ISSN: 0976-3376

Available Online at http://www.journalajst.com

ASIAN JOURNAL OF SCIENCE AND TECHNOLOGY

Asian Journal of Science and Technology
Vol. 08, Issue, 01, pp.4197-4208, January, 2017

RESEARCH ARTICLE

HAEMOCYTE COUNT IN SILKWORM BOMBYX MORI L.: A COMPARATIVE STUDY IN DIFFERENT MULTIVOLTINE RACES

[1]Yojana S. Munivand [2*]Ganesh P.Bhawane

[1]Changu Kana Thakur Arts, Commerce and Science College, New Panvel
[2]Department of Zoology, Shivaji University, Kolhapur, India

ARTICLE INFO

Article History:
Received 18th October, 2016
Received in revised form
24th November, 2016
Accepted 28th December, 2016
Published online 31st January, 2017

Key words:

Bombyxmori, Haemocytes, Kolar gold, Nistari, Pure Mysore, THC, DHC.

ABSTRACT

The total and differential haemocyte counts were carried out in three different multivoltine races of *Bombyxmori*L. from 4th moult day upto the last day of 5th instar larvae. Total haemocyte count was found more at 5th day of 5th instar larvae in all the races under study but the more count was found in Nistari race (13785.50 ± 470.23) than Pure mysore (10713.50 ± 1723.22) and Kolar gold (10048.50 ± 246.78). In differential count seven types of haemocytes were found viz., Prohaemocyte (PR), Granulocyte (GR), Spherulocyte (SP), Plasmatocyte (PL), Adipohaemocyte (AD), Coagulocyte (CO) and Oenocytoid (OE). In Pure mysore high Adipohaemocyte and Coagulocyte count were observed; Nistari having high count of Prohaemocyte, while Kolar gold having high Plasmatocyte, Spherulocyte, oenocytoid and Granulocyte count.

INTRODUCTION

The loose cells in the fluid of body cavity of insects are called haemocytes or blood cells. The blood or haemolymph circulates in the body cavity bathing the tissues directly. It consists of fluid plasma in which haemocytes are suspended. Their primary functions are coagulation, phagocytosis, encapsulation, detoxification and storage and distribution of nutritive materials. The plasma which bathes all the tissues constitutes 5-40% of the total body weight of an insect and contains many organic and inorganic constituents like free amino acids, proteins, lipids, carbohydrates, uric acid etc. The chemical composition of haemolymph is highly variable among the species and at different developmental stages of the same species (Florkin and Jeuniaux, 1974). Certain phytophagous insects, among them the giant silkworm, possesshaemolymph characterized by much lower sodium concentration and higher potassium and magnesium concentrations than those found in the majority of other animals (Weevers, 1966). High temperature affects nearly all biological processes including the structure of protein biological membranes and rates of biochemical and physiological reactions (Hazel 1995, Somen 1995, Willmer*et al.,* 2004). In terrestrial insects, glucose is of little importance as blood sugar is replaced by disaccharide trehalose. The use of trehalose instead of glucose as a blood sugar appears to be an adaptation to overcome the problems of osmotic pressure and chemical reactivity that would result of glucose was the major form of fuel in the haemolymph (Wheeler, 1989). The first study of total haemocyte count in insects was made by Tauber and Yeager (1934). Haemocyte classification in insects based on the morphology, functions and staining or histochemical reactions of haemocytes (Gupta, 1979). The insect haemocyte classification, which is generally used has evolved over more than half a century. According to Cuenot (1896), Millara (1947) were the first to classify insect haemocytes into four categories and was latter followed in this attempt by Hollande (1909, 1911) and others. There is disagreement among insect haematologist about the number of hemocytes types in various insects (Takada and Kitano, 1971; Kim, 1980; Gaikwad, 2007). Ultrastructurally, only seven types have so far been identified in various insects. Prohaemocyte (PR), Plasmatocyte (PL), Granulocyte (GR), Spherulocyte (SP), Adipohemocyte (AD), Oenocytoid (OE) and Coagulocytes (CO) of these seven, Co has been reported by Goffinate and Gregoiren (1975) and Ratcliffe and Price (1974), AD has been reported only by Devauchelle (1971). Podocyte (PO) and Vermicyte (VE) have not been recognized as distinct types in electron microscope studies, because ultrastructurally they appear similar to PLs (Devauchelle, 1971). Haemocytes types mostly studied in Lepidoptera, Hymenoptera, Coleoptera and Diptera (Gupta 1985). Numerous light microscope observations concerning the classification of insect haemocyte have been published (Yeager 1945; Nitton, 1960; Jones 1962; Arnold 1972). There

Corresponding author: Ganesh P. Bhawane,
Department of Zoology, Shivaji University, Kolhapur, India.

are some remarkable differences exhibited between the haemocytes of different insect orders. In *Bombyxmori* Nitton (1960) reported five classes of haemocytes PRs, PLs, GRs, SPs and OEs. There is an inherent variability of haemocytes within a species as well as among closely related species (Arnold, 1974; Gupta, 1979). Haemocytes of various types have been investigated in the german Cockroach, *Blattelagermanica* both in the nymphs and adults (Hazarika and Gupta 1987; Chiang *et al.,* 1988). Wigglesworth (1933) classified four kinds of haemocytes in *Rhodniusprolixus* nymphs and in 1955 he identified two additional types. In the silkworm, haemocytes does not enter the heart through the ostia so that only cell free hamolymph can said be truly circulate (Akai and Sato, 1973). Qamar and Jamal (2009) reported differential count of 5th instar nymphs and adults of *Dysdercuscingulatus* Fabr (Hemiptera: Pyrrhocoridae) treated with an organophosphorus insecticide i.e. acephate.

MATERIAL AND METHODS

Total haemocyte count and differential haemocyte count was done by using the method as described by Praful, (1994). For the haemocyte study, the haemolymph was obtained from the 5th instar larvae of all the races of *Bombyxmori* under study. First of all, the neubauers chamber and coverslip were cleaned and the cover slip was put on that slide. The haemolymph was collected from larvae by amputation of legs for the quantitative study of haemolymph. The haemolymph was taken on this Neubaurs double lined haemocytometer and allowed to settle for a minute. Under light microscope the haemocytes were counted. While making the observations of THC five squares of 1mm size (four big corner squares and central big squares) were counted. For calculation of the total haemocyte (THC) Jones (1962) formula was used.

For THC the following formula used:

$$\text{THC} = \frac{\text{No. of cells counted X 10}}{\text{No. of 1 sq. mm counted}}$$

To study the morphological structure, the differential haemocyte count was carried out. For this purpose, the haemolymph was firstly fixed as per the method of Hazarika and Gupta (1987), followed by fixation of whole insect in hot water at 56-60^0 C for 2-3 mins. Heat can serve to fix haemocytes very rapidly within the insects, without appreciable change to their shape and size and also prevents the coagulation of the haemolymph. Consequently, after the heat fixation the blood can be withdrawn from insect by cutting the any proleg with fine scissor and haemolymph allowed to fall on the clean, grease free slide containing 2% versene ringer solution (2% EDTA in 100 ml of insect ringer solution), mixed well. A smear was made by drawing a second slide across the first one at a 45^o angle. The smear is allowed to dry and was stained with Wrights stain (used dilution with 1:1 with phosphate buffer of pH 7.2). The stained slide then rinsed with distilled water, followed by acetone wash, cleared in xylene and mounted in DPX. The haemocytes were identified by following the identification key of Gupta (1979). The identification and count of different haemocyte types were made in all the races under study.

RESULTS

The blood cells which were dispersed in the blood i.e. haemolymph. The haemolymph, a clear fluid, which was colourless in Kolar gold and Pure mysore while, it was yellow coloured in Nistari. It filled all the sinuses of body cavity, where it freely bathes the various internal organs and also enters the appendages and the tubular cavities of the wing veins in adult. The haemolymph consist of the liquid part known as plasma and cellular part known as the haemocytes.

In the present study, the total and differential count of haemocyte in all the races of *B. mori* under study was carried out. The counts were done from day of 4th moult to the 7th day of 5th instar and the observed variations in the total count and differential count in three multivoltine races were mentioned in the Table no. 5.2 and Fig no. 5.1. The total haemocyte count carried out in the races under study showed day wise variations. On the moult day, all the races showed maximum cell count. In Kolar gold, on moult day the count observed was 7377.00 ± 49.50/mm^3, in Pure mysore 10515.50 ± 275.06/mm^3 while in Nistari greater count was observed as compared to other two races i.e. 11113.50 ± 112.43/ mm^3. On the 1st day of 5th instar larvae the total count was 4885.69 ± 493.85/mm^3 in Kolar gold, in Pure mysore it was 6806.25 ± 496.74/ mm^3 while in Nistari 7441.50 ± 641.35 mm^3 cell count was observed. On the 2nd day of 5th instar larvae the total cell count observed were 5357.31 ± 88.53/ mm^{3}in Kolar gold, 6462.00±152.74/ mm^3 in Pure mysore while 3470.00 ± 36.77/mm^3 in Nistari. On the 3rd and 4th day of 5th instar larvae, the Kolar gold showed total cell count about 5332.64 ± 1847.87/mm^3 and 5723.94±385.99/mm^3, in Pure mysore was about 7476.88 ± 667.33/mm^3 and 5558.75 ± 15.91/mm^3, while in Nistari about 4271.00 ± 371.94/mm^3 and5817.50 ± 229.81/mm^3 respectively. On the 5th day of 5th instar larvae of *B. mori*races under study, highest cell counts were reported. In Kolar gold, 10048.50 ± 246.78/mm^3 cell counts was observed and in Pure mysore and Nistari about 10713.50 ± 1723.22/mm^3 and 13785.50 ± 470.23/mm^3 cell counts was observed. When compared in the races, on the 5th day maximum count was observed in Nistari followed by Kolar gold and then Pure mysore. On 6th day the cell counts got decreased and on the 7th day it got increased in all the races under study. On 6th day of 5th instar larvae, in Kolar gold it was observed 6510.50 ± 95.46/mm^3, in Pure mysore 4657.88±465.45/mm^3 and in Nistari it was 5526.50 ± 226.98/mm^3. On the 7th day the cell count observed were 6709.50 ± 221.32/mm^3, 6277.50 ± 218.50/mm^3 and 6455.50 ± 123.74/mm^3 in Kolar gold, Pure mysore and Nistari respectively.Along with total count, differential count also done day wise and in all the three multivoltine races of *B. mori*under study which showed seven types of haemocytes were observed in all the three races under study. The differential count of the haemocytes from 4th instar moult day upto 7th day of 5th instar was given in Table no. 5.1 and Fig no. 5.2 to 5.8.

1. Prohaemocyte (Pro)
2. Granulocyte (Gra)
3. Spherulocyte (Spe)
4. Plasmatocyte (Pla)
5. Adipohaemocyte (Adi)
6. Coagulocyte (Coa)
7. Oenocytoid (Oen)

Table 1. Comparative study of differential haemocyte count in Kolar gold, Pure mysore and Nistari races of *B. mori* L.

Days		Moult	1	2	3	4	5	6	7
Granulocyte	Kolar gold	33.40 ± 5.62	29.7 ± 0.63	10.29± 1.21	16.7 ±2.92	20.0 ±2.04	31.9 ± 1.38	27.8 ±1.07	26.8 ± 1.67
	Pure mysore	31.74 ± 16.90	21.4 ± 0.9	30.8 ± 2.15	31.2 ± 1.48	0.0 ± 0.0	11.1 ±0.63	7.4 ± 1.08	23.76 ± 2.87
	Nistari	26.79 ± 1.07	27.2 ±1.2	0.0 ± 0.0	21.7 ± 2.92	10.3 ± 0.09	30.8 ± 2.17	31.4 ± 2.89	20.6 ± 1.60
Prohaemocyte	Kolar gold	15.42 ± 2.43	7.9 ± 0.92	30.1 ± 1.71	40.0 ± 3.13	35.0 ± 0.73	12.0 ± 1.25	12.5 ± 1.59	20.1 ± 1.33
	Pure mysore	11.11 ± 1.24	25.1 ± 1.2	2.2 ± 0.82	10.7 ± 2.9	16.7 ± 0.85	5.6 ± 0.16	16.7 ± 1.44	32. 76 ± 4.56
	Nistari	12.50 ± 2.00	31.1 ± 0.75	54.2 ± 0.98	20.8 ± 2.10	21.4 ± 2.84	10.5 ± 1.27	19.5 ± 1.19	15.1 ± 1.44
Spherulocyte	Kolar gold	8.72 ± 1.76	30.6 ± 2.72	10.0 ± 9.2	16.7 ± 2.92	6.7 ± 1.95	15.7 ± 0.64	23.9 ± 1.85	20.2 ± 1.83
	Pure mysore	26.19 ± 2.08	11.1 ± 1.2	15.3 ± 0.41	14.8 ± 1.30	13.3 ± 1.19	5.6 ± 1.62	16.7 ± 2.79	10.93 ± 1.54
	Nistari	12.50 ± 1.96	12.5 ± 0.23	33.3 ± 3.03	15.0 ± 1.42	37.3 ± 1.83	12.6 ± 1.63	11.4 ± 1.33	29.4 ± 1.13
Plasmatocyte	Kolar gold	12.91 ± 2.89	0.0 ± 0.0	24.4 ± 1.23	8.9 ± 0.94	0.0 ± 0.0	0.0 ± 0.0	23.9 ± 1.55	10.4 ± 1.70
	Pure mysore	5.55± 0.62	17.4 ± 2.82	0.0 ± 0.0	2.8 ± 0.68	21.7 ± 2.22	5.6 ± 0.65	0.0 ± 0.0	0.00 ± 0.00
	Nistari	0.00 ± 0.00	4.2 ± 1.31	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Adipohaemocyte	Kolar gold	10.47 ± 1.32	12.0 ± 1.18	22.3 ± 0.77	0.0 ± 0.0	19.2 ± 1.48	24.1 ± 1.6	20.6 ± 2.39	19.5 ± 1.33
	Pure mysore	14.28 ± 2.74	16.2 ± 0.56	45.0 ± 2.68	34.8 ± 1.29	16.7 ± 1.32	5.6 ± 0.69	42.6 ± 3.40	27.06 ± 3.15
	Nistari	36.90 ± 1.78	26.0 ± 22.1	6.3 ± 0.34	25.8 ± 2.44	15.9 ± 1.07	44.3 ± 3.04	27.6 ± 2.07	30.2 ± 1.53
Coagulocyte	Kolar gold	7.16 ± 3.09	10.1 ± 0.91	2.8 ± 0.86	5.6 ± 0.62	4.2 ± 0.23	12.5 ± 1.65	6.7 ± 1.53	3.0 ± 0.22
	Pure mysore	5.55 ±0.62	2.6 ± 0.43	6.7 ± 1.53	3.3 ± 0.8	20.0 ± 2.15	72.2 ± 4.72	0.0 ± 0.0	5.76 ± 1.03
	Nistari	0.00 ± 0.00	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.8 ± 0.95	0.0 ± 0.0	0.0 ± 0.0
Oenocytoid	Kolar gold	14.78 ± 0.98	7.6 ± 0.66	0.0 ± 0.0	12.2 ± 1.07	15.0 ± 1.29	3.7 ± 0.4	0.0 ± 0.0	0.0 ± 0.0
	Pure mysore	13.89 ± 1.73	6.3 ± 0.78	0.0 ± 0.0	2.4 ± 0.14	11.7 ± 1.06	0.0 ± 0.0	16.7 ± 1.64	1.98 ± 0.99
	Nistari	11.18 ± 1.87	2.1 ± 0.27	6.3 ± 0.32	16.7 ± 2.92	14.9 ± 1.53	0.0 ± 0.0	10.0 ± 1.09	4.8 ± 0.82

Table 2. Comparative study of total haemocyte count in Kolar gold, Pure mysore and Nistari races of *B. mori* L.

Race	Days							
	moult	1	2	3	4	5	6	7
Kolar gold	7377.00 ± 49.50	4885.69 ± 493.85	5357.31 ± 88.53	5332.64 ±147.87	5723.94±385.99	10048.50±246.78	6510.50±95.46	6709.50 ± 221.32
Pure mysore	10515.50 ± 275.06	6806.25 ± 496.74	6462.00±152.74	7476.88 ± 667.33	5558.75±15.91	10713.50±1723.22	4657.88±465.45	6277.50 ± 218.50
Nistari	11113.50 ± 112.43	7441.50 ± 641.35	3470.00 ±36.77	4271.00 ± 371.94	5817.50±229.81	13785.50±470.23	5526.50±226.98	6455.50 ±123.74

1. Prohaemocyte

These were small, round or oval cells in Kolar gold (Plate no3. fig no. 1), Pure mysore (Plate no.1 fig no. 1) and Nistari (Plate no. 2 fig no. 1&9). Its nucleus was centrally placed, larger as compared with other haemocyte type. These are having relatively small amount of smooth, homogenous, cytoplasm. The differential haemocyte count showed that, high count prohaemocyte was observed in Nistari on the 3rd day i.e. 54.2% (±0.98) while minimum was observed on the 5th day and was 10.5% (±1.27). In Kolar gold the maximum count was observed on 3rd day i.e. 40.0% (±3.13) while the minimum was observed on 1st day of 5th instar larvae about 7.9% (±0.92). In Pure mysore race, on the 7th day maximum count was observed i.e. 32.76% (±4.56) while the minimum count was observed on 2nd day i.e. 2.2% (±0.82). In Nistari highest prohaemocyte count and in Pure mysore the lowest prohaemocyte count was observed (Fig no.3).

2. Granulocyte

Granulocytes were the most common haemocytes. These were spherical or oval cells in allthe races under study (Plate no. 1, 2, 3 fig no. 2). Their nucleus is relatively small, round or elongated and centrally placed. The cytoplasm is charastically granular and the granules were mostly spherical or ovoid in shape. When compared in all the races, these cells were found maximum in Kolar gold as compared to PM and Nistari but Nistari showed low count Fig no. 2. In Kolar gold (33.40% (±5.62) and Pure mysore (31.74 % (±16.90) the maximum granulocyte count was observed on the day of 4th moult while in Nistari it was observed on 6th day of 5th instar (31.4 % (±2.89), while the minimum count was observed on 2nd day in Kolar gold i.e. 10.29% (±1.21) and in Pure mysore and Nistari no granulocyte was observed on 4th and 2nd day respectively in the preperation.

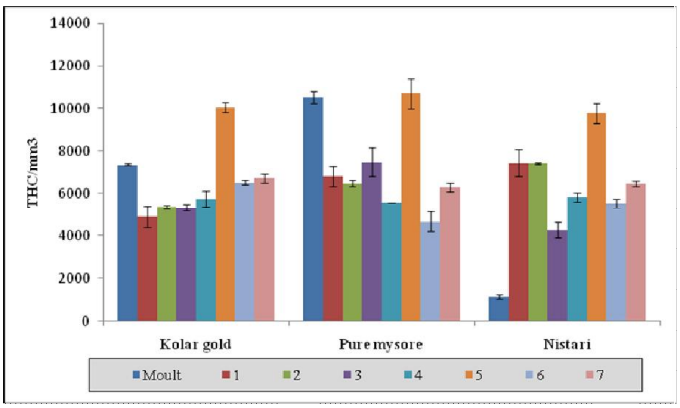


Fig. 1. Total haemocyte count (THC) of fifth instar larvae of *Bombyx mori* L.

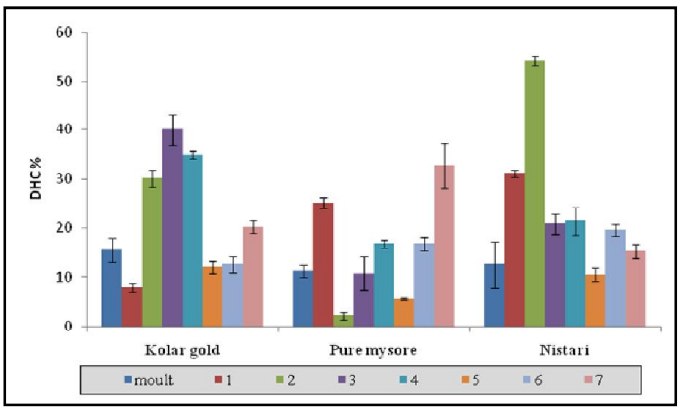


Fig. 3. Prohaemocyte count in fifth instar larvae of *B. mori* L.

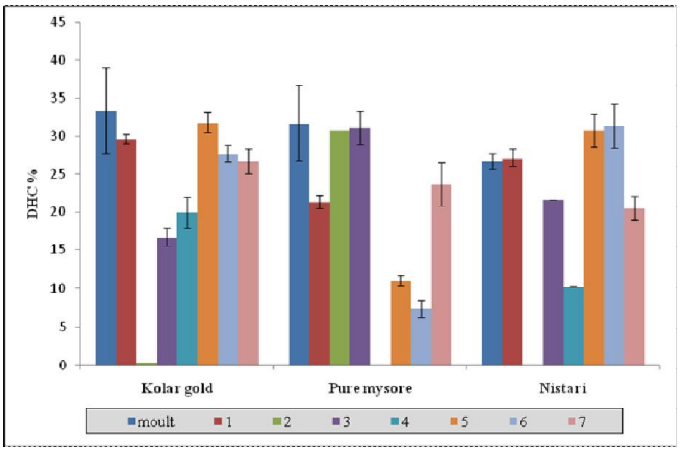


Fig. 2. Granulocyte count in fifth instar larvae of *B. mori* L

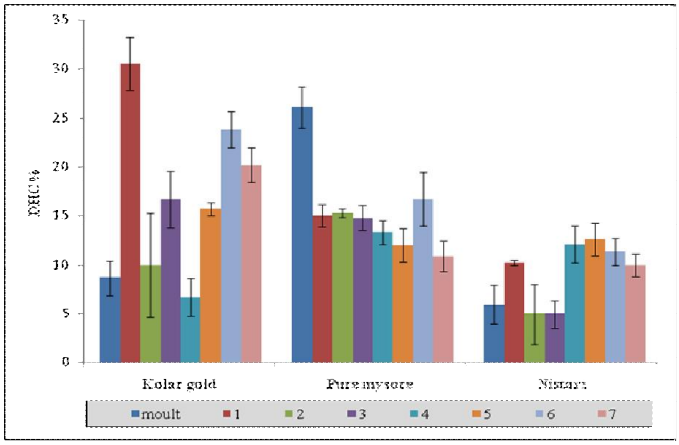


Fig. 4. Spherulocyte count in fifth instar larvae of *B. mori* L

3. Spherulocytes:

These cells were oval in shape. They were having small, round or elongated nuclei eccentrically located in all the races under study (Plate no. 1, 2, 3 fig no. 4). Their cytoplasm was thick and having numbers of spherules of variable sizes located around their nuclei. The maximum spherulocyte count was observed in Nistari, followed by Kolar gold and Pure mysore. On the 4th day of 5th instar larvae of Nistari count found 37.3% (±1.83) and minimum count was found on 6th day about 11.4% (±1.33). In Kolar gold and Pure mysore, the maximum spherulocyte count was observed on 1st day of 5th instar (30.6% (±2.72) and day of 4th moult (26.19% (±2.08) respectively, while the minimum count was observed on 4th day and 5th day of 5th instar larva of Kolar gold (6.7% (±1.95) and Pure mysore (5.6% (±1.62) respectively (Fig no. 4).

4. Plasmatocyte

They were small to large polymorphic cells. Usually they were spindle shaped (Plate no. 1, 2, 3 fig no.3a) or round (Plate no. 2 fig no. 3b; Plate no. 3 fig no. 8a) about 10µm in diameter. Their nuclei were centrally located, rounded or elongated. They contained abundant cytoplasm. The maximum plasmatocyte count was observed in Kolar gold as compared to the other two races and it was on the 2nd day of 5th instar larvae (24.4% (±1.23) (Fig no. 5). On 1st, 4th and 5th day the plasmatocyte were not observed in the preperation.

On 2nd, 6th and 7th day of Pure mysoreplasmatocyte were not observed and the maximum cell count was observed on 4th day about 21.7% (±2.22). In Nistari the plasmatocytes were observed only on 1st day of 5th instar larva was 4.2% (±1.31).

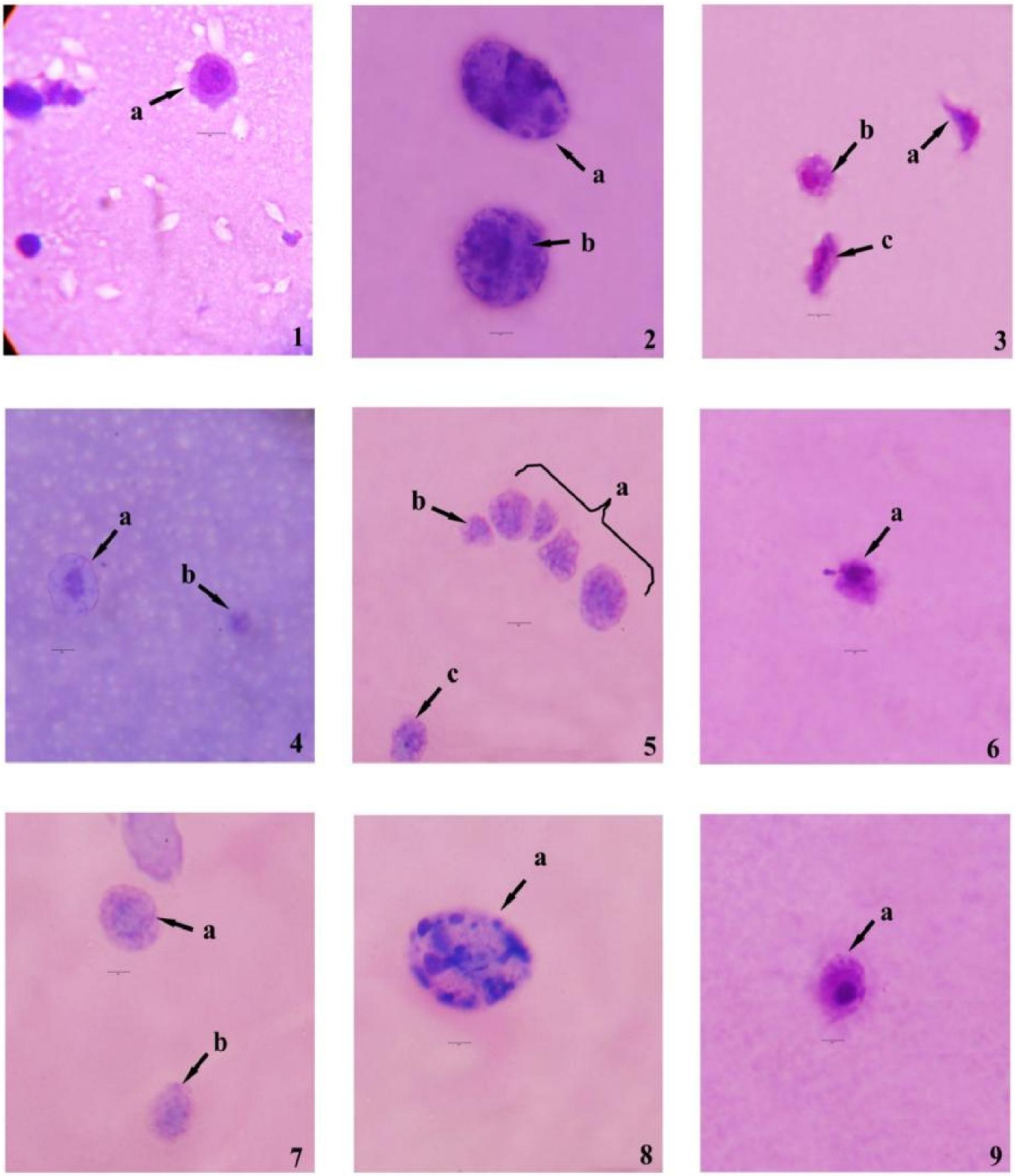
5. Adipohaemocytes

They were small to large, oval or spherical cells with centrally placed nucleus in all the races under study (Plate no. 1, 2, 3 fig no. 5). These cells should be termed as adipohaemocyte, only when they can be distinguished from fat body cells. These cells were known to contain the lipid like globules. The maximum adipohaemocytes were observed in Pure mysore than Kolar gold and Nistari (Fig no. 6). The maximum cell count was observed on 5th day in Kolar gold (24.1% (±1.6) and Nistari (44.3% (±3.04), while in Pure mysore (45.0% (±2.68) it was observed on 2nd day of 5th instar larvae. The minimum cell count was observed on the 5th and 2nd day of Pure mysore and Nistari, while in Kolar gold on 3rd day adipohaemocytes were not observed in the preperation.

6. Coagulocyte

Coagulocytes were generally small to large, spherical hyaline cells. These cells were having combined features of granulocyte and oenocytoid. Their nuclei were small, oval, eccentrically located (Plate no. 1, 2, 3 fig no. 7). In Nistari the coagulocyte count was observed only on 5th day of 5th instar, while in Pure mysore maximum count was observed on the 5th

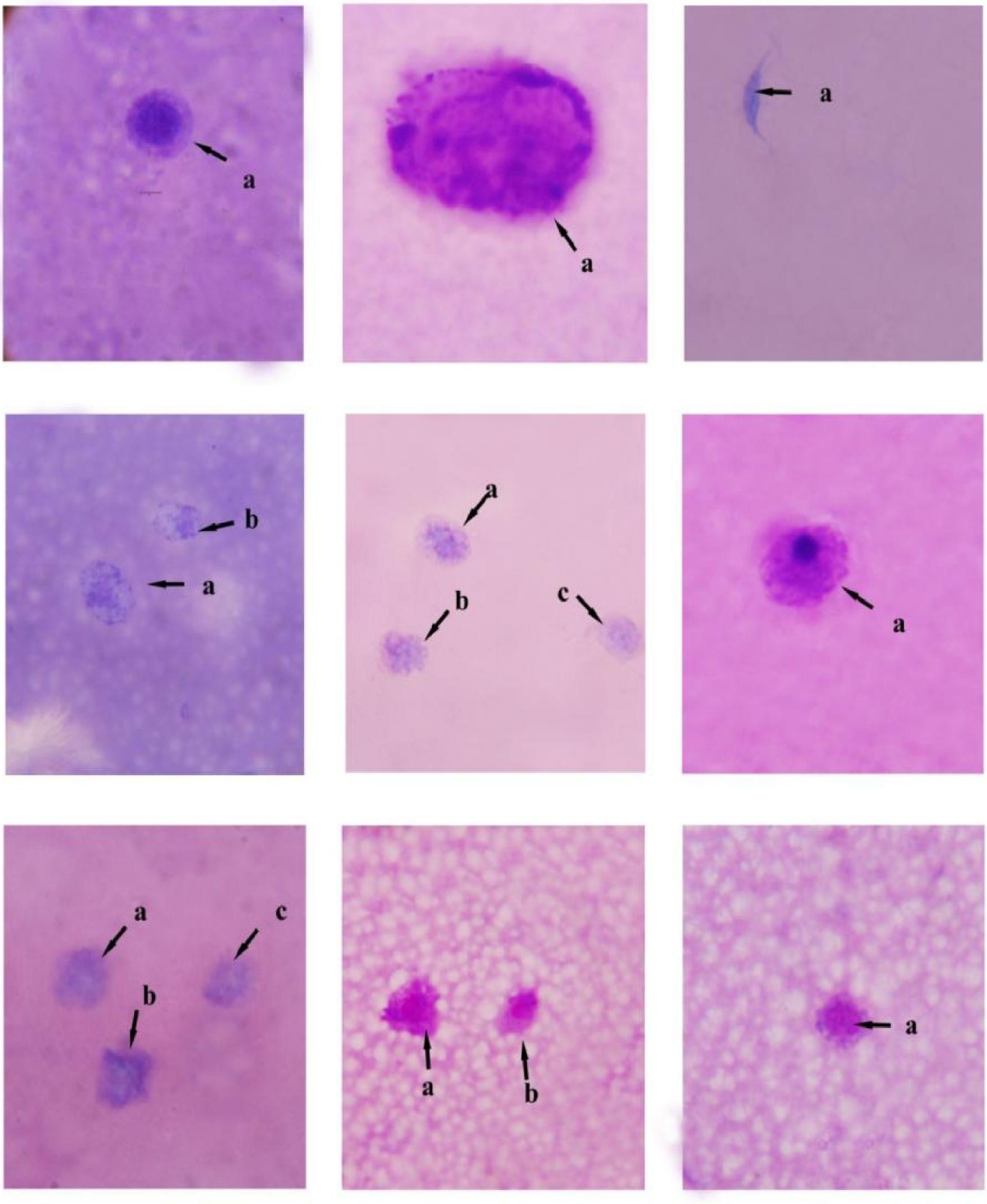
PLATE 1



Haemocytes of 5th instar larvae of Pure mysore race of *Bombyx mori* L.

Fig. 1. a. Prohaemocyte **Fig. 2. a. Granulocyte** **Fig. 3. a. b. Plasmatocyte**
Fig. 4. a. Spherulocyte **Fig. 5. a, b Adipohaemocyte** **Fig. 6. a. Oenocytoid**
Fig. 7. a. Coagulocyte **Fig. 8. a. Granulocyte** **Fig. 9. a. Oenocytoid**

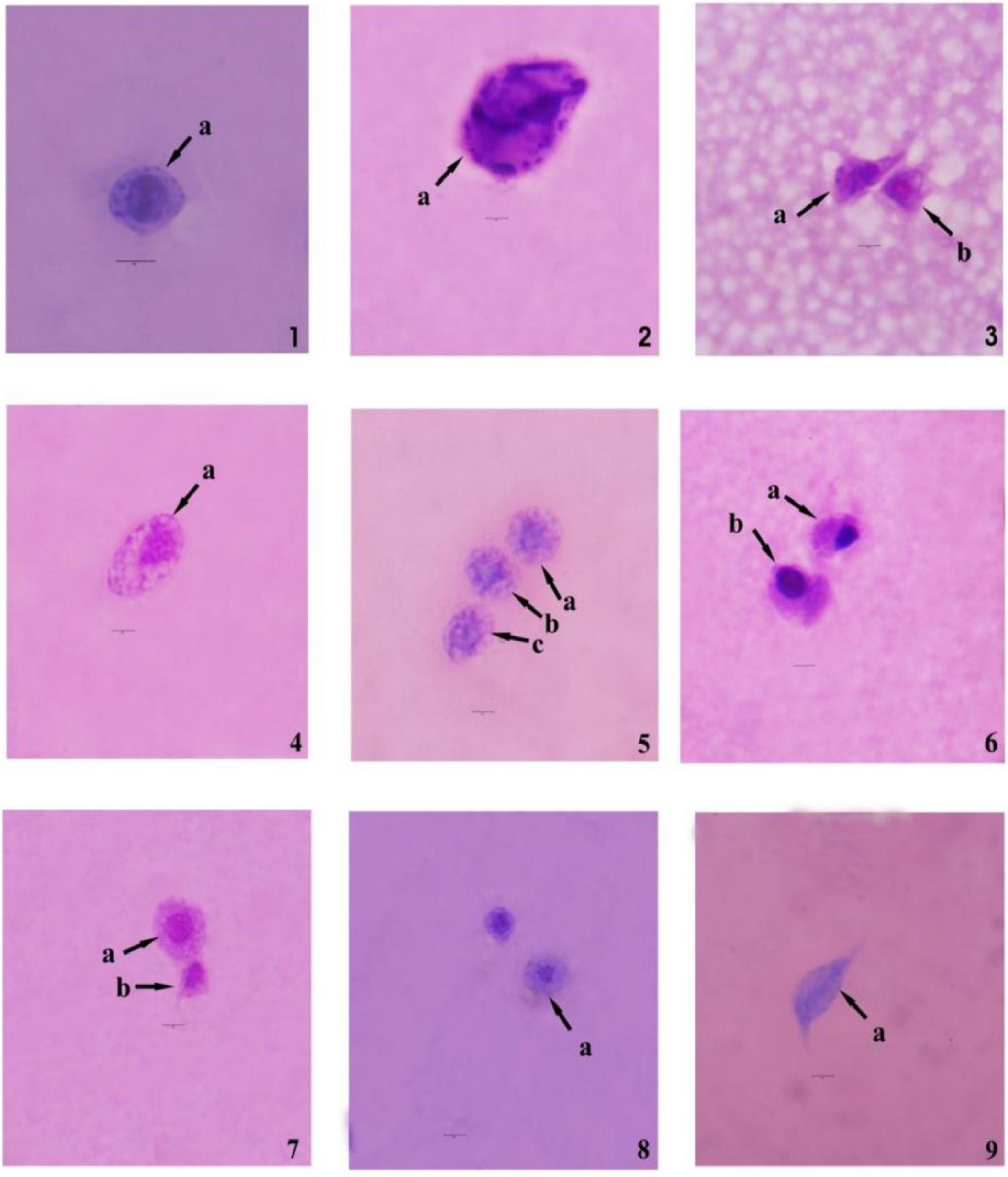
PLATE 2



Haemocytes of 5th instar larvae of Nistari race of *Bombyx mori* L

Fig. 1. a. Prohaemocyte
Fig. 2. a. Granulocyte
Fig. 3. a. b. Plasmatocyte
Fig. 4. a, b, c Spherulocyte
Fig. 5. a. Adipohaemocyte
Fig. 6. a. Oenocytoid
Fig. 7. a, b, c Coagulocyte
Fig. 8. a, b Plasmatocyte
Fig. 9. a. Prohaemocyte

PLATE 3



Haemocytes of 5th instar larvae of Kolar gold race of *Bombyx mori* L.

Fig. 1. a. Prohaemocyte
Fig. 2. a. Granulocyte
Fig. 3. a. b. Plasmatocyte
Fig. 4. a. Spherulocyte
Fig. 5. a, b, c Adipohaemocyte
Fig. 6. a, b Oenocytoid
Fig. 7. a, b Coagulocyte
Fig. 8. a. Sperulocyte
Fig. 9. a. Plasmatocyte

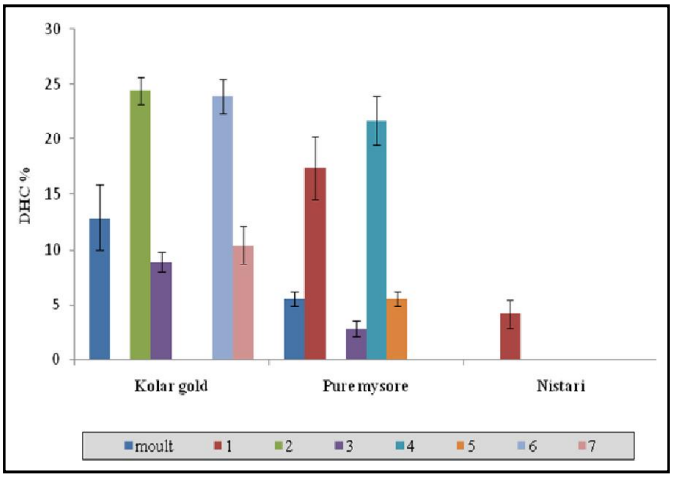


Fig. 5. Plasmatocyte count in fifth instar larvae of *B. mori* L

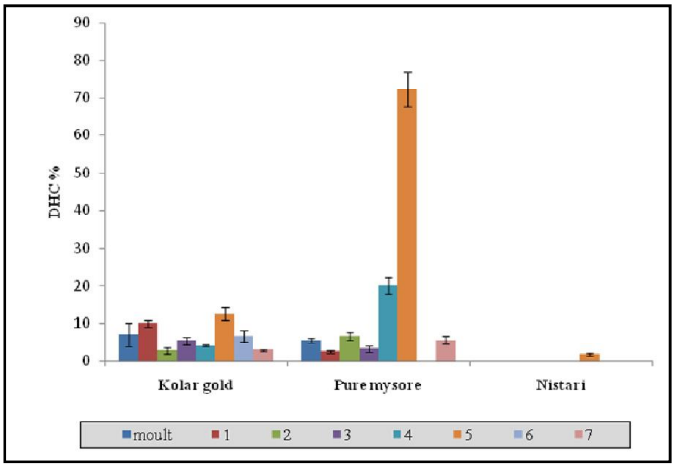


Fig. 7. Coagulocyte count in fifth instar larvae of *B. mori* L.

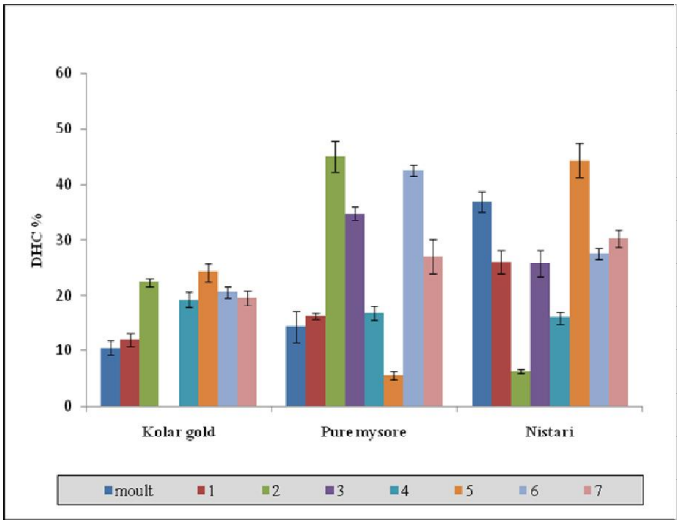


Fig. 6. Adipohaemocyte count in fifth instar larvae of *B. mori* L.

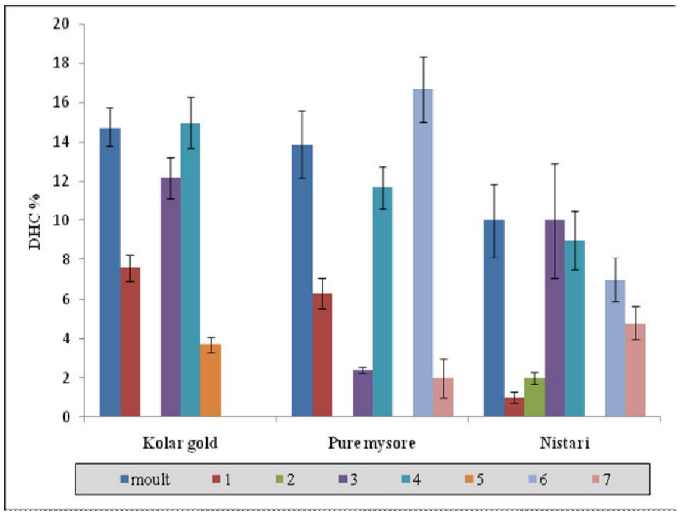


Fig. 8. Oenocytoid count in fifth instar larvae of *B. mori* L

day about 72.2% (±4.72) and on the 6th day cell was not observed. In Kolar gold, these cells were found on each day where the maximum observed on 5th day (12.5% (±1.65%) and minimum observed on the 2nd day (2.8% (±0.86) of 5th instar larvae. Means, here on the 5th day the maximum coagulocytes were found in all the races and Pure mysore showed maximum count as compared to Kolar gold and Nistari (Fig no. 7).

7. Oenocytoid

These cells were large and spherical in shape. Their nuclei were eccentrically placed in Kolar gold (Plate no. 3 fig no. 6), Pure mysore (Plate no. 1 fig no. 6&9) and Nistari (Plate no. 2 fig no. 6). The higher oenocytoid count was observed in Pure mysore and Nistari on 6th(16.7% (±1.64) and 4th (16.7% (±2.92) day respectively. In Kolar gold the maximum count was observed on 4th day of 5th instar (15.0% (±1.29), while on 2nd, 6th and 7th day these cells were not observed. In Pure mysore on 2nd and 5th day, while in Nistari only on 5th day oenocytoids were not observed in preparation.From the above observation, it cleared that the spherulocyte, plasmatocyte and oenocytoid were predominant in Kolar gold and while in Nistari race prohaemocyte was predominant. In Pure mysore the adipohaemocyte was predominant.

DISCUSSION

The haemocytes were highly polymorphic and their status depends on the age, sex, stages of development, state of nutrition, physiological state and insect species because, they showed variations in different insect species and within the same species also (Sanjayan*et al.,* 1996). Jones (1962) proposed the haemocyte classification and classified the haemocytes into nine distinct types; prohaemocyte, plasmatocyte, granulocyte, cystocyte, spherulocyte, oenocytoid, podocyte and vermicyte. In *Rhodnius*one more additional type i.e. granulocytophagus reported by Jones (1976). There was disagreement among the haematologists about the haemocyte types in various insects. There was one to as many as nine or more types were described by light microscopy in insect species, 3 types in mosquito larvae (Hall and Avery, 1978); 4 types in *Dysdercuscingulatus*(Berger and Slavickova, 2008); 5 types in *Dysdercuscingulatus* (Qamar and Jamal, 2009); 6 types in *Adelgestsugae* (Gouli*et al.,* 2000). Ultrastructurally, only seven types were identified in various insects prohaemocyte, plasmatocyte, granulocyte, sherulocyte, adipohaemocyte, coagulogyte and oenocytoid (Gupta, 1979). In the present investigation, also seven types of haemocytes were observed in all the races of *Bombyxmori* under study. Gaikwad (2007) also found seven types of haemocytes in *P.*

polytespolytes. Of these seven types adipohaemocyte was firstly reported by Devauchelle (1971) and coagulocytes by Goffinate and Gregoire (1975) and Ratcliffe and Price (1974). In*Rhodniusproxilus* six types of haemocytes were present (Jones, 1965). In *Papiliodemoleus* in addition to these cells types two additional types were reported viz. vermicyte and podocyte (Jalali and Salehi, 2008). But Podocyte and vermicyte could not be considered as distict type, because ultrastructurally they appear similar to plasmatocyte (Devauchelle, 1971).

Ultrastructural studies of *B. mori*haemocytes was made by Akai and Sato (1971, 1973, 1976) and Sato and Akai (1977). According to them, there are five types of haemocytes present prohaemocyte, plasmatocyte, granulocyte, spherulocyte and oenocytoid, where in present study, two additional haemocytes were found i.e. adipohaemocyte and coagulocyte. Similar observations were made by Bhaisare (2007).The total haemocyte count were carried out from the day of 4thmoult day to 7th day of 5th instar larvae. The total haemocyte count of the silkworm *B. mori*reached to its peak at each moult. Nittono (1960) observed highest cell density (8000/mm3) during the 5th instar and subsequently declined in *Bombyxmori*. In the present study, it was found that during the moult period, total haemocyte count got decreased on 1st day and 2nd day of the fifth instar and again from 3rd day it was increased and on the 5th day highest haemocyte count was found in all the races in present study. In all the races, there was large changes occurred in cell number from day of fourth moult to last day of 5th instar of *B. mori*, and it was 5332.64 ± 147.87/mm^3 to 10048.50 ± 246.78/mm^3 for Kolar gold, 4657.88 ± 465.45/mm^{3}to 10713.50 ± 1723.22/mm^3 for Pure mysore. The cell population changes during moult period were also noted in *Locusta* (Webley, 1951), *Sacrophaga*(Jones, 1956), *Rhondnius* (Wigglesworth, 1955), *Bombyx*(Nittono, 1960), *Periplanteta* (Wheeler, 1963). In *Prodeniaeridania,* total haemocyte count did not vary significantly in sixth instar intermoult period (Rosenberger and Jones, 1960). Yeager (1945) described 10 cell classes and 32 haemocyte types and studied their changes from 5th instar larvae to adult of *Prodeniacridania*. In *Trichoplusiani*, Laigo and Paschke (1996) reported counts varied from 14,000/mm3 to 25000/mm3 with no apparent trend. In *Heliothiszea* larvae, little changes in cell numbers were observed during 6 to 10 period of larval development which was between25000/mm3 to 31000/mm3 (Shapiro *et al.,* 1969). Associated with the changes in total haemocyte count, there were also a change in differential haemocyte count were observed in the *B. mori* in all the races. The maximum spherulocyte count was observed in Nistari, followed by Kolar gold and Pure mysore. On the 4th day of 5th instar larvae of Nistari minimum count of spherulocyte was found on 6th day. In Kolar gold and Pure mysore, the maximum spherulocyte count was observed on 1st day of 5th instar and day of 4thmoult respectively, while the minimum count was observed on 4th day and 5th day of 5th instar larva of Kolar gold and Pure mysore respectively.

In the armyworm, *Pseudaletiaunipuncta*, granulocytes reached to its peak during the 6th instar and then decreased while the plasmatocyte count showed increased trend during 6th instar and also during pupation (Witting, 1965). In the present study granulocytes count was found higher on the day of 4th moult day in Kolar gold and Pure mysore while in Nistari it was higher on 6th day of the fifth instar. The plasmatocytes count observed was maximum in Kolar gold race as compared to the other two races and it was on the 2nd day of 5th instar larvae. In Nistari the plasmatocytes were observed only on 1st day of 5th instar larva.In the present investigation, the highest adipohaemocytes count was observed in Pure mysore than Kolar gold and Nistari. The maximum cell count was observed on 5th day in Kolar gold and Nistari while in Pure mysore it was observed on 2nd day of 5th instar larvae. In Kolar gold on 3rd day adipohaemocytes was not observed, while the minimum cell count was observed on the 5th and 2nd day of Pure mysore and Nistari. In Nistari the coagulocytes were observed only on 5th day of 5th instar, while in Pure mysore maximum count was observed than two races and it was on the 5th day and on the 6th day these cells were not observed. In Kolar gold, maximum coagulocytes number was observed on 5th day and their minimum number was observed on the 2nd day of 5th instar larvae. In larvae of *Pectinophoragossypeilla,* proportions of prohaemocytes, plasmatocyte, adipohaemocytes and coagulocytes varied from instar to instar (Clark and Chandbourne, 1960). He felt that, these changes were due to differential haemocyte functions of food transport, storage, and metabolism during the development of different instars.

Nittono (1960) observed that, in *B. mori*plasmatocytes occurred in high numbers during active growth period of each instar. These cells decreased during 5th instar, increased before adult emergence and attained its maximal value (60-70%) in adults. Granulocytes reached a peak at each moult (60-70%) but get reduced to 10% in adult. Prohaemocyte and spherulocyte observed only in larval stage but not observed after pupation. He examined 301 silkworm strains for larval blood, where in 26 strains spherulocytes were absent and surprisingly these strains produced less silk than those strains having the spherulocyte in their blood. In the present study, the maximum spherulocyte count was observed in Nistari, followed by Kolar gold and Pure mysore. In Kolar gold and Pure mysore, the maximum spherulocyte count was observed on 1st day of 5th instar and on the day of 4thmoult respectively, while the minimum count was observed on 4th day and 5th day of 5th instar larva of Kolar gold and Pure mysore respectively while in Nistari the high count of these cells was found on the 4th day of 5th instar larvae and minimum was found on 6th day. Ling *et al.,* (2005) isolated hemocytes from the hematopoietic organs of fifth larva of *Bombyxmori*, and observed that most these cells were prohemocytes (60%–70%) and oenocytoids (30%–40%). Granulocytes comprised only about 0.5%–1% and no spherulocytes or plasmatocytes were found. In *Helitohiszea* (Shapiro *et al.,* 1969) spherulocyte increased in numbers in 7-9 day old larvae, then get decreased. In *H. virescencens,* spherulocytes increased from 38% in 5th day old larvae to 59% at 8th day and then it gets decreased. The prohaemocytes and plasmatocytes decreased from 5th to 8th day and increased upto pupation. The oenocytoid remained fairly constant upto 1-2% through the duration of the final instar. In the present study, the prohaemocytes were found higher in Nistari than Pure mysore and Kolar gold. The maximum count was observed on the 3rd day in Kolar gold and minimum was on 1st day of 5th instar and in Nistari also on the 3rd day maximum prohaemocyte count was observed but the minimum count was observed on 5th day. In Pure mysore the minimum prohaemocyte count was observed than the other two races. On the 7th day maximum count was observed while the

minimum count was observed on 2nd day. The higher oenocytoid count was observed in Pure mysore on 6th and in Nistari on 4th day. In Kolar gold also the maximum count was observed on 4th day of 5th instar while on 2nd, 6th and 7th day these cells were not observed. In Pure mysore on 2nd and 5th day, while in Nistari only on 5th day oenocytoids were not observed. In *Rhodniusprolixus*, prohaemocytes prior to moult decreased but adipohaemocytes increased. At the moultplasmatocytes and oenocytoids increased and granulocyte decreased (Jones and Liu, 1961).

In *Hylophoracercopia* silkworm plasmatocytes and granulocytes made up more than 90% of the total haemocyte population (Lea 1964). Plasmatocytes were present at high amount in late 5th instar larvae, granulocytes were predominant in 4th and 5th instars, oenocytoids were scarece and spherulocytes occurred at the time of cocoon spinning. In *Tenebriomolitor* larvae, plasmatocytes and granulocytes were the principle haemocytes (Jones, 1950) and differential haemocyte count was not changed as larvae increased in weight. In *D. melanogaster,*plasmatocytes accounted for 90-95% of haemocyte population (Rizki, 1957). Jones (1956, 1967b), studied in detailed haemocyte of the blow fly, *Sacrophagabullata* and observed that prohaemocytes were found in all stages but were rare. Plasmatocytes got decreased as larval development processes whereas granulocytes increased. Haemocytes plays very important roles in defence mechanisms against microorganisms in the haemocoel and this cellular defences refer to haemocyte mediated responses such as phagocytosis, nodulation, encapsulation and haemolymph coagulation (Schmidt *et al*., 2001). The plasmatocyte is the predominant cell type involved in phagocytosis in insects (Salt, 1970) both in vivo (Witting, 1965) and in vitro (Ratcliffe and Rowley, 1975) plays important role in defence against biological agents. The granulocytes of *Galleria mellonella*and *P. brassicae* also have limited phagocytic powers in vitro (Cameron, 1934). However, in *Calopodesethlius* granulocytes were the main phagocytic blood cells and plasmatocytes appear to be non-phagocytic (Neuwirth, 1974). The release of substances from other haemocyte type may, however, coat the foreign particles and facilitate their uptake. Ling *et al.,* (2005) reported that in the *B. mori*least fraction of the prohaemocytes contained within the hematopoietic organs have the capacity to phagocytise the foreign particles. Some prohaemocytes turned into plasmatocytes. When larval hematopoietic organs were cultured in vitro, the newly discharged spherical haemocytes changed into elongated hemocytes after agitation (Nakahara *et al.,* 2003).

These elongated haemocytes were considered to be plasmatocytes according to the standard criteria of hemocyte classification (Nittono, 1960; Beaulaton, 1979; Wago, 1991; Yamashita and Iwabuchi, 2001) and these newly formed plasmatocytes also take part in phagocytosis. In the present study, in Kolar gold the high plasmatocyte and granulocyte count was observed than Pure mysore and Nistari and so that Kolar gold was sturdier than the two. Nistari was having very less plasmatocyte count hence seems to be susceptible to the disease as compared to other two races. This was observed during rearing of these races.Although the haemocyte types in larvae was same in all the races under study but the racial differences were existed in the total and differential cell count. In Kolar gold race plasmatocyte, spherulocyte and oenocytoids were predominant followed by Pure mysore having high resistant power against diseases. While in Nistari minimum numbers of these cells were observed. So Nistari race was found to be susceptible to the diseases during rearing as compared to Kolar gold and Nistari.

Acknowledgement

The authors are thankful to the Head, Department of Zoology, Shivaji University, Kolhapur for providing necessary laboratory facilities and Rajiv Gandhi National Fellowship for providing financial support carry out research work.

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