Differential Count of Haemocyte from the Haemolymph of Muga Silkworm (*Antheraea assamensis* H.) reared on the host plant

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Abstract Five types of circulating haemocytes have been identified from the haemolymph of 5th instar larvae of the Muga Silkworm (*Antheraea assamensis*) reared on Som leaves (*Machilus bombycina*) at Govt. Basic Muga Seed Farm, Khanapara, Assam, India. Haemocytes were identified by means of light microscopy as prohaemocytes, plasmatocytes, granulocytes, spherulocytes and oenocytoids. Differential count of haemocyte from the haemolymph of fifth-instar larvae of Muga Silkworm were also studied and calculated. Among them, prohaemocytes were found to be the most abundant cell types in the haemolymph, whereas spherulocytes the rarest.

***Keywords* - DHC, Haemocytes, Haemolymph, Prohaemocytes, Plasmatocytes, Granulocytes, Spherulocytes, Oenocytoids.**

I. INTRODUCTION

The haemolymph of insects and other invertebrate groups have cellular inclusions called haemocytes. These haemocytes plays an important role in the physiology of the organism to which they belong, being responsible for coagulation of haemolymph[6],[7], connective tissue synthesis[12],[14], wound healing, self recognition[13], general and specific immune response and opsonisation[6],[11], cellular immune reactions like phagocytosis and encapsulation[13], melanisation and discharging elements of the phenoloxidase system[7],[8] and production and storage of the respiratory pigments in some arthropods[12]. As such, it is evident that haemocytes as well as their numbers in the haemolymph play a significant role in indicating the overall physiological condition of the insect.

The free haemocytes in the haemolymph of insects are responsible for the defence reactions against foreign agents that penetrate the haemocoel[11].

The present study involves the 5th instar larval stage of Muga Silkworm *Antheraea assamensis* H.; a sericigenous insect native to the state of Assam, India, and is world famous for producing the golden-hued muga silk fibre[4]. And aims to investigate on the differential count or relative proportions of haemocytes of 5th instar larvae of Muga silkworm reared on Som leaves (*Machilus bombycina*).

II. MATERIALS AND METHODOLOGY

Disease-free eggs of Muga silkworm were collected from Govt. Basic Muga Seed Farm, Khanapara, Assam, India, during March-April. Rearing was done in the Khanapara farm in outdoor conditions on Som leaves (*Machilus bombycina*) in the farm itself. The other experiments were carried out in the Department of Zoology, Cotton University, Assam.

The general method of outdoor rearing of Muga silkworm was followed as recommended by Bharali 1970[1] and Choudhury 1982[5]. The rearing was conducted in Chotua crop/season. First of all, rearing plot was selected in little shady area with plants having quality foliage. Stagnant water was drained out from the plot. Then individual plant was cleaned by removing dry twigs and leaves, spider nets, ant's nest etc. Nylon nets were erected over food plants (trees of Som) to prevent pests and predators. Rearing equipments *viz.* bamboo chalani (sieve), rearing net etc. were disinfected with 5% bleaching powder solution before the commencement of rearing. The Khorikas (1.5-2 feet long stick made of dried straw) with the hatched worms / larvae were fed with tender leaves to prevent them from crawling off and to let them settle on the leaves. Newly hatched larvae along with the tender leaves were transferred to the food plants early in the morning. The larvae immediately crawled and started feeding on leaves. When the leaves exhausted, the larvae crawled down and were collected on triangular bamboo sieves with long handles, i.e., 'Chaloni', which are again hanged on a fresh tree. This process was repeated till the larvae attained 5th instar. A band of straw with little sand was tied around the tree trunk, which were again wrapped by an aluminium foil, 1-1.2 m above the ground to prevent the worms from crawling down the ground.

In the final stage (5th instar), larvae becomes greenish blue and it stops feeding and discharge green coloured semi-solid excreta and comes down from the plant. Now, for haemolymph collection, healthy well-fed fifth instar larvae of *A. assamensis* were taken and put in 56-60°C hot water for 2-3 minutes to fix the haemolymph of the larvae

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(Baishya *et. al*, 2015)[2]. After heat fixation, the insects were removed and rapidly dried on a filter paper. One of the proleg was punctured by a very fine needle at the tip and first two-three drops of pale greenish haemolymph was collected on a clean glass slide. After that, a smear was prepared on the slide and allowed to dry. The dried smear was then stained with May-Gruenwald's solution modified for microscopy (Eosine - methylene blue solution, containing methanol) and kept for 5 minutes and then it was rinsed in distilled water. After that the stained films were mounted in DPX and was observed under the microscope for the different types of haemocyte and for the differential haemocyte count. For differential haemocyte count, cell categories were counted in 200 cells chosen from random areas of the stained smear by a laboratory blood cell counter in each smear using a compound light microscope and different types of haemocytes were observed, counted and recorded. The percentage of different haemocyte types were calculated on the basis of the total number of all the haemocyte which had been obtained in a number of haemolymph smears[9].

III. RESULTS AND DISCUSSION

When the prepared slides were observed under the light microscope (40X), it revealed the presence of basically 5 types of haemocytes in *A. assamensis* haemolymph. These includes: prohaemocytes (PRs), plasmatocytes (PLs), granulocytes (GRs), spherulocytes (SPs) and oenocytoids (OEs). (Fig. 1 and Fig. 2).

i. **Prohaemocytes:** Prohemocytes are mostly round or oval and the smallest of the hemocyte types[10]. The nucleus is large as compared to the cell volume.

ii. **Plasmatocytes:** Plasmatocytes are the most readily distinguishable haemocyte types, because of their spindle-shaped structure[10]. These are significantly larger than prohaemocytes. The nucleus is the largest among all haemocytes and is mostly elongated or oval with an evident nucleolus. The surface shows few small projections. They exhibit phagocytic behaviour.

iii. **Granulocytes:** Granulocytes are mostly round and are larger than prohaemocytes but smaller than plasmatocytes[10], and can be identified by the presence of numerous cytoplasmic granules, as observed under the microscope. The nucleus shows an irregular profile with an evident central nucleolus.

iv. **Spherulocytes:** Spherulocytes have characteristic spherule-like protrusions on the cell surface and are generally spherical/elliptical in shape[10].

These spherules are formed due to the presence of large electron translucent vacuoles/granules in the cytoplasm.

v. **Oenocytoids:** Oenocytoids are the largest of the haemocyte types and also the less frequent cell type in *A. assamensis*. They have smooth cell surfaces without any cytoplasmic projections. Oenocytoids have a large central nucleus and a few, small, electron dense granules in the cytoplasm.

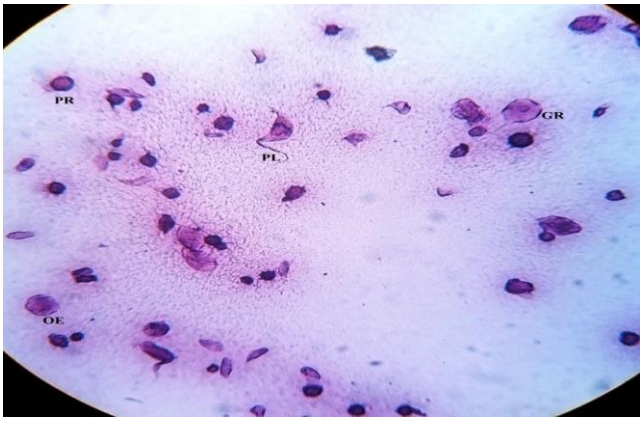

Fig. 1: Haemocytes showing are: PR – Prohaemocytes, PL – Plasmatocytes, GR – Granulocytes, OE- Oenocytoids

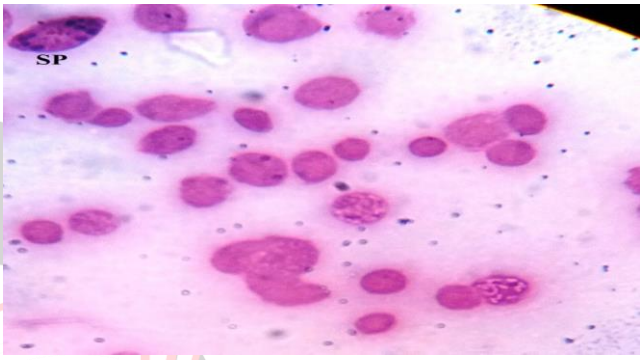

Fig. 2: Haemocytes showing are : SP-Spherulocytes of 5th instar larvae

For differential haemocyte counting profile, a particular area in the slide containing approximately 200 cells were taken. The percentage of each type of haemocyte are listed below (Table 1 and Fig. 3).

Table 1: DHC of different types of haemocytes of muga

Sl.No.	Different types of haemocytes	Percentage(%) ± SE
1	Prohaemocytes	60.3 ± 0.2
2	Plasmatocytes	23.5 ± 0.2
3	Granulocytes	9.6 ± 0.2
4	Spherulocytes	3 ± 0.1
5	Oenocytoids	3.6 ± 0.1

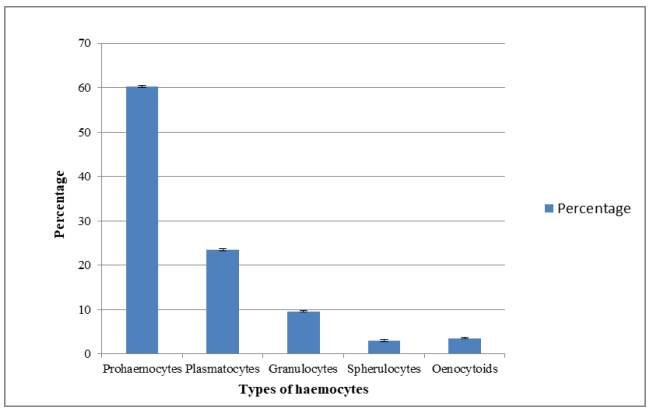

Fig. 3: Percentage of haemocytes of Muga silkworm

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Thus, from the result it is evident that five different types of haemocytes have been observed from the haemolymph of *A. assamensis*, i.e, prohaemocytes (PRs), plasmatocytes (PLs), granulocytes (GRs), spherulocytes (SPs) and oenocytoids (OEs). The findings of Baishya *et al*., 2015[3] regarding the light and electron microscopic studies revealed the presence of basically 5 types of hemocytes in *A. assama* hemolymph, which is comparable to our result. Moreover, from the fig. 3, it is seen that prohaemocytes were found to be the most abundant cell types in the haemolymph, whereas spherulocytes the rarest.

IV. CONCLUSION

India is a tropical country and environmental conditions are the limiting factors of muga silkworm rearing. Majority of muga silkworm rearers encounters the various environmental problems and lose their crops or produce inferior quality of cocoon and silk. Haemocytes number in haemolymph of any particular insect may vary depending on various factors – disease, environmental factors, etc. Therefore, it can be concluded that haemocytes play an important role in the physiology of the insect and moreover, as they are the vital components of immune system. Any change in the physiological condition of insect will have an impact on the haemocyte number. So, therefore, having a baseline idea about different kinds of haemocytes and their percentage will help us to understand the physiological condition of the insect. This work can further be carried out in future for advanced research, to understand its silk production, difference in the rearing seasons, etc.

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REFERENCES

[1] Bharali N. 1970. A new method of nursery Muga rearing. Indian Silk. 9(1):15.

[2] Baishya B.P., Bardoloi S. and Bharali R. 2015. Investigation into the Effect of Altitude on the Differential Hemocyte Count of Circulating Plasmatocytes and Granulocytes of Larval Stage of Antheraea assama (Lepidoptera: Saturniidae). Journal of Insect Science. 15(1): 1-3.

[3] Baishya B.P., Bardoloi S. and Bharali R. 2015. Ultrastructure of the hemocytes of Muga Silkworm larva *Antheraea assama* Westwood (Lepidoptera; Saturniidae): a phase-contrast and electron microscopic study. International Journal of Pure & Applied Bioscience. 3(3): 234-240.

[4] Bardoloi S. and Hazarika L.K. 1992. Seasonal variation of body weight, lipid reserves, blood volumes and haemocyte population of Antheraea assama. Environ. Entomol. 21: 1398-1409.

[5] Choudhury S.N. 1982. Muga Silk Industry, Directorate of Sericulture, Government of Assam, Shillong (Meghalaya), India.

[6] Gregoire C. 1955. Blood coagulation in arthropods V. Studies on hemolymph coagulation on 420 species of insects. Archs. Biol. 66: 104-148.

[7] Gregoire C. 1957. Studies by phase-contrast microscopy on distribution patterns of hemolymph coagulation in insects. Smithson. Misc. Collns. 134: 1-35.

[8] Jalali J. and Salehi R. 2008. The hemocyte types, differential and total count in Papilio demoleus L. (Lepidoptera: Papilionidae) during post-embryonic development. Munis Entomology and Zoology. 3(1): 199-216.

[9] Mall S.B. and Gupta G.S. 1982. Haemocyte picture during metamorphosis of *Atteva fabriciella* (Swed.). Ind. J. Entomol. 44: 101-112.

[10] Tembhare D.B. 2016. The Circulatory System. *In* Modern Entomology. Himalaya Publishing House. 90-103.

[11] Tepass U., Fessler L.I., Aziz A. and Hestensein V. 1994. Embryonic origin of hemocytes and their relationship to cell death in Drosophila. Development. 120: 1829-1837.

[12] Wigglesworth V.B. 1955. The role of hemocytes in the growth and moulting of an insect- Rhodnius prolixus (Hemiptera). J. Exp. Biol. 32: 649- 663.

[13] Wigglesworth V.B. 1957. The hemocytes and connective tissue formation in an insect- Rhodnius prolixus (Hemiptera). Quart. J. Micr. Sci. 97: 89-98.

[14] Wigglesworth V.B. 1973. Haemocytes and basement membrane formation in Rhodnius. J. Insect. Physiol. 19: 831-844.

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