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Research Article

Investigation into the effect of altitude on the Total Hemocyte Count (T.H.C.) of larval stage of Muga Silkworm *Antheraea assama* Ww.

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Abstract: Total Hemocyte Count (T.H.C.) of 5th instar larvae of Muga Silkworm *Antheraea assama* Ww reared at four different sericulture farms situated at different altitudes, viz, Khanapara State Sericulture Farm, Assam, altitude 55.5m above sea level (ASL); Nongpoh (Central Silk Board farm), Meghalaya, altitude 464m ASL; Tura (Central Silk Board farm), West Garo Hills, Meghalaya, 657m ASL; and Kalimpong (Central Silk Board farm), West Bengal, altitude 1247m ASL, were calculated and compared to investigate the effect of altitude on the number of hemocytes per mm^3 of larval hemolymph. The investigation showed that larval T.H.C.s were highest at lower altitudinal broods i.e. at Khanapara (55.5m ASL) whereas their numbers gradually declined in broods reared in higher altitudes, lowest T.H.C.s being recorded at Kalimpong (1247m ASL).
Keywords: *Antheraea assama* Ww, hemocyte, total hemocyte count, hemolymph

INTRODUCTION

The hemolymph of insects and other invertebrate groups have cellular inclusions called hemocytes. These hemocytes play important role in the physiology of the organism to which they belong, being responsible for coagulation of hemolymph [1-2], connective tissue synthesis [3-5], wound healing, self recognition, general and specific immune response and opsonisation [6-10], cellular immune reactions like phagocytosis and encapsulation [11], melanisation and discharging elements of the phenoloxidase system and production and storage of the respiratory pigments in some arthropods [10]. As such, it is evident that hemocytes as well as their numbers in the hemolymph, (i.e. hemocytes per mm^3 of hemolymph) play a significant role in indicating the overall physiological condition of the insect.

However, hemocyte numbers in the hemolymph of any particular insect may vary depending on various factors, such as disease and meteorological factors, including altitude. Our present study involves the 5th instar larval stage of Muga Silkworm *Antheraea assama* Ww; a sericigenous insect native to the state of Assam, India, and is world famous for producing the golden-hued muga silk fibre[12]. Our investigation aims to ascertain whether the different altitudes at which these larvae are reared have any effect on the total hemocyte count (T.H.C.s) of the larvae and in turn on their physiological conditions. Any affect on the physiological condition would ultimately have its impact on the yield of Muga silk.

MATERIALS AND METHODS:

Field investigations were carried out at different sericulture farms located at different altitudes in order to realize the objective of the present investigation. These farms were randomly selected and include the Khanapara State Sericulture Farm, Assam, situated at an altitude of 55.5m above sea level (ASL); Nongpoh (Central Silk Board farm), Meghalaya, altitude 464m ASL; Tura (Central Silk Board farm), West Garo Hills, Meghalaya, altitude 657m ASL; and Kalimpong (Central Silk Board farm), West Bengal, altitude 1247m ASL.

Insects: Fifth-instar larvae were directly collected from the four different sericulture farms as mentioned above, situated at different altitudes, and transported to the laboratory for conduction of the experiments.

Host Plant: It was ensured that all larvae of *A. assama* collected from each farm had been reared on Som plants *(Machilus bombycina*).

Measurement of Total Hemocyte Count (T.H.C.): For Total Hemocyte Count (T.H.C.), 48-hr post-moult 5-instar larvae were considered which were collected

directly from the farms at the time of rearing, and transported back to the laboratory and prepared for T.H.C. experiments. Quantitative estimation of hemocytes per cubic millimeter of hemolymph (T.H.C) from healthy well-fed fifth instars (48-hr post moult) were carried out as per the method of Hazarika and Gupta[13], followed by fixation of whole insect in hot water at 56-60^0 C for 2-3 mins[14]. After heat fixation, the insects were removed and rapidly dried on a filter paper. A metathoracic proleg was severed at the tip and first two-three drops of pale greenish blood were allowed to flow into a clean glass slide. A portion of the blood was quickly drawn to a 0.5 mark of a white blood cell (WBC) diluting pipette, the tip was carefully wiped clean and the blood then diluted to the 11 mark (i.e. 20 times dilution) with physiological saline (NaCl 0.9 gm, KCl 0.041 gm, $CaCl_2$ 0.048 gm, $NaHCO_3$ 0.002 gm, distilled water 100 ml) containing acetic acid (1%). After filling, the pipette was shaken vigorously for several minutes; the first drop was discarded and a hemocytometer was filled. Using a levy double line hemocytometer with improved Neubauer ruling, cells were counted in the four corner squares and total numbers were counted per cubic millimeter by the following formula-

$$\frac{\text{Hemocytes counted in x 1mm squares X dilution X depth of chamber}}{\text{Number of 1mm squares counted}}$$

RESULTS AND DISCUSSION

Statistical analysis of the data (one-way ANOVA) was performed using the statistical software OriginPro8. From the experimental data, it was observed that the total hemocyte count (T.H.C.) of 5th instar larvae was highest at Khanapara situated at the lowest altitude and gradually decreased in larvae reared at Nongpoh, Tura and Kalimpong, even though their numbers were not significantly different statistically (fig 1a and 1b).

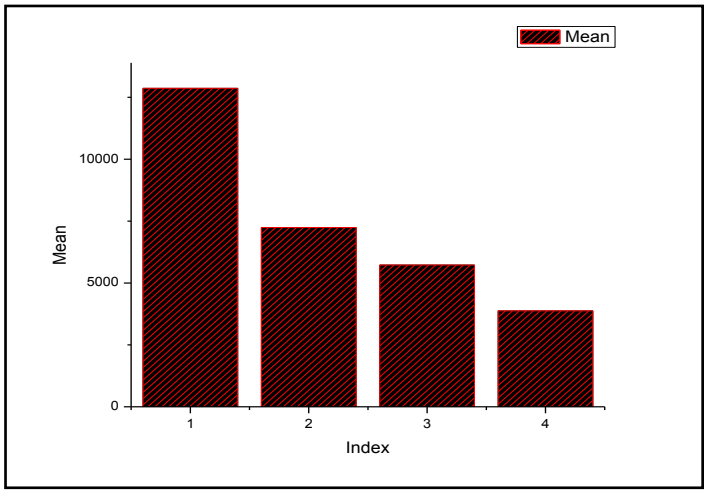

Fig 1a: Comparison of mean T.H.C.s at Khanapara (1), Nongpoh (2), Tura (3) and Kalimpong (4).

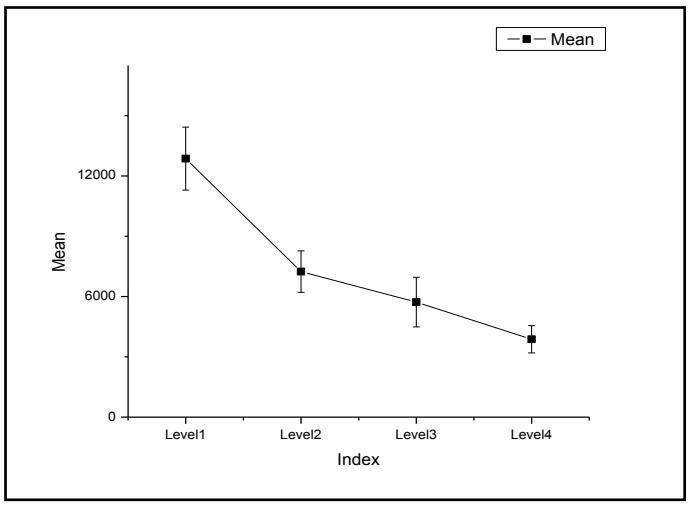

Fig 1b: Plot of means of T.H.C.s at Khanapara, Nongpoh, Tura and Kalimpong

From the given figures, it is evident that silkworms reared at Khanapara, having the lowest altitude, showed the highest mean T.H.C., whereas, those reared at Kalimpong, having the highest altitude showed the lowest mean T.H.C.

THCs of fifth instar larvae collected from farms located at different altitudes, viz. Khanapara (55m ASL), Nongpoh (464m ASL), Tura (657m ASL) and Kalimpong (1247m ASL) were observed to be significantly different from each other. Moreover, it has been observed that at higher altitude THC decreases, i.e., with increase in altitude from Khanapara to Kalimpong, there is a gradual decrease in cell count. This may be attributed to the effect of low environmental temperature at high altitude. It has been reported by various workers that temperature does have an effect on THCs; it has been reported that low temperature treatment to insects decreases THC, whereas at high temperature increase in THC was observed [14-16]. It has been also reported that in a number of insects, as temperature increases the growth rate increases and the developmental period is shortened [17-18]. In *Antheraea mylitta* THC was found to increase in higher temperature treatment but was significantly lower in low temperature [19]. The high hemocyte load at higher temperature may be attributed to loss of body fluid due to dessication. Another explanation suggests that at higher temperature, silkworms being more prone to infections (since microbial growth increases during hot and humid seasons), therefore, probably as a defence mechanism, hemocytes get detached from tissue surfaces and there is a higher rate of multiplication or production of hemocytes to promote cellular defence to the silkworm larvae [19]. Similarly, declining THCs in lower temperature (higher altitudes) may be attributed to clumping of hemocytes due to chilling stress and thus making the hemocytes unavailable in circulating hemolymph[19]. Clumping of hemocytes may be taken as a physiological response of hemocytes in transport system. Since insects, or in that matter, silkworms are cold blooded in nature, low temperature might induce clumping of hemocytes. Moreover, it has been reported in *A. mylitta* that prohemocytes (PRs) increased at high temperature, and since they have been reported to serve as stem cells by many workers [20], these PR cells probably undergo mitotic divisions at higher temperature giving rise to other hemocyte types, thereby resulting in overall increase in the total number of circulating hemocytes which is evident in our study.

CONCLUSION

With the increase in THCs, the components hemocyte types in the silkworm hemolymph also increase in number. This leads to the increased numbers of circulating immunocytes i.e. plasmatocyes (PLs) and granulocytes (GRs), thereby providing stronger cellular immunity to the silkworms. Thus, from this study, it can be concluded that broods and races of plains are better equipped in terms of cellular immunity, than their counterparts in higher altitudinal areas, which make the lower altitudinal broods better suited to adverse pathological conditions than broods at higher altitudes. This information may be very useful in planning rearing strategies for commercial crops. The findings become more important because it suggests genetic rearrangement as the cause of such variations resulting from environmental variability in study areas.

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