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SPECIFIC CELL COUNT ESTIMATES IN SOME BREEDS OF SILKWORM, *BOMBYX MORI* L. DURING DIFFERENT SEASONS IN KASHMIR

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ABSTRACT
The present study on six silkworm breeds of *Bombyx mori* L. revealed six types of haemocytes *viz*., Prohaemocytes, Plasmatocytes, Granulocytes, Spherulocytes, and Oenocytoids. The differential haemocyte count indicated higher number of Granulocytes, followed by Plasmatocytes, Prohaemocytes, Spherulocytes and Oenocytoids in all the breeds under study. The number of Granulocytes ranged from 46.37 to 65.50, Plasmatocytes from 20.75 to 26.87, Prohaemocytes from 5.25 to 13.62, Spherulocytes from 2.87 to 12.62 and Oenocytoids from1.12 to 6.62 per cent during spring, while during summer, the DHC ranged from 43.50 to 64.00, 19.37 to 30.62, 5.25 to 13.25, 3.75 to 16.00 and 2.25 to 11.87 per cent for Granulocytes, Plasmatocytes, Prohaemocytes, Spherulocytes and Oenocytoids respectively.

KEY WORDS: Plasmatocytes, Prohaemocytes, Spherulocytes , Haemolymph, seasons, DHC.

INTRODUCTION
The silkworm, *Bombyx mori* L. is an important economic insect which feeds mainly on mulberry leaves and converts the leaf protein into silk protein (Babu *et al.*, 2009). Insect blood or haemolymph is best described as the intracellular circulating fluid that fills the body cavity or haemocoel. The heamolymph circulates freely within the body of the insects bathing different tissues (Jones, 1979). It is physically isolated from direct contact with the body tissues by a thin permeable membrane, which lines the haemocoel (Ashhurst, 1979). In insects, the haemolymph, like the blood of higher animals comprises of two main components, the plasma and the corpuscles or haemocytes (Kerenhap *et al.*, 2005). The circulating cells in the haemolymph are called "haemocytes" which comprise a complex of several types of mesodermal cells, which though nucleated cannot be directly equated with the vertebrate leucocytes (Pandey and Tiwari, 2012). There are three well defined types of haemocytes in most of the insect's namely prohaemocytes, plasmatocytes and granulocytes and one or more of four other types in some insects which include coagulocytes, spherulocytes, adipocytes and oenocytoids (Arnold, 1979 and Jones, 1979). Gupta (1979) investigated the haemocytes of the larvae of *Bombyx mori* L., which were only differentiated as prohaemocytes, plasmtocytes, spherulocutes and oenocytoids. However they also described two subclasses of Prohaemocytes as macronucleocytes and micronucleocytes depending on their size. Ahmad (1988) described the haemocytes of the middle aged larvae of third, fourth, fifth and sixth instar, prepupae, pupae and adults of *Spilosoma obliqua*. These haemocytes were only characterized as prohaemocytes (PR's), Plasmatocytes (PL's), podocytes (PO's), oenocytoids (OE's) and adipocytes (AD's) which were uniformly found in all the stages. But in *Heliothes armigera* larvae PR's, PL's and OE's were common with both *S. litura* and *S. obliqua*. Mohandes *et al.* (2010) evaluated the influence of different feeding diets (pollen grains and pollen substitutes) on the differential haemocyte counts (DHC) in the haemolymph of the newly emerged worker honey bees. Six haemocyte types were observed in the haemolymph of honey bees feeding on these diets. They were classified as prohaemocytes, plasmatocytes, granulocytes, coagulocytes, oenocytoids and binucleated cells.

MATERIALS & METHODS
Haemolymph was obtained by puncturing the abdominal legs with a sterilized surgical blade and collected in eppendorff tubes. Thin and uniform film of haemolymph was prepared on the clean and grease free slides by the edge of another slide. The smear was examined under microscope and 200 cells per slide were differentiated on the basis of their morphological features as described by Nittono (1960) and Gupta (1979). In addition certain cells found in the microscopic field, which could not be identified, were not counted.

RESULTS
Differential (specific) haemocyte counts were carried out to record the population of identified cells as percentage of 200 cells counted in each of the slides prepared for different breeds of silkworm, *Bombyx mori* L. The clearly identified types of haemocytes were Prohaemocytes, Plasmatocytes, Granulocytes, Spherulocytes, and Oenocytoids. The cells which could not be classified under the above types were not considered for the calculation purposes. The results of the study are presented in Table-1 and Table-2.

Differential haemocyte count (%) during spring season

Prohaemocytes

The mean per cent values of the population of prohaemocytes of different multivoltine and bivoltine breeds of silkworm, *Bombyx mori* L was subjected to analysis of variance (one way ANOVA) to determine the difference among all the breeds studied. Significant differences were recorded in the percentage of prohaemocytes in between most of the breeds during spring. During the pooled analysis of the spring data (2011 and 2012), it was revealed that 13.62±1.14 per cent of haemocytes were comprised of prohaemocytes in SH_6 breed which was significantly high from the prohaemocyte percentage of all other breeds except SKAU-R-6 (11.37±0.31 %) with which it was found at par. The lowest percentage of prohaemocyte (5.25±0.47 %) was recorded in SKUAST-28 during the same season. The two multivoltine breeds *viz*., Pure Mysore and Nistari with their respective prohaemocyte population of 10.62 ±1.63 and 6.87±0.23 per cent also differed significantly from each other (Table-1).

Plasmatocytes

During spring, it was found that plasmatocytes comprised of 26.87±0.98 per cent of total cell population in Nistari and 22.37±0.47 per cent in Pure Mysore breeds of silkworm, *Bombyx mori* L. Among the bivoltine races, highest population was recorded in SKUAST-28 (25.25±0.25 %) and the lowest in SH_6 (20.75±0.47 %). One way ANOVA was carried out in between plasmatocyte per cent values of different silkworm breeds and the decrease or increase of mean population percentage was recorded to be significant between Pure Mysore and Nistari, Pure Mysore and NB_4D_2, Pure Mysore and SKUAST-28, Nistari and SH_6, Nistari and SKAU-R-6, NB_4D_2 and SH_6, SH_6 and SKAU-R-6, SH_6 and SKUAST-28. Differences were non-significant between Pure Mysore and SH_6, Pure Mysore and SKAU-R-6, Nistari and NB_4D_2, SKUAST-28 and

TABLE 1: Differential haemocyte count (DHC) in different breeds of silkworm, *Bombyx mori* L. during spring seasons (Data pooled over same seasons of two years 2011 and 2012)

Race	DHC (%) ± S.E				
	Prohaemocytes	Plasmatocytes	Granulocytes	Spherulocytes	Oenocytoids
Pure Mysore	10.62±1.63 (3.375)	22.37±0.47 (4.834)	50.37±3.79 (7.153)	11.00±2.24 (3.407)	5.50±0.73 (2.536)
Nistari	6.87±0.23 (2.805)	26.87±0.98 (5.277)	58.50±1.09 (7.713)	5.00±1.02 (2.426)	2.62±0.55 (1.887)
NB_4D_2	10.62±0.42 (3.408)	25.12±1.84 (5.102)	49.62±1.73 (7.112)	9.62±0.47 (3.257)	4.50±0.20 (2.344)
SH_6	13.62±1.14 (3.815)	20.75±0.47 (4.663)	46.37±1.39 (6.881)	12.62±0.82 (3.686)	6.62±0.58 (2.753)
SKAU-R-6	11.37±0.31 (3.517)	23.75±0.14 (4.975)	55.87±0.42 (7.541)	7.62±0.96 (2.924)	2.37±0.42 (1.826)
SKUAST-28	5.25±0.47 (2.495)	25.25±0.25 (5.123)	65.50±0.45 (8.139)	2.87±0.55 (1.955)	1.125±0.12 (1.456)
$C.D_{(p\leq0.05)}$	0.387	0.265	0.390	0.586	0.337

Each value is the mean ± S.E of four replications

Figures in parenthesis represent square root transformed values

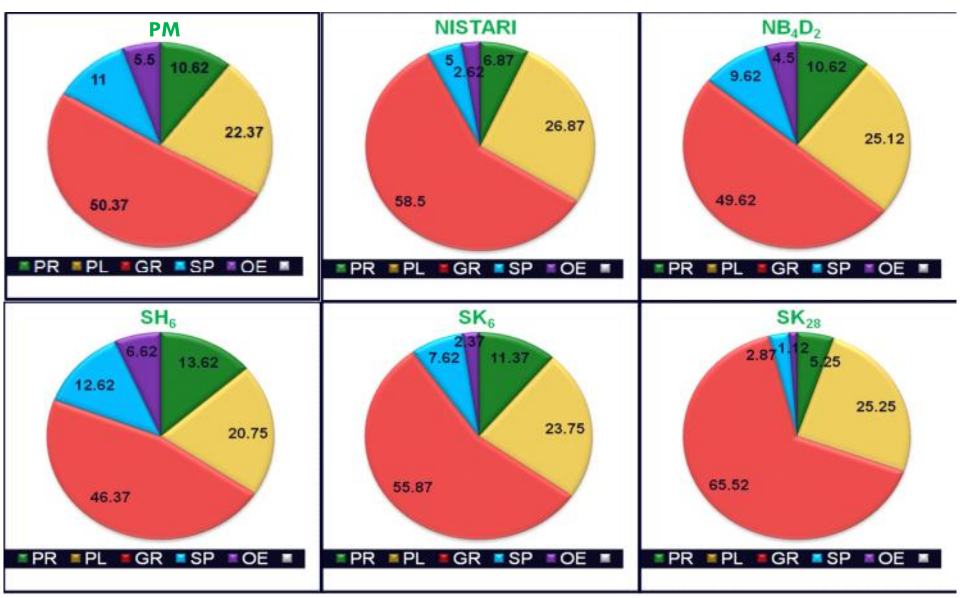

FIGURE 1: Differential Haemocyte count in different breeds of silkworm (*Bombyx mori* L.) during spring

Nistari, NB_4D_2 and SKAU-R-6, SKUAST-28 and NB_4D_2 (Table-1).

Granulocytes

The differential haemocyte count (DHC) analysis of different silkworm, *Bombyx mori* L breeds during the present study indicated higher number of granulocytes among all the cell types observed. During spring the per cent population of granulocytes was recorded significantly high (65.50±0.45 per cent) in SKUAST-28 whereas the lowest percentage of granulocytes (46.37±1.39 %) was recorded in SH_6. Among the multivoltine breeds, Nistari recorded 58.50±1.09 per cent population of granulocytes which was found significantly different from the granulocyte population of NB_4D_2 (49.62±1.73 %) and SH_6 (46.37±1.39 %) respectively, whereas Pure Mysore with 50.37±3.79 per cent population of granulocytes was found statistically different from that of Nistari (58.50±1.09 %) and SKAU-R-6 (55.87±0.42 %). NB_4D_2 differed significantly from SKAU-R-6 and SKUAST-28 and also the difference between the two i.e SKAU-R-6 and SKUAST-28 was found significant (Table-1).

Spherulocytes

The studies on the per cent population of spherulocytes in different silkworm breeds during spring revealed that mean per cent population of spherulocytes was significantly higher in SH_6 (12.62±0.82%), which was significantly different from the spherulocyte population of Nistari, SKAU-R-6 and SKUAST-28. Pure Mysore with spherulocye population of 11.00±2.24 per cent differed significantly from the per cent population of spherulocytes of Nistari (5.00±1.02%). SKUAST-28 which recorded lowest per cent population of spherulocytes (2.87±0.55%) differed significantly from the spherulocyte population of SKAU-R-6 (7.62±0.96%), NB_4D_2 (9.62±0.47%) and Pure Mysore (11.00±2.24%) but was found at par with Nistari (Table-1).

Oenocytoids

While carrying out the differential haemocyte count analysis of different breeds of silkworm, *Bombyx mori* L, and scanty percentage of population of oenocytoids was recorded. Among the multivoltine breeds per cent population of oenocytoids existed to the tune of 5.50±0.73 and 2.62±0.55 per cent in Pure Mysore and Nistari breeds respectively during the spring season. The per cent population of oenocytoids was recorded highest (6.62±0.58%) in SH_6 and the lowest percentage of oenocytoids (1.125±0.12%) was recorded in SKUAST-28 during the same season. NB_4D_2 and SKAU-R-6 recorded oenocytoid population of 4.50±0.20 and 2.37±0.42 per cent respectively (Table-1).

Statistical analysis of the data revealed that the difference in the mean values of the oenocytoid percentage in the haemolymph was observed to be significant in between Pure Mysore and Nistari, SKAU-R-6 and Pure Mysore, SKUAST-28 and Pure Mysore, SKUAST-28 and Nistari, NB_4D_2 and Nistari, Nistari and SH_6, NB_4D_2 and SH_6, NB_4D_2 and SKAU-R-6, SKUAST-28 and NB_4D_2, SH_6 and SKAU-R-6, SKUAST-28 and SH_6, SKAU-R-6 and SKUAST-28, however non-significant differences were also observed in between NB_4D_2 and Pure Mysore, SH_6 and Pure Mysore, Nistari and SKAU-R-6 with respect to the mean per cent values of oenocytoid population.

In the present investigation on differential haemocyte count, it was observed that higher number of granulocytes followed by plasmatocytes, prohaemocytes, spherulocytes and oenocytoids was recorded in all the breeds under study during spring (Fig-1).

Differential Haemocyte Count (%) during summer season

Prohaemocytes

During summer, NB_4D_2 was found to record higher percentage of prohaemocytes (13.25±0.85%) followed by SKAU-R-6 (12.25±1.61%), SH_6 (11.75±11.10%), Pure Mysore (8.50±1.02%), Nistari (7.87±0.71%) and SKUAST-28 (5.25±0.32 %) respectively. NB_4D_2 was found statistically superior to Pure Mysore, Nistari and SKUAST-28 with respect to per cent prohaemocyte population. Significant difference was also observed in between Nistari and SKUAST-28. However, no significant differences were recorded in between Pure Mysore and Nistari, NB_4D_2 and SH_6, SH_6 and SKAU-R-6, NB_4D_2 and SKAU-R-6 (Table-2).

TABLE 2: Differential haemocyte count (DHC) in different breeds of silkworm, *Bombyx mori* L. during summer seasons (Data over same seasons of two years 2011 and 2012).

Race	DHC (%)±S.E				
	Prohaemocytes	Plasmatocytes	Granulocytes	Spherulocytes	Oenocytoids
Pure Mysore	8.50±1.02 (3.069)	30.25±1.70 (5.584)	56.62±0.74 (7.591)	3.75±0.77 (2.159)	2.25±0.43 (1.792)
Nistari	7.87±0.71 (2.980)	30.62±0.31 (5.623)	54.00±1.13 (7.432)	4.95±0.12 (2.425)	2.56±0.18 (1.885)
NB_4D_2	13.25±0.85 (3.770)	24.37±2.24 (5.023)	47.12±1.80 (6.934)	9.00±0.61 (3.158)	5.62±0.12 (2.574)
SH_6	11.75±1.10 (3.561)	19.37±0.87 (4.511)	43.50±2.34 (6.664)	16.00±1.78 (4.107)	11.87±2.57 (3.586)
SKAU-R-6	12.25±1.61 (3.617)	20.75±0.25 (4.663)	51.37±0.37 (7.237)	9.75±0.32 (3.278)	4.62±0.12 (2.371)
SKUAST-28	5.25±0.32 (2.497)	23.87±0.55 (4.987)	64.00±1.88 (8.060)	4.62±0.71 (2.357)	2.50±0.61 (1.848)
$C.D_{(p\leq 0.05)}$	0.457	0.362	0.324	0.412	0.275

Each value is the mean±S.E of four replications

Figures in parenthesis represent square root transformed values

Plasmatocytes

During summer, gradual increase as compared to spring in the population percentage of plasmatocytes of multivoltine races was observed and it was recorded as 30.62±0.31 and 30.25±1.70 per cent in Nistari and Pure Mysore respectively, though the difference between the two was non-significant. In the larvae of bivoltine races, the percentage of plasmatocytes went down, however NB_4D_2 recorded the highest plasmatocyte population of 24.37±2.24 per cent which was significantly higher from SH_6 (19.37±0.87%) and SKAU-R-6 (20.75±0.25%) but at par with SKUAST-28 (23.87±0.55%) (Table-2).

Granulocytes

Pooled analysis of the summer data of 2011 and 2012 revealed that mean per cent population of granulocytes was significantly high in SKUAST-28 (64.00±1.88%) and low in SH_6 (43.50±2.34%). Among the multivoltine breeds, Nistari recorded the granulocyte population of 54.00±1.13 per cent, whereas in Pure Mysore, it was recorded 56.62±0.74 per cent, though the difference between the two was found non-significant. Among the tropical bivoltine breeds NB_4D_2.

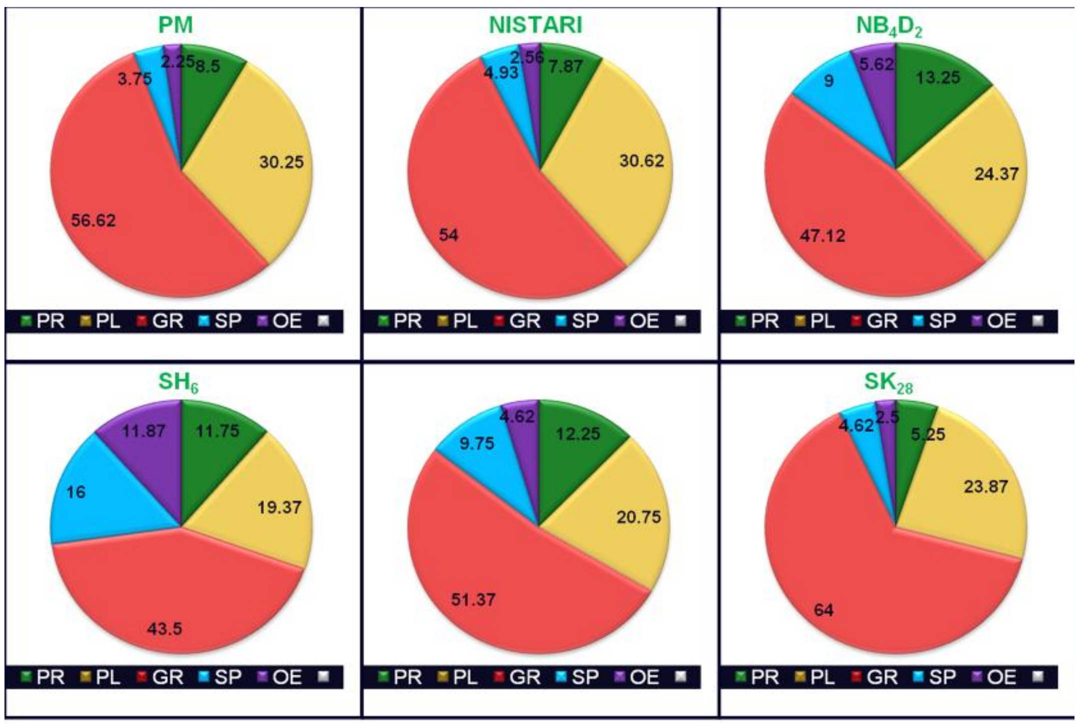

FIGURE 2: Differential Haemocyte count in different breeds of silkworm (*Bombyx mori* L.) during summer

Recorded granulocyte population of 47.12±1.80 per cent, whereas among the temperate bivoltine breeds of silkworm, SKAU-R-6 recorded 51.37±0.37 per cent population of granulocytes during the same season. Significant differences were recorded in between Pure Mysore and NB_4D_2, Pure Mysore and SH_6, Pure Mysore and SKAU-R-6, Pure Mysore and SKUAST-28, NB_4D_2 and Nistari, SH_6 and Nistari, SKUAST-28 and NB_4D_2, SH_6 and SKAU-R-6, SKUAST-28 and SH_6, SKAU-R-6 and SKUAST-28 with respect to their respective per cent population of granulocytes (Table-2).

Spherulocytes

During summer Spherulocyte population was found significantly higher in SH_6 (16.00±1.78%), followed by SKAU-R-6 (9.75±0.32%), NB_4D_2 (9.00±0.61 %), Nistari (4.95±0.12%), SKUAST-28 (4.62±0.71%) and the low mean per cent of spherulocyte population was recorded in Pure Mysore (3.75±0.77 %). Statistical analysis of the data revealed that Pure Mysore was significantly different from NB_4D_2 and SKAU-R-6 but at par with Nistari and SKUAST-28. Nistari differed significantly from NB_4D_2, SKAU-R-6. Likewise NB_4D_2 differed from SKUAST-28 but was at par with SKAU-R-6. Significant differences were also observed in between SKAU-R-6 and SKUAST-28 with respect to the per cent population of spherulocytes (Table-2).

Oenocytoids

During summer significantly high percentage of oenocytoid population was recorded in SH_6 (11.87±2.57%), whereas low per cent value (2.25±0.43%) was registered in Pure Mysore. NB_4D_2 with 5.62±0.12 per cent population of oenocytoids was the second best and was found significantly superior to Pure Mysore. The differential oenocytoid count registered in other breeds during the same season include: Nistari (2.56±0.18%), SKAU-R-6 (4.62±0.12%) and SKUAST-28 (2.50±0.62%) (Table-2).

During summer, DHC analysis revealed higher number of granulocytes followed by plasmatocytes, prohaemocytes, spherulocytes and oenocytoids in all the breeds under study except SH_6 in which granulocytes were followed by plasmatocytes, spherulocytes, oenocytoids and prohaemocytes (Fig-2).

DISCUSSION

In the present study five types of haemocytes viz., Prohaemocytes, Plasmatocytes, Granulocytes, Spherulocytes and Oenocytoids were observed in the haemolymph of silkworm, *Bombyx mori* L. races under investigation (Table-1 & 2). Similar findings have been reported by Akai and Sato (1973), Balavenkatasubbaiah *et al.* (2001), Ling *et al.* (2003) and Kerenhap *et al.* (2005) in *Bombyx mori*. However, Nittono (1960) has classified the blood cells in the silkworm, *Bombyx mori* into six types, *viz*., prohaemocytes, plasmatocytes, granulocytes, spherulocytes, imaginal spherulocytes and oenocytes., while Han *et al.* (1998) reported four types of haemocytes; prohaemocytes, plasmatocytes, granulocytes, and oenocytes in the larvae of *Bombyx mori* L. These findings have slight variation with the present findings due to the difference in the growth turnover of the cells. Some work has also been carried out by some researchers on the haemocytes of other insects like that of silkworm *Antheraea pernyi* in which five types (prohaemocytes, plasmatocytes, granulocytes, spherulocytes and oenocytoids) of haemocytes were reported by Beaulaton and Monpeyssin (1976) and by Bardoloi and Hazarika (1995) in *Antheraea assama*. While scanning the literature, it was found that seven types of haemocytes like prohaemocytes, plasmatocytes, granulocytes, spherulocytes, adipocytes, coagulocytes and oenocytoids have been reported by Gupta (1985) in lepidopteran insects and six types namely prohaemocytes, plasmatocytes, granulocytes, spherulocytes, adipohaemocytes and oenocytoids have been reported in the lepidopteran larvae of *Papilio demoleus* L. by Jalali and Salehi (2008). Six types of haemocytes *viz*., prohaemocytes, plasmatocytes, granulocytes, coagulocytes, oenocytoids and binucleated cells were also observed in the haemolymph of newly emerged worker bees, *Apis mellifera* L. by Mohandes *et al.* (2010). Patil and Shah (2011) also found seven distinct types of haemocytes *viz*., prohaemocytes, plasmatocytes, granulocytes, spherulocytes, adipocytes, oenocytoids and coagulocytes in Scorpion, *Mesobuthus tumulus tumulus.* These findings are by and large in conformity with the present findings with slight variations and such a variation could be attributed to the racial character.

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