Molecular and biochemical characterization of the nematode-insect relationship

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ABSTRACT

In the present study, four nematode species, two belong to the genus Steinernema (S. glaseri, and S. carpocapsae) and two belong to Heterorhabditis (H. bacteriophora and H. megdis), were tested for their ability to kill the third larval instars of white grub (Pentodon bispinosos). The mortality percentage increased with increasing the nematode concentration. S. glaseri proved to be the most effective species against the third larval instars of white grub, 90% mortality followed by H. bacteriophora (83%) and S. carpocapsae (56%), while H.megdis gave the lowest mortality percentage (37%). Based on the data obtained, mortality percentages were higher when the soil surface was contaminated with the nematode suspension. The effect of entomopathogenic nematodes on protein and esterase profiles of the larval hemolymph were investigated. The nematode infection induced some additional bands, while disappearance of other bands was recorded also; an increase in the esterase activity at 48 hr post infection was observed, The genetic polymorphism between the entomopathogenic nematode species used was resolved by RAPD analysis. A total of 104 and 80 bands with 65.35% and 66.2% genetic polymorphism were obtained for the nematode species belongs to genus Steinernema and the genus Heterorhabditis, respectively. The highest number of species-specific markers was recorded for H. megdis (21) followed by S. carpocapsae (15) then S. glaseri (10), while H. bacteriopra showed 7 specific makers only. These markers can be considered as useful markers for the four entomopathogenic nematode species used and can be used to design a very effective biological control program to control white grub in sugarcane plantations in Upper Egypt.

Key word: *Nematode species, white grub, application methods, hemolymph protein, RAPD marker.*

INTRODUCTION

White grubs (*Pentodon bispinosus)*, the root-feeding larvae of scarab beetles (*Coleopteran: Scarab-aeidae*), are important pests of agricultural plants world wide (Koppenhofer and Eugene, 2008). In Egypt, larvae of white grubs are a major pest of sugarcane. The white grub species have an annual life cycle with adults emerging in summer to lay eggs in the soil among the roots of the host plants (Potter, 1998). By late summer, most larvae have developed into the third instar. The larvae continue feeding until pupation in late spring, extensive feeding activity of the larger larvae kills large areas of sugarcane especially under warm dry conditions. The insecticides have

been the primary means for managing grubs for long time (Potter *et al.*, 1996). Increasing concerns about the environment and human safety, effects on non-target organisms, reduced efficacy due to microbial degradation or insecticide resistance, and tolerance have created a need for alternative control strategies (Gaugler, 1987).

Entomopathogenic nematodes (*Heterorhabditidae* and *Steinernematidae*) over an environmentally safe and IPM compatible alternative to chemical insecticides for the control of white grubs and other soil inhabiting insects (Kaya and Gayugler, 1993; Grewal *et al.*, 2005) have been applied. When the nematode was applied under conducive conditions, it has been as effective as chemical insecticides against *Popillia japonica* larvae (Georgis and Gaugler, 1991). Entomopathogenic nematode from the genus *Steinernematidae* and *Heterorhabditidae* was characterized by a symbiotic association with bacteria of the genera *Xenorhabdus* and *Photorhabdus,* respectively. The bacteria are contained in the intestine of the free- living infective juveniles (IJS) of these nematodes, which are capable of seeking out hosts (Ansari *et al.*, 2003). The IJS are enter the hemocoel of the insect and release the symbiotic bacteria (Kaya and Gaugler, 1993) that multiply in the hemolymph causing insect death within 48-72 hr which and establishing conditions for nematode development in the cadaver by providing nutrients (Nickel and Welch, 1984) , they also initially prevent the growth of other microorganism (Forst and Nealson , 1996). Since the hemolymph is the main site of action, biochemical changes in its components are expected (EL-Bishry *et al.*, 1997). EL-Bishry and Eid (1992) reported that hemolymph protein of *Spodoptera littoralis* sixth instar larvae is markedly reduced after 30 hrs of the infection with *Steinernema carpocapsae*. Enzyme markers have been found to be especially suitable (Patnaik and Datta, 1995). In the present study, two entomopathogenic nematode species, belonging to the genus *Steinernema (S. glaseri and S. carpocapsae)*, and two of the genus *Heterorhabditis* (*H. bacteriophora*, *H. megdis*) were used to control the *Pentodon bispinosos* larvae under laboratory conditions. These four nematode species are finger printed using RAPD analysis in trial detect species specific markers useful for the biological control program of these nematode species. The optimum concentration and the effective application method were determined. The effects of entomopathogenic nematodes on the white grub hemolymph protein and esterase banding patterns of third instar was investigated.

MATERIALS AND METHODS

Among the field-collected insects, the third-instar larvae were used in all experiments. White grub (*Pentodon bispinosus*) was collected from sugarcane cultivated areas at Nagaa Hamady and Nakada, Quina governorate, Egypt. None of the sites had been treated with insecticides during the previous year. Larvae were kept individually at 27°C for 1-4 weeks in a mixture of organic compost and loamy sand. *S. glaseri*, *S. carpocapsae* , *H. bacteriophora* and *H. megdis* were cultured in the last instar larvae of the greater wax moth, *Galleria mellonella* (L.) according to Dutky *et al.* (1964). The emerging infective juveniles (IJs) were harvested from White traps and stored in distilled water at 15°C and 4°C for the nematodes belonging to the genus *Heterorhabditis* and *Steinernema,* respectively. The soil used in the laboratory experiments was sandy loam (69% sand, 14% silt, 16% clay, and 1% organic matter, pH 6.4) that had been pasteurized (3 hr at 70°C) and air-dried before

use. The following experiments were conducted under controlled laboratory conditions (27°C) in plastic cups (10 cm diameter x 15 cm depth) filled with 125 g of moist soil provided with pieces of sugarcane as a food.

I- Effects of different nematode concentrations on the mortality of grub larvae

The experiment was conducted to examine the lethal effect of four entomopathogenic nematode *S. glaseri*, *S. carpocapsae* , *H. bacteriophora* and *H. megdis* on white grub. The 3rd larval instars were inoculated with the nematodes with concentrations of 0, 100, 200, 300, 400 and 500 IJs/cup. Each concentration contained 3 replicates of 15 larvae each. All cups were kept under a controlled room temperature (27°C). Cups were inspected daily after treatment; dead larvae, were counted, washed and kept in extraction chambers.

2-Application methods

Four methods were tested with *S. glaseri*, *S. carpocapsae*, *H. bacteriophora* and *H. megdis* at concentration of 300 IJs\cup. The first method: adding the nematode suspension directly to the soil surface before transferring the larvae. In the second; a single cadaver of *Galleria mellonella* containing infective juveniles (IJs) was buried in the soil moistened around 20 % R.H. The third was carried out by placing the white grub larvae in a Petri-dish (10 cm diameter) containing a Whatman filter paper wetted by nematode suspension. The last one was accomplished by adding nematode suspension directly on the white grub larvae. Each method was represented with four replicates each comprised 10 cups. All cups were covered and kept under the room temperature (27°C). Cadavers from each treatment were counted and placed in the extraction chambers. Controls received water only.

3- The effects of nematode on white grub hemolyph protein and esterase banding pattern

The hemolymph were collected from the healthy (non-infected) and infected larvae 24, 36 and 48 hrs post infection through a puncture in one of the prolegs of larvae with a fine needle. Hemolymph was received in clean sterilized vials provided with few crystals of phenylthiourea (PTU) to prevent melanization. The hemolymph samples were centrifuged at 10,000 rpm for 10 min at 4ºC to remove hemocytes and cell debris. The supernatant was recovered in a new vial and stored at -20°C until use.

SDS-protein electrophoresis

Protein extraction was performed using the hemolymph samples isolated from white grub larvae infected with *H. bacteriophora* and S. *glaseri*. SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed for total storage proteins according to the method described by Laemmli (1970).

Isozyme analysis

Isozyme extraction was performed using hemolymph samples isolated from white grub larvae infected with *H. bacteriophora* and S. *glaseri*. Hemolymph samples were extracted by 2 ml extraction buffer (0.1% (w/v) Tris-citric acid, pH 7.5; 1% (w/v) polyvinyl pyrolidone (PVP); 0.1% (w/v) ascorbic acid and 0.1% (w/v) cysteine) and centrifuged at 5333 xg (JS - 5.2 rotor), at 4 °C for 5 min. Twenty µl of the extracted samples were used for electrophoresis on polyacrylamide gel (SDS-PAGE) according to the method of Stegmann *et al.* (1983), using Pharmacia electrophoresis apparatus (GE-4). Estrase was detected by incubating the gel in darkness for one hour at 37°C in a mixture of 100 ml

phosphate buffer (0.15 M pH 7.2) , 20 mg 1-naphthylacetate dissolved in 2 ml acetone and 50 mg fast blue RR salt. After the incubation period, the gel was rinsed in distilled water and fixed in 50% glycerol for one hour. Rf value of each band was calculated as follows:

$$Rf = \frac{\text{Distance travelled by the band from the top of the running gel}}{\text{Distance travelled by the tracking dye}}$$

RAPD analysis

Total genomic DNA was isolated using the CTAB method described in Rogers and Bendich (1985). PCR reactions were conducted using arbitrary 10-mer primers Operon Technology Inc., Alameda, CA, USA). The names and sequences of the primers that give clear bands are as follows:

Primer name	Sequence
OPA-01	5` - CAGGCCCTTC -3`
OPA-04	5` - AATCGGGCTG -3`
OPB-10	5` - CTGCTGGGAC -3`
OPC-05	5` - GATGACCGCC -3`
OPC-08	5` - TGGACCGGTG -3`
OPD-05	5` - TGAGCGGACA-3`
OPD-10	5` - GGTCTACACC -3`
OPF-15	5` - CCAGTACTCC -3`
OPK-04	5` - CCGCCCAAAC-3`
OPK-10	5` - GTGCAACGTG -3`
OPP-03	5` - CTGATACGCC -3`

The reaction mixture (20 µl) contained 10 ng DNA, 200 µM dNTPs, 1 µM primer, 0.5 units of Red Hot Taq polymerase (AB-gene Housse, UK) and 10-X Taq polymerase buffer (AB-gene House, UK). Samples were heated to 94°C for 5 min and then subjected to 35 cycles of 1 min at 94°C; 1 min at 35°C and 1 min at 72°C. The amplification products were separated in 1% (w/v) agarose gel in 1 x TBE buffer and visualized by staining with ethidum bromide. Reproducibility of DNA profiles was determined by replicating all RAPD reactions at least three times. Variations among entomopathogenic nematode across the primers used were evaluated from pairwise comparison for the proportion of shared bands amplified (Nei 1987).The similarity coefficients was calculated by using statistical software package STATISTICA_SPSS (Stat Soft Inc.).

RESULTS AND DISCUSSION

Entomopathogenic nematodes (EPN) are a ubiquitous group of obligate and lethal parasites of insects. They are widely used as biological control agents of many insect pests (Kaya and Gaugler, 1993). Koppenhofer and Fuzy (2003) isolated a new nematode species, *Steinernema scarabae,* from white grubs *Exomala orientalis* and *Popillia japonica* (*Coleoptera, Scarabaeidae*) in New Jersey and they could use them to control the economically important white grub species. Grewal *et al.* (2004) reported that the potential for biological control of *Heterorhabditis zealandica* X1(H2-X1) and *H. bacteriphora* GPS11 (Hb-GPH11) were 73-98 and 34-97% control of *P. japonica* and 72-96 and 47-83% control of *Cyclocephala borealis,* respectively. When *Steinernema glaseri* was used against the same insects, 6-58% control of *P. japonica* was recorded with no control for the *C.*

borealis. The four nematode species, two belong to the genus *Steinernema* (*S. glaseri,* and *S. carpocapsae*), and the others to *Heterorhabditis* (*H. bacteriophora* and *H. megdis*) were tested for their ability to kill the third larval instar of white grub *(Pentodon bispinosos)*. The data illustrated in Fig. 1 show that, the mortality percentages after 7 days from treatment *at* 27 °C, increased with increasing the nematode concentration and the highest mortality percentage was achieved by using 500 IJs /insect in all cases. Using 500 IJS/insect, *S. glaser*i was the most effective against the third larval instars of white grub 90% mortality followed by *H. bacteriophora* (83%) and *S. carpocapsae* (56%) while *H. megdis*, gave the lowest mortality rate (37%).

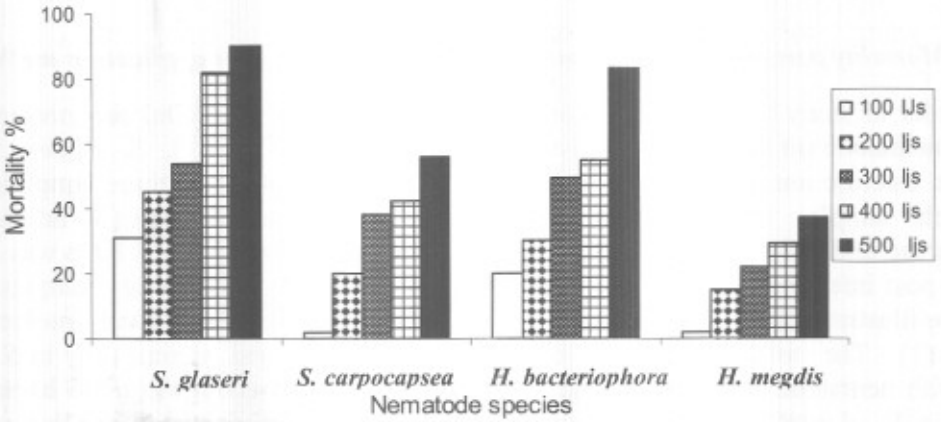

Fig. (1): Mortality percentages of white grub larvae treated with different nematode species at different concentrations (100, 200, 300, 400, and 500 IJS/ insect).

To determine the best treatments for controlling white grub under laboratory conditions four entomopathogenic nematode species were applied to the third larval instar of white grub through four application methods and data were illustrated in Fig. 2. The mortality percentages of white grub larvae were higher when the soil surface was contaminated with the nematode suspension. On the other hand, nematode wetted filter paper method was unsuitable, having the lowest mortality percentage, these results are in agreement with those reported by Ibrahim (2005) in which contaminated was the most effective. Referring to the other two methods, it is clear that the direct spraying of infected on the grub was less efficient followed by burying of the infected cadaver into the soil, but both were of comparably of lower actively than soil contamination. It may be mentioned that, irrespective of method, the nematode species are arranged according to their activity against the grub as *S. glaseri, H. bacteriophora, S. carpocapsae* and *H. megdies*.

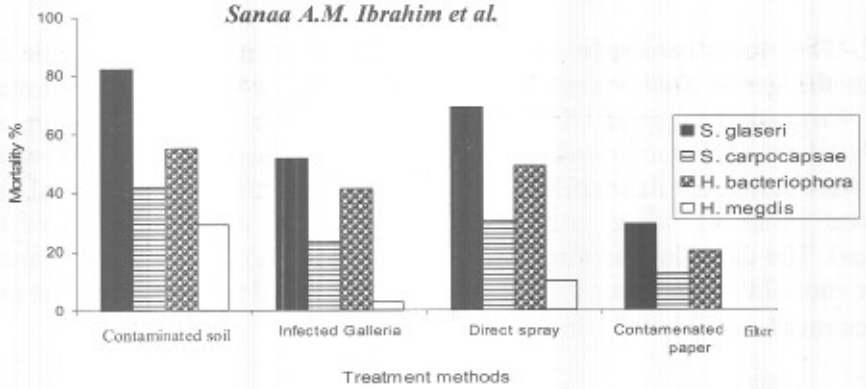

Fig. (2): Mortality percentages of four nematode species under four application methods.

In order to study the effect of entomopathogenic nematode on the larval hemolymph, protein and esterase analyses were performed. Hemolymph samples were collected from infected larvae and from the control at 24, 36 and 48 hr post infection. The protein banding patterns are illustrated in Fig. (3) and recorded in Table (1). The total number of bands differed with nematode infection, the number increased in *S. glaseri* and *H. bacteriophora* (48 hr) (10 bands each) comparing with the control (8 bands), however, the numbers lower in both treatments at 24 or 36 hr. The nematode infection induced some new bands, but in the other cases caused other bands to disappear. The bands with molecular weights (60.5 and 88.46 kDa) are present with high intensity in control, *S. glaseri* (48 hr), *H. bacteriophora* at the three sampling periods. In the *S. glaseri* treatment (36 hr) the band with molecular weight 58.58 KDa was absent but it appeared in all other samples with low intensity. The band with molecular weight 43.05 KDa was present only in *S. glaseri* (48 hr) and *H. bacteriophora* (48 hr) and this band completely disappeared in other samples .The protein band with molecular weight 26.36 KDa was absent in *S. glaseri* and *H. bacteriophora* 36 hr but found in the other samples. The band with molecular weight 14.42 KDa was present with low intensity in all samples except in *S. glaseri* (36 hr).

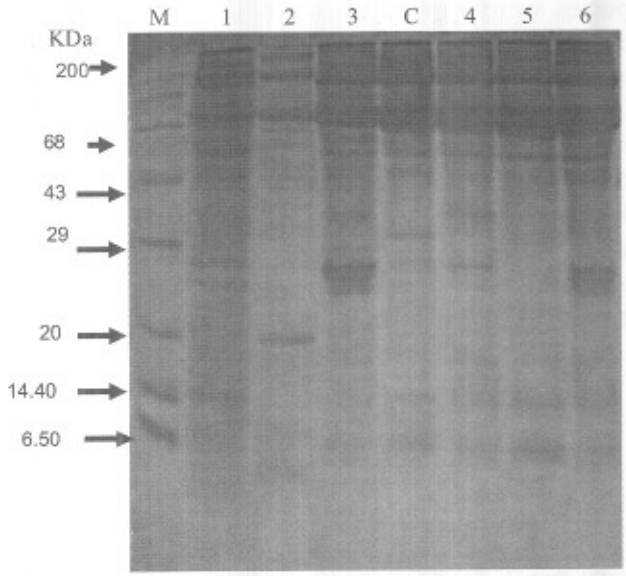

Fig.(3): The SDS-PAGE of total protein extracted from the white grub hemolymph samples collected at 24, 36 and 48 hr post infection, M : Marker ,lanes 1-3 are the white grub treated with S. glaseri at 24, 36 and 48 hr, C; control and lanes 4-6 white grub treated with H. bacteriophora at 24, 36 and 48 hr, respectively.

Table (1): Survey of SDS-protein bands in the white grub hemolymph, broad range protein marker was used to detect M.W. of extracted protein.

M.W (KDa)	Control	*S. glaseri*			*H. bacteriophora*		
		24h	36h	48h	24h	36h	48h
160.50	+	+	+	+	+	+	+
88.46	+	+	+	+	+	+	+
68.00	-	-	+	-	-	-	-
58.58	+	+	-	+	+	+	+
43.05	-	-	-	+	-	-	+
35.50	-	+	-	+	-	-	-
31.61	+	-	-	-	-	-	+
26.36	+	+	-	+	+	-	+
24.02	-	+	-	+	-	-	+
20.00	-	-	+	-	-	-	-
14.42	+	+	-	+	+	+	+
6.50	+	+	+	+	+	+	+
4.00	+	-	-	+	+	+	+
Total	8	8	5	10	7	6	10

The effect of entomopathogenic nematode infection on the larval hemolymph esterase isozyme banding pattern was detected at three different treatment times. Fig. (4) and Table (2), show the esterase profiles and the Rf values of the control and infected hemolymph. A total of four bands were detected with variation in their intensity depending upon the infection and time of sampling. The band with Rf 0.163 was found with the same intensity in the control and other samples except with *S. glaseri* which appeared in low intensity (36 hr) and completely disappeared in the last sampling (48 hr). The band with RF 0.072 showed low intensity in control, *S. glaseri* (48 hr) and *H. bacteriophora* (24 and 48hr) and was absent in *S. glaseri* (24 and 36 hr), and *H. bacteriophora*(36 hr). The band with RF 0.227 was presented in high intensity in *S. glaseri* (48 hr) and *H. bacteriophora* (24 and 48hr) but absent in control and *S. glaseri* (24 and 36hr) and *H. bacteriophora* (36hr). The band with Rf 0.68 was present in low intensity only in *S. glaseri* and *H. bacteriophora* at the second sampling as well as in control.

Table (2): Rf value of the esterase bands in larvae hemolymph of control and infected white grub at 3 sampls.

Rf	Control	S.g(24h)	S.g(36h)	S.g(48h).	HB (24h)	HB (36h)	HB (48h)
0.0727	+	-	-	+	+	-	+
0.163	+	+	+	-	+	+	+
0.227	-	-	-	+	+	-	+
0.68	+	-	+	-	-	+	-
Total	2	1	2	2	3	2	3

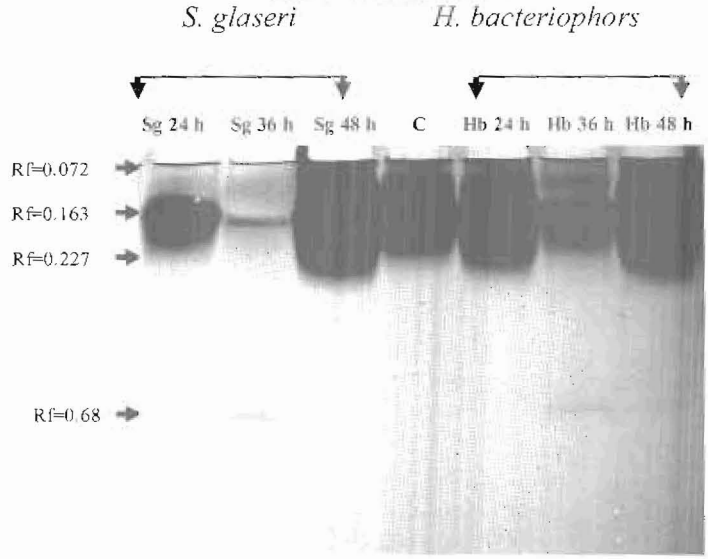

Fig.(4): Esterase banding pattern and Rf values in control and hemolymph samples of white grub larvae treated with two entomopathogenic nematode for 24, 36 and 48 hr.

Random amplified polymorphic DNA (RAPD)

In order to find more variation among the species of nematode, they were exposed to the random amplified polymorphic DNA (RAPD) analysis. DNA samples were extracted and analyzed by RAPD-PCR technique using 11 oligonucleotides primers (Table 3). All the 11 primers produced reproducible PCR products with a clear pattern for each species and showing informative RAPD profiles (Fig. 4).As in Table 3, the highest number of bands (12 bands) was generated by using the primers OPA-01, OPK-04 and OPK-10 in *Steinernema* genus, while the lowest number was 4 bands in *Heterorhabditis* genus and generated with primer OPP-03.The total number of generated bands in *steinernema* genus was 104; 68 out of them were polymorphic (65.35%). The highest number of polymorphic bands (9 bands) was obtained with primer OPP-03, with 90% polymorphism and the lowest number was 3 bands with primer OPD-10 representing 60% polymorphism, while the primer OPD-05 recorded the lowest percentage of polymorphism (44.4%). In the *Heterorhabditis* genus the total number of generated bands was 80 ; 53 out of them were polymorphic (66.2%). the highest number of polymorphic bands (9 bands) was obtained with primer OPD-05 ,with 81.8% polymorphism and the lowest number was 2 bands with primer OPK-04 representing 40% polymorphism (Fig.4 and Table 3). In the present study, the genotype–specific bands were determined (Table 4). The highest number of specific marker was recorded for the genus *S. carpocapsae* (15) followed by *H. megdis* (12) then *S. glaseri* (10) while *H. bacteriopra* showed 7 markers only.

Taking all data together, it can be concluded that increasing activity of white grub increases the opportunity of random encounter between them and the pathogen. Also, moistened soil is the best environment for nematode IJs, where it can move easily guided by certain cues produced by their insect hosts heading toward these hosts (Nguyen and Smart, 1990). These two reasons are most probably the main cause of the

superiority of contaminating soil surface as a method of application to white grub control as found in the present study. Burying infected *Galleria* larvae cadaver in the soil as a source of nematode IJs has its own advantages and disadvantages. *Galleria* cadaver provides some protection for nematode IJs against their natural enemies in the soil (such as mites, fungi), it guarantees a continuous source of IJs in the soil for some time and nematode IJs can stay inside the cadaver larvae if soil moisture is not enough to allow nematode cruising.

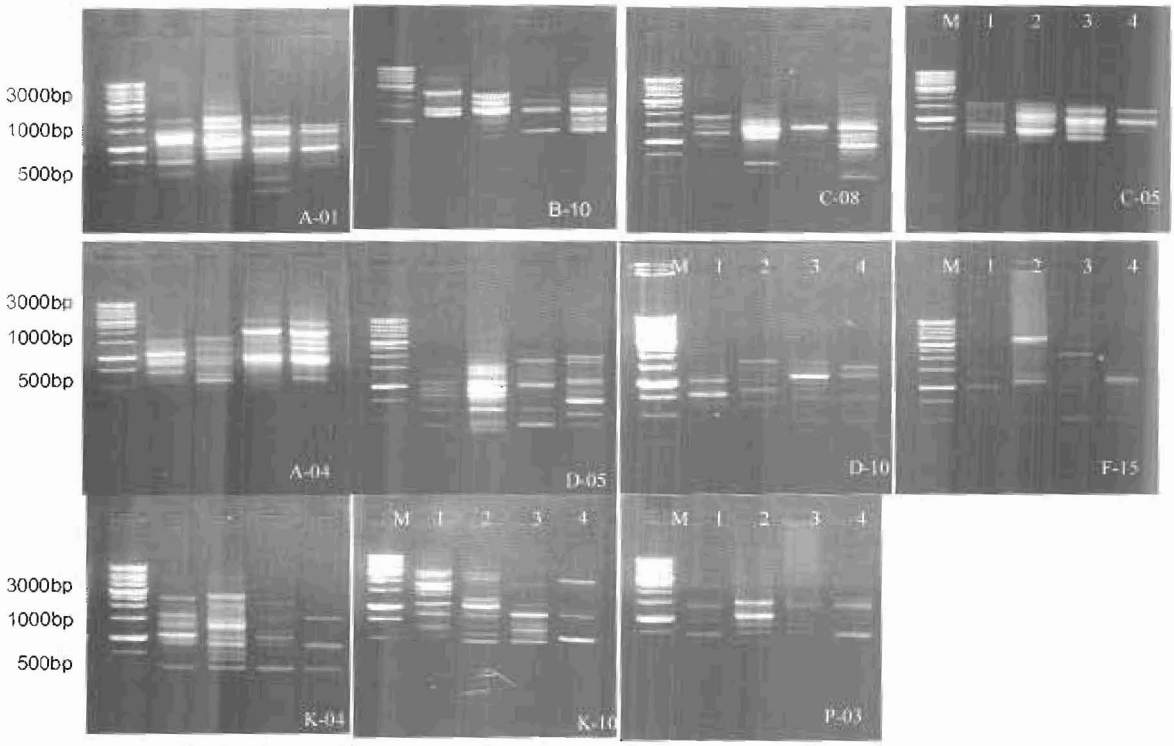

Fig. (5): RAPD banding patterns of the four different Entomopathogenic nematode genotypes using 11 selected random primers, M: 1 kbp plus DNA ladder, 1-4: S. glaseri, S. carpocapsae, H. bacteriopra, H. megdis .

On the other hand, the slow release of nematode IJs from the infected *Galleria* larvae may not produce the adequate IJs concentration needed for reasonable mortality rate among the target insect. This may be the reason for the decrease in mortality rate among the target insect. The present data indicate that the entomopathogenic nematode caused changes in insect hemolymph protein banding pattern. Also an increase in the esterase activity at 48 hr post infection was observed.

The genetic polymorphism between the entomopathogenic nematode species used was clarified by RAPD analysis and the genotype-specific RAPD markers were determined. These markers can be considered as useful markers for the four entomopathogenic nematode species examined and can be used to design a very effective biological control program to control white grub in sugarcane plantations.

Table (3): The numbers of total polymorphic bands and polymorphic frequency in the two Entomopathogenic nematode genera using 11primers.

Primers	*Steinernema*			*Heterorhabditis*		
	Generated bands	polymorphic bands	Polymorphic frequency%	Generated bands	polymorphic band	Polymorphic frequency%
OPA-01	12	8	66.6	10	7	70
OPA-04	11	6	54.5	9	6	66.6
OPB-10	10	7	70	10	6	60
OPC-05	9	6	66.6	6	3	50
OPC-08	9	8	88.8	7	6	85.7
OPD-05	9	4	44.4	11	9	81.8
OPD-10	5	3	60	6	3	50
OPF-15	5	4	80	5	3	60
OPK-04	12	6	50	5	3	40
OPK-10	12	7	58.3	7	5	71.4
OPP-03	10	9	90	4	3	75
Total	104	68	65.3	88	53	66.3

Table (4): The genotype specific RAPD marker in four entomopathogenic nematode species.

Genotype	RAPD markers	Number of markers
S. glaseri	OPA01-600,OPA04-730,OPA04-2500 , OPB10-2300, OPC08-1900, OPD10-790,OPK04-990,OPK10-1400, OPP03-250, OPF15-1000	10
S. carpocapsae	OPA01-1870, OPA01-2900,OPA04-690, OPB10-500, OPB10-730, OPC05-250,OPC05-630,OPC05-1100, OPC08-680, OPD10-1900, OPF15-3090,OPK04-700, OPK10-600, OPK10-1280,OPP03-680.	15
H. bacteriopra	OPA01-400,OPA01-500,OPA04-3050,OPC08-750, OPD05-550, OPD05-1000, OPF15-2200.	7
H. megdis	OPA01-700,, OPA04-1500,OPA04-3000, OPB10-380, OPB10-1100,OPB10-2880,OPB10-5000, OPC08-450, OPD05-580,OPD05- -750, OPD10-1500, OPF15-1500,,	12

REFERENCES

Ansari, M.A., Tirry, L. and Moens M. (2003). Entomopathogenic nematodes and their symbiotic bacteria for the biological control of *Hoplia philanthus* (Coleoptera: Scarab-aeidae). Biological control, 28: 111-117.

Dutky, S.R., Thompson J.V., and Cantwell, G.E. (1964). A technique for the mass propagation of the DD-136 nematode. J. Insect. Pathol., 6: 417-422.

EL-Bishry, M.H. and Eid, M.H., (1992). Studies on the mode of action of the entomopathogenic nematode *Steinernema carpocapsae*. III effect of infection of hemolymph protein and esterase activity Egypt. J. Appl. Sci., 7(11):408-417. Agric. Res., 85(6):2121-2138.

Forst, S. and Nealson, K. (1996). Molecular biology of the symbiotic pathogenic bacteria *Xenorhabdus spp.* and *photorhabdus spp.* Microbiol. Rev., 60: 21-43.

Gaugler, R. (1987). Entomogenous nematodes and their prospects for genetic improvement. In "Biotechnology in Invertebrate Pathology and Cell Culture" (K. Maramorosch, Ed.), pp.. Academic Press, San Diego CA, 457-484

Georgis, R., and Gaugler R. (1991). Predictability in biological control using entomopathogenic nematodes. J. Econ. Entomol., 84: 713–720.

Grewal, P.S., Power K.T., Grewal S.K., Sugars, A. and Haupricht, S. (2004) Ehanced consistency in biological control of white grubs(*Coleoptera: Scarabaeidae*) with new strains on entomopathogenic nematodes. Biological control, 30: 73-82.

Grewal,P.S. , Koppenhofer A.M. and Choo, H.Y. (2005). Turfgrass and pasture pests. CABI publishing,Wallingford,UK 115-146.

Ibrahim S .A.M. (2005). Laboratory experiments on the control of *Pentodon bispinosus* last instar larvae with *Heterorh-abditidae* and *Steinernematidae* nematodes. *Egyptian J. of Biological control*.**1: 1-7**

Kaya, H.K. and Gayugler R. (1993). Entomop-athogenic nematodes. Annu. Rev. Ento-mol., 38:181- 206.

Koppenhofer, A.M., and Fuzy E.M. (2003). *Steinernema scarabaei* for the control of white grubs. Biological control, 28:47-59.

Koppenhofer , A.M., and Eugene, M.F. (2008) .Attraction of four entomopathogenic nematodes to four white grub species J. of Invertebrate pathology, 99 (2):227-234.

Laemmli, U. K. (1970). Cleavage of structural protein during the assembly of the head of bacteriophage T_4. Nature, 227:680-685.

Nei, M. (1987) .Molecular Evolutionary Genetics, Columbia University Press,New York, USA.

Nickle, W.R. and Welch, H.E. (1984). Nematode parasites of *Lepidoptera*. In :Plant and Insect Nematodes, Marcel Decker Inco. NewYork and Basel ,655-696.

Nguyen, K.B. and Jr-Smart, G.C. (1990). Studies on the *Steinernema scapterisci*. J. Nematol., 22 : 187-199.

Potter, D.A., (1998). Destructive Turfgrass Insects: Biology, Diagnosis,and Control. Ann Arbor Press, Chelsea.

Patnaik A, and Datta, R.K. (1995). Amylase – its genetics and prospects as a marker in silkworm breeding. Indian Journal of Sericulture, 34: 82 - 89.

Rogers S.O. and Bendich, A.J. (1985). Extraction of DNA from milligram amounts of fresh herbarium and mummified plant tissues. Plant Mol. Biol., 5: 69 – 76.

Stegmann, H.; Burgermeister W., Francksen H. and Krogerrecklen, F. (1983). Manual of gel electrophoresis and isoelectric focusing with the apparatus PANTA-PHOR INST. Biochem., Messewg 11, D-3300 Braunschweig West-Germany.

الملخص العربي

التوصيف الجزيئى و الكيميائى للعلاقة بين النيماتودا و الحشرات

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تم فى هذه الدراسة اختبار كفاءة اربعة انواع من النيماتودا الممرضة للحشرات اثنان يتبعان جنس *Steinernema* : *(S.glaseri و S. capapsae)* و اثنان يتبعان جنس *Heterohabditis* (*H. megdis* و *H. bacteriophora*) لقتل العمر اليرقى الثالث من حشرة الجعل ذو الظهر الجامد. اوضحت النتائج ان النسبة المئوية للموت تزداد بزيادة تركيز النيماتودا. و قد تبين ان النوع *S.glaseri* أكثر كفاءة لمقاومة العمر الثالث للجعال حيث سجلت نسبة الموت 90% ، يليها *H. bacteriophora* 83% ثم *S. capapsae* 56% بينما سجل النوع *H. megdis* اقل نسبة موت(37%) . اتضح طبقا للنتائج المتحصل عليها فى هذه الدراسة ان النسبة المئوية لموت الحشرات كانت عالية عندما تم رش سطح التربة بمعلق يحتوى على النيماتودا.و بالاضافة فقد تم دراسة تأثير النيماتودا الممرضة لحشرات الجعل على انماط شرائط البروتين و أنزيم الاستريز فى الهيموليمف المستخلص من اليرقات . و اظهرت النتائج ان الاصابة اظهرت شرائط جديدة و تسببت فى اختفاء بعض الشرائط الاخرى. لوحظ وايضا زيادة نشاط انزيم الاستريز بعد 48 ساعة من العدوى بالنيماتودا. هذا و قد تم دراسة التباين الوراثى بين 4 انواع من النيماتودا الممرضة للحشرات عن طريق تحليل الـ RAPD و اظهرت النتائج ان عدد الواسمات الوراثية تقدر بـ 104 و 80 واسم اظهرت فيما بينها 65.35% و 66.2 % تباين وراثى لانواع النيماتودا التابعة لجنس *Steinernema* ، *Heterohabditis* على التوالى. تم تحديد الواسمات الوراثية لكل نوع و كان هناك 21 واسم وراثى يميز *H. megdis* و 15 واسم يميز *S. capapsae* و 10 واسمات تميز *S.glaseri* بينما 7 واسمات فقط تميز *H. bacteriophora*. يمكن اعتبار هذه الواسمات مميزة للاربع انواع من النيماتودا المستخدمة و يمكن استخدامها فى عمل برامج فعالة للمكافحة الحيوية للجعل ذو الظهر الجامد فى حقول قصب السكر فى صعيد مصر.