non-infected	Infected	Non-infected	infected	Non-infected	infected	Non-infected	infected
2	4.6 ± 1.2^a	4.9 ± 2.2^a	21.4 ± 2.1^a	28.2 ± 4.1^a	48.64 ± 2.6^a	45.33 ± 0.3^a	18.6 ± 1.6^a	16.3 ± 0.7^a	5.4 ± 1.1^a	5.7 ± 1.4^a
12	5.0 ± 1.3^a	2.0 ± 0.5^b	38.1 ± 2.0^b	32.7 ± 4.1^b	41.2 ± 2.1^a	46.6 ± 4.9^a	10.5 ± 1.6^b	12.7 ± 1.8^b	6.2 ± 0.8^a	6.1 ± 0.9^a
24	1.4 ± 0.3^b	8.4 ± 1.0^c	39.3 ± 6.3^b	16.8 ± 0.9^a	53.0 ± 5.8^b	58.3 ± 2.9^b	11.5 ± 2.0^b	3.1 ± 0.6^c	3.6 ± 1.3^b	8.9 ± 1.6^c

*Means followed by the same letter, in the same column of the parameter are not significant differ (P > 0.05).

a- Effect of EASD77 isolate on the Total Haemocyte Count (THC).

From table (5) it is evident that after 2 hours of infection the THC of the haemolymph of the infected *S. littoralis* larvae was reduced by 13.6% as compared to that of the control larvae. By increasing the time after infection to 12 hours a marked increase in the THC of the infected larvae was obtained as compared to that of the non-infected larvae as well as with that of the infected larvae at 2 hours post-infection (P<0.05). This was followed by a percent reduction reached (70.77%) at 24 hours post-infection. In case of the non-infected larvae, the THC decreased significantly in comparison with that of the infected ones being 656.5 ± 931 and 1502.7 ± 611.1, respectively.

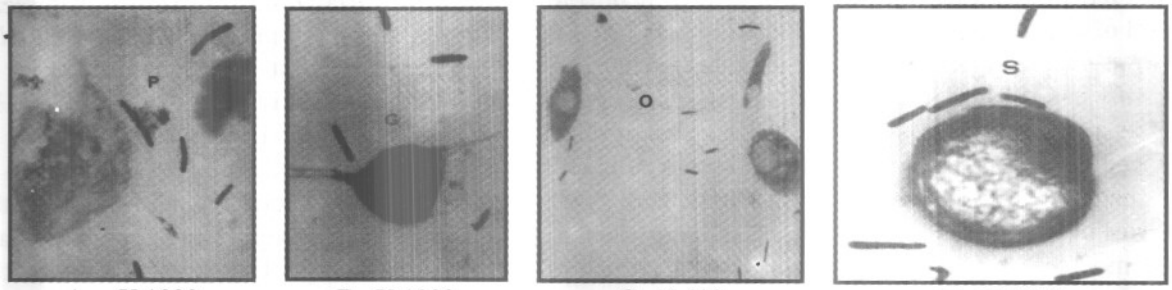
b- Effect of EASD77 isolate on the Differential Haemocyte Count (DHC).

As shown in table (6), a significant reduction in the percentage of the prohaemocytes was observed at 12 hours post-infection which was followed by a significant increase at 24 hours post-infection with *H. indica* (EASD77 isolate). A significant difference between the percentage of prohaemocytes in the infected and non-infected larvae at both 12 and 24 hours post-infection was found. The percentage of plasmatocytes in the infected larvae significantly increased at 12 hours post-infection then decreased at 24 hours post-infection (P<0.05). The percentage of plasmatocytes in the control larvae increased significantly to about 1.9 folds at 12 and 24 hours.

The percentage of the granulocytes in both infected and non-infected larvae showed a significant increase only at 24 hours. The percentage of the spherulocytes showed a gradual decrease reaching a very low percentage at 24 hours post-infection as compared to the non-infected larvae. A significant increase in the percentage of Oenocytoids was evident at 24 hours post-infection whereas their percentage was significantly reduced in the non-infected larvae.

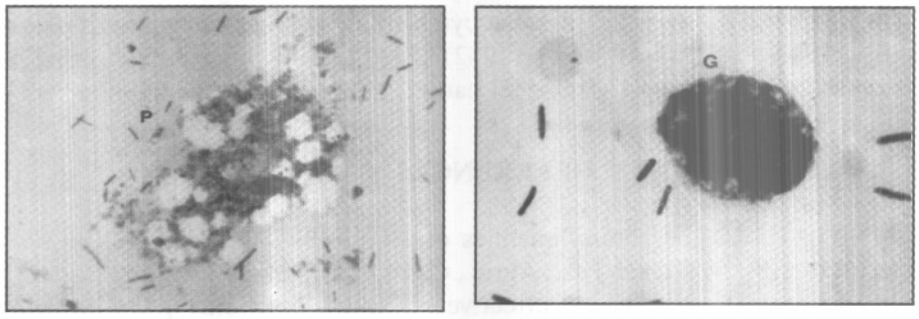
From the above data, it may be concluded that, *S. littoralis* showed decreases in prohaemocytes and spherulocytes at 12 hours post-infection, while plasmatocytes, granulocytes and oenocytoides increased in numbers. On the other hand, prohaemocytes, granulocytes and oenocytoides increased in numbers at 24 hours post-infection while plasmatocytes and spherulocytes were decreased. Nematodal bacteria *Xenorhabdus nematophilus* started to get out and release from nematodes at 12 hours post-infection. Plasmatocytes, granulocyte and spherulocytes which are responsible for immune responses such as phagocytosis and nodule formation tried to react with these bacteria.

Plasmatocyte forms a pseudopodial to engulf the bacteria also some of them have a very irregular outline and the bacteria bending at the cell boundary (fig. 2) plasmatocyte after 24 hours post-infection swallow and plasma membrane ruptured as a result of toxins secreted by bacteria. Granulocyte has very long filaments



A X 1000 B X 1000 C X 400 D X 1000

Fig. (2) Haemocytes of *S.littoralis* larvae infected with *H. indica* nematode (EASD77 isolate) and its released bacterium *X.nematophilus* at 12 hours post infection.



A B

Fig. (3) Haemocytes of *S.littoralis* larvae after infected with *H.indica* nematode (EASD77 isolate) and its released bacterium *X.nematophilus* at 24 hours.

due to the effect of bacteria, increased attachment of bacteria to granular cell and increased phagocytic activity; vacuole enclosing the bacteria fig. (2 and 3). Spherulocyte attached with bacteria then damaged at 24 hours post-infection fig. (2).

Entomopathogenic nematodes play an important role in the early development of insect pathology, and subsequently they are considered as pest suppressive agent. The changes in total haemocyte counts (THCs) and differential haemocyte counts (DHCs) following exposure to entomopathogenic nematodes are important criteria determining cellular defence reactions.

The present study attempted to investigate the capability of *H. indica* nematode (EASD 77 isolate) to parasitize the larvae of *S. littoralis*. The immune reactions of the haemocytes of these larvae against the invading nematodes and their released bacteria (*X.nematophilus*) were investigated. The studied cellular immune reactions were changes in total haemocyte counts (THCs), percentage of differential haemocyte, the activities of the larval haemocytes to phagocytose nematodal bacteria.

The dynamics of reactions of *S. littoralis* larvae against *H. indica* nematode (EASD77 isolate) showed a considerable changes in each of the THCs and percentage of DHCs. Decreases in THCs at 2 hours post infection (although insignificant) may be due to damage and death of haemocytes by the haemocytotoxin lipopolysaccharide released from the outer membrane of *X. nematophilus* bacteria into the haemolymph; these toxins contain fatty acid that damage the haemocytes (Dunphy and Webster, 1988) or it may follow the suggestion that the initial haemocyte reaction could be analogues to a margination effect, where the haemocytes would tend to adhere to the wall of the haemocoel and to each other, after which some cells return into circulation. Similar results were reported by Ratcliffe and Gagen (1976) and Chain and Anderson (1982). On the other hand, the increase in THCs of the infected larvae of *S. littoralis* larvae at 12 hours post infection may be suggested to be due to stimulation of the insect's defences (haemopoiesis and haemopoietic organs) (Serysznska and Kamionek, 1974) or as a result of the activation of the immune reactions. This haemogram decreased again at 24 hours and continued till the cells died gradually followed by death of the larvae themselves. The differential haemocyte count; the decrease in prohaemocyte numbers after 2 hours of *S. littoralis* post-infection with *H. indica* nematode (EASD 77 isolate) may be due to the great acceleration of other haemocyte types or to temporary block of haemopoiesis in prohaemocyte types accord also at 12 hours post-infection but it started to increase again at 24 hours and this may be due to enhanced activation of the haemopoietic organs to produce prohaemocytes to compensate the storage of the other blood cell types

which were damaged in the phagocytosis reaction against the released bacteria and these reactions may lead to the death of haemocytes which are involved in lysis and melanization of foreign materials. *X.nematophilus* bacteria were released from *H.indica* nematode (EASD 77 isolate) at 12 hours post-infection or just prior to it. *S. littoralis* larval haemocytes showed weak phagocytic activity by 12 hours post-infection and the THC decreased as a result of the death of the haemocytes or this haemocytes would tend to adhere to the lining of the haemocoel and to each other, after which some cells return into circulation. At 24 hours post-infection *S. littoralis* phagocytic response increased as indicated by the decrease in plasmatocyte type as well as the spherulocytes percentages accompanied by a sharply decrease of the THC which may be a result of the death of the haemocytes during phagocytic response, which may explain the high bacterial density in the haemolymph at 24 hours post-infection. At 48 hours post-infection all *S. littoralis* larvae were dead and their haemolymph was sticky, and brownish in colour and full of bacteria. It may be concluded that the weakness in the cellular immune reactions may be due to the decrease in plasmatocyte and spherulocyte percentages post nematode infection. Götz (1986) mentioned that the cellular response seemed to involve the granulocytes and plasmatocytes. Whatever the plasmatocytes or granular cells function as phagocytes depend mainly on the insect species. Chain and Anderson (1982) stated that the injection of a suspension of gram positive *Bacillus cereus* into *G.mellonella* haemocoel caused rapid decrease in the numbers of circulating plasmatocytes.

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الملخص العربى

تأثير عزلات مصرية من النيماتودا الممرضة للحشرات فى مكافحة بعض الحشرات الأقتصادية

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تهدف هذه الدراسة لتقييم قدرة العزلات المختلفة لنوعين من النيماتودا المصرية *Heterorhabditis bacteriophora* و *H.indica* ودورهما في مكافحة أهم الآفات الحشرية الإقتصادية. دودة ورق القطن *Spodoptera littoralis* والدودة القارضة *Agrotis ipsilon* ودودة القصب الكبيرة *Sesamia cretica* ودودة درنات البطاطس *Phthorimaea operculella* والدودة الخضراء *Spodoptera exigua* . وقد وجد أن درجة الحرارة (المرتفعة والمنخفضة) من العوامل الهامة، كما وجـد أن هناك رد فعل دفاعي من خلايا دم يرقات دودة ورق القطن المصابة بالنيماتودا لنوع *H.indica* وسلالاتها المستخدمة (EASD77 isolate) التي تتميز بخاصية تبادل المنفعة مع بكتريا *Xenorhabdus nematophilus* .