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Estimation of Proteins in Body Fluids (Serum) Of Male and Female "Red Pumpkin Beetle", *Aulacophora Foveicollis* (Chrysomelidae, Coleoptera)

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Abstract
Proteins are the important constituent of body fluids or serum of insects. In the present work, the protein content of the serum of Aulacophorafoveicollis, commonly called "red pumpkin beetle" has been estimated by Biuret method. First of all the serum of insects was separated by centrifugation and the protein content of the serum was estimated by colorimeter. Then the total serum protein content was calculated.
The average total serum protein content in male insects of Aulacophorafoveicollis was 5.91 gm/dl and in female insects it was 3.21 gm/dl which is much less than that in the male.

Key Words: *Proteins, serum extract, estimation, Aulacophorafoveicollis, red pumpkin beetle, Biuret method, colorimeter, male, female, centrifugation.*

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I. Introduction

Aulacophorafoveicollis, commonly called "red pumpkin beetle" is the pest most destructive of vegetable plants belonging to the family, Cucurbitaceae e.g. pumpkin or kaddu, tinda, ghia tori, cucumber, etc. but has special liking for kaddu or pumpkin. The adult insects feed voraciously on the leaves, flowers and fruits of host plants. For the present work. The insects both male & female were collected from pumpkin or kaddu plants grown in NandlalChhapra village, situated south of New Bypass Road, Patna.

The adult insects are coloured deep orange dorsally and black ventrally. The body measures 5-8 mm long and 3.5-3.75 mm broad.

II. Material And Methods

Adult male and female insects of *Aulacophorafoveicollis* were collected from the host plants, brought to the laboratory and kept in the breeding chambers. The breeding chambers consisted of plastic jars. The tops of plastic jars were closed by fine mosquito net to prevent the escape of insects but allowing ventilation and observation from outside. The food and water were also placed in the breeding chambers. Every morning fresh leaves, flowers and tender shoots of pumpkin or kaddu were provided into the breeding chambers as food for insects. The rearing of insects was essential for making them easily available for experiments.

For manual or automated determination of serum or plasma total proteins, Biuret method was used. A group of 24 male insects of *Aulacophorafoveicollis* wereweighed and the weight was noted in Table-I. The insects were then narcotized in chloroform and were then macerated in 3 ml of double distilled water. The macerate was then centrifuged at about 4000 rpm (revolutions per minute) for 15 minutes. The protein content of the serum extract from the centrifuge was kept for 10 minutes in the freeze.

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The protein content of the serum extract was estimated by colorimeter. For this purpose, three tubes were taken. In one tube 1 ml of Biuret reagent was taken and adopted in colorimeter. In another tube 1 ml of Biuret reagent and 10 microliter or 5 drops of protein standard (6.5 gm/dl) were taken and shaken so that the colour of the solution changed to violet. The violet mixture was put into colorimeter and its value was noted. In the third tube 1 ml of Biuret reagent and 10 microliter or 5 drops of serum extract were mixed and its protein amount is obtained in colorimeter. For taking 10 microliter or 5 drops of protein standard or serum extract U40 insulin syringe was used.

The total serum proteins were calculated by the following method:

Serum proteins by Biuret method:gm/dl= $\frac{OD\ TEST}{OD\ Std.} X6$

OD = Depth of colour formed; the colour depth is directly proportional to the concentration of proteins present in the specimen. Std. = Standard.

The experiments were repeated 5 times and the mean value was calculated. All data were noted in Table-I.

Similarly the serum proteins of 20 female insects of *Aulacophorafoveicollis* were worked out. The various required measurements were noted in Table-II.

III. Observation

The weight of 24 male insects of *Aulacophorafoveicollis* was 620 mg, Biuret reagent + protein standard was 0.63 nm, Biuret reagent of Test Serum was 0.42 nm and serum proteins, gm/dl was calculated to be 4.00 gm/dl by applying the following formula.

Serum proteins (gm/dl) $= \frac{OD\ TEST}{OD\ Std.} X6$

OD = Depth of colour formed; Std. = Standard.

The experiment was repeated 5 times and the mean value of serum proteins in male was 5.91 gm/dl or shown in Table-I.

Similarly the serum proteins of 20 females insects of *Aulacophorafoveicollis* was worked out. The various measurements are noted in Table-II. The mean value of serum proteins of 20 female insects was 3.21 gm/dl. Thus the serum protein content females was lower than that in the male.

Table-I
Estimation of serum (or plasma) Proteins of Male
Aulacophorafoveicollis

Sl. No.	No. of insects	Wt. of insects in mg	Biuret reagent + Protein Standard	Biuret reagent + Test serum	Serum Proteins: gm/dl by formula $\frac{OD\ TEST}{OD\ Std.} X6$
1.	24	620	(i) 0.63 nm (ii) 0.50 nm (iii) 0.34 nm (iv) 0.63 nm (v) 0.50 nm	(i) 0.42 nm (ii) 0.56 nm (iii) 0.43 nm (iv) 0.59 nm (v) 0.47 nm	(i) 4.00 gm/dl (ii) 6.72 gm/dl (iii) 7.58 gm/dl (iv) 5.61 gm/dl (v) 5.64 gm/dl
Mean			0.52 nm	0.49 nm	5.91 gm/dl

Table-II
Estimation of serum (or plasma) Proteins of Female
Aulacophorafoveicollis

Sl. No.	No. of insects	Wt. of insects in mg	Biuret reagent + Protein Standard	Biuret reagent + Test serum	Serum Proteins: gm/dl by formula $\frac{OD\ TEST}{OD\ Std.} X6$
1.	20	420	(i) 0.59 nm (ii) 0.55 nm (iii) 0.69 nm (iv) 0.55 nm (v) 0.54 nm	(i) 0.27 nm (ii) 0.32 nm (iii) 0.32 nm (iv) 0.32 nm (v) 0.29 nm	(i) 2.74 gm/dl (ii) 3.49 gm/dl (iii) 3.14 gm/dl (iv) 3.49 gm/dl (v) 3.22 gm/dl
Mean			0.58 nm	0.30 nm	3.21 gm/dl

IV. Results And Discussion

Like any other animal, the chief biochemical constituents of the insect body are water, proteins, lipids and carbohydrates which play important role in the metabolic activities, growth and development of insects. The biochemical constituents greatly vary in their percentage of compositions of the body weight of different insects and in the male and female of the same species.

Proteins are the most abundant substance of protoplasm after water. The functional proteins or enzymes and different hormones including proteinoid hormones control the metabolic activities and growth of different insects (Williams, 1952; Bodenstein, 1953; Wigglesworth, 1954; Gilbert, 1964).

Qualitative and quantitative changes in contents of proteins and amino acids in many insects have been worked out by many scientists. Extensive investigations have been done on *Calliphoraerythrocephala*and *Pholerabucephala*(Angrel, 1949); *Galleriamallonera*(Auclair and Dubrevil, 1952; Amanieu et al., 1956; Wyatt

et al. 1956); *Anomalaorientalis* (Pro-chadley, 1956); *Drosophilamelanogaster* (Hadorn and Mitchell, 1951; Chen and Hadorn, 1954); *Calliphoraangur* (Hackman, 1956) and *EphestiaKuhniella*(Chen and Kuhne, 1956). Similar studies have been on the desert locust, *Schistocercagregaria*(Benassi et al, 1961); the bug, *Rhodniusprolixus*(Harigton, 1961) and Southern army worm, *Prodeniaeridania*(Levenbook, 1962) and the rice moth, *Corcyra cephalonica.*(Ganti and Saumugasundaran, 1963).Tripathi, A.K (1986) described that the protein content decreases significantly in the middle of the larval period and again increased in the V^{th} instar larvae. Kathryn, E Boes et al. (2014) identified and characterised the seminal fluid proteins in the Asian tiger mosquito, *Aedesalbopictus.* Oibiokpa, Florence Inji et al. (2018) investigated the protein quality of four indigenous edible insects species in Nigeria.

In the female there is a continuous deposit of yolk at the time of egg formation whereas in the male different proteins are synthesized in connection with spermatogensis and secretion of the accessory glands (Novalk et al., 1960). As such there is bound to be differences in the quality and quantity of proteins in male and female insects. Pandey, D.P (1993) has observed that in *Chrysocorisstolliit*he percentage of protein content in the body of male is greater than that in female.

In the present study the protein estimations in the body fluids of male and female insects of the species, *Aulacophorafoveicollis* were made and it was found that the total serum protein content of the male was greater than that of the female. It is most probably due to the fact that the experiments were mostly carried out in the months of breeding season during which there is continuous deposition of yolk in the developing eggs in female. In the yolk proteins are important constituent. This observations is in full agreement with the observation of Pandey, D.P. (1993) but contrary to the observation of Novak et al. (1960).

Reference

[1]. **Agrel, I. (1949) :** Occurrence and metabolism of free amino acid during insect metamorphosis. Acta Physiol. Scand. 18: 247-258.

[2]. **Amanieu,M., Duchateau, G., Florkin, M., &Jeunianx, C. (1956) :** Systems all acids-amines non-proteins duplasma de I' Haemolymphe, aucours de In view larvaire et nymphale de Bombyxmori, Archs-ent. Physiol. 64: 518-519.

[3]. **Auclair, J.L. &Dubreuil, R. (1952) :**A simple ultra micromethod for the quantitative estimation of amino acids by paper partitions chromatography. Can J. Zool. 30: 109-113.

[4]. **Ganti, &Shaumugasundaran E.R.B. (1963) :** A study on the free amino acids during growth and metamorphosis of *Corcyra cephalonica.* J. exp. Zool. 152: 1-4.

[5]. **Hackman, R.H. (1956) :** Changes in the free amino-acids of the blood of blowfly larvae at metamorphosis. Aust. J. Biol. Sci. 9: 400-405.

[6]. **Hadorn, E. & Mitchell, H.K. (1951) :** Properties of mutants of *Drosophila melanogaster* and changes during development as revealed by paper chromatography. Proc. North. Acad. Sci. U.S.A. 37: 650-665.

[7]. **Kathryn, E. Boes, Jose M.C., Ribeiro Alex Wong et al. (2014) :** Identification and characterization of seminal fluid proteins in the Asian Tiger Mosquito, Aedes*albopictus*. https://doi.org/10.1371/ journal.Pntd.0002946.

[8]. **Levenbook, L. (1962) :** The distribution of free amino-acids, glutamine and glutamate in the Southern army worm, Prodeniaeridania. J. Insect, Physiol. 8: 559-567.

[9]. **Novak, A.F., Blum M.S. Taber, S. &Liuzzo, J.A. (1960) :** Separation and determination of seminal plasma and spern amino-acids of the honey bee, *Apismellifera.* Ann. Ent. Soc. Am. 53: 841-843.

[10]. **Oibiokpa, Florence Inji, Akanya et al.(2018) :** Protein quantity of four indigenous edible insect species in Nizeria. Food Science and Human Wellness, Pages 175-183. Dept. of Biochemistry, Federal University of Technology, M.B65, Minna, Nigeria.

[11]. **Pandey (1993) :** Experimental studies in Relation to some aspects of the Bioogy of *Chrysocorisstollii.*P.D Thesis Magadh University, Bodh Gay.

[12]. **Tripathi, A.K. (1986) :** Studies on some aspects of the biology of *A. mylitta.* Thesis for Ph.D. degree from magadh University. Bihar.

[13]. **Wigglesworth, V.B. (1954) :** "The physiology of Insect metamorphosis" Cambridge University Press, Cambridge.

[14]. **Williams, C.M. (1952) :** Morphogenesis and metamorphogenesis of Insects. Harvey Lect. 47:126-155.

[15]. **Wyatt, G.R., Lougheed, I.C. & Wyatt, S.S. (1956) :**The chemistry of Insect haemolymph. Organic components of the haemolymph of the Silk worm, *Bombyxmori*and two other species. J. Gen. Physiol., 39: 853-868.

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