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A Study on the Haemocytes Profile of *Spodoptera mauritia* Boisd. (Lepidoptera: Noctuidae)

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ABSTRACT

Larval and pupal haemocytes of Spodoptera mauritia (Lepidoptera:Noctuidae) are presented and classified based on morphological characteristics. Haemolymph sample collected from fourth larval stage to pupal stage were observed using light microscopy. Six general haemocytes: Plasmatocytes (PLs), Granulocytes (GRs), Prohaemocytes (PRs), Spherulocytes (SPs), Oenocytoids (OEs), and Adipohaemocytes (ADs), besides two additional haemocytes, Podocytes (Pos) and Vermicytes (VEs) were observed. PR was smallest with large nucleus, PLs was polymorphic and abundant. The GRs was abundant with cytoplasmic granules, SPs was large with spherules. The ADs was within variable size and shape, OEs was large cell with large nucleus. The POs was triradiate with three cytoplasmic extension and VEs was very elongate with elongated anterior and posterior ends. Total and differential haemocytes count (THC & DHC) was also calculated in present investigation.

Key words: *Noctuidae, DHC,THC*

INTRODUCTION

Haemocyte science is a very vast, evergreen and interesting subject for scientific community. The ability to isolate and identify haemocytes is essential for studies in insect cellular immunity. For last few decades, research on insect haemocyte has received much attention because they are the cells that mediate insect cellular immunity. Further, it is regarded as an excellent model system for the study of cell development, differentiation and communication.

Eclosion to adult is an important event in the life of insects and this is accompanied by a number of internal and external changes in the haemolymph. It is well established that the haemolymph picture is one such internal phenomenon and insect haemocytes respond to such changes during development. The number and type of haemocytes vary with insect species, developmental stage and physiological state. Generally, insect haemocytes are categorised into several types: Prohaemocytes (PRs), Plasmatocytes (PLs), Granulocytes (GRs), Spherulocytes (SPs), Oenocytoids (OEs) and Adipohaemocytes (ADs). Besides, Vermicytes (VE) and Podocytes (PO) were occasionally observed in specific stage of insect life[1,2]. Our present knowledge of insect haemocytes is limited to studies of not more than 200 insect's species in about 100 genera[3]. Haemocytes have been studied mostly in Lepidoptera, Hymenoptera, Coleoptera, and Diptera[4]. There is an inherent variability of haemocytes within a species as well as among closely related species[5,6].

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The haemocytes perform various physiological functions in the body of insect. They direct nutrients to various tissues and store them, perform phagocytosis, encapsulation of foreign bodies in the insect body cavity, coagulation to prevent loss of blood, nodule formation, transport of food materials, hormones and detoxification of metabolites and biologically active materials[7]. Haemocytes migrate towards and engulf several targets such as apoptotic bodies, cell debris from damaged tissues and pathogens[8]. Jones[9] suggested that haemocytes of insects are comparable in their morphology, embryonic origin, amoeboid movements and phagocytic activity to white blood cells of mammals. Knowledge 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 biochemical processes. Although, in last several years, a lot of research has been conducted on haemocytes, but the haematological profile of a major sporadic pest of paddy, the rice swarming caterpillar *Spodoptera mauritia* remains unexplored. Hence, in the present context, this study has been carried out to evaluate the haemocyte profile, in order to characterize the cells based on their morphology and further, for quantification of haemocytes through differential and total count in *S. mauritia*.

MATERIALS AND METHODS

Collection and maintenance of insect culture: The adult moths of *S. mauritia* were collected at night using fluorescent lights. They were kept in glass beakers covered with muslin cloth and were fed with a dilute solution of honey and maintained at the Laboratory of Entomology, Department of Applied Zoology, University of Calicut under laboratory condition of 28 $\pm$ 2^{o}C, RH90 $\pm$ 30% under 12 hour light: 12 hour dark photoperiod. They were allowed to lay eggs and the larvae hatched out after 3-4 days. The larvae were reared in plastic tubs, fed with fresh leaves of paddy plants or leaves of the grass *Ischaemum aristatum*.

The experimental animals, 4th instar larvae (day 1 and day 2) to 6th instar larvae and pupae were taken from the laboratory stock colony reared and maintained as described above. The age of the larvae were abbreviated to day'n' where day'0' denotes the day of ecdysis to a particular instar. Larvae showing synchronous development from single egg mass were utilized for various experiments in order to avoid variation on the intermoult duration. 10 numbers of various instars day zero larvae were used for the study.

For blood smear slide preparation, a small drop of hemolymph was obtained by clipping of the proleg present on the 7th 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[10] 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 blood smear.

To study total hemocyte counts (THC), the hemolymph was drawn into a Thoma white blood cell pipette up to 0.5 marks and diluted up to the 11th mark with tauber–yeager fluid[11]. The pipette was then shaken for several minutes and the first three drops were discarded. A double line with improved Neubauer ruling Hemocytometer was filled with diluted hemolymph and the hemocytes counted in its four corner and one central (1mm^2) squares under a microscope (Olympus, Japan). If the distribution of cells in all the squares were not even, the sample was discarded. The number of circulating hemocytes per cubic millimetre (mm^3) was calculated using the following formula of Jones[9].

$$\frac{\text{Hemocytes in five } 1\text{mm}^2 \times \text{Dilution} \times \text{Depth factor of chamber}}{\text{No. of squares counted}}$$

Statistical Analysis

All data were presented as means ± SD and Standard error (SE) by using SPSS version 16.0.

RESULTS

Haemocyte types of *Spodoptera mauritia*

The haemocytes types were identified by using established morphological characters presented by Jones[9] and Gupta[4].

1. Plasmatocytes: Highly polymorphic cells with variable size are identified. Majority of the cells is spindle shaped. Bean and barrel shaped plasmatocytes are also present. When observed under light microscope, the nucleus was round or elongate and is centrally located. The majority of plasmatocytes were mononucleated.(Plate: 1; Fig. 1, 2, 3)
2. Granulocytes: These are round, oval in shape. The nucleus is round and centrally located. Cytoplasm is filled with immense number of small granules. (Plate : 1; Fig. 4)
3. Prohaemocytes: These are small, round cells. A thin layer of cytoplasm surrounds a centrally located nucleus and the nucleus almost fills the cell. The cytoplasm is normally homogenous, lacking granules or vacuoles.(Plate :1; Fig. 7)
4. Oenocytoid: These are large cells, may be oval, or elongated in shape, with occasional rounded form. The nucleus is often eccentrically positioned.(Plate:1; Fig. 8)
5. Spherulocytes: These are oval cells and packed with large spherical granules,which tend to obscure the cytoplasm and the nucleus.(Plate : 1; Fig. 9)
6. Adipohaemocytes: These are small to large, spherical or oval cells. Compared with that of plasmatocytes, the nucleus is relatively small, rounded or elongate and is mostly eccentrically located. The cytoplasm contains small fat and lipid droplets and vacuoles.(Plate:1; Fig. 5)
7. Vermicytes: A vermiform cell was fusiform in shape. The nucleus was elongated and centrally located; also, these cells possessed elongated anterior and posterior ends.(Plate:1; Fig. 6)
8. Podocytes: Many filamentous and lamelliform processes separate out from these haemocytes. Normal number of their cytoplasmic extensions is three.(Plate:1; Fig. 10,11,12)

Differential Haemocyte Count (DHC) of *Spodoptera mauritia*

A cytological approach was attempted to evaluate the variation in the total number of haemocytes present in the developmental stage of *S.mauritia.* THC was performed during the metamorphosis stage from fourth instar to pupal period.

In fourth larval stage, plasmatocytes maintain their predominance, followed by granulocytes. Number of prohamocytes, vermicytes and podocytes were significantly higher in fourth larval stage. The population of oenocytoids, spherulocytes are always in lower frequencies (Table:1; Fig. A,C,D,E).

Granular haemocytes are the most abundant cells in fifth larval stage. A general decline in the plasmatocytes was noticed when compared to fourth instar.Major increase in the population of spherulocytes and also in number of oenocytoids was observed. The noticeable feature was, that adipohaemocytes starts to elevate in fifth larval stage i.e., day 1 onwards (Fig. B,D,G,H).

In sixth instar, granular haemocytes maintain their predominance, followed by spherulocytes, oenocytoids and adipohaemocytes. The population of plasmatocytes, prohaemocytes, podocytes and vermicytes were less abundant. In late sixth instar, drastic reduction in plasmatocytes and prohaemocytes with an increase in adipohaemocytes was noticed. Oenocytoids and spherulocytes significantly increase in this stage. Podocytes and vermicytes are always marginal in number (Fig. A- H).

Total haemocyte count (THC)

THC was significantly higher in sixth larval stage as when compared to that of early larval stages. The mean value of THC were 12,32000 cells/mm^3 (day 0),12,54000 cells/mm^3 (day 1), 13,86000 cells/mm^3 (day 2), 14,74000 cells/mm^3 (day 3) respectively.In fifth larval instar, the THC range considerably form a value of 16,84000 cells/mm^3 (day 0), 20,38000 cells/mm^3 (day 1),20,62000cells/mm^3 (day 2) during their successive days. In last instar larva, considerable variation in the number of THC starting from day 0 to prepupal stage were evident as depicted in (Table : 2., Fig. I)

Plate : 1

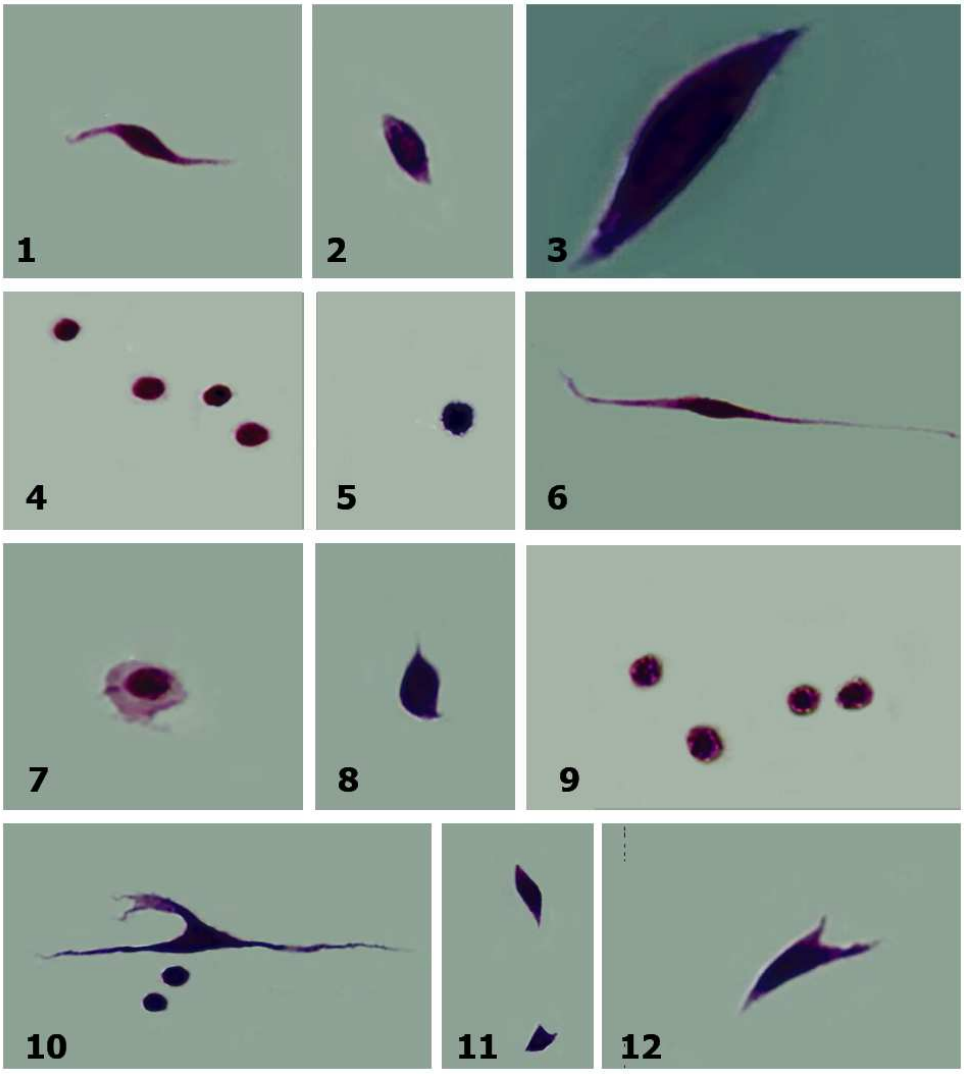

Fig. 1,2, 3: Plasmatocytes 400X; **Fig. 4:** Granulocyte 400X; **Fig. 5:** Adipohaemocytes 400X
Fig. 6: Vermicytes 400X; **Fig. 7:** Prohamocytes 400X; **Fig. 8:** Oenocytoid 400X
Fig. 9: Spherulocyte 400X; **Fig. 10,11,12 :** Podocytes 400X

Table 1: Haemogram of the Plasmatocytes of Spodoptera Mauritia in Relation to the Developmental Stages

INSTAR	DAY 0	DAY 1	DAY 2	DAY 3
4th INSTAR	152.70± 4.785 (1.513)	180.30±16.04 (5.073)	352±5.981 (1.892)	312.00±5.754 (1.820)
5th INSTAR	244.80 ±37.97 (12.009)	206.10±3.604 (1.140)	115.20±8.162 (2.581)	100.50±2.461 (0.778)
6th INSTAR	100.90±2.998 (0.948)	97.50±4.062 (1.285)	97.20±34.22 (10.824)	95.20±34.22 (10.824)
WANDERING STAGE (DAY 4)	50.50±19.472 (0.948)	---	----	----
PREPUPA (DAY 5)	43.50± 19.472 (1.843)	---	----	----
PUPAE	40.00±18.382 (1.703)	35.58±11.813 (1.547)	27.93±9.762 (0.675)	20.89±5.431 (0.453)

Mean± Standard deviation (Standard error), n=10

Table 2: Total Haemocytes Count During the Developmental Stages of Spodoptera Mauritia

INSTAR	DAY 0	DAY 1	DAY 2	DAY 3
4 th INSTAR	1.2320± 4.04332 (1.27861)	180.30±16.04 (5.073)	352±5.981 (1.892)	312.00±5.754 (1.820)
5 th INSTAR	1.9480 ±6.01494 (1.90209)	206.10±3.604 (1.140)	115.20±8.162 (2.581)	100.50±2.461 (0.778)
6 th INSTAR	2.134±2.94249 (9.30496)	97.50±4.062 (1.285)	97.20±34.22 (10.824)	95.20±34.22 (10.824)
WANDERING STAGE (DAY 4)	1.6990±4.18064 (1.32204)	-----	-----	-----
PREPUPA (DAY 5)	1.2942±6.08198 (1.92329)	-----	-----	-----
PUPAE	2.0234±2.18632 (1.43261)	1.09524±1.04328 (1.85607)	1.0084±1.02687 (1.56549)	1.01693±1.00967 (1.16284)

Mean± Standard deviation (Standard error), n=10

DIFFERENTIAL HAEMOCYTES COUNT OF *Spodoptera mauritia*

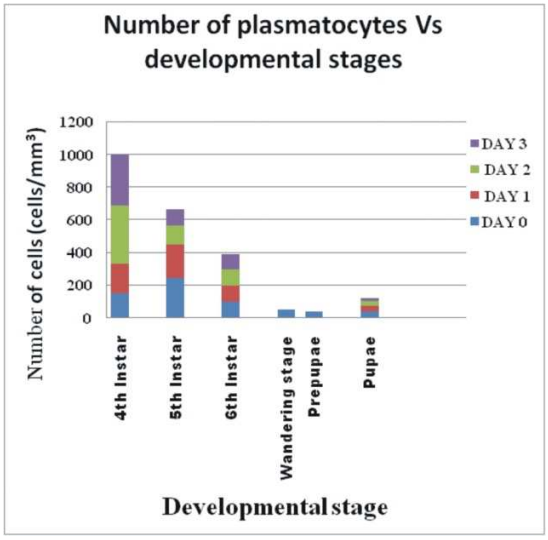

Fig: A

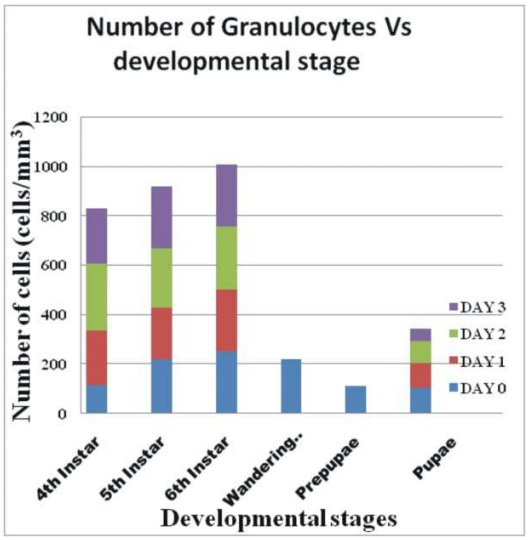

Fig: B

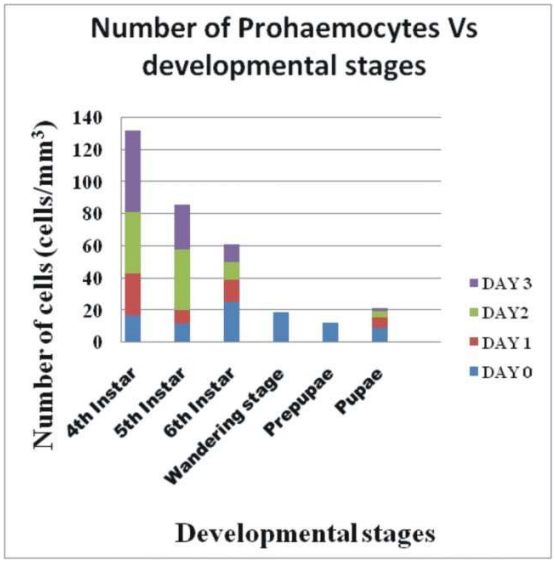

Fig: C

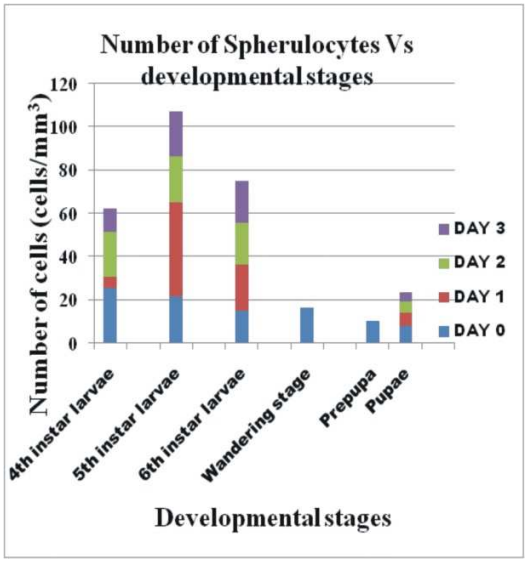

Fig: D

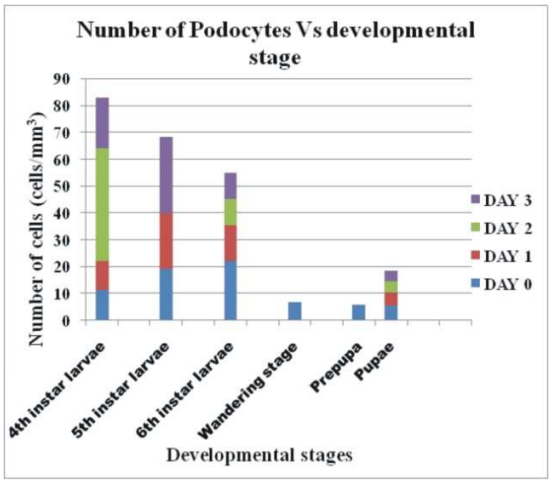

Fig: E

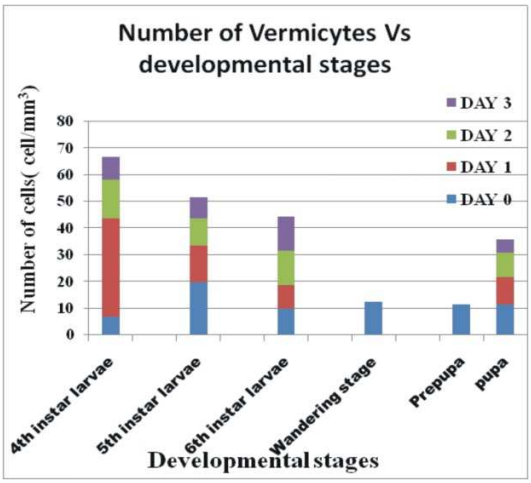

Fig: F

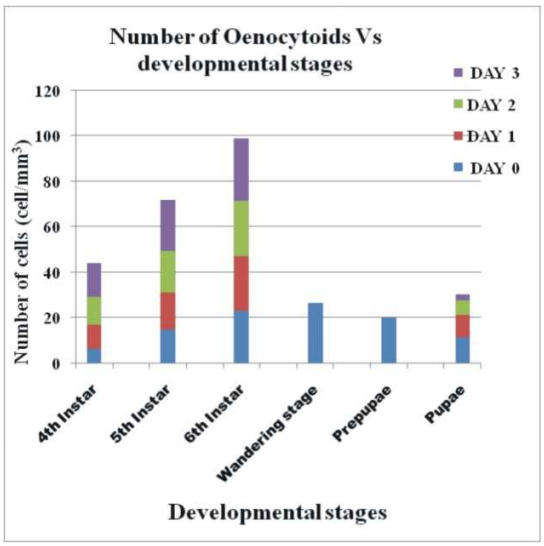

Fig: G

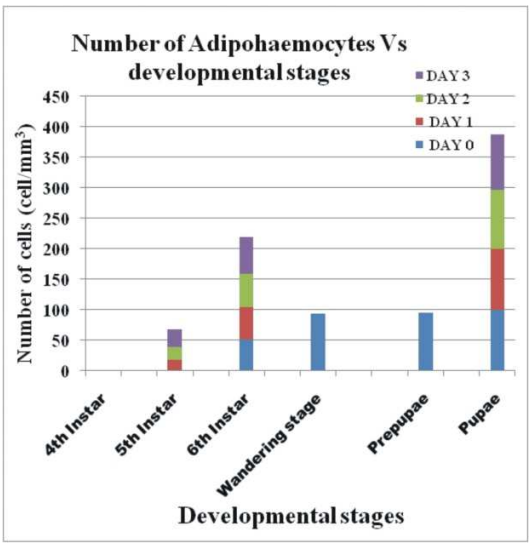

Fig: H

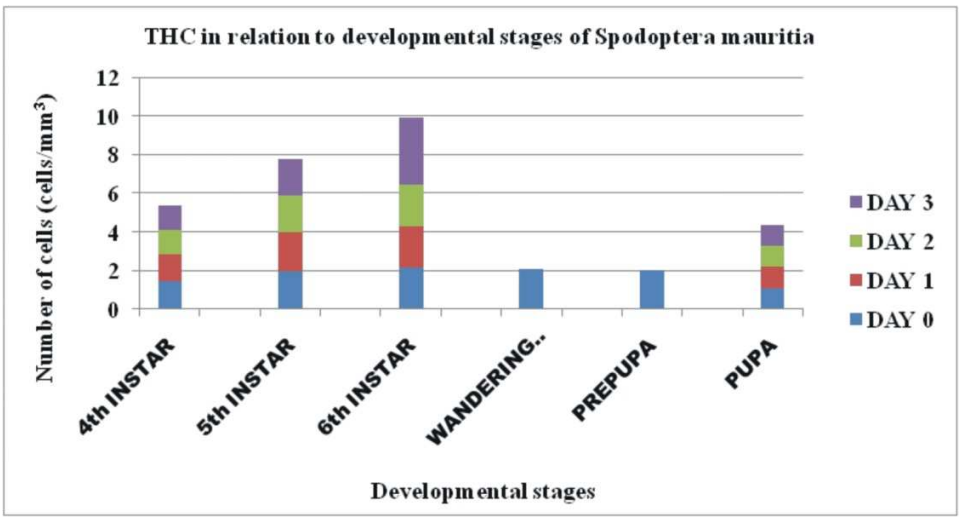

Fig: I

DISCUSSION

The present investigation was aimed to categorize the haemocytes types and numbers during the developmental stages of *S. mauritia*, based on distinctive morphological and cytological features revealed by light microscopy. Six morphological types, namely, plasmatocytes, granulocytes, prohaemocytes, oenocytoids, spherulocytes, adipohaemocytes and additional two haemocytes such as podocytes and vermicytes were identified during the developmental stage of *S. mauritia*. Similar studies have been observed in *S. littoralis*[12], *Papilio demoleus*[13].

Our result indicated that plasmatocytes populations were high in each larval instar and it was significantly higher in fourth larval instar as compared to others. Plasmatocytes have been reported in all insect orders[4]. Lea[14] recorded large variation in cell number from the fourth instar to the adult in *Hyalophora cercopia*, but at pupation the count decreased and remained low in newly emerged adults. In *S. mauritia*, plasmatocytes are known to present themselves in pleomorphic forms. Majority of plasmatocytes are spindle shaped with their variants. The present results are in agreement with that of[13] in *Papilio demoleus*.

The granulocytes are the most abundant haemocytes in the haemolymph of *S. mauritia*. They are the only haemocyte type that has been reported from all major arthropod groups[4]. A phagocytic role has been assigned to granulocytes by several authors[15,16,17].The granulocytes in the present work corresponds to that described by Mesherif *et al*.,[17]. The prohaemocytes have been reported in all insect orders studied except for Thysanura and Odonata[4]. The populations of prohaemocytes were higher in earlier instars and a decline was observed in late larval stages of *S.mauritia*. It seems that most of the larval cell disintegrates in the earliest stages; the prohaemocytes divide mitotically and increased in number to give rise to other types of haemocytes.

Spherulocytes were the other distinguishing cells observed. They are similar to the spherule cells. Recent findings of Mesherif *et al*.,[20] in *S .littoralis* support the above observation. Oenocytoids are reported to be round to ovoid, rod shaped, elongated and fusiform cells in different species of Noctuidae[18]. In *Anticarsia gemmtalis*, these cells are the largest cellular types observed. The decline in the percentage of granulocytes, plasmatocytes and prohaemocytes and corresponding increase in the population of adipohaemocytes, in late sixth instar stages of this insect tends to support many of the earlier works. In *S. mauritia*, the vermicytes populations were present throughout all instars and pupal stage, but there were no drastic changes in the numbers during the developmental stages. Similar observations were recorded in *S. litura*[12].

Total haemocyte count of *S. mauritia*, increased during late larval instar and declined during the pupal period. The THC peaked at every moult in the larvae of *Bombyx mori*, but the highest THC was attained in fifth instar[19]. Further, it later declined and reached its lowest level after adult emergence. The number of haemocytes per unit volume of blood increases throughout larval development, but with additional variation within each developmental stage.

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