Biochemical Changes in *Parasarcophaga. aegyptiaca* and *Argas (persicargas) persicus* Haemolymph Infected With Entomopathogenic Nematode

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Abstract: The physiological changes in *Parasarchofaga aegyptica* larvae and *Argas (persicargas) persicus* adult haemolymph had been investigated resulting in entomopathogenic nematode infection. It was dramatically declined in total protein and total lipids in both *P.sarchophaga* and *A.persicus*. The Amino acid was fluctuated between increase in aspertine, glutamine, serine and decrease in glycine, histadine, argnine, porline, tyrosine, valine, isoleucine, leucine, methonine, and phenylalanine. Also, there was a significant increase in protease and lipase activity in larval and Adult haemolymph of both studied hosts after nematode infection. [Nature and Science. 2009;7(6):70-81]. (ISSN: 1545-0740).

Key words: Entomopathogenic nematodes; Steinernema; Heterorhabditits; Parasarchophaga; Argas; Ticks; Biochemical.

INTRODUCTION

With the exception of some lipids and carbohydrates, information on biosynthetic processes in entomopathogenic nematodes is extremely limited(Wright and Perry, 2002). Knowledge of which amino acids cannot be synthesized by nematodes ('essential' amino acids) has been based partly on nutritional studies on species that have been grown axenically in fully defined culture media (Vanfleteren,1980). For example, *Caenorhabditis elegans* and *C.briggsae* require arginine pluse nine amino acids that are usually essential in mammals. The requirement for entomopathogenic nematodes to be produced monoxenically has precluded such studies. Like most organisms, nematodes can synthesize purine and pyrimidine bases but studies on the biosynthesis on nucleoside monophosphates are lacking. Anderson and Kimble (1997). have reviewed the translational mechanisms involved in protein synthesis in *C.elegans*. Nutrial lipids include triacylglycerols, diacylglycerols, free fatty acids, sterol esters of fatty acids, and free sterols. Phospholipids, which provide the major proportion of cellular membranes, are usually derived from a molecule of glycerol, two fatty acids, phosphoric acid and and asecond alcohol. In freshly-emerged infective juveniles of *Steinernema* species (Patel and Wright, 1997). Other lipids include glycolipids, lipoproteins and proteolipids (Chitwood,1998). For entomopathogenic nematodes, the quality and quantity of lipids in infective juveniles produced on a commercial scale is paramount since this has a critical influence on nematode viability and infectivity (Wright and Perry, 2002).

The physiological information of the entomopathogenic nematode pathogenisity on veterinary insects are lacked.So, this investigation aims to study the evaluation of the nematodes *Steinernema riobrave* and *Heterorhabditis bacteriophora* Hp88 role in degradation haemolmph proteins, amino acids and lipids in infected flesh-fly larvae *Parasarcophaga. aegyptiaca* and chicken ticks *Argas (persicargas) persicus* through quantitatively determination of protease and lipase enzymes present in the haemolymph of control and infected hosts.

MATERIAL AND METHODS

Biochemical studies: Five larvae of *Parasarcophaga aegyptiaca* and five adult *Argas (persicargas) persicus* were homogenized separately as a whole body in 5 ml of extraction solvents. The samples were centrifuged for 20 minutes at 10,000 rpm, and then the supernatant was collected for the biochemical

studies. The experiment was done at 10, 20, and 40 hr post injection of *P. aegyptiaca* and 10, 20, and 30 hr after infection of *A.(P) persicus*.

***P. aegyptiaca*:** The third instar larvae were divided into 4 groups ;
1)Control negative group. 2) Control positive group, injected with 10µ water.3) Injected group with 40 IJs/larva of *Heterorhabditits bacteriophara.* 4) Injected group with 40 IJs/larva of *Steinernema riobrave*.Every group contain 10 larvae.

***A. persicus*:** In this case adult were frankly exposed to 500 infected juveniles (IJs) which were suspended in 1.5 ml water and sprayed on 15gm clean sand. The used plastic pots were 25cm^3 .This experiment divided into 3 groups.1) Control group. 2) Infected group with *H. bacteriophara*. 3) Infected group with *S. riobrave.* Every group contains 10 pots every pot contains 3 adults.

Determination of total protein: Total protein was determined using kit supplied by Diamond, according to Doumas (1975) at 546 nm wave length.

Sample preparation: Protein was extracted from the haemolymph by the homogenization the hall body in NaCl 0.15 Molar according to Marion (1976).

Free amino acid analysis:
HPLC determination of the free amino acid content in the haemolymph:
Haemolymph free amino acids were detected by high performance liquid chromatography (HPLC) using the precolumn PTC derivatization technique according to the method of Heinrikson and Meredith (1984). The HPLC system of Perkin- Elmer consisted of quaternary pump; a column oven, Rheodine injector and 20µ / loop, UV variable wavelength detector. The report and chromatogram taken from data acquisition program purchased from Perkin- Elmer. PICO- TAG column (Waters) for free-amino acid analysis 3.9 × 30 cm.; Eluent (1) and Eluent (2), Phenylisothiocyanate (PITC), Triethylamin, *Amino acids standard. (standards and eluents are Waters chemistry package for free amino acids). 46 ^{0}C; wave-length: 254 nm; flow rate: 1ml/min.

Preparation of the sample: *Parasarcophaga aegyptiaca* larvae were injected with 40 IJs /larva and *Argas (p) persicus* was infected with 500 IJs / one, and then let them for 40 hr post infection. Control samples were considered in all experiments. The first step in determination of amino acids by HPLC method involved weighting and homogenization of the haemolymph in 1/10 weight/volume of 75% aqueous HPLC grade methanol. The homogenate was spun at 3000 r.p.m. for 10 min and the supernatant was divided into two halves; the first was dried using vacuum (70 millitore) at room temperature, whereas the second half was used for monoamine determination.

Derivatization procedure: The derivatization started by re drying the sample under test using drying solution consisted of 2:2:1 mixture (by volume) of methanol: 1M sodium acetate trihydrate: triethylamine (TEA). The drying solution was added to the dry sample, shook well and then put under vacuum till complete dryness.

The derivatizing agent consisted of 7:1:1:1 mixture (by volume) of methanol: TEA: water: PITC (Phenylisothiocyanate). The derivatizing solution was added to the redried sample, shook well and left to stand at room temperature for 20 min, then applied to vacuum (70 millitore) till dryness. The dry sample was then diluted by a sample dilution composed of 0.71-g di sodium-hydrogen phosphate adjusted to a pH of 7.4 by 10% phosphoric acid. Acetonitrile was then mixed, as 5% by volume with the resulting solution.
Derivatized amino acids standard and derivatized sample were injected, (the injected volume is 20µl), into the column for separation by HPLC. The resulting chromatogram identified each amino acid position and concentration from the sample as compared to that of the amino acids standard and finally the determination of the µ mole content of each amino acid per gram brain tissue was achieved.

Determination of total lipid: Total lipid was determined using kit supplied by Diamond, according to Knight *et al*., (1972), at 525 nm wave length.

Sample preparation: Lipids were extracted from the haemolymph according to Abu-Hatab and Gaugler (1997) as homogenization in 2:1 chloroform : methanol (v/v).

Determination of protease enzyme: The protease activity of *A.(P) persicus* adult or *P. aegyptiaca* larva was determined by the casein digestion method at 280 nm wave length according to Birk, ***et al***., (1962).

Sample preparation: The enzyme solution was prepared by the extraction method of Abu-Hatab ***et al***., (1995). The samples were homogenized in phosphate buffer 0.1 Molar at PH 7.6. The method is based on casein substrate since it is considered to be supplemented protein.

Procedures: The reaction mixture was prepared by addition of 0.2 ml glycine-NaOH buffer (0.075 Molar, PH 10) and 0.4 ml 1.5 % casein solution, to 0.2 ml enzyme solution. After 60 minute incubation at 37 C°, the enzymatic activity was determined by adding 1.2 ml of 5% trichloroacetic acid solution. The reaction mixture was centrifuged at 18,000 rpm for 15 minute. The supernatant was taken for enzymatic activity evaluation.

Determination of lipase enzyme:

Sample preparation: The enzyme solution was prepared by the extraction method of Abu-Hatab ***et al***., (1995)**.** The samples were homogenized in phosphate buffer 0.1 Molar at PH 7.6. The method depends on titration of liberating fatty acids with alkaline reagent.

Procedures: Lipase activity was measured according to the method of Tietz and Fiereck (1966, 1972). Substrate emulsion were prepared by mixing 0.2 gm sodium benzoate with 7 gm gum acacia in 100 ml water in mechanical blender at slow speed, and then 1.3 gm triolin was added slowly and mixed for 10 minute at maximum speed. The 3 ml assay reaction mixture contained 2 ml of substrate emulsion, 0.4 ml 20 mM Tris-HCl, PH 8.0, 0.4 ml 5 mM $CaCl_2$, and 0.2 ml enzyme solution. The reaction mixture was incubator 1 hr at 37 C°. The reaction was stopped by addition of 0.6 ml 96% ethanol. Then titrated with 5 mM NaOH in presence of an indicator (phenolphthalein).

Statistical Analysis: The data were subjected to statistical analysis using T. test and F test (one way classification least significant differences "L.S.D.") according to (Snedecor and Cochron, 1967). The significance of the main effects was determined by analysis of variance (ANOVA). The significance of various treatments was evaluated by Duncan's multiple range test ($P<0.05$, 0.01). All analysis was made using a software package "Costat", a product of Cohort Software Inc. Berkeley, California.

Results

Total protein content: There were dramatically declined in total protein content of *P. aegyptiaca* larvae 10hr and 20hr post injection. It was also found that, the lowest decline was recorded at 40 hr post injection with *H. bacteriophora* and *S. riobrave* 0.12±0.08 and 0.142±0.01 respectively (Table 1). Results of *A.persicus* haemolymph gave highly significant declined in total protein content in the same intervals time. The data declared that, the infection with *S. riobrave* induce more decrease in total protein content than *H. bacteriophora* (Table 2).

Amino acids:

The data indicated that, the free amino acids aspertine, glutamine, and serine increased 35.5, 19, and 82% respectively in infected larval haemolymph of *P. aegyptiaca*, but the other amino acids glycine, histadine, argnine, porline, tyrosine, valine, isoleucine, leucine, methonine, and phenylalanine decreased -65.8, -36, -73,
-8.9, -66.4, -97, -24, -15.7, -94.4, and -63.5 % respectively in infected larval haemolymph of *P. aegyptiaca* (Table 3). There were clearly changes in free amino acids concentration in control and infected haemolymph of *A. (p) persicus*. These changes demonstrated as increasing in concentrations of aspartine, glutamine, and serine 57.6, 16, and 92.9% respectively post infection of *S. riobrave* nematode, and sharply decline in concentrations of glycine, histadine, theronine, alanine, valine, isoleucine, and leucine, -48.2, -96.3, -92.4, -87.2, -75.2, -82.1,and -69% respectively in infected haemolymph of *A.(p) persicus*. But argnine and phenylalanine were disappeared in infected groups (Table 4).

Total lipid content: The results obtained in table (5) showed that, there was reduction in total lipid content of the 3rd instar larvae of *P. aegyptiaca* after 10 hr post injection with *H. bacteriophora* and *S. riobrave* 3.83±0.09 and 3.62±0.03 respectively as compared with the two types of control 4.61±0.12 and 4.3±0.17**.** The lowest decrease in total lipid content was recorded at 40 hr post injection. From the present data we noticed that, the injection with *S. riobrave* induced more reduction in total lipid content. The reduction in total lipid was clearly observed in the infected groups of *A. (p) persicus* which measured at10 and 20 hr after infection. The lowest decrease was recorded at 30 hr post infection with *H. bacteriophora* and *S. riobrave* as compared with control group. The present data represented that, the infection with *S. riobrave* induce more decrease in lipid content than *H. bacteriophora* (Table 6).

Protease activity: The results revealed that, there was significant increase ($P \leq 0.001$) in protease activity after 10 hr of injected groups of *H.bacteriophora* and *S.riobrave* 363.26±2.107 and 345.56±2.8 respectively, compared with control groups 214.3±4.1 and positive control groups 289±2.It also observed that, the protease activity of *P. aegyptiaca* was increased by the increasing of the time post injection (Table7). The results in table (8) revealed that, there was significant increase ($P \leq 0.001$) in protease activity of *A. persicus* after 10 hr post infection with *H. bacteriophora* and *S. riobrave* 167.53±2.83 and 252.43±1.5 respectively compared with control group 70.46 ±.38. The same results were obtained in the infected groups at 20 hr post infection. Thus the highest increase in protease activity was recorded at 30 hr post infection with the two strains of nematodes *H. bacteriophora* and *S. riobrave* 586.1± 6.65 and 564.43±7.17 respectively as compared with control group 80.8±0.18.

– Lipase activity: The data demonstrated that *S. riobrave* more increase in lipase activity as compared with *H. bacteriophora* in both two studied hosts. Table (9) revealed that, there was a significant increase ($P \leq 0.001$) in lipase activity of 3rd instar larvae of *P. aegyptiaca* after 10 hr of injected groups with *H. bacteriophora* and *S. riobrave* 33.5±0.28 and 35.66±0.166 respectively as compared to the two control groups. Such changes were clearly observed in infected *P. aegyptiaca* estimated at 20 and 30 hr post injection. Similar changes were clearly observed in infected *A.(p) persicus* estimated at10 and 20 hr post infection. The highest increase in lipase activity was recorded at 30 hr post infection with *H. bacteriophora* 586±6.65 and *S. riobrave* 564.43±7.17 compared with the control group 80.8±0.18 (Table10).

Table (1): Effect of *H. bacteriophora* and *S. riobrave* on total protein content (gm/dl)of non infected and infected *P. aegyptiaca*, n = 5

Time (hours) Post infection	Contro Negative	Control Positive	*H bacteriophora*	*S. riobrave*
10	1.58±0.03	1.49±0.02	0.9±0.02 ***	1.05±0.089 ***
20	1.49±0.01	1.43±0.02	0.43±0.02 ***	0.45±0.058 ***
40	1.35±0.01	1.34±0.03	0.12±0.08 ***	0.142±0.01 ***

Values represented mean of three separated groups ± SE
***** P < 0.001 highly significance**

Table (2): Effect of *H. bacteriophora* and *S. riobrave* on total protein content (gm/dl) of non- infected and infected *A. (p) persicus.* n=*5*

Time (hours) post infection	Control	*H. bacteriophora*	*S. riobrave*
10	**2.17±0.03**	**1.68±0.02 *****	**1.29±0.26 *****
20	**2.02±0.02**	**1.37±0.037 *****	**1.004±0.005 *****
30	**2.01±0.02**	**0.84±0.039 *****	**0.91±0.0134 *****

Values represented mean of tree separated groups± SE
***** P < 0.001 very highly significance**

Table (3): Effect of *Steinernema riobrave* on the concentrations of free amino acids in the haemolymph of the 3rd instar larvae of *Parasarcophaga aegyptiaca*.

Free amino Acids	Control mg /dl	Treated mg /dl	% of change from control
Aspertine	14	18.97	35.5
Glutamine	314	373.66	19
Serine	24	43.68	82
Glycine	5	1.71	-65.8
Histadine	1	0.64	-36
Argnine	8	2.16	-73
Proline	16	14.58	-8.9
Tyrosine	23	7.73	-66.4
Valine	15	0.45	-97
Isoleucine	8	6.1	-24
Leucine	7	5.9	-15.7
Methonine	7	0.39	-94.4
Phenlalanine	7	2.56	-63.5

Table (4): Effect of *Steinernema riobrave* on the concentration of free amino acids in the haemolymph of the adult *A. (p) persicus.*

Free amino Acids	Control (mg /dl)	Treated (mg /dl)	% of change from control
Aspertine	6.34	10	57.6
Glutamine	80.24	93.16	16
Serine	8.12	15.66	92.9
Glycine	7.01	3.6	-48.2
Histadine	8.43	0.31	-96.3
Theronine	7.5	0.57	-92.4
Argnine	Trace	-	-
Alanine	4.01	0.5	-87.2
Valine	24.92	6.2	-75.2
Isoleucine	7.44	1.33	-82.1
Leucine	5.35	1.66	-69
Phenylalanine	Trace	-	-

Table (5): Effect of *H. bacteriophora* and *S. riobrave* on total lipid content (gm/dl) of non infected and infected *P. aegyptiaca*, n = 5

Time (hours) Post infection	Control negative	Control Positive	*H. bacteriophora*	*S. riobrave*
10	4.61±0.12	4.3±0.17	3.83±0.09 ***	3.62±0.03 ***
20	3.97±0.04	3.9±0.05	3.48±0.1 ***	3.25±0.02 ***
40	3.67±0.01	3.6±0.02	1.8±0.07 ***	1.36±0.15 ***

Values represented mean of three separated groups ± SE
***** P < 0.001 very highly significance**

Table (6): Effect of *H. bacteriophora* and *S. riobrave* on total lipids content of non infected and infected *A. (p) persicus* n=5

Time (hours) Post infection	Control	*H. bacteriophora*	*S. riobrave*
10	**2.97±0.029**	**1.25±0.06 *****	**1.11±0.052 *****
20	**2.96±0.035**	**0.983±0.02 *****	**0.88±0.014 *****
30	**2.72±0.15**	**0.761±0.031 *****	**0.671±0.031 *****

Values represented mean of tree separated groups± SE
***** P < 0.001 very highly significance**

Table (7): Effect of *H. bacteriophora* and *S. riobrave* on protease activity units 10^3x no of non infected and infected *P. aegyptiaca* n=5

Time (hours) Post infection	Control negative	Control Positive	*H bacteriophora*	*S.riobrave*
10	241.3±4.1	289±2.1	363.26±2.107 ***	345.6±2.8 ***
20	274.8±3.5	319.7±3.1 *	544.2±3.1 ***	590.5±4.9 ***
40	315.7±2.9	387.4±3.2 *	686.7±7.8 ***	811.03±5.8 ***

Values represented mean of three separated groups ± SE
***** P < 0.001 very highly significance**

Table (8): Effect of *H. bacteriophora* and *S. riobrave* on protease activity units x 10^3/no. of non infected and infected *A. (p) persicus.* n=*5*

Time (hours) Post infection	Control	*H.bacteriophora*	*S. riobrave*
10	**70.46±0.38**	**167.53±2.83 *****	**252.43±1.5 *****
20	**71.7±0.46**	**325.2±11.76 *****	**416.1±8.68 *****
30	**80.8±0.18**	**586.1±6.65 *****	**564.43±7.17 *****

Values represented mean of tree separated groups± SE
***** P < 0.001 very highly significance**

Table (9): Effect of *H.bacteriophora* and *S. riobrave* on lipase activity units no. of Mm of fatty acid/hr of non infected and infected *P. aegyptiaca* n = 5.

Time (hours) Post infection	Control Negative	Control Positive	*H.bacteriophora*	*S.riobrave*
10	25±0.0	25.7±0.2	33.5±0.28 ***	35.7±0.2 ***
20	25.66±0.2	26.5±0.3	37±0.6 ***	38.8±0.16 ***
40	26.5±0.5	27.2±0.2	44.16±0.6 ***	47.2±0.16 ***

Values represented mean of three separated groups ± SE
***** P < 0.001 very highly significance**

Table (10): Effect of *H. bacteriophora* and *S. riobrave* on lipase activity unit (no of Mm fatty acid/hr of non infected and infected *A. (p) persicus* n=5.

Time (hours) Post infection	Control	*H.bacteriophora*	*S. riobrave*
10	**70.46±0.4**	**165.53±2.83 *****	**252.43±1.5 *****
20	**71.7±0.46**	**325.2±11.76 *****	**416.1±8.68 *****
30	**80.8±0.18**	**586.1±6.65 *****	**564.43±7.17 *****

Values represented mean of tree separated groups± SE
***** P < 0.001 very highly significance**

DISSCUSION

The present study has been shown that, the nematodes *H. bacteriophora* and *S. riobrave* severely deplete proteins in the haemolymph of larval *P. aegyptiaca* and adult *A. (p) persicus.* This finding agree with that obtained by Mckinstry and Steinaus (1970) who were able to separate electrophoretically septicaemic and fresh plasma of *G. mellonella* infected with *pseudomonas aeurginosa* and detected a reduction in the amount of protein. Also, Pare *et al.,* (1977) studied the effect of *Bacillus thuringiensis* on the plasma protein of *Choristeneura fumiferana* and reported reduction in number of slow moving proteins.

Similar results were observed by Abdel- Kawy (1981 & 1985), El-Bishry (1989) who reported that, *N. carpocapsae* caused a reduction and or disappearance of haemolymph slow moving protein fractions in both *Schistocerca gregaria* and *S. littoralis*. Johns *et al.,* (1998) found that, the bacterial infection in the hard tick *Dermacentor variabilis* reduced the protein content non significantly by 24 hr, but the mean protein concentration was even lower at 48 hr which was highly significant. Moreover Gillespie *et al.,* (2000) observed that, there was reduction in total protein content of the haemolymph of desert locust *Schistocerca gregaria* during the course of infection with the entomopathogenic fungus, *Mertarhizium anisopliae* var *acridum*.

The losses of soluble protein from the host' haemolymph during parasitism may be explained in three ways. (1) The parasite may secrete proteolytic enzemes into the haemocoel of the insect and hydrolyze the host's proteins. Muller (1931) suggested that, mermithids produce a secretion that serves for the predigestion of the host haemolymph. In addution, Gordon and Webster (1971) could not find proteolytic enzyme activity in homogenates of *Mermis nigrescens* and concluded that this nematode affects the protein metabolism directly or indirectly in the fat body of the host. (2) Protein metabolism in the host fat body may be altered by nutritional stress or endocrine manipulation brought on by parasitism. Protein metabolism, transport of amino acids, and excretion are all regulated hormonally (Highnam and Hill, 1969) and disturbance of the host's endocrine balance by the parasite could be responsible for reduced protein concentrations in the host haemolumph. (3) The nematodes may absorb intact protein directly from the hosts' haemolymph; however, research by Gordon and Webster (1972) showed that. *Mermis nigrescens* was unable to incorporate a dipeptide, tritiated L-histihyl-L-leucine, or tritiated haemolymph proteins of *Schistocerca gregaria* into its protein, whereas labeled amino acids were rapidly utilized.

On the other hand our results do not agree with Andreadis and Hall (1976) who studied the defense reaction of *Aedes aegyptii* against the nematode *Neoaplactana carpocapsae* elactrophoretically and showed a shift in certain bands, a reduction in intensity of others and him presence of an additional protein fraction. They also found that, some proteins released by the host or by the parasite in response to parasitism, and were unable to understand the function of this protein fraction in the defense reaction. Hulbert *et al.,* (1985); Spies and Spence, (1985); Dunn, (1986); Spies *et al.,* (1986); Dimarcq *et al*., (1990) found that, the injection of forgein molecules into the insect haemolymph induce the synthesis of immune protein, these finding confirmed with Ayaad *et al.*, (2001).They observed that, the stimulated newly protein bands particularly at 40 hr post injection of *P. surcoufi* with *H. bacteriophora* are probably immune proteins.

Amino acids play an important role in maintaining the proper osmotic balance in haemolymph of arthropods (Sutcliffe, 1963). The effect of parasitism on the total and individual amino compounds varies depending on the host and parasite considered.

The entomopathogenic nematodes parasitism caused sharply changes in free amino acids of *P. aegyptiaca* and *A. (p) persicus* haemolymph. In this study, results induced dramatically increased in 3 amino acids in *P. aegyptiaca* and *A. (p) persicus* haemolymph after infection with *S. riobrave* nematode. On the other hand there was depletion in 10 amino acids in both hosts after infection. Our results confirmed with Gordon *et al.*, (1978) who reported variable results between two black fly species. In *Prosimulium mixtum / fuscum* most amino compound concentrations were reduced by parasitism, while nematode infections of *Simulium venustum* induced increases in the concentration of almost half of the ninhydrin positive substances. However, these infected and control black flies. Moreover, Rutherford and Webster (1978) found that parasitism of *Schistocerca gregaria* by *Mermis nigrescens* caused the haemolymph levels of 10 amino acids to increase and 3 to decrease significantly.

Rutherford *et al.,* (1977) explain the variable effects on amino acids encountered in nematode infections reflect the dynamic relationship between host and parasite. The importance of maintaining an osmotic balance in the insect haemolymph necessitates an efficient system of amino acids regulation. Hormones are responsible for control of excretion of nitrogenous compounds, transport of amino acids into the fat body, protein synthesis, and proteolysis. A simpler alternative is that the mermthid absorbs the amino acids selectively across the cuticle. Schmidt and Platzer (1980) reported that, the reduction in the

haemolymph amino acids are replenished by increased host proteolysis, decreased excretion or transport into the fat body, or reduced protein synthesis, these explanation agree with our results in determination of total protein and protease activity of *P. aegyptiaca* and *A. (p) persicus* post infection with nematode, where we showed sharply decrease in total protein which were replenished by increasing in protease activity in both hosts *P. aegyptiaca* and *A. (p) persicus*.

In our study the entomopathogenic nematodes *S. riobravus* belongs to the family Steinernematidae have symbiotic bacteria *Xenorhabdus spp*. This bacteria play an important role in enhance and proliferate the host haemolymph for the nematode reproduction. So, we can explain the sharply changes in the free amino acids of the two studied hosts *P. aegyptiaca* and *A. persicus* haemolymph, that, it may be due to the interference between the nematode and symbiotic bacteria inside the host. In addition knowledge of which amino acids can not be synthesized by nematodes (essential amino acids) has been partly on nutritional studies on species that have been grown axenically in fully defined culture media (Vanfleteren, 1980). For example, *Caenorhabditis elegans* and *Caenorhabditis briggsae* require arginine plus nine amino acids that are usually essential in mammals. Rutherford and Webster (1978) reported changes in the individual carbohydrates and amino acids in the haemolymph of infected locusts. It has been also noticed from this present study that, there was actual decrease in total lipid of infected hosts after 10, 20, 30, and 40 hr from infection with the nematodes. These results were agreed with Milstead (1979) while studied the pathophysiological influences of nematode *H. bacteriophora* complex on the sixth instar larvae of *G. mellonella*, he reported that, shortly after the nematode penetration into the haemocoel of the larvae begin feeding upon the fat body. Thompson and Barlow (1983) reported that, an extreme depression of glyceride synthesis would allow the parasite to use its host's fat after partial digestive hydrolysis and its own fatty acids for rapid triglyceride synthesis, thereby minimizing the energy cost of fat synthesis. Moreover, our results were agreed with Ghally *et al.,* (1988) who observed that, the lowest decline in total lipids was after 18 hr post infection of *Ceratitis capitata Wiedmann* with *Steinernema feltiae* Filipjev nematode. This finding is in consort with the findings of Hawlitzky and Boulay (1986) who reported that, the significant declines in lipids of parasitized larvae *Anagasta kuehniella* Zell by *Phanerotoma flavitestacea* are a normal feature with the general scope of endoparasite action on host chemical composition.

The higher level of protease enzyme in infected hosts compared with control may be due to the parasite may secrete proteolytic enzymes into the haemocoel of the insect and hydrolyzed the hosts proteins once hydrolyzed the free amino acids may be absorbed by the nematode and utilized for protein synthesis Rubstov (1967), who suggested that the parasitic stage released proteases for the digestion of the host fat body and absorbed the partially dissolved products through cuticle. Production of a toxins by axenic nematodes was discussed also by Goetz et al. (1981), Burman (1982), Goetz and Guelzon (1982), Boeman et al., (1983) and El-Bishry (1989). Poinar (1979) stated that, insect death probably arises from production of proteolytic enzymes which explain the relative lack of resistance to *Achromobacter nematophilus* when just one to three cell are injected in the body cavity of *G. mellonella* larvae Abdel Kawy (1985) mentioned that proteinase may produced by *X. nematophilus* since the addition of potato extract as proteinase inhibitor to infected haemolymph filtrate decreased the lethal effect of that filtrate when injected in healthy *Spodoptera* larvae. But protease may be released from disrupted fat body tissue and in part may account for the substantial proteolytic activity found in otherwise protein-depleted haemolymph of *Culex pipiens*. Digestion of proteins in the haemolymph by proteases present in the haemolymph may provide additional amino acids (Schmidt and Platzer,1980). However Gordon and Werbster (1971), reported that, proteolytic enzymes were absent in homogenates of *M. nigrescens*.

It is well obvious from the present work that, lipase enzyme of parasitized hosts *P. aegyptiaca* and *A. (p) persicus* is higher than controls; this finding may be provided by the reduction of total lipids. Spasski *et al.*, (1977) determined the lipase, pepsin, trypsine, pectinase and invertase activity in Hydromermis and observed that, the enzymic activity was much higher in pre parasitic than in post parasitic larvae, these enzymes are important in the extra intestinal digestion of the mermithids and probably also effect the lyses of the chitinous covering the host.

Metabolite depletion by the parasite could cause physiological imbalances in the host that lead to a reduction in haemolymph protein and lipid concentrations, and increase in protease and lipase enzymes activity. Mechanisms by which the nematode parasite could bring about such changes are unknown.

Generally, the purpose of this study was to evaluate the role of nematodes *H. bacteriophora* and *S. riobrave* in physiological imbalances in the hosts *P. aegyptiaca* and *A. (p) persicus*. This imbalance causes homolysis of haemolymph of the host and then death of the host. Thus we can use the entomopathogenic

nematodes as biological control agent against some veterinary insect pest which spend all or part from their life in soil.

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