International Journal of Science and Research (IJSR)
ISSN: 2319-7064
ResearchGate Impact Factor (2018): 0.28 | SJIF (2019): 7.583

Estimation of Proteins in Body Fluids (Serum) of Male and Female "Ladybird Beetle", *Coccinella Septempunctata* (Coccinellidae, Coleoptera)

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Abstract: ***This paper is concerned with the estimation of proteins in the body fluids of insect. For estimation of proteins in the body fluids of Coccinella septmpunctata, commonly called "Ladybird beetle" Biuret method is used. The weighed insects were narcotized and macerated in 3 ml of double distilled water. The macerate was centrifuged to separate serum extract. The protein content of the serum extract was estimated by colorimeter. Total serum protein content was calculated by the following formula:*** $Serum\ Protein, gm/dl = \frac{OD\ Test}{OD\ Std.} X6$ ***. O.D. Test = depth of the colour formed by the testing fluid. O.D. Std. = depth of the colour of the standard fluid. The average total serum protein content in male insects of C. septempunctata was 5.99 gm/dl and that in female insects was 6.8 gm/dl which is slightly greater than that in males.***

Keywords: Estimation, serum extract, protein content, male, female, *Coccinella septempunctata*, Biuret method, centrifuge, colorimeter

1. Introduction

Coccinella septempunctata, commonly called ladybird beetle, belongs to the family Coccinelidae of Insect order - Coleoptera. It is a very beneficial insect, being natural enemy of many agricultural pests, especially aphids, coccids, and other soft bodied insects that damage plants by feeding on their sap. A single ladybird beetle can consume a large number of aphids in its lifetime, perhaps as many as 5,000 or more. *Coccinella septempunctata*,the seven spotted ladybird beetle, sometimes called 'C-7', is a medium sized, orange coloured beetle with seven black spots on the back. Both adults and larvae are well known primarily as predators of aphids (plant lice), but they prey also on other pests such as soft scale insects, mealy bugs, spider mites, etc. A few feed on plant and pollen mildews. C. *septempunctata* may be a more effective predator than some active ladybird beetle species, displacing them in some areas.

The adults are comparatively large (7-8 mm) with a white or pale spot on either side of the head. The body is oval, and dome-shaped. The spot pattern is usually 1-4-2, black on the orange or red forewings. Larva is dark and alligator like with three pairs of prominent legs, growing to 7-8 mm in lengths. Eggs are spindle shaped and small, about 1 mm long.

2. Material & Methods

For both manual and automated determinations, Biuret method was used. 40 male & female insects of *C. septempunctata* were weighed and then narcotized in chloroform. The narcotized insects were macerated in 3 ml of double distilled water. The macerate wasthen subjected to centrifugal force in a centrifuge rotated at about 4000 rpm (revolution per minute) for 15 minutes. The serum extract from the centrifuge was kept for 10 minutes in the freeze.

The protein content of the serum extract was estimated by colorimeter. For this purpose, three tubes were taken. In one tube 1 ml Biuret reagent was taken and adopted in colorimieter. In another tube 1 ml of Biuret reagent and 10 micro liter of 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 micro liter or 5 drops of serum extract were mixed and its protein amount is obtained in colorimeter. For taking 10 micro liter or 5 drops of protein standard or serum extract U 40 insulin syringe was used.

The total serum proteins were calculated by the following method:

$$\text{Serum Proteins by Biuret method: Gm/dl} = \frac{OD\ Test}{OD\ Std.} X6$$

O.D. = Depth of colour formed; the colour depth is directly proportioinal to the concentration of Proteins presents in the specimen.

The experiments were repeated five times and the mean values were calculated.

3. Observation

A group of 40 male individuals of *Coccinella septempunctata* were weighed and the weight is noted in **Table-1.** The insects were macerated in 3 ml of Double distilled water and macerate was centrifuged at about 4000 rpm for 15 minutes. The Protein content of the serum extract was estimated by colorimeter and the different readings are noted in the above Table. The average serum Total Protein content is 5.99 gm/dl calculated by the following formula:

$$\text{Serum Protein: gm/dl} = \frac{OD\ Test}{OD\ Std.} X6$$

O.D. = Depth of the colour formed; Std. = Standard.

Similarly the serum Protein of the 40 females of *Coccinella septempunctata* were worked out. The various required measurements were noted in **Table-II.** The average serum Protein content of the female was found to be 6.81 gm/dl, which is slightly greater than that in the male.

Table I: Estimation of serum (or plasma) Proteins of Male *Coccinella septempunctata*

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.	40	1210	(i) 0.33 nm (ii) 0.34 nm (iii) 0.37 nm (iv) 0.56 nm (v) 0.48 nm	(i) 0.36 nm (ii) 0.33 nm (iii) 0.40 nm (iv) 0.55 nm (v) 0.42 nm	(i) 6.54 gm/dl (ii) 5.82 gm/dl (iii) 6.48 gm/dl (iv) 5.89 gm/dl (v) 5.25 gm/dl
Mean			0.41 nm	0.41 nm	5.99 gm/dl

Table II: Estimation of serum (or plasma) Proteins of Male *Coccinella septempunctata*

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.	40	880	(i) 0.54 nm (ii) 0.48 nm (iii) 0.58 nm (iv) 0.52 nm (v) 0.50 nm	(i) 0.57 nm (ii) 0.55 nm (iii) 0.69 nm (iv) 0.65 nm (v) 0.52 nm	(i) 6.33 gm/dl (ii) 6.87 gm/dl (iii) 7.13 gm/dl (iv) 7.50 gm/dl (v) 6.24 gm/dl
Mean			0.52 nm	0.59 nm	6.81 gm/dl

4. Results and Discussion

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 constituent greatly vary in their percentage compositions of the body weight of different insects and in the male and female of the same insect.

Proteins, the polymers of amino acids, are the most abundant substance in cell protoplasm. The functional Proteins or enzymes and different hormones including Proteinaceous 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 *Calliphora erythrocephala* and *Pholera bucephala* (Agrel, 1949); *Galleria mellonera* (Auclair and Dubrevil, 1952); *Bombyx mori* (Florkin, 1937, 1959 Sarlet et al, 1952)(Amanieu et al, 1956; Wyatt et al. 1956; *Anomala orientalis* (Po-chadley, 1956)*Drosophila melanogaster* (Hadorn and Mitchell, 1951; Chen and Hadorn, 1954); *Calliphora angur* (Hackman. 1956) and *Ephestia kuhniella* (Chen and Kuhne 1956). Similar studies have been made on the desert locust, *Schistocerca gregaria* (Benassi et al. 1961), the bug *Rhodnius prolixus* (Harigton, 1961) and Southern army worm, *Prodenia eridania* (Levenbook, 1962) and the rice moth, *Corcyra cephalonica.*(Gantiand 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, *Aedes albopictus.* 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 (Novak 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 *Chrysocoris stollii* the 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, *Coccinella septempunctata* were made and it was found that the total serum protein content of female was greater than that in the male. It is most probably due to the fact that the experiments were carried out in the months of breeding season during which there is continuous deposition of yolk in the formation of eggs in female. This observation is in full agreement with the observation of Novak et al. (1960) but contrary to the observation of Pandey D.P. (1993).

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