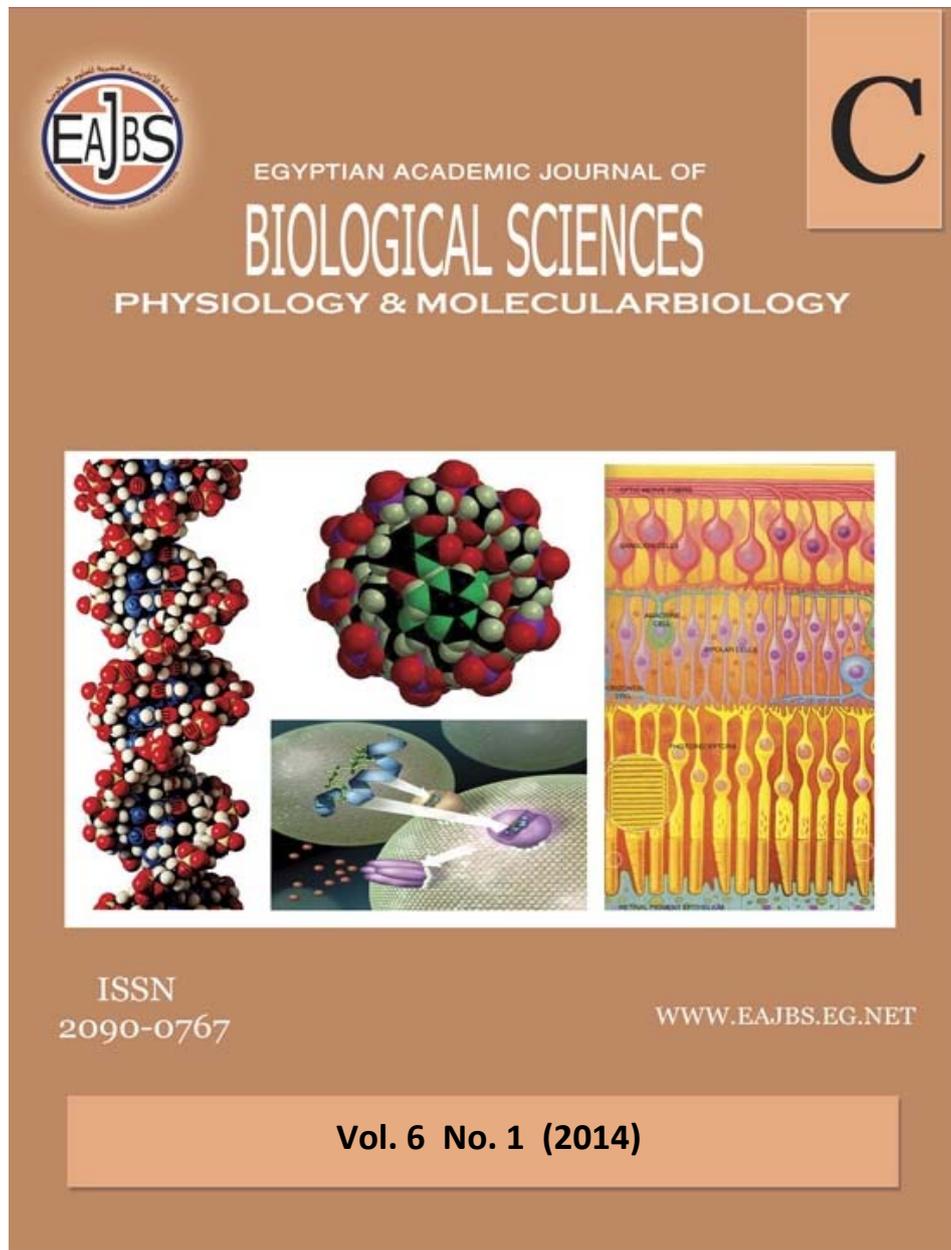


Provided for non-commercial research and education
use.



Egyptian Academic Journal of Biological Sciences is the official English language journal of the Egyptian Society for Biological Sciences, Department of Entomology, Faculty of Sciences Ain Shams University.

Physiology & molecular biology journal is one of the series issued twice by the Egyptian Academic Journal of Biological Sciences, and is devoted to publication of original papers that elucidate important biological, chemical, or physical mechanisms of broad physiological significance.

www.eajbs.eg.net



Biochemical effects of *Steinernema feltiae*, *Steinernema riobrave* and *Heterorhabditis bacteriophora* on *Spodoptera littoralis* larvae

Naglaa, F. Ahmed^{1&3}; Amna. M. H. Maklad¹; Samia, A. Yassin¹ and Shaker M. Abolmaaty²

1- Plant Protection Research Institute, Agricultural Research Center, Dokki, Egypt.

2- The Central laboratory for Agriculture Climate, Agriculture Research Center, Dokki, Giza, Egypt.

3- Biology Department ,Faculty of science ,King Khalid University ,Abha- KSA

ARTICLE INFO

Article History

Received: 25/12/2013

Accepted: 23/1/2014

Keywords:

Steinernema feltiae
Steinernema riobrave
Heterorhabditis bacteriophora
Spodoptera littoralis

ABSTRACT

In the present study, the infectivity of the three entomopathogenic nematodes, *Steinernema feltiae*, *Steinernema riobrave* and *Heterorhabditis bacteriophora*, on the cotton leafworm, *Spodoptera littoralis* was studied. Moreover, the effect of these pathogens on certain biochemical and physiological aspects of the host was also studied. *H. bacteriophora*, appeared to be more pathogenic than *S. riobrave* and *S. feltiae* to the *S. littoralis* larvae.

The highest production (7000 infective juveniles) was obtained, where infective juvenile production from cadavers infected with *H. bacteriophora* was higher than that produced from cadavers infected with *S. riobrave* and *S. feltiae*.

The principle nutrients (total protein, carbohydrate and lipid) of the host larvae were highly decreased post-infection with the nematodes *S. riobrave* and *H. bacteriophora*.

The activity of some larval enzymes was also affected due to infection by these nematodes. Thus, the activity of carbohydrate hydrolyzing enzymes (amylase, invertase and trehalase) changed depending on the species of the pathogen and the enzyme. Amylase activity decreased with the infection by *H. bacteriophora*, and the reverse was obtained with the infection by *S. riobrave* and *S. feltiae* where such activity increased. Invertase and Trehalase activity increased with the infection by three nematode species. The highest increase was obtained in case of infection by *S. riobrave*. Activities of acid and alkaline phosphatases increased due to infection by *S. riobrave*, *H. bacteriophora*. The only exception was a non-significant decrease in the alkaline phosphatase activities of larvae infected with *S. feltiae*. Whereas, the activity of transaminases (GOT and GPT) was highly decreased with the infection by *H. bacteriophora* and *S. riobrave*.

INTRODUCTION

The Egyptian cotton leaf worm, *S. littoralis* (Boisd) is one of the most destructive phytophagous insect pests in Egypt, not only to cotton, but also to other field crops and vegetables (Kandil *et al.*, 2003). These caterpillars are very polyphagous, causing important economic losses in both greenhouses and open field on a broad range of ornamental, industrial and vegetable crops.

Besides many populations have acquired resistance towards most insecticide groups (Alford, 2000). Therefore, there is always need for finding out new material shaving specific modes of actions to replace the conventional insecticides.

Among the most suitable biological control agents for controlling the cotton leaf-worm are the entomopathogenic nematodes of the families Steinernematidae and Heterorhabditidae, which are considered as good biocontrol agents because they cause rapid death of the insect host without side effects on mammals or plants (Poinar, 1986). Infective third-stage juveniles of these nematodes, which are capable of long-term survival without feeding, carry symbiotic bacteria, *Xenorhabdus sp.* In their intestine to be released into the host's haemocoel leading to septicemia followed by death of the host insect species, the nematodes, then reproduce within the cadaver (Molyneux *et al.*, 1983).

The present study aimed to evaluate the pathogenic action of the three nematode species, *S. feltiae*, *S. riobrave* and *H. bacteriophora* on *S. littoralis* larvae and to study the physiological and biochemical activities of some enzymes in fourth larval instars in laboratory.

MATERIALS AND METHODS

Insect rearing technique:

The stock colony of *S. littoralis* was maintained in the laboratory at 25±2°C and 65±5% RH. Adults were fed on 20% sucrose solution, while larvae were fed on castor oil leaves, *Ricinus communis*.

Nematodes used:

Three entomopathogenic nematodes, *S. riobrave*, *S. feltiae*, (Steinernematidae) and *H. bacteriophora* (Heterorhabditidae) were obtained from Pest Physiology Department, Plant Protection Research Institute, Egypt.

Pathogenicity of the nematodes to *S. littoralis* larvae

Bioassay procedure:

The 4th larval instar of *S. littoralis* was used for this purpose. The inoculum of IJs from *S. riobrave*, *S. feltiae* and *H.*

bacteriophora was carried out by placing 4th larval instar in 1.5 ml Eppendorf tube lined with filter paper. The latter was contaminated with 5, 10, 20 and 40 IJs. Each concentration level was replicated five times, ten larvae per each replicate. Control experiment of non-infected larvae was also carried out. Mortality records were made after 48 hr, and were corrected against natural mortality that was obtained from control using Abbott's formula (Abbott, 1925). The data were statistically analyzed according to Finney (1971) to obtain estimate of LC₅₀ value. The cadavers were dissected for nematode development and progeny production.

Biochemical Studies:

The biochemical studies of 4th larval instars were measured after 48 hours of treatment. Total protein, lipid and carbohydrate and protein contents were measured according to the methods described by Singh and Sinh (1977) and Bradford (1976), respectively. The total lipids were determined by the phosphovanilin method of Barnons and Blackstock (1973).

Determination of amylase, invertase and trehalase enzymes according to the method described by Ishaaya and Swiriski (1970). Acid and alkaline phosphatase activities were determined by the method described by Laufer and Schin (1971).

Glutamate-oxaloacetate transaminase (GOT) and glutamate-pyruvate transaminase (GPT) were determined by the method described by Reitman. Frankel (1957).

RESULTS AND DISCUSSION

Susceptibility of *S. littoralis* larvae to *S. feltiae*, *S. riobrave* and *H. bacteriophora* nematodes:

The Pathogenicity of *S. feltiae*, *S. riobrave* and *H. bacteriophora* nematodes against the fourth larval instar of *S. littoralis*. *H. bacteriophora*. seemed to be comparatively more pathogenic than *S. feltiae* and *S. riobrave* to the tested instar larvae (Table 1). Estimated of LC₅₀ values

were 5.01, 8.57 and 16 IJs/larva for *H. bacteriophora*, *S. riobrave*, and *S. feltiae* respectively. Thus, *H. bacteriophora* was

about 3 times as pathogenic as *S. feltiae* to *S. littoralis* larvae at the LC₅₀ level.

Table 1 : Pathogenicity of *S. feltiae*, *S. riobrave* and *H. bacteriophora* against 4th larval instar of *S. littoralis*

Nematode Species	LC ₅₀ (IJs/larva)	95% confidence limits (Lower-Upper)	Slope ± S.E.
<i>S. feltiae</i>	16.0	13-19.4	2.33±0.33
<i>S. riobrave</i>	8.57	5.28-9.96	1.51±0.16
<i>H. bacteriophora</i>	5.01	3.25-6.59	1.47±0.22

Infective juvenile production

As shown in Fig. (1), the total number of juveniles produced /a single *S. littoralis* larvae varied between the nematode species.

The highest progeny was produced from larvae infected by *H. bacteriophora* (at conc. 40 IJs/larva) which gave 7000 IJs/larva).

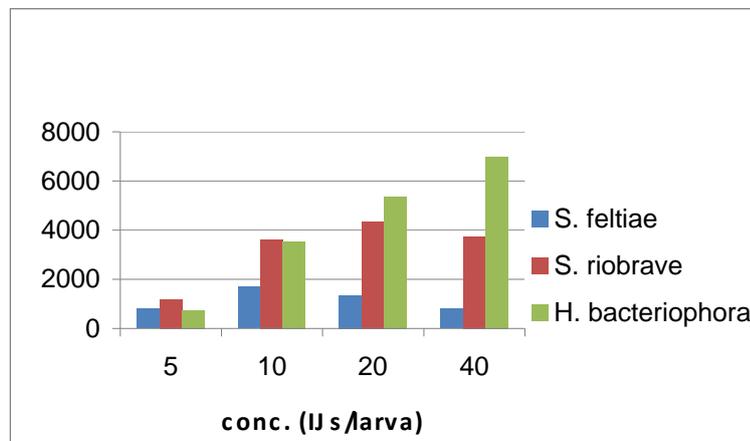


Fig. 1: Number of juveniles emerging from *S. littoralis* larvae infected by *S. feltiae*, *S. riobrave*, *H. bacteriophora*.

In the present investigation, the mortality percentage increased with the increase of the parasite density. This is in accordance with the findings of Sikora *et al.* (1979) who stated that most developmental stages of *S. littoralis* were highly susceptible to *N. carpocapsae* infection, and the mortality was positively correlated with the parasite density. Similar findings were also reported by several authors (Ahmed, 1982; Abdel-Kaway, 1985; Kondo and Ishibashi, 1987; Choo *et al.*, 1988 and Ghally *et al.*, 1988). Ghally *et al.* (1991) found that the rate of development of *S. feltiae* was faster and the rate of reproduction was higher in *S. littoralis* than in *Musca domestica*. Also, Hatsukade and Grey (1996) showed a higher infectivity of *S. carpocapsae* to larvae of *S. littoralis*. Khlibsuwan (1996) obtained a relationship between *S. carpocapsae* concentration and the number of nematodes

invading *S. litura* larvae, percentage invasion increased with the exposure time. Likewise, Mogahed (1996) showed that the efficacy of *H. heliothidis* and *H. bacteriophora* increased with the increase of concentration and period after treatment of different stages of *S. littoralis*.

The efficacies of *H. bacteriophora* (HP88), *H. bacteriophora* (EASD98), *S. riobrave* and *H. indicus* (EAS59) against *S. littoralis* were tested by Shamseldean *et al.* (1996). All the tested nematodes attained almost 100% mortality at 4, 10 and 25°C, but at 35°C *H. bacteriophora* (HP88) achieved the least mortality (64%). Also, Reyad (2001) showed that the tested ionculum levels of *S. carpocapsae* and *H. bacteriophora* were effective against the larval instars of *S. littoralis*, and the level 40 infective juveniles/ml distilled water caused 100% mortality of the host.

Elawad *et al.* (1997) isolated *S. abbasi* from soil in alfalfa fields and showed that this nematode species could be used as a biological control agent in high temperature against *S. littoralis*, with LD₅₀ value of 60.3 IJs/larvae. Also, Abbas and Saleh (1988) studied the efficiency of *S. riobrave* against 4th instar larvae of the same insect species, with LD₅₀ value of 49.6 IJs/larva. The highest mortality (91.7%) was obtained in the 3rd day post-treatment.

S. littoralis larvae infected by *H. bacteriophora* produced infective juvenile more higher than these infected by *S. feltiae* and *S. riobrave*. These may be due to that the nutritional requirements of *H. bacteriophora* nematodes were more higher than those of *S. feltiae* and *S. riobrave* as evidenced in the present study. Obtained results agree with the work of Selvan *et al.* (1993) who reported that percentage of penetration of *S. carpocapsae* and *H. bacteriophora* to *G. mellonella* larvae declined in spite of an increase in the number of invading nematodes with the increase of the dose. Infective juvenile production was reduced at densities above and below 100 nematodes/host. They thought that the effects of increased density of nematodes resulted from competition for limited nutrients within the host. Shannag *et al.*

(1994) found that larval mortality and penetration of infective juveniles of *S. carpocapsae*, *S. feltiae* and *H. bacteriophora* into pickle worm *Diaphania nitidalis* were positively correlated to host exposure time. Smaller nematodes were more infective and induced mortality more quickly. Further, Shannag and Capinera (1995) determined that *S. carpocapsae* was the most pathogenic nematode species to the same insect species, followed by *H. bacteriophora*, *S. feltiae*, *S. anomaly* and *S. glaseri*. Infection of *Phthorimaea operculella* larvae with *S. feltiae*, *S. biobionis*, *S. carpocapsae* at a rate of 20000 infective juveniles per one larvae resulted in 95.5, 93.4 and 93.1% mortality, respectively (Ivanova *et al.*, 1994).

Biochemical influences of *S. feltiae*, *S. riobrave* and *H. bacteriophora* nematodes on *S. littoralis* larvae:

Total protein, lipid and carbohydrate contents:

The data obtained (Table 2) show that 48 hr post-infection of 4th instar larvae of *S. littoralis* by the three nematode species significantly decreased the total content of protein, lipid and carbohydrate of larvae, as compared to control. The highest decrease was recorded in case of infection by *S. riobrave* and *H. bacteriophora*. *Nematodes*.

Table 2: Effect of LC₅₀ of *S. feltiae*, *S. riobrave* and *H. bacteriophora* on the total content of protein, lipid and carbohydrate 48h post-infection of 4th instar larvae of *S. littoralis*.

Pathogen	Mean total content (mg/ml)±S.E.		
	Protein	Lipid	Carbohydrate
<i>S. feltiae</i>	12.1±1.44*	20.27±1.15*	1.53±0.09*
<i>S.riobrave</i>	9.95±0.071*	14.48±0.44**	1.14±0.14**
<i>H. bacteriophora</i>	8.13±0.32**	16.77±0.64**	1.17±0.09**
Control	17.47±0.39	37.28±1.97	2.029±0.11

* Significant at P< 0.05

**Highly significant at P< 0.01

Carbohydrases activity (amylase, invertase and trehalase):

The effects of LC₅₀ of *S. feltiae*, *S. riobrave* and *H. bacteriophora* on the activity of the carbohydrate digestive enzymes, 48 hr post-infection of 4th instars larvae of *S. littoralis* were shown in Table (3). The results revealed that amylase activity was significantly increased, as

compared to control, due to infection of 4th instar larvae of *S. littoralis* by *S. feltiae*, *S. riobrave*. Whereas, the activity of this enzyme was decreased insignificantly in case of infection by *H. bacteriophora*.

Infection by the two nematode species, *S. riobrave* and *H. bacteriophora* significantly increase the activity of invertase of *S. littoralis* larvae, as compared to control.

The highest increase was recorded in case of infection by *H. bacteriophora*. Infection by the three nematode species increased

significantly trehalase activity as compared to control.

Table 3: Effect of LC₅₀ of *S. feltiae*, *S. riobrave* and *H. bacteriophora* on the carbohydrate digestive enzymes 48h post-infection of 4th instar larvae of *S. littoralis*.

Pathogen	Mean carbohydrases activity \neq (IU/ml) \pm S.E.		
	Amylase	Invertase	Trehalase
<i>S. feltiae</i>	545.03 \pm 7.69**	304.13 \pm 6.12 ^{ns}	527.53 \pm 26.58*
<i>S. riobrave</i>	473.07 \pm 11.16*	480.74 \pm 9.24**	873.26 \pm 13.59**
<i>H. bacteriophora</i>	304.70 \pm 12.25 ^{ns}	603.47 \pm 11.51**	823.63 \pm 17.89**
Control	339.72 \pm 11.63	262.23 \pm 6.22	470.35 \pm 14.29

^{ns} : Not significant

** : Highly significant at P< 0.01

* : Significant at P< 0.05

\neq IU: International unit (the amount of enzyme which under defined assay conditions will catalyze the conversion of micromole of substrate per minute).

It is well known that the pathology of the entomopathogenic nematodes beings immediately after reaching the insect's haemocoel. The symbiotic bacteria when released into the haemocoel, rapidly multiply causing a lethal septicemias to the insect host (Dutley, 1959; Nickle and Welch, 1984). So, biochemical changes in the haemolymph composition are expected, since the haemolymph is the main site of action. In the present study, the total protein content decreased due to parasitism of two nematodes to the fourth instar larvae of *S. littoralis*. This result agrees with that obtained by El-Bishry *et al.* (1997) who found that 30 hr post-infection of 6th instar larvae of *A. ipsion* markedly produced the haemolymph protein in case of the three tested isolates; HP88 strain, Also isolates (*H. bacteriophora*) and AS2 isolate *CH. Indicus*). Also ,Thong and Webster (1975) found that the total protein level decreased with the parasitism of the sphaerulariid nematode *Contortylenchus reverses* during the maturation of female scolytid beetle *Dendroctonus pseudotsugae*. Also, they found that the parasitism by this nematode did not affect haemolymph trehalose. Level. This finding is in contrast to somewhat with the results obtained in the present study where the nematode species tested decreased the total carbohydrate in *S. littoralis* larvae. Sahota (1970) obtained depletion of protein in the haemolymph of mature, but not in

callow adult females of *D. pseudotsugae*, following infection by the nematode *C. reverses*. This suggests that during the maturation of the latter, the normal active incorporation of haemolymph protein into ovarian protein occurs concurrently with nematode withdrawal of host haemolymph protein, thus upsetting the haemostatic mechanism for the maintenance of the haemolymph protein levels. Nematode utilization of host protein probably also occurs in the callow female, but the homeostatic, control of haemolymph protein levels is able to cope with the rate of depletion, without the developing eggs drawing from the available protein. Alternatively, during the beetle diapause, the nematode may enter a state of inactivity and decrease its rate of metabolism and, hence, of protein utilization. Such effects have been observed in the nematode *Heterotylenchus autumnalis* parasitizing the face fly, *Musca autmnalis*.

It is also possible that the protein depletion measured may be the indirect consequence of changes in the insect fat body caused by the nematode *Mermis nigrescens*, for example, done not affect the taotal haemolymph protein level in the desert locust, *Schistocerca gregaria*, but depletes both fat body protein and amino acids (Gordon and Webster, 1971).

Gordon *et al.* (1978) showed that the mermithid nematode, *Neomesomermis*

flumenalis reduced the level of most amino compounds and depleted most protein fractions in haemolymph of both larval blackflies, *Prosimulium mixtum/fuscum* and *Simulium venustum*, together with a significant decrease of haemolymph glucose levels. However, blood trehalose concentration was not affected. This effect contrast with *M. nigrescens* which caused an overall reduction of blood carbohydrates in *S. gregaria* (Gordon and Webster, 1971), and which may be attributed to lowered trehalose levels.

Glucose, but not trehalose, is assimilated from the host's haemolymph in a transcuticular manner by *M. nigrescens* (Rutherford and Webster, 1974; Rutherford, Webster and Barlow, 1977). Thus, depletion of blood glucose and exhaustion of fat body glucogen (Candon and Gordon, 1977) in mernithid-parasitized simuliids results from the nematode's nutritional demands for glucose and are symptomatic of accelerated glucogenolysis and/or impaired glycolysis by the host fat body. They added also that the utilization of haemolymph glucose by mernithid parasites could favor the production of more glucose via increased trehalose activity of the host. This activity, in turn, could increase fat body glycolysis and/or lower glycolysis to maintain adequate concentrations of trehalose in the haemolymph.

Dahlman and Greene, (1981), Thompson (1982 a & b), Kawai *et al.* (1983), Cook *et al.* (1984), and Karnavar (1984) stated that haemolymph proteins and lipids exhibited quantitative variations by endoparasitism. Milstead (1979) while studying the path physiological influences of the nematode, *Heterorhabditis bacteriophora* complex on the seventh instar larvae of *Galleria mellonella*, reported that shortly after the nematode penetration into haemocoel of larvae began feeding upon the fat body. Thompson and Barlow (1983) reported that an extreme depression of *de novo* glyceride synthesis would allow the parasite to use host's fat after partial digestive hydrolysis and its own fatty acids

for rapid triglyceride synthesis, thereby minimizing the energy cost of fat synthesis.

Schmidt and Platzer (1979) found that the concentrations of total carbohydrates, protein, glucose and trehalose in the haemolymph of 4th instar of *Culex pipiens* infected by the nernithid nematode *Romanomermis culicivorax* were reduced. These results agrees with those obtained in the present study regarding total carbohydrate and protein in nematode-parasitized *S. littoralis* larvae.

The flight ability of *Locusta nigratoria* was reduced by infection with *M. nigrescens*. Concomitantly, haemolymph level of carbohydrates was elevated and protein concentration was lowered during parasitism. Fat body carbohydrate, protein, and lipid were also reduced as was the amount of fat body tissue in *L. nigratoria*.

Based on the forgoing findings, it can be concluded that the interaction the nematodes tested in this study with *S. littoralis* larvae appears to be primarily nutritional. The parasite absorbs small molecular weight components from the host depriving the larvae of nutrients necessary for development. Growth of the nematode proceeds while the nutritional status of the host larvae deteriorates, i.e., the host become in a state of physiological starvation.

Many nematodes secrete chemicals that facilitate penetration and migration through host tissues, feeding, and avoidance of host immune responses. These chemicals include digestive enzymes and toxins (Lee and Atkinson, 1976). Proteases are digestive enzymes that catalyze the cleavage of peptide bonds in proteins. Some animal parasitic nematodes secrete proteases to assist in skin and tissue penetration (Von Brand, 1973). It has been proposed that these proteases are essential for the Pathogenicity of *Steinernema kraussei*. An inhibitor present in the haemolymph of *Galleria mellonella* inhibits both *S. kraussei* and its symbiotic bacteria proteases unevenly. The inhibitor is produced during the second period of infection when the larval defense system has already been overcome and

infection is established (Kucera and Mracek, 1989). Moreover, Abu Hatab *et al.* (1995) found that when the nematodes *S. glaseri* were treated with protease inhibitors and injected into *G. mellonella* gut, the percentage mortality of *G. mellonella* was reduced as compared to control, and nematode penetration of *G. mellonella* gut was reduced.

Phosphatases activity (acid and alkaline).

The activity 48 hr post-infection of 4th instar larvae of *S. littoralis* by *S. feltiae*, *S.*

riobrave and *H. bacteriophora*. was significantly increased as compared to control (Table 4). The highest increase was recorded in case of infection by *S. riobrave*. The same pattern was also obtained for the activity of alkaline phosphatase in the larvae infected by *S. riobrave* and *H. bacteriophora*. Whereas, alkaline phosphatase activity in the larvae infected by *S. feltiae* was insignificantly decreased.

Table 4: Effect of LC₅₀ of *S. feltiae*, *S. riobrave* and *H. bacteriophora* on phosphatases activity, 48h post-infection of 4th instar larvae of *S. littoralis*.

Pathogen	Mean carbohydrates activity \neq (IU/ml) \pm S.E.	
	Acid phosphates	Alkaline phosphates
<i>S. feltiae</i>	8.13 \pm 0.32 ^{ns}	1.333 \pm 0.244 ^{ns}
<i>S.riobrave</i>	17.53 \pm 0.755 ^{**}	7.51 \pm 0.41 ^{**}
<i>H. bacteriophora</i>	13.07 \pm 0.292 ^{**}	5.23 \pm 0.04 ^{**}
Control	7.256 \pm 0.512	1.97 \pm 0.04

^{ns} : Not significant

^{**} : Highly significant at P< 0.01

\neq IU: International unit (the amount of enzyme which under defined assay conditions will catalyze the conversion of one micromole of substrate per minute).

Xia *et al.* (2000) suggested that acid phosphates, as a lysosomal enzyme, may have a role in autophagy and cell turn over as well as defense. Therefore, it appears that the enhancement of acid phosphates activity in *S. littoralis* larvae infected with *S. riobrave*, *Heterorhabditis sp.* and *B. bassiana* is an attempt by the insects to deferred or them self was against the invasion of the three pathogens. These authors also added that phagocytosis is known to stimulate the production of lysosomal enzymes of which acid phosphates is a key component. Acid phosphate had been found in insect haemocytes and shown to be released into the plasma (Lai-Fook, 1973; Rowley and Ratcliffe, 1979). Cheng (1983) reported hyper synthesis of acid phosphates by haemocytes of the mollusk, *Biomphalaria glabrata* during phagocytoses. The enzyme was subsequently released into the plasma where its role is unknown although alteration of surface procedures of foreign particles recognition although a direct role of acid phosphates in cell killing can not be ruled out.

On the other hand, alkaline phosphates of secreting products across cell boundaries.

In the present study, acid phosphates activity was higher than Alkaline phosphates activity in non-infected nematode larvae. The predominance of acid phosphates activity could be correlated to an active range (Pant and Lacy, 1969).

In agreement with our results, Soliman (2002) found that acid and alkaline phosphates activity in last instar *Ceratitis capitata* infected with *S. riobrave* and *Heterorhabditis bacteriophora*.

Transaminases activity (GOT & GPT):

Data in table (5) show the effect of the nematode species, *S. feltiae*, *S. riobrave*, *H.bacteriophora* on the activity of glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT), 48hr post-infection of 4th instar larvae of *S. littoralis*. The results indicated that infection by the nematode species *S. riobrave*, *H.bacteriophora* (decreased significantly the activity of GOT and GPT as compared to control. Whereas, insignificantly decreased. in the larvae infected by *S. feltiae*.

Table 5: Effect of LC₅₀ of *S. feltiae*, *S. riobrave* and *H. bacteriophora* on transaminases activity, 48h post-infection of 4th instar larvae of *S. littoralis*.

Pathogen	Mean specific activity ($\mu\text{g pruvate/min/ml}$) \pm S.E.	
	GOT	GPT
<i>S. feltiae</i>	20.55 \pm 0.264 ^{ns}	30.06 \pm 2.49 ^{ns}
<i>S. riobrave</i>	18.65 \pm 0.26 ^{**}	20.067 \pm 0.762 ^{**}
<i>H. bacteriophora</i>	16.35 \pm 0.27 ^{**}	18.287 \pm 0.849 ^{**}
Control	27.48 \pm 1.27	37.22 \pm 2.69

^{ns}: Significant at P < 0.05

^{**}: Highly significant at P < 0.01

Activities of glutamate- pyruvate transaminase (GPT) and glutamate-oxaloacetate transaminases (GOT) are correlated with protein anabolism in some instances (Chen, 1966; Gilbert, 1967; Plant and Morris, 1972) and with protein catabolism in certain others (Asmore *et al.*, 1964; Wergedal *et al.*, 1964; Knox and Greengard, 1965). In the present work, the significant decline of GOT in *S. littoralis* larvae after 48 hr post-infection by *H. bacteriophora* and *S. riobrave*, as compared to control treatment, may be attributed to the significant decline in free amino acids content, as has been pointed out by Kaur *et al.* (1985). They added that the quantum of free amino acids directly influenced the activity of transaminase at the time of protein synthesis. Thus, both GOT and GPT may play a direct role in protein synthesis of *S. littoralis* larvae and may explain the coincidence in the activity of this transaminase with the total protein content in non-infected *S. littoralis* larvae in the present study. The increased ratio in GPT: GOT in *S. littoralis* larvae showed that GPT was comparatively more active than GOT in non-infected by nematode infected larvae reflecting that there was a better rate of interplay between alanine and glutamate, as has been suggested by Kaur *et al.* (1985). They added that the fact that higher activities of GOT and GPT were simultaneous to the increased deposition of glycogen content is suggestive of the possible role of these two enzymes in incorporating free amino acids into carbohydrates via transamination reactions to bring about the metabolism of waste nitrogen products and in gluconeogenesis.

Soliman (2002) reported that GOT and GPT activities decreased in *C. capitata* last instar infected with *S. riobrave* and *Heterorhabditis* sp.. This result agrees with that obtained in the present study.

REFERENCES

- Abbas, M. S. T. and Saleh, M. M. E. (1998): Comparative pathogenicity of *Steinernema abbasi* and *S. riobrave* to *Spodoptera littoralis* (Lepidoptera: Noctuidae). *Inter. J. Nematol.*, 8 (1): 43-45; Ilref.
- Abbott, W. S. (1925): A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.*, 18: 265-267.
- Abdel-Kawy, A. M. (1985): Biological control of some lepidoprous pests by the nematode, *Neoaplectana carpocapsae* and the physiological changes associated with it. Ph. D. Thesis. Fac. Agric. Cairo, Univ.
- Abu Hatab, M.; S. Selvan and R. Gaugler (1995): Role of proteases in penetration of insect gut by the entomopathogenic nematode, *Steinernema glaseri* (Nematoda: Steinernematidae). *J. Invertebr. Pathol.*, 66: 125-130.
- Ahmed, N. M. (1982): Comparative studies on the effect of pesticides and the entomogenous nematodes in controlling some economic pests. Ph.D. Thesis, Fac. Agric., Cairo Univ., pp. 157.
- Alford, D. V. (2000): Pest and disease management hand book British crop protection council, Blackwell Science, Oxford, 615 pp.
- Asmore, J.; S. P. Wagle and T. Uete (1964): Studies on gluconeogenesis. In *Advance*

- in Enzyme Regulation (Edited by Weber G.P, 2:101-114. Pergamon Press, Oxford.
- Baronos, H. and Blackstock, S. (1973): Estimation of lipids in marine animals and tissue: Detailed investigations of the sulphophos phovanilin method for total lipids. *J. Exp. Mar. Biol. Ecol.*, 12: 103-118.
- Bradford, M. M. (1976): A rapid and sensitive method for the quantization of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, 72 (V): 248-254.
- Chen, P. S. C. (1966): Amino acid and protein metabolism in insect development. In *Advance in Insect Physiology*, 3:53-132. Academic Press, New York.
- Cheng, T. C. (1983): Role of lysosomes in molluscan inflammatron. *Amer., Zool.*, 23: 23: 129-144.
- Choo, H. Y.; Kaya, H. K. and Reed, D. K. (1988): Biological control of onion maggot and tobacco cutworm with insect parasitic nematodes *Steinernema felita* and *Heterorhabditis heliothidis*. *Korean J. Appl. Ent.*, 27 (4): 185-189.
- Condon, W. J. and R. Gordon (1977): Some effects of mermithis parasitism on the larval black flies *Prosimulium mixtum/fuscum* and *Simulium venustum*. *J. Inverteber. Pathol.*, 29: 56-62.
- Cook, D. I.; D. B. Stoltz and S. B. Vinson (1984): Induction of a new haemolymph glycoprotein in larvae of permissive hosts parasitized by *Campoletis sonorensis*. *J. Insec Phsiol.*, 20: 1515-1528.
- Dahlman, D. L. and J. R. Greene (1981): Larval haemolymph protein patterns in tobacco hornworms parasitized by *Apanteles congregatus*. *Ann. Ent. Am.*, 47: 103-133.
- Dutly, S. R. (1959): *Insect microbiology*. *Adv. Appl. Microbial.*, 1: 175-200.
- Elawad, S. A.; Ahmed, W. and Reid, A. P. (1997): *Steinernema abbasi* sp. (Nematoda: Steinernematidae) from the Sultanate of Oman. *Fund. App. Nema.*, 20(5): 435-442.
- El-Bishry, M. H.; Al-Elimi, M. H.; Eid, A. M. H. and Korashy, M. A. (1997): Studies on the mode of action of the entomopathogenic nematodes of the genus *Heterorhabditis*. I. Effect on haemolymph protein, amino acids content and esterase activity. *Egypt.J. Biolo. Pest Contr.*, 7: 2, 79-83.
- Finney, D. J. (1971): "Probit Analysis". Cambridge University Press. Cambridge, UK. 3rd. pp. 333.
- Ghally, S. E. (1991): Some factors affecting the activity and pathogenicity of *Heterorhabditis heliothidis* and *Steinernema carpocapsae* nematodes. *J. Egypt Soc. Parasitol.*, 25(1):125-135 22ref.
- Ghally, S. E.; Kamal, E. G. and Nasr, N. M. (1988): Study on the influence of entomophilous nematodes on *Spodoptera littoralis* (Boisd.). *J. Egypt. Soc. Parasitol.*, 18 (1): 119-127.
- Gilbert, L. I. (1967): Lipid metabolism and function of insects. *Adv. Insect Physiol.*, 4: 69-211.
- Gordon, R. and Webster, J. M. (1971): *Meris nigrescens*: Physiological relationship with its host, the adult desert locust *Schistocerca gregaria*. *Exp. Parasit.*, 29: 66-79.
- Gordon, R.; Condon, W. J.; Edgar, W. J. and Babie, S. J. (1978): Effects of mermithid parasitism on the haemolymph composition of the larval blackflies, *Prosimulium mixtum fuscum* and *Simulim venustum*. *Parasitol.*, 77l: 367-374.
- Hatsukade, M. and Grey, G. (1996): Control of turf grass insect pests with entomopathogenic nematodes in Japan. *Biological pest control in systems of Integrated Pest Management. Proceedings of the International Symposium on "The use of Biological control Agents under IPM"*. 78-85; 4 ref.
- Ishaaya, I. and Swirski, E. (1970): Invertase and amylase activity in the armoured scales, *Chrysmophalus aonidium* and *Aonidella aurantii*. *J. Insect Physiol.*, 16: 1599-1606.

- Ivanova, T. S.; Borovaya, V. P. and Danilov, L. G. (1994): A biological method of controlling the potato moth. *Zashch.-Rast.-Moskva*. No. 2, 390.
- Kandil, M. A.; Abdel-Aziz N.F. and Sammour E.A. (2003). Comparative toxicity of chlorofluazron and leufenuron against cotton leaf worm, *Spodoptera littoralis* (Boisd). *Egypt. J. Agric. Res. NRC*, 2: 645-661
- Karnavar, G. M. (1984): Studies on the influence of parasitoid, *Apanteles glomeratus* on the metabolic levels of the host *Pieris brassicae*. *Insect Sci. Appl.*, 5: 99-100.
- Kaur, S. P.; D. S. Sidhu; S. S. Dhillon and N. K. Kumar (1985): Transaminases during development and aging of the bruchis *Zabrotes subfasiatus* (Boh.) (Coleoptera : Bruchidae). *Insect Sci. Applic.*, 6(5): 585-590.
- Kawai, T.; S. Maeda; H. Ono and H. Kai (1983): Parasitic effects of wasp, *Apanteles glomeratus* on haemolymph storage proteins in host cabbage worm, *Pieris rapae*. *J. Fac. Agric. Tottori Univ.*, 18: 18-26.
- Khlibsuwan (1996): Pathogenicity of the entomopathogenic nematode, *Steinernema carpocapsae*, in armyworm, *Spodoptera litura* F. Kaen-Kaset-Khon-Kaen-Agric. J. 24(1): 35-40, ref.
- Kondo, E. and N. Ishibashi (1987): Comparative infectivity and development of the entomogenous insect larvae, *Spodoptera litura* (Noctuidae) and *Galleria mellonella* (Galleridae). *Jpn. J. Nematol.*, 17: 35-41.
- Konx, W. E. and O. Greengard (1965): The regulation of some enzymes of nitrogen metabolism, an introduction to enzyme physiology. In *Advances in Enzyme Regulation* (Edited by Weber G.), Vol. 3, pp. 247-313. Pergamon Press, Oxford.
- Kucera, M. and Mracek, Z. (1989): Proteolytic enzymes of the invasive larvae of entomopathogenic Steinernematidae nematodes. *Acta Entomol. Bohemoslov.*, 86:193-202.
- Lai-Fook, J. (1973): The structure of the haemocytes of *Calpodes ethlius* (Lepidoptera). *J. Morphol.*, 139:79-88.
- Laufer, H. and Schin, K. S. (1971): Quantitative studies of hydrolytic enzymes activity in the salivary gland of *Chironomus tentans* (Doptera : Chironomidae) during metamorphosis. *Can. Entomol.*, 103: 454-457.
- Lee, D. L., and Atkinson, H. J. (1976): *Physiology of Nematodes*, 2nd ed. Columbia Univ. Press, New York.
- Milstead, J. E. (1979): Pathophysiological influences of the *Heterorhabditis bacteriophora* complex on the seventh instar of the greater wax moth. *Galleria mellonella*: Changes in the haemolymph refractive index. *J. Inverteber. Pathol.*, 33: 274-277.
- Mogahed, M.I. (1996): Evaluation of the impact insect parasitic nematodes and essential oils for control of *Spodoptera littoralis*. *Bull. ent. Soc. Egypt, Econ. Ser.*, 23:1-10.
- Molyneux, A. S.; R. A. Bedding and R. J. Akhurst (1983): Susceptibility of larvae of sheep blowfly, *Lucilia cuorina* to various *Heterorhabditis spp.* and *Neoplectana spp.*, and an unidentified steinernematid. *J. Inverteber. Pathol.*, 42: 1-7.
- Nickle, W. R. and Welch, H. E. (1984): Nematode parasites of Lepidoptera. In: *Plant and Insect Nematodes*. Marcel Decker Inco., New York and Basel : 655-696.
- Pant, R. and P. S. Lacy (1969): Phosphatase activity in *Philosamia ricini* during development. *Ind. J. Biochem.*, 6: 154-156.
- Plant, R. and I. D. Morris (1972): A comparative study on the variation of aminotransferase activity and its total free amino acids in the fat body, haemolymph, intestine and haemolymph protein content in *Philosamia ricini* during larval-pupal development. *Ind. J. Biochem. Biophys.*, 9: 199-202.
- Poinar, G. O. (1986): Entomogenous nematode. In "Biological plant and

- Health Protection" (B. D. Franz, ED.), pp. 95-121. Fisher-Verlag. Stuttgart.
- Reitman, S. and S. Frankel (1957): A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. *Ann. J. Clin. Pathol.*, 28: 56-63.
- Reyad, N. F. (2001): Abundance and control studies of *Spodoptera littoralis* (Boisd.) infecting certain vegetables. M. Sc. Fac. Sci., Cairo Univ.
- Rowley, a. F. and Rakcliffe, N. A. (1979): An ultra structural and cytochemical study of the interaction between latex parenchyma cells and the haemocytes of the wax moth, *Galleria mellonella*, in vitro. *Cell and Tissue Res.*, 199: 127-138.
- Rutherford, T. A. and Webster, J. M. (1974): Transcuticular uptake of glucose by the entomophilic nematode *Mermis nigrescens*. *J. Parasitol.*, 60, 804-808.
- Rutherford, T. A.; J. M. Webster and J. S. Barlow (1977): Physiology of nutrient uptake by the entomophilic nematode, *Mermis nigrescens* (Mermithidae). *Can. J. Zool.*, 55: 1773-81.
- Sahota, T. S. (1970): Haemolymph and ovarian proteins in the bark beetle, *Dendroctonus pseudotsugae* in relation to ovarian development. *Can. J. Zool.*, 48, 1307-1312.
- Schmidt, S. P. and Platzer, E. G. (1979): Changes in body tissues and haemolymph composition of *Culex pipiens* in response to infection by *Romanomermis culicivorax*. *J. Invertebr. Pathol.*, 36, 240-254.
- Selvan, S.; Campbell, J. F. and Gaugler, R. (1993): Density-dependent effects on entomopathogenic nematodes (Heterorhabditidae and Steinernematidae) within an insect host. *J. Invertebr. Pathol.*,
- Shamselden, M. M.; Abd-Elgawad, M. M. and Atwa, A. A. (1996): Evaluation of four entomopathogenic nematodes against *Spodoptera littoralis* (Lep., Noctuidae) larvae under different temperature. *Angeiger für Schadlingskunde*, Pflanzenschutz, Umweltschutz, 69 (5): 111-113; 21 ref.
- Shannag, H. K. and J. L. Caprina (1995): Entomopathogenic nematode effect on pickleworm (Lepidoptera: Pyralidae) under laboratory and field conditions. *J. Econ. Entomol.*, 87(5): 1205-1212.
- Shannag, H. K.; Webb, S. E. and Capinera, J. L. (1994): Entomopathogenic nematode effect on pickle worm (Lep. Pyralidae) under laboratory and field conditions. *J. Econ. Entomol.*, 87: 5, 1205-1212; 34 ref.
- Sikora, R. A., Salem, I.E. and Kingauf, F. (1979): Susceptibility of *Spodoptera littoralis* to *Neoaplectana carpocapsae* and the importance of environment factors in an insect control program. *Med. Fac. Landbouww Rijk. Gent.*, 44:309-321.
- Singh, and Sinha, (1977): Carbohydrates, lipids and protein in the developmental stages of *Sitophilus oryzae* and *Sitophilus granaries* (Coleoptera: Cuculionidae). *Ann.*
- Soliman, N. A. (2002): Pathological and biochemical effects of some entomopathogenic nematodes on Mediterranean fruit fly, *Ceratitidis capitata* (Wiedemann) (Diptera : Tephritidae). Ph.D. Thesis, Fac. Sci., Cairo Univ., Egypt.
- Thompson, S. N. (1982a): Effects of parasitization by the insect parasite *Hyposoter exigua* on the growth, development and physiology of its host *Trichoplusia ni*. *Parasitol.*, 84: 491-510
- Thompson, S. N. (1982b): Effects of insect parasite *Hyposoter exigua* on the total body glycogen and lipids levels of its host, *Trichoplusia ni*. *Comp. Biochem., Physiol.*, 72: 233-237.
- Thompson, S. N. and J. S. Barlow (1983): Metabolic determination and regulation of fatty acids composition in parasitic Hymenoptera and other animals. In "Metabolic Aspects of Lipid Nutrition in Insects" (T.E. Mittler and R. H. Dadd. Ed.), pp. 73-106. Westview, Boulder.

- Thong, C. h. S. and J. M. Webster (1975): Effects of *Contrylenchus reverses* (Nematoda: Sphaerulariidae) on the haemolymph composition and oocyte development in the beetle *Dendroctonus pseudotsugae* (Coleoptera : Scolytidae). J. Inverteber. Pathol., 26: 91-98.
- Von Brando, T. (1973): "Biochemistry of parasites" 2nd ed. Academic Press, New York.
- Xia, Y.; Dean, P.; Judge, A. J.; Gillespie, J. P. Clarkson, J. M. and Charnely, A. K. (2000): Acid phosphatases in the haemolymph of the desert locust, *Schistocerca gregaria*, infected with the entomopathogenic fungus, *Metarhizium anisopliae*. J. Inverteber. Pathol., 80, 139-147.
- Wergedal, J. E.; Y. K. U. and A. E. Harper (1964): Influence of protein intake on the catabolism of ammonia and glycine *in vivo*. In *Advances in Enzymes Regulation*" (Edited by Weber G.), 2:289-310. Pergamon Press, Oxford.

ARABIC SUMMARY

التأثير البيوكيميائي لثلاثة أنواع من الديدان الممرضة للحشرات وهي *S. feltiae* , *S. riobrave* and *H. bacteriophora* على يرقة دودة ورقة القطن

نجلاء فتحي رياض^{1,3} - أمينة محمد حسن عثمان مقلد¹ - سامية عبد الفتاح يسن¹ - شاكر محمد أبو المعاطي²

1- معهد وقاية النباتات

2- المعمل المركزي للمناخ الزراعي

3- قسم الاحياء - كلية العلوم - جامعة الملك خالد - ابها - المملكة العربية السعودية

في هذه الدراسة، تم دراسة إصابة ثلاثة أنواع من الديدان الممرضة وهم *Steinernema feltiae* ، *Heterorhabditis bacteriophora* ، *Steinernema riobrave*، علي دودة ورق القطن *Spodoptera littoralis*. بالإضافة الي ذلك، تم دراسة تأثير الديدان الممرضة السابقة علي بعض الأوجه الكيميائية الحيوية والفسولوجية لحشرة العائل. ولقد أظهرت الدراسة أن الديدان *H. bacteriophora* كانت أكثر قدرة علي احداث الإصابة للحشرة عن نوعي الديدان المستخدمين وكانت أيضا مميزة بقدرتها علي اعطاء أعلى إنتاج من اليرقات المعدية الشبائية للديدان (7000 يرقة معدية نيماتودية / يرقة العائل).

كذلك نقصت المغذيات الرئيسية (المحتوي الكلي للبروتين والكربوهيدرات والليبيدات) لليرقات المصابة نتيجة للإصابة بالديدان. أيضا تغير نشاط بعض الانزيمات في يرقات دودة ورق القطن نتيجة لأصابتها بالديدان. فعلي هذا تغير نشاط الأنزيمات الهاضمة للكربوهيدرات تبعا لنوع الإنزيم والديدان المستخدمة. فمثلا نقص نشاط أنزيم الأميليز مع الإصابة بالديدان *H. bacteriophora* ، وعلي العكس فقد زاد نشاطه مع الإصابة بالنوعين الاخرين. أما أنزيم الأنفرتيز فقد زاد نشاطه مع الإصابة بانواع الديدان المستخدمة، غير أن نشاط أنزيم التريهاليز قد زاد بدرجة عالية مع الإصابة بانواع الديدان وخاصة عند الإصابة بالديدان *S. riobrave*. أيضا زاد نشاط الأنزيمات الفوسفاتية الحامضية والقلوية نتيجة للإصابة بأنواع الديدان، وكان الاستثناء الوحيد هو حدوث نقص طفيف في نشاط الأنزيمات الفوسفاتية القلوية لليرقات المصابة بالديدان *S. feltiae*. وعلي العكس من ذلك فقد نقص بشكل ملحوظ نشاط الأنزيمات الناقلة لمجموعة الأمين (GOT & GPT) نتيجة للإصابة بأنواع الديدان المستخدمة.