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LABORATORY AND FIELD STUDIES ON THE PATHOGENICITY OF SOME ENTOMOPATHOGENIC NEMATODE STRAINS TO BLACK CUTWORM *AGROTIS IPSILON* (HUFN.).

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ABSTRACT

Three entomopathogenic nematode strains, namely FIN, SAR and Hbs1 belonging to *Heterorhabditis bacteriophora* were selected and tested for pathogencity against newly moulted 6th instar larvae of *Agrotis ipsilon* (Hufn.). All strains were pathogenic to the mentioned instar. Analysis of data revealed that the nematode strains, exposure period (24, 48 and 72 hours) dose (10, 20, 40, 70 and 100 of infective juveniles/larva) in laboratory experiments, (150, 300, 500, 700 and 900 of infective juveniles/larva) in field trial and application method (surface and subsurface of the soil) were significantly ($P<0.05$) affected larval mortality. It was found that the mortality rates were directly correlated with the applied doses, exposure period and different strains. Under controlled laboratory conditions the LC_{50} values of treated 6th instar larvae were 17.1, 30.9 and 41.1 infective juveniles (IJs) per insect for Fin, SAR and Hbs1 strains, respectively after 72h post-infection. Fin strain was the most virulent causing the maximum mortality; An inoculum level of 100 IJs/larva caused 98%, 80 and 72% after 72h post infection for Fin, SAR and Hbs1, respectively in laboratory. Under field conditions 93.3%, 73.3% and 63.3% mortality results were achieved at the subsurface application of nematodes versus 86.6%, 63.3% and 56.6% at the soil surface application with FIN, SAR and Hbs1 nematode strains respectively at the highest concentration (900 IJS/Larva), after 72h from infection. The LC_{50} values of treated 6th instar larvae were $6.0x10^2$, $3.9 x10^2$ and $6.2 x 10^2$ at the subsurface of nematode application against $3.3 x 10^2$, $5.2 x 10^2$ and $6.9 x 10^2$ at the soil surface of nematode application with Fin, SAR and Hbs1 nematode strains, respectively, 72h post-infection. It is clear that the newly moulted 6th instar larvae of *A. ipsilon* were more susceptible to Fin and SAR strains than Hbs1 when treated at the subsurface of the soil and the soil surface, respectively.

INTRODUCTION

Entomopathogenic nematodes (Families Steinernematidae and Heterorhabditdae) include many species with relatively wide host ranges that many suppress a variety of insect pest populations belonging to various Orders and Families (Grewal *et al.*, 2005). Most biocontrol agents require days or weeks to kill their hosts. Yet nematodes, working with their symbiotic bacteria, kill insects in 24-48hr. (Salem *et al.*, 2007). The specialized third stage juvenile, called the infective juvenile, is only stage that survives outside of a host and searches for a susceptible insect host (Susurluk, 2006). The infective juvenile (IJs), the only free-living stage, enter hosts through natural openings (mouth, anus, and spiracles). *Heterorhabditis* also possess a large terminal tooth that may be used to penetrate soft intersegmental areas of the

insect cuticle (Bedding and Molyneux, 1982). After entering the host's hemocoel, nematodes release their symbiotic bacteria, which are primarily responsible for killing the host, defending against secondary invaders, and providing the nematodes with nutrition (Dowds and Peters, 2002). The nematodes molt and complete up to three generations within the host after which IJs exist the cadaver to search out new hosts (Kaya and Gaugler, 1993). EPNs are currently marketed world-wide for the biological control of insect pests. Wide host range, high efficacy, and the availability of techniques for economic mass production have led to the rapid increase in the use of these biological control agents in recent years (Grewal and Georgis, 1998). EPNs possess the ability to search for hosts actively, present no hazard to mammals and are except from registration and regulation requirements by the Environmental Protection Agency (Georgis and Manweiler, 1994). EPNs should be useful in the management of soil-inhabiting insects, because the soil provides a good environment for their survival (high humidity, temperature, moderation, and protection from ultra violet radiation) (Gaugler, 1988 and Kaya, 1990).

Black cutworm, *A. ipsilon* (Lepidoptera: Noctuidae), is polyphagous insect which attacks the seedlings of many crops and causes great damage to cotton, rice, potato, tobacco, crereals and cruciters (Hill, 1975). Larvae of this insect feed on grasses and other agricultural crops at or near the soil surface (Tashiro, 1987). The early larval instars feed on foliage, but the late instars may tunnel to a soil depth between 2.5-5 cm, cut the plants at the soil surface at night, and pull them into their burrows in the ground before feeding (Rings and Musick, 1976). This insect is considered as one of the most serious pests on the cotton. In Egypt, this pest is polyphagous and damages many winter and summer crops (Abdel-Salam, 1987). Chemical management of black cutworm in fields is often erratic. One alternative to chemical pesticides is Entomopathogenic nematodes used as a bioinsecticides. Efficiency of EPNs were studied against the black cutworm *A. ipsilon* using different techniques under laboratory conditions (Caroli *et al.*, 1996). Black cutworm larvae are susceptible to *Steinernema carpocapsae* in both laboratory and field trials (Capinera *et al.*, 1988; Levine and Oloumi-Sadeghi, 1993). EPNs have been shown to be pathogenic toward the black cutworm in the laboratory and in semi field on seedling corn plants (Hammad, 1996). Therefore, the main objective of the present work was to study the pathogenicity of some EPNs strains against the black cutworm *A. ipsilon* under laboratory and field conditions.

MATERIALS AND METHODS

Insect:

Standard laboratory colonies of the black cutworm *A. ipsilon* were provided by Cutworms Department. Plant Protection Resarch Institute, ARC, Ministry of Agriculture, Larvae have been grown on castor bean leaves in our laboratory at the Department of Plant Protection, Faculty of Agricultgure, Al-Azhar University, as described by Khalil *et al.* (1994) insects were reared

away from any insecticidal contamination in controlled rearing room at 27 ± 2°C and 65 ± 5% RH. Larvae were reared individually from 4th instar to avoid cannibalistic behaviour. Adults were paired in big glass jars (3 kg) with a sex ratio of (1:1) and fed on (20%) honey solution.

Entomopathogenic nematode strains:

EPNs strains used in the present investigation were *Heterorhabditis bacteriophora*. Three strains were used, HbS1 strain (obtained from department of pest physiology, Plant protection research Institute, Dokki, Giza, Egypt), SAR strain (provided by the department of plant protection Faculty of Agriculture, Ain Shams University, Egypt) and FIN strain (supplied by Department of Agriculture Zoology and Nematology, Faculty of Agriculture, Al-Azhar University, Nasr City Cairo, Egypt).

IJs of *H. bacteriophora* strains were propagated in wax moth larvae, *Galleria melonella* (L.) according to the culture techniques of (Dutkey *et al.*, 1964). Larvae were starved for several hours before infection. Every five healthy late-instar larvae were placed on a filter paper in a Petri dish where 1000 of nematode juveniles in 3 ml distilled water was added. The dish was completely closed. Dishes were kept at 25 ± 2°C in an incubator. After two days of inoculation, the larval cadavers were placed on white traps, according to the technique of (White, 1927). The infective juveniles were harvested and stored in sterilized distilled water at 10°C for 7-12 days before use (Woodring and Kaya, 1988). Nematode viability was checked when determining the dosage. Using a microscope, nematodes that did not move after being probed with a needle were considered dead.

Laboratory Experiment:

For the laboratory studies, a black cutworm larvae 6th instar were starved for 8 hours prior to experiment. All treatments were kept at 25 ±1C° and 70± 5 RH and natural photo period. Bioassay tests were carried out using conical plastic cups (4cm diameter x 3cm lower surface x 4cm height) with perforated covers for aeration, lined with satirized, moistened sandy loam soil. For each cup 1ml of a suspension containing 10, 20, 40, 70 and 100 IJs/larva was applied to the soil. One larva were placed in each cup to avoid cannibalistic behavior. Every replicate contain 10cup. This treatment was replicated five times for every tested nematode concentration. Castor leaves was provided as food. Cups with sterilized, moistened sandy loam soil with distilled water free of nematodes used as check ones. Larvae were observed, larval mortality was recorded at 24, 48 and 72 hours post treatment.

Field Trial:

Studying the pathogenicity of the (EPNs) strains as a bio-control agent against *A. ipsilon* in cotton field was carried out in Minufya Governorate. The area had not been treated with pesticides or fertilizes and was free of indigenous nematode. Samples from soil of cotton field were taken and mechanically analysed at Soils and Water Resource Department, Faculty of Agric., Al-Azhar University. The soil type was clay with a pH 7.1. Temperature in average was 15±2°C and 60 ± 5% RH.

Seeds were sown on February 11 in 2007. After approximately two weeks, seedlings were thinned to two plants per hill. The experimental design

was complete randomized blocks with three replicates for every concentration. Plot size was 0.75 m^2. Plots were separated by 0.50m. Each plots were surrounded by woody square barrier, the height of it was 20 cm to prevent the movement of larvae inside or outside the plot. Larvae of insect were collected from the laboratory rearing, then 10 larvae were gently placed into each treated in addition to plot as control.

The treatments consisted of three nematode strains *H. bacteriophora* (SAR), (FIN) and (HbS1). Nematode suspension was dispersed at five concentrations of 150, 300, 500, 700 and 900 IJS per larva. Control was treated with water only. Little of water were sprayed to each plot after treatment to enhance the distribution of nematode in the treated area. Each plot was replicated three times for each concentration. Experiments were repeated for application method, spraying at the surface of the soil and the sub-surface approximately (7cm depth). Alive and dead larvae of the tested insect were counted after 24, 48 and 72h after application. Dead larvae were examined for nematode infection.

Statistical analyses:

Data presented in percentage were normalized using arcsine transformation to calculate LC values. Analysis of mortality data from bioassays of Fin, SAR and Hbs1 nematode strains against the 6th instar larvae of *Agrotis ipsilon* was conducted using SPSS (ver11.0) computer software (SPSS for Windows, SPSS Inc., 1997). The significance of main effects was determined by one way analysis of variance (ANOVA). Significance of different treatments was evaluated by multiple comparison tests (Sheffé) using SPSS (ver11.0) computer software (SPSS for Windows, SPSS Inc., 1997).

RESULTS

The pathogencity of three EPNs strains (FIN, SAR and HbS1) to the 6^{th} larval instar of black cutworm *A. ipsilon* was investigated.

Three strains were selected to determine the median lethal concentrations (LC_{50}) under controlled laboratory conditions and cotton seedlings field conditions. The results in Table (1) present the LC_{50} and LC_{90} for the three tested strains under laboratory conditions. It is clear that the strains were pathogenic to the newly moulted 6^{th} larval instar of *A. ipsilon*. Larvae were susceptible to the applied concentrations. The determined LC values illustrate that positive correlation was found between insect mortality and inoculum concentration of EPNs. The increase in nematode concentration caused increase in percent larval mortality. EPNs strains differed in their virulence toward *A. ipsilon* based on LC_{50}. *H. bacteriohora* (FIN) had the lowest LC_{50} compared with the two other strains and was approximately 2 and 3 times more pathogenic than SAR and HbS1 strains), respectively after 72h post-infection. The same trend was observed after 48h. Beside that, the (FIN strain) was the most pathogenic than any other tested strains based on the LC_{90} 72h post infection. It could be arranged descendingly as follows: FIN (LC_{90}=67.4) followed by SAR

(LC_{90}=1.9x10^2), Hbs1 came in the third rank (LC_{90}=2.7x10^2). The differences in the virulence of the three strains after 48h could be arranged as follows: Fin, SAR and Hbs1 with LC_{90} of 3.5x10^3, 5.3x10^3 and 5.4 x 10^3, respectively. Positive correlation between larval mortality and the length of exposure time to *H. bacteriophora* strains.

Table (2) demonstrated that after 24 and 48 h from treatment in the open field, the LC_{50} of FIN strain is lower than any other strains. So it was more pathogenic than Hbs1 and SAR strains. Statistical analysis clarified that only the differences between LC_{50} and LC_{90} of Fin and SAR and between Fin and Hbs1 were significant ($P<0.05$) after both 48 and 72 h based on non overlapping in 95% confidence intervals.

The infectivity of the tested strains after 72h from infection could be arranged descendingly as follows: FIN> Hbs1 > SAR based on LC_{90} were 1.6 x 10^3, 3.9 x 10^3 and 4.5 x 10^3, respectively. Although the LC_{90} of FIN was four times lower than that of SAR, 72h post-infection, there was a substantial overlap in the 95% confidence limits of the two strains. Statistical analysis revealed that only the difference in LC_{50} Fin and Hbs1 was significant ($P<0.05$) after 72h based on non overlapping in 95% confidence intervals.

When sub-surface of soil was treated, obtained results indicated that the LC_{50} of FIN strain was approximately two times greater than Hbs1 at 48h post-infection (Table 3). The above mentioned difference was considered non-significant due to a substantial overlap in the 95% confidence limits of the two strains. Although SAR strain could be considered as the most pathogenic strain based on LC_{50}, statistical analysis revealed that only the difference between Fin and Hbs1 is significant ($P< 0.05$) due to non overlap in the 95% confidence intervals (LC_{50} = 3.9 x 10^2 for SAR, LC_{50}=6.0x10^2 for Fin and LC_{50} = 6.2 x 10^2 for HBs1 strain). It was observed that the LC_{90} of FIN strain was nearly four and five times greater than Hbs1 and SAR strains, respectively at 72h post-infection. These differences were considered non-significant due to a substantial overlap in the 95% confidence limits of the three tested stains. LC_{90} of the tested strains after 48h from infection were 5.0 x10^4 for FIN, 1.2 x 10^4 for Hbs1 and 2.5 x 10^6 for SAR.

Data in Tables (4 – 6) show the percentage larval mortality in relation to concentration for the three tested strains under controlled laboratory conditions, as well as open field conditions. Under laboratory conditions, no significant differences ($P>0.05$) in percentage larval mortality was observed between the three tested nematode strains for all applied concentrations, 24h post-infection Table (4). After 48h post-infection, significant differences ($p<0.05$) in the percentage larval mortality was observed between FIN and SAR strains for the concentrations 20 Ijs and 100 Ijs/larva. Non significant differences ($P>0.05$) were observed in the other concentrations Table (4).

On the other hand, significant differences ($P<0.05$) were recoded between FIN and SAR for the concentration 100 Ijs/larva Table (4). Non significant differences ($P>0.05$) were observed in the other concentrations 72h post-inoculum. Furthermore, significant differences ($P<0.05$) were observed between FIN and Hbs1 for all applied concentrations, 72h post-infection.

Table (1): Comparative susceptibility of newly moulted 6th instar larvae of *A. ipsilon*, to three nematode strains under controlled laboratory conditions.

Nematode strain	LC_{50} (95% confidence limits)*			LC_{90} (95% confidence limits)			Model fitting information		
	24 h	48 h	72 h	24 h	48 h	72 h	X^{2**}	*df*	*P***
Fin	2.5×10^4	1.0×10^2 (6.2×10-4.1×10^2)	17.1 (13.0 – 21.0)	2.6×10^7	3.5×10^3 (6.8×10^2-9.1×10^5)	67.4 (52.1-99.0)	11.3, 9.1, 14.5	23	0.98, 0.99, 0.91
SAR	3.3×10^3 (3.8×10^2-5.3×10^{36})	2.0×10^2 (1.0×10^2-1.6×10^3)	30.9 (23.8-39.0)	2.4×10^5 (3.6×10^3-5.6×10^{71})	5.3×10^3 (9.1×10^2-2.2×10^6)	1.9×10^2 (1.2×10^2 – 1.6×10^6)	19.5, 17.0, 34.8	23	0.67, 0.81, 0.05
Hbs1	2.5×10^4	2.7×10^2 (1.3×10^2-2.5×10^3)	41.1 (32.2 – 53.4)	5.7×10^6	5.4×10^3 (9.5×10^2-1.6×10^6)	2.7×10^2 (1.6×10^2-6.5×10^2)	14.8, 15.1, 26.9	23	0.90, 0.89, 0.26

* Significant difference at 95% confidence interval.
**X^2 after 24, 48 and 72 h, respectively.
****P* values after 24, 48 and 72 h, respectively.

Table (2): Comparative susceptibility of newly moulted 6th instar larvae of *A. ipsilon*, to three nematode strains under open field conditions (surface application).

Nematode strain	LC_{50} (95% confidence limits)*			LC_{90} (95% confidence limits)			Model fitting information		
	24 h	48 h	72 h	24 h	48 h	72 h	X^{2**}	*df*	*P***
Fin	1.4×10^4	2.2×10^3 (9.6×10^2-9.6×10^8)	3.3×10^2 (2.3×10^2 – 4.3×10^2)	4.4×10^5	5.3×10^4 (5.5×10^3-4.8×10^{21})	1.6×10^3 (1×10^3 – 4.8×10^3)	3.3, 3.9, 1.2	7	0.85, 0.79, 0.99
SAR	1.6×10^9	2.7×10^4	5.2×10^2 (3.6×10^2 – 8.6×10^2)	3.9×10^{14}	8.7×10^6	4.5×10^3 (1.9×10^3-9.5×10^4)	3.8, 7.2, 6.2	7	0.79, 0.41, 0.52
Hbs1	5.7×10^5	8.6×10^3	6.9×10^2 (5.2×10^2 – 1.2×10^3)	1.6×10^8	2.7×10^5	3.9×10^3 (1.9×10^3 – 2.9×10^4)	6.9, 1.8,1.6	7	0.44, 0.97, 0.98

*LC values with no 95% confidence interval don't fulfill the probit model equation.
**X^2 after 24, 48 and 72 h, respectively.
****P* values after 24, 48 and 72 h, respectively.

Table (3): Comparative susceptibility of newly moulted 6th instar larvae of *A. ipsilon*, to three nematode strains under open field conditions (subsurface application).

Nematode strain	LC_{50} (95% confidence limits) *			LC_{90} (95% confidence limits)			Model fitting information		
	24 h	48 h	72 h	24 h	48 h	72 h	X^{2**}	*df*	P^{***}
Fin	1.7×10^4	2.9×10^3 (1.1×10^3 – 3.6×10^8)	6.0×10^2 (3.6×10^2 – 2.9×10^3)	3.9×10^5	5.0×10^4 (5.6×10^3 – 8.0×10^{17})	1.4×10^4 (2.9×10^3- 7.2×10^9)	1.1, 1.8, 4.5	7	0.99, 0.97, 0.72
SAR	2.1×10^5	1.8×10^4	3.9×10^2 (2.6×10^2 – 5.6×10^2)	3.9×10^7	2.5×10^6	2.9×10^3 (1.4×10^3- 2.2×10^4)	1.9, 1.3, 8.9	7	0.96, 0.99, 0.26
Hbs1	2.1×10^3 (1.2×10^3 – 1.2×10^7)	1.7×10^3 (9.8×10^2 – 1.5×10^4)	6.2×10^2 (4.6×10^2 – 9.7×10^2)	6.2×10^3 (2.2×10^3 – 1.1×10^{11})	1.2×10^4 (3.4×10^3 – 2.9×10^6)	3.5×10^3 (1.8×10^3- 2.4×10^4)	5.3, 1.7, 2.6	7	0.06,0.97, 0.92

*LC values with no 95% confidence interval don't fulfill the probit model equation.
**X^2 after 24, 48 and 72 h, respectively.
***P values after 24, 48 and 72 h, respectively.

Table (4): The percentage larval mortality in relation to concentration for the three tested strains under controlled laboratory conditions.

Concentration IJS/ larva	Percentage mortality (Mean ± SE)								
	Fin			SAR			Hbs1		
	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
10	8±0.20^a	20±0.32^a	38±0.4^a	4±0.24^a	12±0.45^a	24±0.6^a	2±0.20^a	8±0.25^b	18±0.37^b
20	10±0.0^a	26±0.00^a	46±0.73^a	6±0.25^a	16±0.49^b	32±0.51^a	6±0.25^a	14±0.40^b	28±0.94^b
40	10±0.00^a	40±0.32^a	78±0.58^a	10±0.32^a	30±0.45^a	60±0.73^a	8±0.20^a	18±0.32^b	50±0.77^b
70	12±0.20^a	42±0.32^a	90±0.4^a	16±0.25^a	36±0.55^a	72±0.66^a	6±0.25^b	32±0.93^a	66±0.48^b
100	18±0.58^a	50±0.84^a	98±0.0^a	12±0.58^a	36±0.68^b	80±0.60^b	10±0.00^a	32±0.32^b	72±0.37^{bc}

*ANOVA different letters indicate significance level at α= 0.05

Obtained data show that significant difference ($P<0.05$) was observed between SAR and Hbs1 for 100 IJs/larva, 72h post-infection Table (4).

Results of open field conditions which illustrated in Table (5) clarified that after 24 h from infection non significant difference ($P>0.05$) was observed between the three strains for all applied concentrations.

After 48h from infection significant differences ($P<0.05$) were observed between Fin and SAR and Fin and Hbs1 and SAR and Hbs1 at concentration of 900 IJs/larva. In addition, significant differences. ($P<0.05$) were observed between Fin and Hbs1 at the concentrations of 300 and 500 IJs/larva.

At 72h significant differences ($P<0.05$) between Fin and SAR were observed at 150, 300 and 900 IJs/larva. Also the differences between Fin and Hbs1 were significant ($P<0.05$) at all applied concentrations. The differences between SAR and Hbs1 were significant at 150 , 300, 500, 700 and 900 IJs/larva.

In the case of sub-surface application, statistical analyses revealed that, no significant ($P>0.05$) differences between the tested strains were observed at all applied concentrations at 24h post infection (Table 6).

After 48h from infection only the difference between SAR and Hbs1 was significant ($P<0.05$) at the concentration of 700 IJs/larva.

At 72h post infection only the difference between Fin and Hbs1 was significant ($P<0.05$) at the concentration of 150 IJs/larva (Table 6).

Comparing the applied concentrations in relation to method of application FIN subsurface application at the rate of 300, 500 and 900Ijs/larva showed significant increase ($P<0.05$) in percentage mortality when compared to soil surface application ones, at 72 h post infection. Non significant differences ($P>0.05$) were observed in other concentrations. At the same time, SAR and Hbs1 soil subsurface application showed significant increase ($P>0.05$) in percentage mortality at the rate of 900 Ijs/larva when compared to soil surface application ones 72h post infection. Non significant differences ($P>0.05$) were observed in other concentrations.

DISCUSSION

The present study demonstrated that the entomopathogenic nematode strains *Heterorhabditis bacteriophora* (FIN, SAR and Hbs1 strains) were pathogenic to the newly moulted 6th larval instar of Black cutworm *A. ipsilon* under laboratory and filed conditions. 98%, 80% and 72% percentage mortality under laboratory conditions were achieved with Fin, SAR and Hbs1 nematode strains, respectively, at the highest concentration (100 Ijs/larva), and 72h post infection. Under field conditions, 93.3%, 73.3% and 63.3% mortalities were recorded at the soil subsurface application against 86.6%, 63.3 and 56.6% at soil surface application with Fin, SAR and Hbs1 nematode strain respectively, at the highest concentration (900 Ijs/larva), and 72h post-infection.

Table (5): The percentage larval mortality in relation to concentration for the three tested strains under open field conditions (surface application)

Concentration IJS/ larva	Percentage mortality (Mean ± SE)								
	Fin			SAR			Hbs1		
	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
150	3.3±0.33[a*]	13.3±0.33[a]	30.0±0.33[a]	3.3±0.33[a]	10.0±0.00[a]	20.0±0.33[a]	3.3±0.33[a]	6.7±0.67[a]	13.3±0.33[bc]
300	10.0±0.0[a]	23.3±0.33[a]	43.3±0.33[a]	6.7±30.33[a]	20.0±0.58[a]	43.3±0.33[b]	3.3±0.33[a]	10.0±0.00[b]	23.3±0.58[c]
500	10.0±0.0[a]	26.7±0.33[a]	60.0±0.00[a]	10.0±0.0[a]	16.6±0.00[a]	50.0±1.5[a]	6.7±0.33[a]	16.6±0.33[b]	43.3±0.33[b]
700	10.0±0.58[a]	26.6±0.33[a]	66.6±0.33[a]	3.3±0.33[a]	16.6±0.33[a]	53.3±0.33[a]	6.7±0.33[a]	16.6±0.33[a]	50.0±0.33[b]
900	16.7±0.67[a]	40.0±0.33[a]	86.6±0.33[a]	6.7±0.33[a]	23.3±0.33[b]	63.3±0.33[b]	6.7±0.33[a]	20.0±0.33[c]	56.6±0.33[c]

*ANOVA different letters indicate significance level at α= 0.05

Table (6): The percentage larval mortality in relation to concentration for the three tested strains under open field conditions (subsurface application).

Concentration IJS/ larva	Percentage mortality (Mean ± SE)								
	Fin			SAR			Hbs1		
	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
150	3.3±0.0.33[a*]	10.0±0.33[a]	33.3±0.33[a]	3.3±0.33[a]	10.0±0.68[a]	26.6±0.33[a]	0.0±0.00[a]	6.67±0.33[a]	16.6±0.33[ab]
300	3.3±0.58[a]	13.0±0.33[a]	33.3±0.67[a]	6.67±0.58[a]	13.0±0.67[a]	43.3±0.1.2[a]	0.0±0.00[a]	10.0±0.00[a]	30.0±0.57[a]
500	10.0±0.0[a]	26.6±0.33[a]	43.3±0.67[a]	6.67±0.33[a]	23.3±0.33[a]	56.6±1.2[a]	6.7±0.33[a]	20.0±0.00[a]	36.6.3±1.2[a]
700	6.67±0.33[a]	20.0±0.33[a]	50.0±1.2[a]	6.67±0.33[a]	13.3±0.33[a]	60.0±0.58[a]	10.0±0.00[a]	30.0±0.00[ac]	56.6±0.88[a]
900	13.3±0.33[a]	33.3±1.2[a]	93.3±1.8[a]	10.0±0.00[a]	23.3±0.33[a]	73.3±1.2[a]	13.3±0.33[a]	33.3±0.33[a]	63.3±0.33[a]

*ANOVA different letters indicate significance level at α= 0.05

There was a close positive correlation between insect mortality and exposure time with all concentrations under both laboratory and filed conditions. Similar increases in host mortality associated directly with increase of exposure time in case of *S. littoralis* (Glazer *et al.*, 1991), *Spodoptera. frugiperda* and *A. ipsilon* (Huf.) (Epsky and Capinera 1993). Hammad and Abdel-Hamid (2007). Reported that house fly larvae were susceptible to the two EPNs. Nematode species/strain, *Steinernema carpocapsae* (All strain) and *H. bacteriophora* (TK strain) nematode inoculums level and incubation temperature all have been reported to significantly affect the number of IJs infecting host insects. EPNs in the families Heterorhabditidae and Steinernematidae are lethal of insects and have been proven effective against *A. ipsilon* (Hammad 2001).

In this respect Jin (1989) found that the larvae of *P. rapae* and *A. ipsilon* were very sensitive to the nematode; and both insects died 3-5 days after inoculation Wu and Chow (1989) reported that larvae of *P. rapae* infected with *S. feltiae* exhibited mortality 3 days after exposure ranging from 75 to 97.5%. Also, Saleh (1995) found that, *H. tayserae* nematode, at concentration of 5-100 Ijs/larva of *P. rapae* induced 30-100% and 55-100% within 24 and 48 hours, respectively.

Obtained results also showed that larval mortality differed according to nematode strains. In this respect, (Bedding *et al.*, 1983) also reported that infectivity of the EPNs varies widely according to nematode species and strains. Data also indicated that the higher nematode inoculum levels; however, caused higher and faster mortality than the lower levels. The death of the treated insect larvae is caused mainly by the effect of the nematode associated bacteria. Thus, it could be suggested that the higher concentrations of nematodes will elaborate much more bacteria which in turn multiply rapidly producing huge numbers of bacterial cells which in turn kill the insect larvae more rapidly. This may be explain why the higher concentrations caused faster and higher mortality than lower ones (Salem *et al.*, 2007).

In brief, our results show that the three selected EPNs strains (FIN, SAR and Hbs1) of *H. bacteriophora* may be pathogenic against 6th larval instar of *A. ipsilon.* The virulence of nematode strains for it could be arranged as follows: Fin > SAR then Hbs1. Results also proved that sub-surface application of EPNs is preferable and effective because it help to avoid the adverse environmental effects of UV and high temperature (Gaugler *et al.*, 1992).

EPNs hold promise in controlling black cutworm in seedling cotton field, particularly when the application of nematodes was at the subsurface of the soil. This efficacy difference is because below the soil surface, conditions may enhance nematode survival and efficacy (Duncan and Mccoy, 2001).

REFERENCES

Abdel-Salam, N.M. (1987): Bionomic studies on the greasy cutworm, *Agrotis ipsilon* (Hufn.), (Lepidoptera: Noctuidae), Ph.D. Thesis, Fac. Agric., Al-Azhar Univ., Cairo, Egypt.

Bedding, R.A. and Molyneux, A.S. (1982): Penetration of insect cuticle by infective juveniles of *Heterorhabditis* spp. (Heterorhabditidae: Nematoda). Nematologica, 28, 354-359.

Bedding, R.A., A.S. Molyneux and R.J. Akhurst, (1983): *Heterorhabditis spp. Neoaplectana spp.* and *Steinernema kraussei* interspecific and interspecific difference in infectivity for insects. Exp. Parasit, 55 (2): 249-257.

Capinera, J.L., D. Pelissier, G.S. Menout and N.D. Epsky, (1988): Control of black cutwork, *Agrotis ipsilon* (Lepidoptera: Noctuidae), with entomogenous nematodes (Nematoda: Steinernematidae, Heterorhabditidae). J. Invertebr. Pathol., 52:427-435.

Caroli, L.; I. Glazer and R. Gaugler (1996): Entomopathogenic nematode infectivity assay: Comparison of penetration rate into different hosts (Biocontrol Sci. Technol., 6(2):227-233)).

Dowds, B.C.A. Peters, A. (2002): Virulence mechanisms. In: Gaugler, R. (Ed.), Entomopathogenic Nematology. CABI, New York, NY, pp. 79-98.

Duncan L.W., Coy C.W. (2001): Hydraulic lift increases herbivory by *Diaprepes abbre* viatus larvae and persistence of *Steinernema riobrave* in dry soil. J. Nematol. 33, 142-146.

Dutky, S.R., Thompson, J.V. and Cnatwell, G.E. (1964): A technique for the mass propagation of the DD-136 nematode. Journal of Insect. Pathology., 6, 414-422.

Epsky N. D, and Capinera J.L. (1993): Quantification of invasion of two strains of *Steinernema carpocapsae* (weiser) into three lepidopteran larvae. J. Nematol. 25: 173-180.

Gaugler R., Bednarek A. Bednarek and Compbell J.F. (1992): Ultra violet inativation of heterorhabditid and steinernematid nematodes. J. Invertebr. Pathol. 59: 155-160.

Gaugler, R. (1988): Ecological considerations in the biological control of soil-inhabiting insect pests with entomapathogenic nematodes. Agric. Ecosyst. Environ., 24:351-360.

Georgis, R. and S.A. Manweiler, (1994): Entomopathogenic nematodes: a developing biological control technology, pp. 63-94. In: K. Evans (ed.), Agricultural Zoology, Reviews, Intercept, UK.

Glazer I.S., Glaper S., and Sharon E. (1991): Virulence of the nematode (setinernematids and Heterorhabditis) bacteria (*Xenorhabdus spp*) complex to the Egyptian cotton leaf worm, *Spodoptera littoralis* (Lepidoptera: Noctuidae). J. invertebr. Pathol. 57: 94-100.

Grewal, P.S. and Georgis, R. (1998): Entomopathogenic nematodes. *Methods In Biotechnology*, Vol. 5, *Biopesticides: Use and Delivery* (Eds. F.R. Hall and J.J. Menn), pp. 271-279. Huaman Press Inc., Totowa.

Grewal, P.S., Ehlers, R.U. and Shapiro-Ilan, D.I. (2005): Nematodes as Biocontrol Agents. CABI, New York, NY (in press).

Hammad, S.A.M. (1996). Studies on the effect of some pathogenic organisms on insect pests. M.Sc. Fac. Agric., Al-Azhar Univ., 110 pp.

Hammad, S.A.M. (2001): Studies on the biological control of some insect pests of berseem using Entomopathogenic nematodes, Ph.D. Thesis, Fac. Agric., Al-Azhar Univ. 189 pp.

Hammad, S.A.M. and Abdel –Hamid, Y.M (2007): Factors affecting susceptibility of the house fly larvae to infection with two Entomopathogenic nematodes under laboratory conditions. Ain Shams Science Bulletin, 45: 43-55.

Hill, D.S. (1975): Agricultural insect pests of the topics and their control (Printed in Great Britain, Cambridge University Press, 499 pp.).

Jin, D.C., (1989): A parasitic nematode isolated from *pieris rapae L. sichuan*. J. Agri. Science, 2 (1):87-89.

Kaya, H.K. (1990): Soil ecology, pp.93-115. In Entomopathogenic nematodes in biological control (R. Gaugler and H.K. Kaya, eds.) CRC Press, Boca Raton, Florida.

Kaya, H.K. and Gaugler, R. (1993): Entomopathogenic nematodes. Annu. Rev. Entomol., 38, 181-206.

Khalil, M.E., G.E.S. Abo El-Ghar, and T.M. Eid (1994): The antifeedant and growth disturbing activity of plant extracts on the black cutworm. *Agrotis ipsilon* (Hufn.) (Lepidoptera: Noctuidae). Bull. Ent. Soc. Egypt. Econ. Ser., 21: 135-150.

Levine, E. and H. Oloumi-Sadeghi, (1993): Field evaluation of *Steinernema carpocaspae (Rhabditida: Steinernematidae)* against black cutworm (Lepidoptera: Noctuidae) larvae in field corn. J. Entomol. Sci., 27 (4):427-435.

Rings, R.W. and G.J. Musick, (1976): A pictorial field key to the armyworms and cutworms attacking corn in the north central states. Ohio Agric. Res. Dev. Cen. Res. Circ., 221.

Saleh, M.M.E., (1995): Efficiency of the Egyptian entomopathogenic nematode *Heterorhabditis tayserae* (Nematoda: Heterorhabditidae) in controlling the cabbage worm, *Pieris rapae* (L.) Lepidoptera: Pieridae). Egypt. J. Biol. Pest Control, 5 (2): 103-105.

Salem S.A, Abdel-Rahman H.A., Zebitz C.P.W., Saleh M.M.E., Ali, Fawkia I., El-Koly M.Y. (2007): Evaluation of Entomopathogenic nematodes in controlling some cabbage pests. J. Appl. Sci. Res. 3 (4): 323-328.

Susurluk A, (2006): Effectiveness of the Entomopathogenic nematodes *Heterorhabditis bacteriophora* and *Steinernema feltia* against *Tenebrio molitor* (Yello Mealworm) larvae in different soil types at different temperatures Turk J. Biol. 30: 199-205.

Tashiro, H. (1987): Turfgrass insects of the United States and Canada. Comstock, Ithaca, NY.

White, G.F. (1927): A method for obtaining infective nematode larvae from cultures. Science (Washington, DC), 66:320-303.

Woodring, J.L., and Kaya, H.K. (1988): "Steinernematid and Heterorhabditid Nematodes. A Handbook of Biology and Techniques". Southern Coop. Ser. Bull. 331. Arkansas Agric. Exp. Fayetteville, AR.

Wu, HJ. and Y.S. Chow, (1989): Susceptibility of *Pieris rapae* (Lepidoptera: Pieridae) to the imported entomogenous nematode *Steinernema feltiae*. Bulletin of the Institute of Zoology, Academia, Sinica, 28 (4): 237-244.

دراسات معملية وحقلية على قدرة بعض سلالات من الديدان الخيطية الممرضة للحشرات لإصابة الدودة القارضة

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تم انتخاب ثلاث سلالات من الديدان الخيطية الممرضة للحشرات والتي تنتمي إلى الجنس هيتيرورابدتيس واختبار قدرتها على إصابة العمر اليرقي السادس للدودة القارضة، وقد وجد أن كل السلالات كانت ممرضة لهذه الحشرة. وبتحليل البيانات تبين أن سلالات الديدان الخيطية وفترة التعريض (٢٤، ٤٨، ٧٢ ساعة) والجرعة (١٠، ٢٠، ٤٠، ٧٠، ١٠٠ يرقة معدية/حشرة) وكذلك الجرعة فى التجربة الحقلية (١٥٠، ٣٠٠، ٥٠٠، ٧٠٠، ٩٠٠ يرقة معدية /حشرة) فى تجربة الحقل وطريقة التطبيق (سطح التربة وتحت سطح التربة) تؤثر معنويا ($P<0.50$) على وفيات اليرقات وقد تبين أن معدل الوفاة يرتبط ارتباطاً مباشراً بالجرعة المستخدمة وفترة التعريض واختلاف السلالة. وفى الدراسة المعملية كانت قيم LC_{50} (١٧,١، ٣٠,٩، ٤١,١) فرد يرقة معدية لكل من السلالات الثلاث FIN, SAR, Hbs1 على التوالي بعد ٧٢ ساعة من المعاملة وقد كانت السلالة FIN أكثر قدرة على الإصابة لأنها حققت أعلى نسبة للوفيات فى العمر اليرقي السادس للحشرة فعند مقارنة النسبة المئوية للوفيات التي حققتها السلالات الثلاثة وجد أنهـــا كانت (٩٨% ، ٨٠%، ٧٢%) بعـد ٧٢ ساعة من المعامله وذلك عند تركيز ١٠٠ يرقة معدية/حشرة.

أما نتائج التجربة الحقلية فقد حققت السلالات الثلاث (٩٣,٣%، ٧٣,٣% ، ٦٣,٣%) نسبة منوية للوفيات فى العمر اليرقي السادس والمعاملة (تحت سطح التربة) وذلك فى مقابل (٨٦,٦% ، ٦٣,٣% ، ٥٦,٦%) عند معاملة (سطح التربة) على التوالي السابق عند تركيز ٩٠٠يرقة معدية/حشرة بعد ٧٢ ساعة من المعاملة. وقد كانت قيم LC_{50} (٦×١٠^{٢}، ٣,٩× ١٠^{٢}، ٦,٢؛ ١٠^{٢}) المعاملة (تحت سطح التربة) وذلك فى مقابل (٣,٣× ١٠^{٢}، ٥,٢× ١٠^{٢} ، ٦,٩ × ١٠^{٢}) على التوالي السابق عند معاملة (سطح التربة) بعد ٧٢ ساعة.

وقد أوضحت النتائج أن العمر اليرقي السادس للدودة القارضة أكثر قابلية للإصابة بالسلالات الثلاثة عند التطبيق تحت سطح التربة عنها عند التطبيق على سطح التربة على التوالي.