Quatitative and Qualitative Estimation of Free Amino Acids in the Haemolymph of parasitized bugs (*Leptocoris augur)*.

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Abstract

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 desapping habit loss the viability of seeds. In L. augur a total of 16 amino acids were identified in the control bug's haemolymph (both sexes) by paper chromatogram.

Thus, free amino acids level of haemolymph is greatly affected by the parasitization. In this way the Quantitative level of amino acid in parasitized bug haemolymph significantly altered by mermithid parasitism.

Keywords: *Leptocoris augur, Haemolymph, Free amino acids, Parasitized bug, Quantitative level.*

1. Introduction

Entomophilic nematodes are a group of parasites that causes debilitation, sterility (partial or complete) or even death of a large number of insect orders and families. ***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 desapping habit loss the viability of seeds (Dhiman & Gulati 1986). It is parasitized by a mermithid nematode, *Hexamermis vishwakarma* Dhiman, which naturally checks rapid built up of bug population (Figure 1).

H. vishwakarma Dhiman takes nutrition from the haemolymph and muscles of insect body cavity (Dhiman & Singh 1989). 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. Amino acids play vital role in body metabolism of bugs. After digestion of protein these amino acids reach into haemolymph which transport these to required body organ or tissue.

Thus, in present investigations, distribution of free amino acids in the haemolymph of *Leptocoris augur* Fabr. was studied after 5th, 10th, 15th and just before emergence of parasitic juvenile from the body of host bug in the females and in the males of the corresponding age.

In view of this to explore all, these biochemical aspects of healthy (control) and parasitized bug of *Hexamermis vishwakarma*, present investigations are taken up.

Figure 1. Parasitized bug (L. augur) showing coils of parasitic juvenile (Hexamermis vishwakarma)

2. Materials and Methods

Parasitized nymphs and adults of *Leptocoris augur* collected along with fresh leaves and seeds of kusum plant by hand picking method from the field (Horticulture Experiment and Training Centre, Saharanpur) in polyethylene bags during rainy season, July to September.

Collected bugs were reared in lab, in hurricane glass lamp chimneys (24×36 cm) covered at top by fine muslin cloth. Fresh food supply was maintained daily and stale food was replaced. To maintain necessary relative humidity (70%), a cotton swab was placed in a water filled glass vial and temperature was maintained at 28°C. Behaviour of the bugs was noticed after at 5^{th}, 10^{th}, 15^{th}days and just before emergence of parasitic juvenile, with the aid of hand lens.

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

For qualitative separation of amino acids chromatographic method was used. All the chemicals used were of the analytical reagent grade. Extracts were applied as compact spots on Whatman no. 1 filter paper sheet (40 cm × 40 cm). The paper chromatography was done according to the method described by (Smith & Agiza 1951) and the chromatograms were developed for six hours with n-butanol, glacial acetic acid and distilled water (4: 1: 5). Spots were developed with ninhydrin and identified with the predetermined RF values of the known amino acids.

The concentration of all free amino acids was placed separately in tubes, to which were added 1ml NaoH (neutralized with phenolphthalene), 1ml citrate buffer (pH 3) and 2 ml ninhydrin solution. The tubes were then placed in boiling water, to which was added 1ml stannous chloride solution. This produced red colour which after 15 minutes changed into purple. After it, the tubes were kept in a dark place for 15 minutes, then content was made upto a volume of 10ml with a saturated solution of NaCl, and 5ml of n-butanol was added to each tube; which was then shaken and kept ready for estimation. The optical density of colour was measured with a Carl Zeiss Universal Spectophotometer (model VSU-1) at 570µ and at 440µ (only for proline), against a blank extract prepared from a piece of the same filter paper of the same areas. The concentration of the separated amino acids was calculated from the standard curves, previously prepared by using standard amino acids solutions chromatographed for the same period using the same solvent.

Similarly, the haemolymph was collected from other specimens after the lapae of 5, 10, 15 and just before the emergence of parasitic nema. In both the cases, Chromatographic separation and quantitative estimation was made as described above.

3. Results

Amino acids play vital role in body metabolism of insects. After digestion of protein these amino acids reach into the haemolymph which transport these to required body organ or tissue. In *L. augur* a total of 16 amino acids were identified in the control bug's haemolymph (both sexes) by paper chromatogram. The amino acid contents of the haemolymph of male and female (*L. augur*) bugs were also similar. The haemolymph of these two groups of bugs had Alanine, Arginine, Aspartic acid, Glutamic acid, Glycine, Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Proline, Serine, Threonine, Tyrosine and Valine (Table- 1). But, in parasitized bug (just before emergence of parasitic nema) drastic depletion in most of the free amino acid of haemolymph was recorded.

The levels of total free amino acids in the haemolymph of female and male bugs (*L. augur*) were altered or depleted due to parasitization by *H. vishwakarma.*

Quantitative results showed that in control bug, total free amino acids (FAA) in the haemolymph were 15.9 µmol/100ml in female and 14.1 µmol/100ml in male. While in parasitized bug, these occurred a gradual decrease, *i.e.*, 13.0μ mol/100ml in male and 14.5 μmol/100ml in female of 5 days old parasitized bug, in 10 days parasitized female and male bug, it was 12.6 μmol/100ml and 11.2 μmol/100ml, in 15 days parasitized female and male bug, it was 7.6 μmol/100ml and 7.0 μmol/100ml, just before emergence it was 6.1 μmol/100ml in female and 4.0 μmol/100ml in male (Graph-1) and (Table –1).

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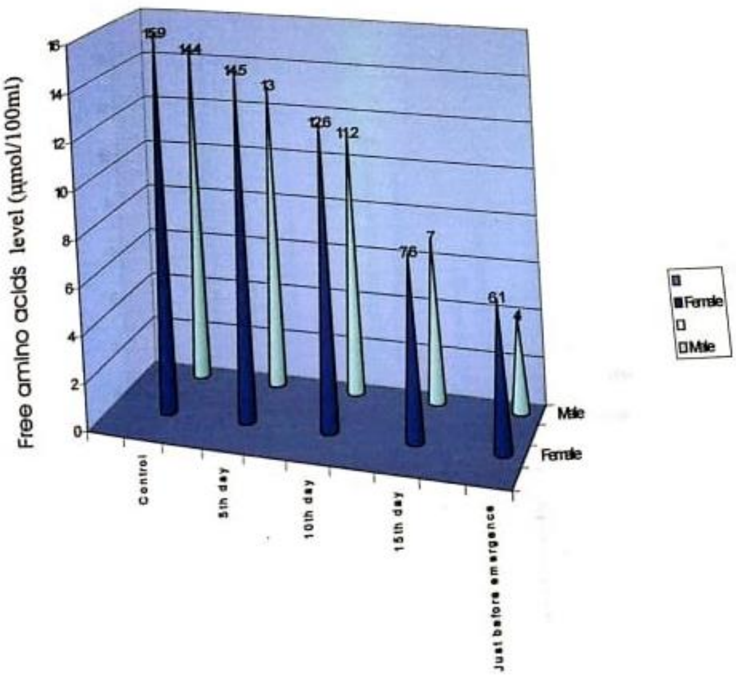
Thus, free amino acids level of haemolymph is greatly affected by the parasitization. In this way the Quantitative level of amino acid in parasitized bug haemolymph significantly altered by mermithid parasitism but the levels of cholesterol increase in host bug.

Table-1. Concentration of the free amino acids in the haemolymph (μmol/ml) of control and parasitized bugs (*L. augur*) in laboratory.

S. No.	**Amino acid (μ mol/ml)**	**Days of parasitization /Sex**									
		Control		**5th**		**10th Day**		**15th Day**		**Just before emergence**	
		(♀)	(♂)	(♀)	(♂)	(♀)	(♂)	(♀)	(♂)	(♀)	(♂)
1	**Alanine**	0.9	0.8	0.8	0.7	0.8	0.8	0.6	0.6	0.5	0.4
2	**Arginine**	1.1	1	1	0.8	0.5	0.7	0.4	0.5	0.2	0.2
3	**Aspartc acid**	0.2	0.1	0.1	0.1	0.2	0.3	0.2	0.2	0.1	0.1
4	**Glutamic acid**	0.8	0.7	0.7	0.6	0.6	0.4	0.5	0.2	0.3	0.1
5	**Glycine**	0.6	0.5	0.5	0.3	0.5	0.3	0.4	0.1	0.3	0.1
6	**Histidine**	0.6	0.4	0.4	0.3	0.8	0.6	0.6	0.4	0.4	0.1
7	**Isoleucine**	1.1	1.1	1	0.9	1	0.6	0.4	0.4	0.2	0.2
8	**Leucine**	0.7	0.8	0.6	0.7	0.7	0.5	0.3	0.3	0.2	0.3
9	**Lysine**	1.4	1.3	1.3	1.2	0.9	0.9	0.5	0.6	0.4	0.4
10	**Methionine**	1.2	1	1.1	0.9	0.6	0.7	0.4	0.5	0.3	0.5
11	**Phenylalanine**	0.8	0.8	0.7	0.7	0.7	0.5	0.3	0.3	0.1	0.3
12	**Proline**	1.8	1.9	1.7	1.8	2	1.2	0.9	0.8	0.5	0.2
13	**Serine**	1.3	1	1.2	0.9	1	0.9	0.5	0.6	0.3	0.3
14	**Threonine**	0.6	0.5	0.5	0.4	0.5	0.5	0.2	0.2	0.1	0.2
15	**Tyrosine**	1.3	1.2	1.2	1.1	1	1	0.7	0.6	0.3	0.4
16	**Valine**	1.5	1.4	1.4	1.3	0.8	1.3	0.2	0.7	0.1	0.3
	Total level	**15.9**	**14.4**	**14.5**	**13**	**12.6**	**11.2**	**7.6**	**7**	**6.1**	**4.0**

Graph-1. Showing concentration of the free amino acids in the haemolymph (μmol/ml) of control and parasitized bugs (*L. augur*).



4. Discussion

In the present studies amino acid in haemolymph,(Female and male) of control and parasitized bug (*L. augur*) have been studied. The amino acids contents of the control bug haemolymph of both male and female bugs were also found similar quantitatively. The amino acids composition of the haemolymph of the control and parasitized bugs, however, was different (Quantitatively as well as qualitatively). These observations suggested that the parasitizing effect on the amino acid composition of their haemolymph.

The variation in the haemolymph of the two groups of *L. augur* is so great that one is tempted to regard them as two physiological strains, control and parasitized. This further supported by the observations of Rakspal & Singh (1972) who reported strikingly different patterns of amino acids in different species of insect haemolymph, *viz;* Otheroptera, Hemiptera, Coleoptera, Hymenoptera, Lepidoptera, Odonata and Diptera. In the muscles contaminated with haemolymph, all the amino acids of the haemolymph were present in control bugs, where as in parasitized bugs muscles washed with distilled water did not have Tyrosine. Patton (1963) stated that the haemolymph, probably, serves as a reservoir of amino acids and appears to be the main source for the supply of amino acids to different tissues. The haemolymph of *L. augur* contains the maximum variety of amino acids, and those which are present in the haemolymph and not in the tissues, were probably used up in the in the synthesis of specific tissue proteins. Prosser & Brown (1965) reported that specific new proteins are formed from amino acids liberated as a result of degradation of already existing tissue proteins. Arginine and lysine of the haemolymph are thus probably used up in the synthesis of proteins of the eggs and sperms.

The free amino acids in the haemolymph of insects are sixty times higher than in the human blood, and all those commonly found in proteins have been identified in the insect haemolymph, although some of them such as Methionine, Cystine, Serine, Hydroxyproline and Tryptophan either occur in low concentration or are completely absent (Gilmour, 1965).

Belogaun has suggested that Proline plays the role of a readily available metabolizable energy reserve for flight and movements. Besides a great number of amino acids like Alanine, Glutamic acid, Glycine and Proline are known to have an important role in the synthesis of cuticle proteins, chitin and other constituents of cuticle. 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.

H. vishwakarma significantly brings depletion in 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 (Graph-1). This is close finding in agreement with the Gordon & Webster (1971) in *Schistoceria gregaria* host, parasitized by *Mermis nigrescens.*

The levels of various amino acids in parasitized hosts (*L. augur*) vary with stage of parasitism. Amino acids level of haemolymph diminishes only during period of very rapid mermithid growth in adult hosts, *L. augur.* This mermithid absorbs the vitellogenic proteins from the ovaries. Subsequent oocyte resorption probably results from a depletion of vitellogenic proteins in the haemolymph due to nutrient requirements of the developing mermithids. This results in sterilization of the female host bug, *L. augur.* Rutherford & Webster (1978) also recorded sterility in female host due to parasitization of mermithid nematode.

5. Conclusion

Parasitism by a mermithid is usually fatal to the host. Mermithid larvae are usually found in the body cavities of all stages of susceptible insect species. The nematode parasite undergoes several moults within the insect body cavity, taking nourishment from the insect's blood and increases in length many folds.

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. Entomophilic nematodes works as a potential biocontrol agent.

Acknowledgement

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.

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