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Comparative studies on the effect of four entomopathogenic nematodes on the protein profile in *Labidura riparia* (Pallas) (Dermaptera: Labiduridae)

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ABSTRACT

In the present study, the infectivity of the four entomopathogenic nematodes, *Steinernema glaseri, Steinernema carpocapsae, Steinernema riobrave* and *Steinernema scarptasci* on the earwig. *Labidura riparia* (Nymph and adult) was studied under laboratory conditions. *S. carpocapsae* exhibited a high virulence against the nymphs of *L.riparia*. On the other hand, *S. scarptasci* showed a higher mortality rate to the *L.riparia* adults. The highest numbers of juveniles emerging from infected insects (adult and nymphs) by nematode species *S. carpocapsae* were 38750 Is/adult and 20750 IJs/nymph. Using SDS-polyacrylamide gel electrophoresis the whole body proteins fractionated into 19, 18, 13, and 15 bands when *L.riparia* adult were infected by *S.glaseri*, *S. scarptasci*, *S. riobrave* and *S. carpocapsae* ,respectively. The most obvious observation was the complete disappearance of the slow moving protein fractions (fractions number 14-17) in samples infected by *S. riobrave*. Also, fractions 16-17 also disappeared in samples infected by *S. carpocapsae*. On the other hand, the disappearance of some protein fractions was accompanied with appearance of new fractions in samples of infected insects, i.e., fractions 18 and 19 in *S.glaseri*- infected insects. Most of these new protein fractions were among the low molecular weight. The tested samples affected the molecular weight, band percentage and protein content of the whole body profile protein. These results suggested the tested four steinernematid species have side effect on the predacious insect *L.riparia* (adults and nymphs), penetrates to its haemocoel causing death 4-days post infection.

Keywords: *Steinernema glaseri, Steinernema carpocapsae, Steinernema riobrave*, *Steinernema scarptasci* , *Labidura riparia*, SDS-polyacrylamide gel

INTRODUCTION

Steinernematid nematodes are considered as good biological agents because they cause rapid death of the insect host without side effects on mammals or plants (Gaugler, 1981; Kaya and Gaugler (1993) and Poinar, 1986).

It is obligatory pathogens in nature and characterized by their mutualistic association with a specific bacterial species in the genera *Xenorhabdus* (Burman, 1982). These symbiotic bacteria are carried in the intestines of the non-feeding infective third-stage juveniles of these nematodes. The infective juveniles (IJs) enter their insect hosts through their natural openings (i.e., mouth, anus, or spiracles) and penetrate into the haemocoel in case of steinernematids (Triggiani and Poinar, 1976; Choo *et al.,* 1988; Smith *et al.,* 1990, Ghally, 1995 and Ghally *et al.,* 1992). On the other hand, the IJs of heterorhabditids can penetrate directly into the haemocoel through the soft intersegmental areas of the integument (Bedding and Molyneux, 1983). Once into the haemocoel, the juveniles release the bacterial cells that multiply and kill the insect within 48 hr. The nematodes feed on the bacterial cells and

degenerating host tissues, produce two or three generations, and emerge from the cadavers as IJs that search for new hosts (Kondo and Ishibashi, 1988).

As a rule, generalist predators are regarded as the principal natural mortality factors of herbivorous insects including management (IPM) programs, therefore, conservation of such generalist predators can be a desirable means for reducing the total amount of pesticide used.

Labidura riparia Pallas (Dermaptera: Labiduridae) is regarded as an important mortality factor of insect pests in various crop fields (Ammar and Farrag, 1974; Buschman *et al*., 1977; Strandberg,1981a, b; Godfrey *et al.,* 1989; Pair and Gross, 1989; Kharboutli and Mack, 1993) and sometimes becomes a dominant species among predatory insects (Kharboutli and Mack, 1991, 1993; Kohno *et al*.); therefore *L. riparia* should be conserved in agricultural ecosystems as a promising natural mortality factor against insect pests.

In the present study, we examined entomopathogenic nematodes susceptibility of *L. riparia* in the laboratory in order to select nematodes species that are compatible with IPM programs and we studied the effect of the four nematodes, *Steinernema riobrave*, *Steinernema glaseri*, *Steinernema carpocapsae* and *Steinernema scarptasci* on protein banding of *L. riparia*, in an attempt to clarify the possible humeral immune response of *L. riparia* adult to the tested nematodes.

MATERIALS AND METHODS

Entomopathogenic nematodes used:-

The four tested nematodes are *Steinernema glaseri, Steinernema carpocapsae, Steinernema riobrave* and *Steinernema scarptasci.* All these species and /or strains were cultured on the last instar larvae of the greater wax moth, *Galleria mellonella* according to the method of Dutky *et al*., (1964).

The infective juveniles (IJs) were harvested by white traps as described by White (1927) at 25 ±1°ć. A stock suspension of the IJs in sterilized water was stored at 10°ć for 2 weeks until used.

As for the *L. riparia*, nymphs were reared in glass Petri-dishes (12 cm. diameter) while the adults were placed in similar dishes containing a thick layer of moisten sand where development, mating, oviposition and maternal care took place.

The individuals were provided daily by sufficient amounts of newly hatched *Spodoptera littoralis* larvae to feed upon, (El-Husseini and Tawfic, (1971).

The rearing was carried out in a room maintained at 34 ±1°Ć under a complete dark condition. To clarify efficacies of the four tested nematodes against *L. riparia* nymphs and female adults, plastic boxes (8×8×10cm), each was provided with an autoclaved sandy clay layer (2 cm height) were used. The sandy layer, in each box, received a nematode suspension at rate of 500 IJs/insect. Directly, a group of five last nymphal instars or newly adults was gently introduced into the box and normally supplied with food. Each nematode treatment as well as control (untreated) was represented with four boxes (replicates). Four days post–treatment, died individuals were counted and cadavers were extracted using White trap technique to determine the number of juveniles emerged. The corrected mortalities were calculated according to Abbott formula (1925). The cadavers were dissected for nematode development and progeny production.

Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE):

The homogenate of treated and non-treated larvae analyzed by SDS-PAGE based on the method of Laemmli (1970). Samples were detected to an equal volume

of 0.5 M Tris-HCl, pH 6.8, containing 10% (w/v) SDS, 10% glycerol and 1% (w/v) bromophenol blue. All samples were then heated at 95ºC for 4 min. and centrifuged (3000 r.p.m. for 5 min.) before applying onto the gel 12% polyacrylamide mini-gels (protein II electrophoresis cell, Bio-Rad) were used. Running conditions were 200 V for 45 min. at room temperature (27-30ºC). The gels were calibrated with a broad range molecular weight (MW) marker protein (Bio-Rad). The silver staining method for protein described by Sammons *et al.* (1981) was used. This method of staining is sensitive and detect as little of protein in a single band in fixative (40% methanol and 10% acetic acid) overnight at room temperature and then destined the following day using several changes of 40% methanol/10% acetic acid. The data was analyzed using gel documentation system (gel pro-analyzer).

RESULTS

Virulence of nematodes

It is evident from Fig. (1) that, *S. carpocapsae* exhibited a high virulence (about 75% mortality) against the nymphs of *L.riparia*, the other three species had significantly a less virulent. On the other hand, *S. scarptasci* showed a higher mortality rate (72.5%) to the *L.riparia* adults.

Infective juvenile production:

The juveniles were emerged from the cadavers, the highest numbers of juveniles emerging from infected insects (adult and nymphs) by nematode species *S. carpocapsae* were 38750 IJs /adult and 20750 IJs/nymph. On the contrary the lowest number of juveniles emerging from infected insects (adult and nymphs) by nematode species *S. glaseri*. Fig (2).

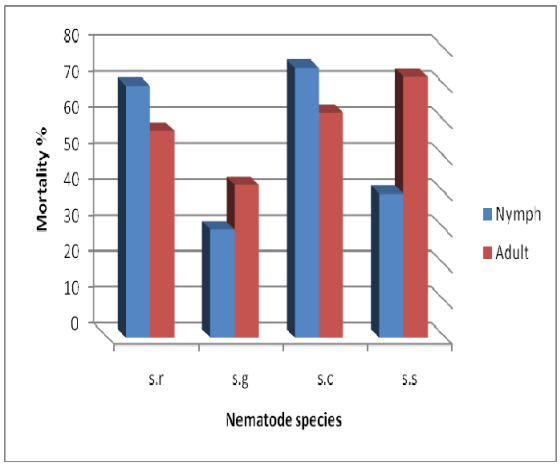

Fig. 1: Virulence of four entomopathogenic nematodes at the rate of 500 IJs / L.riparia adults or nymphs (S.r: S. riobrave; S.g: S. glaseri; S.c: S. carpocapsae and S.s: S. scarapasci).

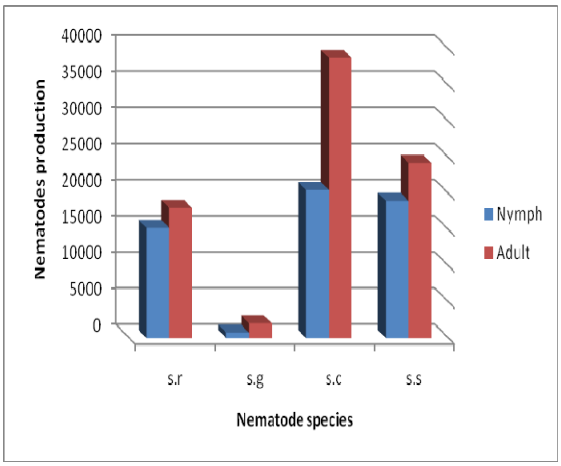

Fig. 2: Number of juveniles emerging from L.riparia (adult and Nymph) infected by (S.r: S. riobrave; S. g: S. glaseri; S.c: S. carpocapsae and S.s: S.scarapasci.

SDS-Protein electrophoresis analysis:

Gel-electrophoresis is still the most widely used protein separation

Sodium polyacrylamide gel electrophoresis (SDS-PAGE) analysis of protein in control, nematodes -infected *L.riparia* is shown in Fig. (3) and Table (1). Scanning of the gels revealed the presence of 17 different protein fractions in the control sample. It was found that there were differences between the protein patterns of infected and

non- infected samples. The most obvious observation was the complete disappearance of the slow moving protein fractions (fractions number 14-17) in samples infected by *S. riobrave*. Also, fractions 16-17 also disappeared in samples infected by *S. carpocapsae*. On the other hand, the disappearance of some protein fractions was accompanied with appearance of new fractions in samples of infected insects, i.e., fractions 18 and 19 in *S. glaseri*-infected insects. Most of these new protein fractions were among the low molecular weight .This change in the protein profile indicates qualitative differences among the tested samples and hydrolysis of protein in the infected insects.

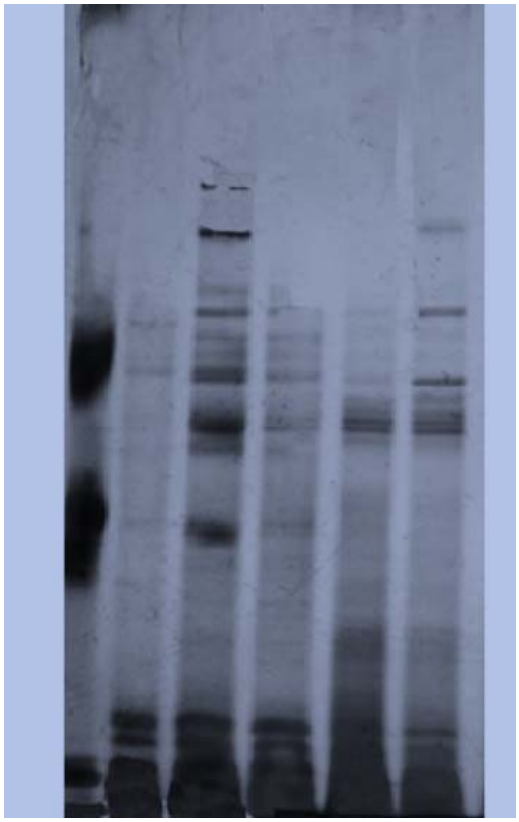

Fig. 3: Protein pattern in control, and S.riobrave (laneA)-, S.scarapsci (laneB)-, S.carpocapsae (laneC)-, S. glaseri (laneD)-infected adult of L.riparia

Increase in the mol. Wt. in bands no. 9-17 in samples infected by *S. glaseri* and *S. scarptasci*, also in band no. 9-13 in samples infected by *S. carpocapsae* as compared to control.

Decrease in the mol. wt. was observed in bands no. 2-13 in samples infected by *S. riobrave* as compared to control.

Band no. 12 was considered as a major band since it had the highest percentage of protein in samples infected *S. riobrave*. Band no. 14 was the major band in samples infected by *S. carpocapsae*. Bands no. 16 and 18 (new band) were the major bands in samples infected by *S. scarptasci*. Also bands no. 17 and 18 (new band) were the major bands in samples infected by *S.glaseri*.

On the other hand band (no. 1) was considered as a major band since it had the lowest percentage of protein in samples infected by *S. carpocapsae*. Band no.19 (new band) was a minor band in samples infected by *S.glaseri*. Meanwhile band no. 2 was a minor band in samples infected by *S. riobrave*. While bands no. 2 and 6 were a minor band in samples infected by *S. scarptasci*.

The largest protein content (normalization quantity) was presented in Table (1) appeared in band no. 12 in case of samples infected by *S. riobrave* (612.7) as copared to control band, at the same time band no. 1 and 9 showed an increase in the protein content more than three time (344.23 and 378.37) the value of the control.

Table 1: Molecular weight, band percentage and normalization quantity of the electrophoretic protein pattern of the whole body homogenate of the adult of L.riparia affected by *Steinernema glaseri, Steinernema carpocapsae, Steinernema riobrave* and *Steinernema scarptasci* under laboratory conditions of (25±2Ć and 40-60 % R.H.).

	control			*S.glaseri*			*S.scaraptasci*			*S.riobrave*			*S.carpocapsae*		
	M.W. (KDa)	Band %	% To Control	M.W.	Band %	% To Control	M.W.	Band %	% To Control	M.W.	Band %	% To Control	M.W.	Band %	% To Control
1	98	1.56	100	98	2.49	159	98	1.98	126.92	98	5.37	344.23	98	0.37	23.71
2	93	0.89	100	92	4.64	521.3	94	0.74	83.14	79	0.65	73.03	96	1.85	207.9
3	91	2.17	100	73	2.47	113.8	93	6.44	296.77	73	2.49	114.74	91	3.64	167.7
4	87	3.54	100	70	1.09	30.79	72	2.78	78.53	58	10.16	287	72	6.99	197.5
5	73	3.86	100	64	2.83	73.31	63	5.05	130.82	56	2.23	57.77	61	6.90	178.8
6	64	5.19	100	59	3.34	64.35	61	0.92	17.72	52	2.71	52.21	58	3.34	64.35
7	58	2.73	100	57	1.49	54.57	56	4.53	165.93	48	2.35	86.08	55	1.34	49.08
8	53	3.76	100	53	2.37	63.03	52	1.28	34.04	43	7.50	199.46	52	3.75	99.73
9	47	2.22	100	49	1.23	55.4	50	1.90	85.58	37	8.40	378.37	49	1.97	88.73
10	40	10.67	100	43	6.98	65.41	44	4.98	46.67	35	6.15	57.63	44	8.33	78.06
11	32	14.59	100	40	1.62	11.15	39	6.28	43.04	31	4.22	28.92	40	6.36	43.59
12	24	7.24	100	38	9.34	129	36	1.86	25.69	19	44.36	612.70	34	9.59	132.5
13	21	1.64	100	32	5.31	323.8	34	2.36	143.9	11	3.42	208.53	31	6.82	415.9
14	19	9.28	100	30	2.92	31.46	29	7.81	84.15	-	-	-	19	36.67	395.2
15	17	9.47	100	26	3.82	40.33	25	8.73	92.18	-	-	-	11	2.08	21.96
16`	14	15.65	100	24	3	19.16	17	18.86	120.51	-	-	-	-	-	-
17	11	5.54	100	15	21.04	379.8	15	8.6	155.23	-	-	-	-	-	-
18	-	-	-	11	23.56	N	10	14.8	N	-	-	-	-	-	-
19	-	-	-	10	0.47	N	-	-	-	-	-	-	-	-	-

Bands no. 2 was the largest protein content in case of samples infected by *S. glaseri* (521.3) as compared to control band, at the same time band no. 17 showed an increase in the protein content more than three time (379.8) the value of the control.

Band no.13 was the largest protein content in case of samples infected by *S. carpocapsae* (415.9) as compared to control band, at the same time band no. 14 showed an increase in the protein content more than three time (395.2) the value of the content.

While bands no.2 showed an increase in the protein content, two times the value in case of samples infected by *S. scarptasci* as compared to control (296.77), also, Band no.4 and 13 in case of samples infected by *S. riobrave* (287 and 208.53, respectively) as compared to control, also band no. 2 in case of samples infected by *S. carpocapsae* (207.9) as compared to control.

Some protein bands had the protein content with nearly the same value as control band, these were: band no. 3 in case of samples infected by *S. glaseri*, band no. 15 in case of samples infected by *S. scarptasci*, band no. 3 in case of samples infected by *S. riobrave* and band no. 8 in case samples infected by *S. carpocapsae*. Other protein bands showed a decrease in the protein content for more than half the value of the control band as in band no. 4-11-14-15-16 with protein content 30.79-11.15-31.46-40.33and 19.16 in case of samples infected by *S. glaseri*, bands no.6-8-12 with protein content (17.72, 34.04, 25.69) in case of samples infected by *S. scarptasci*, bands no. 1 and 15 with protein content 23.71 and 21.96 in case of samples infected by *S. carpocapsae*.

DISCUSSION

These results suggested the tested four steinernematid species have aside effect on the predacious insect *L.riparia* (adults and nymphs), penetrates to its haemocoel causing death 4-days post infection. (Shamseldean *et al*., 1999).

Similarly, Reyad and Ibrahim (2008) found that, the earwig *Euborella annulipes* (adults and nymphs) exposed to *Steinernema glaseri, Steinernema carpocapsae, Steinernema riobrave* and *Steinernema abbassi* at the dose 500 IJs / insect showed a high virulence against this insects. On the contrary of El-Mahdi (1996) who found that, *L. riparia* nymphs and adults exposed *to S. carpocapsae* and *Heterorhabditis bacteriophra* at higher doses (10000 IJs) did not show any death symptoms over 20 days post treatment.

Poinar and Thomas (1966) indicated that differences in the rate of reproduction of entomopathogenic nematodes were due to quality of food reserves and not to the size and/or weight of the tested insect. Both the growth and propagation of *Steinernema* sp. and *Heterorhabditis* sp. are closely associated with their symbiotic bacterium. They also added that since the nematodes feed on the disintegrated insect tissues, therefore, the nematode development is influenced by the available bacteria and insect tissues. The rate of emergence in nematode IJs from insect hosts absolutely depends on the balance between the Pathogenicity of nematode/bacterium complex and the development of a defense mechanism in the insects against them. After pathogens penetrate the insects' structural barriers, they rely solely on an efficient innate immune system which shares many characteristics with the innate immune system of vertebrates. Insect innate immune system comprises both humoral and cellular responses (Pinheiro and Ellar, 2006, Lemaitre and Hoffmann, 2007). Insect humoral defenses include the production of a potent arsenal of antimicrobial peptides (AMPs) (Pinheiro and Ellar, 2006, Lemaitre and Hoffmann, 2007), coagulation, and melanization led by protease cascades (Kanost *et al.*, 2004). Insect cellular defense refers to haemocyte-mediated immune responses, such as phagocytosis, nodulation, and encapsulation (Lavine and Strand, 2002). The encapsulation process involves cell adhesion and melanization (Eslin and Prevost, 2000).

Kondo and Ishibashi (1986; 1987 and 1988) concluded that the lower susceptibility of *S. litura* than *Galleria mellonella* larvae to three *Steinernema* sp. (*S. feltiae, S. bibionis* and *S. glaseri*) was mainly due to the higher resistance level against invading nematode rather than the higher defense against nematode invasion. They also added that the propagation and establishment of these nematodes would be difficult in moist soil containing a large number of fungi and other saprophytic microorganisms.

In accordance with the present findings, Abdel-Kawy (1985) reported that electrphoretic analysis of haemolymph protein in fullgrown larvae of *S. littoralis* infected with the nematode *Steinernema carpocapsae* revealed complete disappearance of some protein fractions, in addition to decreased optical density of some others. However, El-Bishry *et al* (1997) found no qualitative differences in haemolymph protein of *Manduca sexta* and *S. littoralis* infected with the same nematode species, but all the changes were confined to the quantitative ones. Further, El-Bishry (1989) reported that haemolymph protein of *S. littoralis* was markedly reduced 30 hr post-nematode infection. He attributed this reduction to the proteolytic activity detected in the haemolymph of infected larvae, this activity was believed to be the main cause of the host quick death (Pionar, 1966; El-Bishry, 1989). In addition,

Kucera and Mracek (1989) could partially purified 3 proteolytic enzymes from haemolymph of *G. mellonella* larvae infected with Steinernematid nematodes. This proteolutic activity was proved by El-Bishry (1989), where he could have separated 7 protein fractions having this activity from haemolymph of *S. littoralis* larvae infected with the nematode *S. feltiae*. He also found that these fractions were lethal when injected into haemocoel of healthy larvae and a positive relation could be detected between their enzymatic activity and their lethal effect. The source of these enzymes may be the nematode or its symbiotic bateria. Bleakley and Nealson (1988) reported that cells of the secondary from of the bacterium *Xenorhabdus luminescens* were deficient in pigmentation, extracellular antibiotic, protease and lipase activities. Schmidt *et al.* (1988) found that the bacterium *X. luminescens* produces1 only one extracelluar protease. Kucera and Mracek (1989) purified one proteolytic enzyme from the invasive juveniles of *S. kraussei* and two from the associated bacterium, *X. nematophilus.* Protease activity of the infective juveniles is believed to be responsible for disorganizing the gut cells of insects, allowing penetration of the nematode into the haemocoel, and acts as an anhibitor, acting on the immune proteins of the insects (Laumond *et al.* 1979). Rubtsov (1967) suggested that degrade the haemolymph proteins to provide an amino acid source. He added that this has yet to be demonstrated but should be examined along with the hypothesis that the host may initiate protein hydrolysis in response to nutrional stress of endocrine manipulation. The importance of isolating and identifying the toxins released by the entomopathgogenic nematodes and their associated bacteria lies in the possibility of conferring resistance to insects in plants by incorporating the gene an coding for the toxin into the genoplasm of economics crops (Laumond *et al.,* 1989).

Finally, Reyad (2005) also found that there were great differences between the protein patterns of infected 4th instar larvae of *S. littoralis by S. riobrave* and *Heterorhabditis* sp. and non-infected samples. The most obvious observation was the complete disappearance of the slow moving protein fractions (fractions number 1-4) in all samples of infected insects. The disappearance of some protein fractions was accompanied with the appearance of new fractions in samples of infected insects

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ARABIC SUMMARY

دراسات مقارنة على تأثير أربعة أنواع من النيماتودا الممرضة للحشرات على التوصيف البروتينى لحشرة ابرة العجوز الكبيرة

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تم دراسة اصابة أربعة أنواع من النيماتودا الممرضة للحشرات على الطور البالغ من ابرة العجوز الكبيرة بالاضافة الى ذلك تم تحليل البروتينات للحشرة باتباع طريقة التفريد الكهربائى (SDS-PAGE).

ولقد أوضحت الدراسة أن النيماتودا *S.carpocapsae* كانت أكثر قدرة على احداث الاصابة بالطور غير الكامل لحشرة ابرة العجوز الكبيرة بينما النيماتودا*S.scarptasci* كانت أكثر قدرة على احداث الاصابة بالطور الكامل للحشرة ، وأيضا تأثرت أعداد الاطوار المعديةمن النيماتودا الناتجة من الاستخلاص . حيث كان أعلى انتاج من تلك اليرقات المعدية (38750 يرقة معدية/الطور الكامل) و (20750 يرقة معدية /الطور غير الكامل) عند تطبيق النيماتودا *S.carpocapsae* .

ولقد اتضح من خلال تحليل البروتينات للحشرات المصابة باتباع طريقة التفريد الكهربائى SDS-(PAGE) اختفاء أو ظهور بعض أنواع البروتين ، وكانت الظاهرة الملفتة للانتباة هو اختفاء البروتينات ذات الحركة البطيئة فى التفريد الكهربائى نتيجة للاصابة بأنواع النيماتودا المستخدمة