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Differential HaemocyteCount (DHC) in Silkworms Bombyx Mori During Pathogenic Diseases in Akola District (MS)

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Abstract

Differential haemocyte count was studied in Silkworm Bombyx mori larvae infected by pathogenic diseases Grasserie and Flacherie in Akola district. DHC in silkworms infected with, Grasserie, and Flacherie recorded significant changes in the 5 cellular types [Prohaemocytes (PR), Plasmatocytes(PL), Granular cells(GR), Spherule cells(SP), and Oenocytoids (OE)] at early infection I.e. on second daywith Grasserie and with Flacherie as compared to control healthy non infected worm of the same developmental stage.However, late infection i.e. on day five of the 5th instar larva, these diseases led to a more significant changes in DHC as compared to non infected healthy silkworms of the same developmental stages. The values obtained for the DHC of the 5 cellular types in Grasserie infected silkworm larvae, with the relative frequency of these cells determined as (PR> GR> SP> PL> OE), in Bacterial Flacherie infected silkworm larvae, with the relative frequency of these cells were recorded as (PR> GR> SP> PL> OE) and the values obtained for the DHC of the 5 cellular types in Viral Flacherie infected silkworm larvae, with the relative frequency of

these cells determined as (PR> GR> SP> PL> OE) respectively. The results indicating that these cells respond quickly to thepathogenic diseases at both early and late infections and also pointed to the effective role of the hemocytes in the response of Silkworm to Grasserie, and Flacherie pathogens.

Keywords: Silkworms, Bombyx mori, Instar larvae, Grasserie, Flacherie.

Introduction:

Akola is a district in the Indian state of Maharashtra (Vidarbha) with an area of 5,431 km^{2}lie on Latitude 20° 42' 10.59"N Longitude 76°59'57.97" E Altitude 285 m and is largelya fertile plateau. Though the district is well known as cotton district, farmers in manyareas are diverted in opting rearing of mulberry silkworm, using CSR2 and Kolar goldbreeds of silkworm Bombyx mori, and taking commercial crops round the year with smallsericulture units in the farms. Continuous domestication, year after year leads toperpetuation of diseases in the silkworm, causing a great economic loss. Pathogenicdiseases like Grasserie, Flacherie and Muscardine are common and are vary seasonally inVidarbha region (Rashmi et al., 2013). High temperature and the dry climatic conditionsof the district are conductive to the incidence of these diseases. The pathogens causing Grasserie and Flacherie, usually enter per orally through the contaminated food in to thealimentary canal and penetrate midgut wall and then to the haemolymph. Haemolymph isthe first line of pathogenic attack through midgut. Therefore study of Haemolymph issignificant in monitoring and control of diseases at early stages. Hemocytes, inHaemolymph, carry out many essential physiological activities and are chief mediators ofcellular defense response (Nahla et al., 2010). Hemocytes responses during pathogenicattack in insect are good indicators of host-pathogen interaction (Da Silva et al., 2000;Gillespie et al., 1997).Insect haemolymph compared with mammalian blood differs in the lack of erythrocytes and has an elevated concentration of numerous types of free amino acids. It serves significant roles in the immune system and in transport of hormones, nutrients, and metabolites (Sairamalinga et al., 2012). Various changes occur in the biochemical composition of haemolymph during incidence of diseases. These changes be a sign of the molecular and physiological disturbances in the body of the insect, in response to attack by the diseased pathogens. Hemocytes, the major constituents in haemolymph, move and perform various physiological functions in the body of insect including defense against the pathogen and toxins in the body. Their variety and population index is very important. The most abundant haemocyte types typically described in Lepidopteron larvae are granular cells and plasmatocytes, which are capable of adhesion and phagocytosis of pathogenic agents. (Levine and Strand, 2002). Nonadherant haemocyte types include oenocytoids, which synthesize prophenoloxidase, and spherule cells, whose functions are poorly understood. Monoclonal antibodies are very useful reagents for distinguishing lepidopteron haemocyte populations based on antigenicity rather than morphology, (Willott et al., 1994) which can vary considerably, especially for plasmatocytes.

The investigation of changes in either the total or part of insect haemocytes is a appropriate system for studying effects of infectious disease. The diseased condition induces an irreversible cytopoiesis of the host haemocytes, at the same time either inactivating or inhibiting the synthesis of bacteriolytic cationic proteins in the haemolymph of immune insects. Haemocytes of some caterpillars exposed to the effects of sterile homogenates of some insect pathogens shows that either bacteriolytic proteins are not produced at all, or the amount of synthesized proteins is much lower than in controls. The ability of haemocytes to induce synthesis of bacteriolytic proteins could be a reliable test of the functions of infected haemocytes of insects.

Material Methods:

For differential count haemolymph smear slides were prepared, as per the procedure, of Jalal and Rasoul Salehi (2010) a small drop of heat–fixed haemolymph was obtained by clipping of the pro leg present on the abdominal segment of the larva or piercing the cuticle of the pupa. The drop was then drawn into a thin film by the edge of another slide and the film air–dried before staining. For staining, the stock solution of Giemsa stain prepared by the method of Yeager (1945) was diluted 10 times with distilled water. The air dried smear was stained with the diluted stain for 20 minutes and subsequently differentiated in dilute lithium carbonate solution for red staining structures and then in HCL acidified distilled water for blue staining structures. The slide was rinsed in distilled water and mounted in DPX. To determine the DHC, cell categories were counted in 200 cells chosen from random areas of the stained haemolymph smear.

Results and Discussion:

During differential count studies, we found 5 populations of haemocytes observed in the haemolymph of the silkworms. They are Prohaemocytes, Plasmatocytes, granular cells, spherule cells, and oenocytoids found in silkworm, which were as follows: (Micrograph I)

1) Prohaemocytes (PR): They appeared small, circular and globular in shape. Each cell has a large and centrally positioned nucleus, which is surrounded by a small quantity of cytoplasm. In smears stained with Geimsa, nucleus is acidophilic stained pink almost filling the cell and very slender peripheral basophilic bluish cytoplasm about the nucleus(Micrograph I-1)

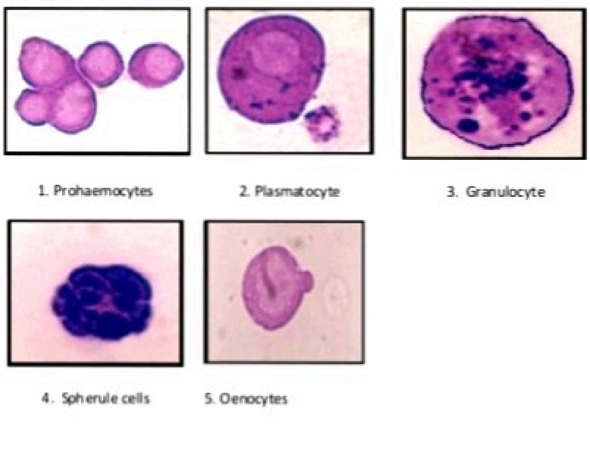
2) Plasmatocytes (PL): The Plasmatocytes appeared polymorphic (round, vermiform, fusiform, and spindle shaped) and changeable in size, generally ovoid or spindle shaped with a basophilic blue cytoplasm. The nucleus was round or elongated and is often centrally positioned. On occasion numerous and large vacuoles are present in Plasmatocytes. (Micrograph I-2)

3) Granular cells (GR): They illustrated dense granules in the cytoplasm. GR disintegrate very rapidly on contact with glass slide. Neither the GR spread like Plasmatocytes on glass surface nor show cytoplasmic Nucleus is comparatively small, compact and centrally located. In smears stained with Geimsa, the cytoplasm look pale blue with faintly pink colored granules, and nucleus is stained pink .The degranulated GR infrequently classed as Coagulocytes (CO). (Micrograph I-3).

4) Spherule cells (SP): Spherule cells appeared rough in shape with variable sizes. The cytoplasm has many small spherules or few large spherules. Nucleus is rather small, central or eccentric. In smears stained with Geimsa, the spherules are weakly basophilic and nucleus stained pink. The cell with large and abundant spherule has nucleus almost concealed. (Micrograph I-4)

5) Oenocytes (OE): The OE (also called as oenocytoide) observed to be large cells, with nucleus usually small in size but bigger than granulocytes and spherule cells, and generally eccentric. In smears stained with Geimsa, cytoplasm is basophilic. (Micrograph I-5).

Micrograph-I : Normal Haemocytes in silkworm



1. Prohaemocytes 2. Plasmatocyte 3. Granulocyte

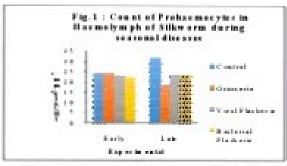
4. Spherule cells 5. Oenocytes

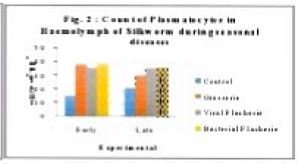
Table 1: Differential Haemocyte Count in Haemolymph of Silkworm during seasonal diseases.

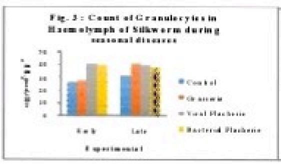
Haemocytes	Differential Count (DHC) during 2010-11 (THCx10³/mm³)							
	Healthy Control		Grasserie Infection		Bacterial Flacherie Infection		Viral Flacherie Infection	
	Early	Late	Early	Late	Early	Late	Early	Late
Prohaemocytes	24.5 ±1.29	32 ±1.39	24.29 ±1.50	18.66 ±2.70	23.27 ±1.40	23.65±2.20	22.55 ±2.60	23.65 ±2.90
Plasmatocytes	14.25 ±1.71	20.23 ±1.41	37.42±3.6	29.84±1.61	34.58 ±1.41	35.22±1.21	38.29 ±1.11	35.59 ±1.11
Granulocytes	26 ±1.83	31 ±1.88	27 ±1.33	41 ±1.53	41 ±1.63	40 ±2.93	40 ±1.23	38 ±1.43
Spherulocytes	19 ±0.82	22 ±0.62	19 ±1.23	35 ±1.79	19 ±0.39	38 ±0.79	20 ±0.59	35 ±1.89
Oenocytes	4.75 ±3.22	9.35 ±2.92	5.67 ±0.75	4.17 ±1.92	6.67 ±1.52	4.67 ±1.92	5.07 ±1.73	4.67 ±1.52

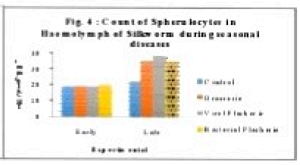
DHC during Grasserie, Bacterial Flacherie and Viral Flacherie: (Table: 1: Fig.1-5)

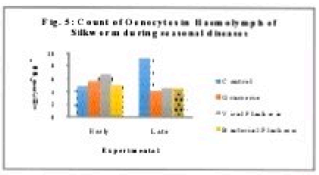
The values obtained for the DHC (Table 1) of the 5 cellular types in Grasserie infected silkworm larvae, with the relative frequency of these cells determined as (PR> GR> SP> PL> OE), in Bacterial Flacherie infected silkworm larvae, with the relative frequency of these cells were recorded as (PR> GR> SP> PL> OE) and the values obtained for the DHC of the 5 cellular types in Viral Flacherie infected silkworm larvae, with the relative frequency of these cells determined as (PR> GR> SP> PL> OE).











Insect haemocytes respond to internal changes during development (at ecdysis) and to conditions such as starvation, wounding, parasitism, diseases, chemicals including insecticides. Ayesha and Khowaja (2009) said that information of normal haemocytes of an insect is necessary to physiologists, toxicologists and biochemists, as alterations in structure, types and number of cells reflects changes in physiological and biomolecular processes. As defensive agents haemocytes migrate towards and engulf several targets such as apoptotic bodies, cell debris from damaged tissues and pathogens (Wood and Jasinto, 2007).

Balavenkatasubbaiah and Nataraju (2005) and Krenhap et al., (2005) reported increase in THC, DHC and period of survival of haemocytes with regard to granulocytes, plasmatocyte and spherulocytes under normal condition and during infective BmNPV infection that causes Grasserie. The present study as Prohaemocytes (PR) and oenocytoides (OE) decreased in number whereas the number of plasmotocytes (PL), granulocytes (GR), and spherule cell (SP) increased as the experimental diseases progresses.

Balavenkatasubbaiah et al., (2001), reported that during the progressive infection, there was gradual decrease in the prohaemocyte. The decrease in prohaemocyte count might be due to the conversion of prohaemocytes to other types of haemocytes that is required for defensive mechanism.In Lepidoptera, granulocytes and plasmatocytes are the only haemocytes types reported to be phagocytic (Jiravanichpaisal 2006). These results may be due to the important role of plasmatocytes and granulocytes in the insect immunity against viral and bacterial infection causing the experimental diseases. Maryam et al.,(2013) suggested that these cells have a major role in the immune system of this insect against foreign particles. Results reported in the present work therefore indicated that plasmotocytes and granulocytes of silkworm also played a role in phagocytosis against invaded pathogens of Grasserie and Flacherie and their toxins. According to study of Zibaee et al., (2011) in S. litura larvae, granulocytes and plasmatocytes were the most sensitive haemocytes to toxins, and on exposure contrastingly the proportion of plasmatocytes decreased while the proportion of granulocytes increased. Other studies showed that toxin led to a decrease in the proportion of plasmatocytes and spherule cells while the proportion of granulocytes increased in S. litura larvae (Sharma et al., 2003, 2008). In Agrotis ipsilon, exposure to toxins decreased the total number of haemocytes, increased the proportion of plasmatocytes, granulocytes, and spherule cells, and slightly decreased the proportion of prohemocytes (Nahla et al., 2009).

Five common populations of haemocytes viz., Prohaemocytes (PR), Plasmatocytes (PL), Granular cells (GR), Spherule cells (SP), and Oenocytes (OE) were characterized in the haemolymph of larvae of silkworm B. mori during incident of diseases like Grasserie, viral Flacherie and bacterial Flacherie. The observation in the present work reported that with progress of experimental disease Grasserie, viral and bacterial Flacherie, there was decrease in the count of Prohaemocytes (PR), and oenocytes (OE), while there was an increase in the number of plasmotocytes (PL), granulocytes (GR), and spherule cell (SP), such variations indicating that these cells have a key role in the interaction against pathogens or foreign particles. Such interactive defensive activities of silkworm with that of pathogenicity of studied diseases therefore depend upon the DHC of haemolymph.

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