Free fatty acids in some tissue of parasitized bug, Leptocoris augur Fabr., a pest of Kusum tree, Schleichera oleosa Lour.

Kumkum
Department of Zoology, Shaheed Mangal Pandey Govt. Girls P.G. College, Meerut (Uttar Pradesh) 250002 India
Entomology Research lab.
kmkmrajput@gmail.com

Abstract

Leptocoris augur is a pest of kusum plant (Schleichera oleosa), which in turn is a host of lac insect. The bug is parasitized by a mermithid nematode, Hexamermis vishwakarma, which naturally checks rapid built-up of bug population. In the present studies free fatty acids content in different tissue such as gonads (i.e., testes and ovaries), muscles and haemolymph of parasitized bugs, L. augur have been studied. H. vishwakarma not only utilize fat body proteins but also the lipid content of the fat as a result of which fat bodies disappears from the haemolymph of L. augur. The fatty acid composition of the host haemolymph is not significantly changed by H. vishwakarma parasitism in L. augur of both the sexes.

H. vishwakarma Dhiman takes nutrition from the haemolymph and muscles of insect body cavity Dhiman and Singh [3]. Availability of free fatty acids in the haemolymph of host bug help the parasitoid to establish in the haemocoel. After establishment, the parasitoid induces changes in the availability of biochemicals and composition of haemolymph, by the secretion of enzymes. The parasitoid's enzymes cause degeneration or dissolution of muscles, adipose tissues, testes as well as ovaries and the material of these organs is made available to the parasitoid. Hence, parasitoid grows at the expense of host tissues.

Keywords: *Leptocoris augur, Hexamermis vishwakarma, Haemolymph, Free fatty acids, Parasitoid.*

1. Introduction

Leptocoris augur is a pest of kusum plant (Schleichera oleosa), which in turn is a host of lac insect. This bug is a gregarious feeder and by its de-sapping habit, viability of the seed is lost Dhiman and Gulati [2]. It is parasitized by a mermithid nematode, Hexamermis vishwakarma, which naturally checks rapid built-up of bug. H. vishwakarma Dhiman takes nutrition from the haemolymph and muscles of insect body cavity Dhiman and Singh [3]. Availability of free amino acids, carbohydrates and fatty acids in the haemolymph of host bug help the parasitoid to establish in the haemocoel. After establishment, the parasitoid induces changes in the availability of biochemicals and composition of Haemolymph, by the secretion of enzymes. The parasitoid's enzymes cause degeneration or dissolution of muscles, adipose tissues, testes as well as ovaries and the material of these organs is made available to the parasitoid. Hence, parasitoid grows at the expense of host tissues.

In the present studies free fatty acids content in different tissue such as gonads (i.e., testes and ovaries), muscles and haemolymph of parasitized bugs, Leptocoris augur have been studied.

2. Materials and Methods

2.1. Morphological study

Both control as well as parasitized bugs were carried out by means of several dissections. The bugs were fixed in wax trays. Dissections were done by using handmade fine blades, fine forceps and thin, pointed as well as hooked needles under the stereoscopic binocular microscope. Lens of different magnification (10X, 12X, 14X, 17X, 1X, 2X, 3X, 5X) and small entomological pins were used. After dissections many permanent slides were made of the whole reproductive organs of control as well as parasitized bugs 30% alcohol was used as excellent media for dissections, because, it cleared the fatty tissue lying around the reproductive organs and was compared with parasitized bug reproductive organs. 1% methylene blue yielded good results in staining the reproductive organs. Figures were sketched of the freshly dissected bugs as well as the mounted slides on graph sheets using graticule. Measurements were taken in mm with the help of stage micrometer using different magnifications of lenses.

2.2. Collection of haemolymph

Pooled haemolymph of each sex formed a sample and three such samples were collected for each group of the bugs, e., control and parasitized at a regular interval of five days, ten days, fifteen days and just prior to emergence of parasitic nema. Small ice-cooled centrifuge tubes which were previously coated with phenylthiourea to inhibit tyrosine activity were used.

The haemolymph was centrifuged at 7,000 rpm to obtain a supernatant free haemocytes aliquot of 0.05ml supernatant were used to determine the protein, lipid, glucose and free fatty acid concentration.

2.3. Free Amino Acids Determination

For the determination of free amino acids concentration of control and parasitized bugs in muscles, testes and ovaries, a known weight of each tissue was homogenized in a mixture of chloroform and methanol and centrifuged at 7,000 rpm to obtain the supernatant. The residue was directly estimated by employing the colorimetric method. The concentration is stated in mg/100mg tissue weight of gonads or muscles.

Free fatty acids were estimated by the method suggested by Lowry and Tinsley [8]. The suitable aliquots of lipid extracts were transferred to seven test tubes except one in which equal amount of chloroform was added, which was used as control. The samples were evaporated to dryness. To each tube 5.0 ml of benzene was added and stirred to dissolve the samples. The mixtures were warmed slightly and subsequently, 1.0 ml of Cupric acetate pyridine *reagent was added. The tubes were mixed well and centrifuged for 5 minutes at 3000 Xu. The lower layer was discarded and the absorbance of upper layer was recorded at 715nm. The amounts of free fatty acids were worked out against the previously prepared standard curve.

Cupric acetate pyridine- 5% aqueous Cupric acetate solution was prepared, filtered and pH was adjusted with the help of Pyridine at 6-6.2.

2.4. For Histopathological studies

For histopathological studies small pieces of samples were removed and fixed in xylene. The material was kept in 50% wax and 50% xylol for 4-6 hours then transferred into small vials with pure paraffin wax at 60° (According to the melting point of wax) in an oven. Block were prepared using ‘L’ pieces. These were trimmed and sectioned (5-6µ thick) on

a rotary microtome and changes were observed under a light research microscope. Histological diagrams were also drawn with the help of graticule on graph sheets. Various suitable magnifications were employed. Photomicrographs were taken by Asahi Pentax S.P.1000 camera with the help of microscopic attachment. The measurements of the nematodes were taken with aid of ocular micrometer and slide micrometer.

3. Results

Parasitism of Hexamermis vishwakarma brings about changes in biochemical's of L. augur specially concerning with the normal growth, circulation, reproduction, moulting and metamorphosis.

Quantitative estimation of free fatty acids in haemolymph of control bug was estimated as 149.96 ± 1.99 mg/100ml in female and 148.96 ± 1.98 mg/100ml in male. But, in parasitized bug the level minutely decreased as 149.64 ± 2.68 mg/100ml in female and 148.96 1.98 mg/100ml in male of 5 days old parasitized bug, after 10 days of parasitization in female and male bug haemolymph, it was 129.98 ± 2.0 mg/100ml 91.23 ± 1.32mg/100ml, on 15 days of parasitization, it was 88.69 ± 3.14 mg/100ml in female haemolymph and 77.89 ± 1.84 mg/100ml in male and just before emergence it was calculated as 74.24 ± 7.21 mg/100ml in female and 61.54 ± 1.98 mg/100ml in male (Table – 1, Graph 1).

Like that of cholesterol level, free fatty acids were estimated in the gonads of control and healthy bugs. Quantitative estimation, is recorded in (Table 3, Graph 3). The results of estimations have shown that the free fatty acids level in control bugs was 215.51 ± 1.95 mg/100mg in ovaries and 112.02 ± 2.13 mg/100mg in testes. The level of free fatty acids was 212.32 ± 1.44 mg/100mg in ovaries and 103.29 ± 0.86 mg/100mg in testes in 5th day old parasitized bugs, (L. augur); in 10 days old parasitized bug it was 184.88 ± 1.99 mg/100mg in ovaries and 88.69 ± 3.44 mg/100mg in testes; in 15 days it was 112.39 ± 1.85 mg/100mg in ovaries and 71.31 ± 2.01 mg/100mg in testes. Prior to emergence of H. vishwakarma from the L. augur body it was 64.85 ± 2.13 mg/100mg in ovaries and 61.54 ± 1.98 mg/100mg in testes. (Figure 1 & Table 3).

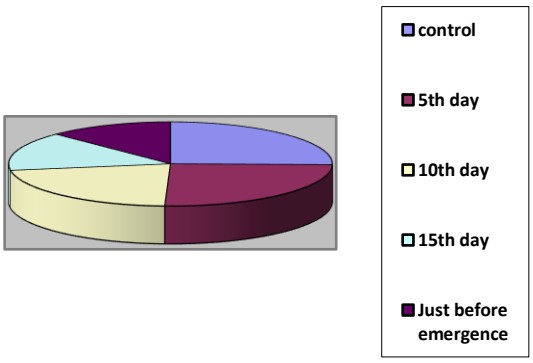
Data of quantitative results of (Figure 2 & Table 2, Graph 2) depict that the quantity of free fatty acids in control bug was 112.02 ± 2.13 mg/100mg tissue in female muscles and 92.17 ± 1.85 mg/100mg in male muscle. While in case of parasitized bug the level was estimated as 95.68 ± 2.06 mg/100mg tissue in female muscle and 91.24 ± 2.94 mg/100mg tissue in male muscles on 5th days of parasitization. But, in 10 days it was estimated as 61.54 ± 1.98 mg/100mg tissue in female muscle and 55.98 ± 2.33mg/100mg tissue in male muscle, in 15 days, it was further minutely decreased as 29.58 ± 3.30 mg/100mg in female and 31.17 ± 4.82mg/100 mg in male. The muscle free fatty acid levels also observed just before the emergence of parasitic nema as 18.98 ± 2.18 mg/100mg in female muscle and 20.84 ± 1.03 mg/100mg in male muscle.

3.1. Tables

Table 1. Average free fatty acids level in haemolymph (mg/100mg) of Control and Parasitized bugs (L. augur) in laboratory.

S.No.	Days of Parasitization	Sex (Female) (♀) Av mean ± SE	Sex (Male) (♂) Av mean ± SE
1.	Control	149.96 ± 1.99	148.96± 1.98
2.	5th day	149.64 ± 2.68	148.96 ± 1.98
3.	10th day	129.98 ± 2.01	091.23 ± 1.32
4.	15th day	088.69 ± 3.41	077.89 ± 1.84
5.	Just before emergence	074.24 ± 7.21	061.54 ± 1.98

Average has been taken of 10 observations of each one.
Abbreviation- Av mean = Average mean
SE = Standard error



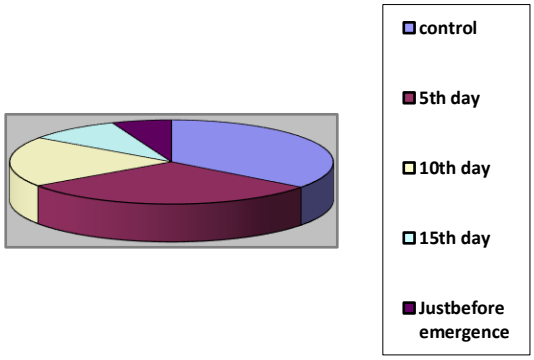
Graph 1. Showing average free fatty acids level in haemolymph (mg/100mg) of Control and Parasitized bugs (L. augur) in laboratory.

Table 2. Average free fatty acids level in muscles (mg/100mg) of Control and Parasitized bugs (L. augur) in laboratory.

S.No.	Days of Parasitization	Sex (Female) (♀) Av mean ± SE	Sex (Male) (♂) Av mean ± SE
1.	Control	112.02 ± 2.13	92.17 ± 1.85
2.	5th day	95.68 ± 2.06	91.24 ± 2.94
3.	10th day	61.54 ± 1.98	55.98 ± 2.33
4.	15th day	29.58 ± 3.30	31.17 ± 4.82
5.	Just before emergence	18.98 ± 2.18	20.84 ± 1.03

Average has been taken of 10 observations of each one.

Abbreviation- Av mean = Average mean
SE = Standard error

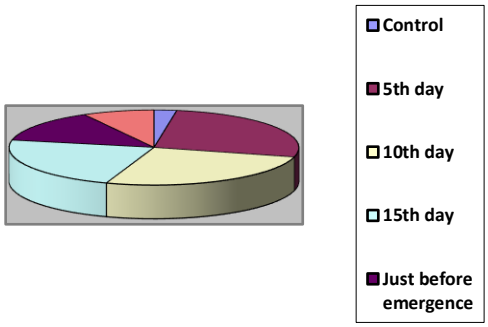


Graph 2. Showing average free fatty acids level in muscles (mg/100mg) of Control and Parasitized bugs (L. augur) in laboratory.

Table 3. Average free fatty acids level in gonads (mg/100mg) of Control and Parasitized bugs (L. augur) in laboratory.

S.No.	Days of Parasitization	Sex (Female) (♀) Av mean ± SE	Sex (Male) (♂) Av mean ± SE
1.	Control	215.51 ± 1.95	112.02 ± 2.13
2.	5th day	212.32 ± 1.44	103.29 ± 0.86
3.	10th day	184.88 ± 1.99	088.69 ± 3.41
4.	15th day	112.39 ± 1.85	071.31 ± 2.01
5.	Just before emergence	064.85 ± 2.13	061.54 ± 1.98

Average has been taken of 10 observations of each one.
Abbreviation- X Av mean = Average mean
SE = Standard error



Graph 3. Showing average free fatty acids level in gonads (mg/100mg) of Control and Parasitized bugs (L. augur) in laboratory.

7.2. Figures

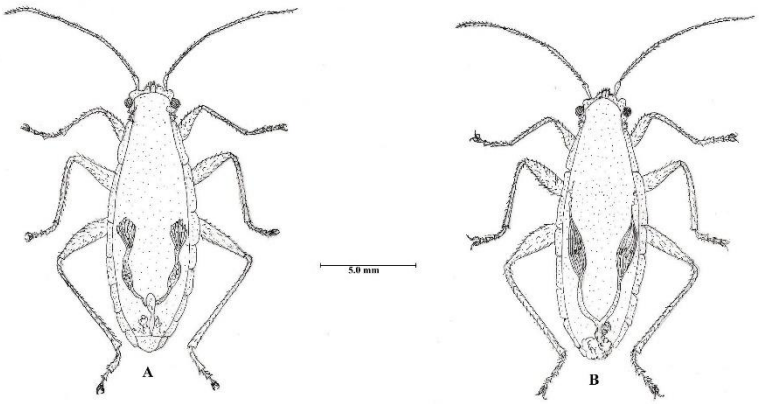


Figure 1. A. Male reproductive organs of healthy bug, Leptocoris augur B. Female reproductive organs of healthy bug

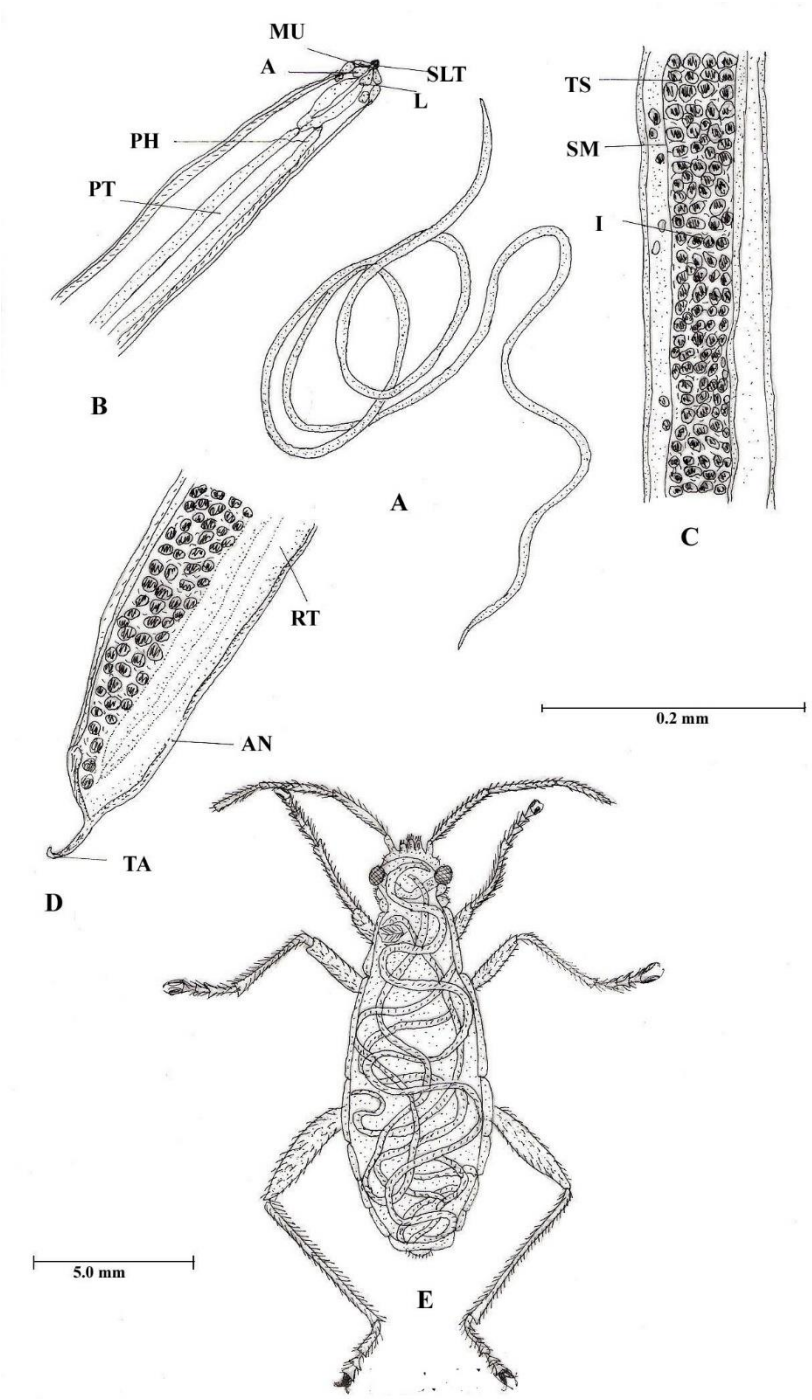


Figure 2. A, B, C & D Parasitic Hexamermis vishwakarma showing different parts
E. Parasitic stage within the body cavity

Abbreviation-

PH- Pharynx, SM- Stichosome, I-Intestine, TS- Trophosome, AN-Anus, TA-Tail, A-Amphid, SLT-Stylet, MU- Mouth, L-Lip. PT-Pharyngeal tube, RT-Rectum

4. Discussion

In the present investigation, however, the parasitized bug showed a marked change in the concentration of the free amino acids is probably a consequence of the higher metabolic activity in the parasitized insects which causes an imbalance between the rates of anabolism and catabolism in them. Cellular differentiation involves a large accumulation of proteins and thus, protein metabolism is of prime importance during insect development.

It is well known that organic substances are required for growth and reproduction on one hand and for energy production on the other. Two factors determine the requirements for either purpose. First, the organic substance must be either directly assimilable component in the digestive system. Secondly, the insect must be able to synthesize from the raw material provide all the complex organic substances in its body.

In parasitized bug, amino acids are taken up in large quantity by developing mermithid, which incorporated these into protein at varying rates during their development. Chitwood and Jacobs, Gordon and Webster [1,5] reported that timing of maximum synthesis coincides with the rapid increase in total dry weight and protein level that occurs mostly between 17 and 21 days after infection as the developing nematode accumulates stored proteins and lipids in the trophosome prior to emergence from the host. Present observations are in agreement of these studies as in H. vishwakarma rounded spheres of proteins and fats are stored in large number as trophosomes.

H. vishwakarma significantly brings depletion in carbohydrates, proteins and amino acids of host L. augur after one week of parasitism and this depletion is not compensated for by increased food consumption by the host. This is close finding in agreement with the Gordon and Webster [4] in Schistocerca gregaria host, parasitized by Mermis nigrescens.

Rutherford and Webster [14] also recorded sterility in female host due to parasitization of mermithid nematode.

Parasitic juvenile of H. vishwakarma within the haemocoel of their host (L. augur) takes nutrients fom haemocoelomic fluid, causing quantitative loss in it. In the late stage of its development antennal intrinsic muscles and some mouth parts muscles of host are also dissolved. Mermithids cause the degeneration of several tissues within the immature and adult of Simullid host Hocking and Pickering, [6]. The closest finding to the author's work appeared to the Monolepta saginata (host) due to Howardula saginata).

From the parasitized host bug's muscles, gonads and haemolymph large quantity of amino acids, lipids and carbohydrates are taken up by the parasitic juvenile of H. vishwakarma. Rubtsav [11] and others suggested that parasitic mermithids may digest the protein reserves of the host fat body by secreting hydrolytic enzymes, it is more likely that mermithids indirectly utilize host fat body proteins by inducing changes in the host's metabolism. The level of haemolymph proteins and amino acids remains relatively constant in adult, S. gregaria parasitized by M. nigrescens, but a significant decrease in fat body proteins and amino acids occurs Gordon and Webster [4].

The lipid content of fat body and muscles of L. migratoria (host) was diminished by Mermis nigrescens infections as indicated earlier by Justum and Goldsworthy.

Ruhm [12]; Nickle, Poinar and Caylor [9,10], reported that bark beetles infected with Parasitylenchus and Neoparasitylenchs are also known to show aberrant gallery construction, reduced longevity, reduced flight activity, reduced fat body and sterilization.

H. vishwakarma not only utilize fat body proteins but also the lipid content of the fat as a result of which fat bodies disappears from the haemolymph of L. augur. The fatty acid composition of the host haemolymph is not significantly changed by H. vishwakarma parasitism in L. augur of both the sexes but the levels of cholesterol appear to be increased. Rutherford and Webster [13] also observed similar changes.

5. Conclusion

H. vishwakarma Dhiman takes nutrition from the haemolymph and muscles of insect body cavity [3]. Availability of free amino acids, carbohydrates and fatty acids in the haemolymph of host bug help the parasitoid to establish in the haemocoel. After eatablishment, the parasitoid induces changes in the availability of biochemicals and composition of haemolymph, by the secretion of enzymes. The parasitoid's enzymes cause degeneration or dissolution of muscles, adipose tissues, testes as well as ovaries and the material of these organs is made available to the parasitoid. Hence, parasitoid grows at the expense of host tissues.

In view of this to explore all, these biochemical aspects of healthy (control) and parasitized bug as well as to record the biocontrol efficacy of Hexamermis vishwakarma, present investigations are taken up Kumkum [7].

Acknowledgments

We are extremely thankful to the principal and head to Zoology Department of M.S. College Saharanpur for providing necessary facilities and to the Deputy Director cum in charge HRI and Training Centre, Saharanpur for field studies. The authors wish to thank Dr S.C. Dhiman for useful discussions on this work.

References

[1] *B. G. Chitwood and L. Jacobs, "Stored nutritive materials in the trophosome of the nematode, Agamermis decaudate (mermithidae)", Journal of the Washington Acad. of Sci.,* ***(1938),*** *28 (1), pp. 12-13.*

[2] *S. C. Dhiman and Gulati, "Aggregation behaviour of Serinetha augur Fabr. (Heteroptera : Coreidae)", Indian Jr. of Bio Research, vol. 2(4),* ***(1986)****, pp. 318-323.*

[3] *S. C. Dhiman and R. K. Singh, "Studies on the parasitism of Leptocoris augur (Fabr.) (Heteroptera : Rhopalidae) by Leptus sp. of mite (Acarina : Erythraeidae)", U.P. J. Zool., 8 (2),* ***(1989),*** *pp. 180-185.*

[4] *R. Gordon and J.M. Webster, "Mermis nigrescence Physiology relationship with its host,the adult desert locusts schistocera gregaria", .Exp. Parasitol., 29,* ***(1971),*** *pp. 66-79.*

[5] *R. Gordon and J. M. Webster, "Nutritional requirements for protein synthesis during parasitic development of the entomophilic nematode Mermis nigrescens", Parasitology, 64,* ***(1972),*** *pp. 161-172.*

[6] *B. Hocking and L. R. Pickering, "Observatios on the bionomics of some northern species of Simullidae (Diptera)", Can. J. Zool., 32;* ***(1954),*** *pp. 99-119.*

[7] *Kumkum, Editor, "An Entomophilic Nematode, Hexamermis SP., a biocontrol agent". 1st edn. Scholars' Press,* ***(2021).***

[8] *R. R. Lowry and T. J. Tinsley, "Rapid Colorimetric determination of free fatty acids", J. Ann. Oil. Chem. Soc., 53,* ***(1976),*** *pp. 235-250.*

[9] *W. R. Nickle, "Behaviour of the shoothole borer, Scolytus rugulosus, altered by the nematode parasite neoparasitylenchus regulosi", Ann. Entomol. Soc. Am.,* ***(1971),*** *pp. 64, 751.*

[10] *G.O. Poinar and Jr. J. N. Caylor, "Neoparasitylenchus amvlocercus sp. n. (Tylenchida : Nematodea) from Conophthorus monophyllae (Scolytidae : Coleoptera) in California with a synopsis of the nematode genera found in bark beetles", J. Invert. Pathol., 24,* ***(1974),*** *pp. 112-119.*

[11] *I. A. Rubtsov, "New and little-known species of nematode from the Danube. Hydrobiologischeski", Zh. 5,* ***(1969),*** *pp. 49-59.*

[12] *W. Ruhm, "Die Nematoden der", Ipiden. Parasitol. Schrift, 6,* ***(1956),*** *pp. 1- 437.*

[13] *T. A. Rutherford and J. M. Webste, "Effects of the nematode Mermis nigrescens on some chemical components of the insect host's hemolymph", Proc. Int. Colloquicm Invert. Pathology, Kingston, Ontario, pp.* ***(1976),*** *pp. 272-275.*

[14] *T. A. Rutherford and J. M. Webster, "Some effects of Mermis nigrescens on the hemolymph of Schistocera gregaria, Can. J. Zool., 56,* ***(1978****), pp. 339-347.*