

Hemocyte variations with emphasis to granulocytes and plasmatocytes in grasshoppers collected from the surrounding areas of Pench Tiger Reserve and Nagzira National Park

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Abstract

In insects, cellular immune responses are performed by granulocytes and plasmatocytes. The author visited Pench Tiger Reserve (PTR), Madhya Pradesh and Nagzira National Park (Maharashtra) during the period of February 23 to 26, 2023. Quadrats, pitfall trap studies and collection of grasshoppers were done along the existing tourist routes and forest rest houses and from surrounding areas of forest. Hemolymph was smeared on glass slides and stained by Giemsa. Presence of lysosomes was indicated by neutral red staining. Activated charcoal particles in normal saline (0.67% NaCl) was used for phagocytosis study. Prohemocytes have been found dominated in all hemocytes. The high abundance of plasmatocytes and granulocytes suggested their importance in the immunity. The presence of vermicytes and podocytes has rarely been found. The granulocytes aggregated after charcoal particles injection but rarely formed large clusters. As a result of these conserved features insects have become popular choices for evaluating and assessing the efficacy of immune cells like hemocytes.

Key words : Hemocytes, Grasshoppers, Granulocytes, Plasmatocytes, Cell aggregation.

Orthopterans are distributed worldwide and many have a negative impact on cereals crop^{5,15}. They can eradicate significant amounts of vegetation during outbreaks¹⁴.

Because of this feeding behaviour, several locust and grasshopper like migratory

locust (*Locusta migratoria*) are considered as the most notorious crop pests. Thus, studies of orthopteran have focused on developing effective control strategies⁵. The capacity to recognize hemocyte types is fundamental to the investigation of insect immunity. Insect hemocytes are responsible for uptake and

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transport of specific materials, phagocytosis, nodulation, encapsulation, and melanisation^{6,17}.

The author visited Pench Tiger Reserve (PTR), Madhya Pradesh and Nagzira National Park (Maharashtra) during the period of February 23 to 26, 2023 during educational visit. Quadrats, pitfall trap studies and collection of grasshoppers were done along the existing tourist routes and forest rest houses and from surrounding areas of forest (Fig 1). Hemolymph

samples were withdrawn from the insects by means of incision made near the 3rd coxae. Hemolymph was smeared on glass slides, fixed by methanol and stained by Giemsa, and neutral red (Fig. 2). For phagocytosis study activated charcoal particles suspended in normal saline (0.67% NaCl) was injected into insect leg and the aspirate was taken for study. Presence of lysosomes was indicated by neutral red staining^{7,8,9,10,11}.



Fig. 1. Sample collection sites

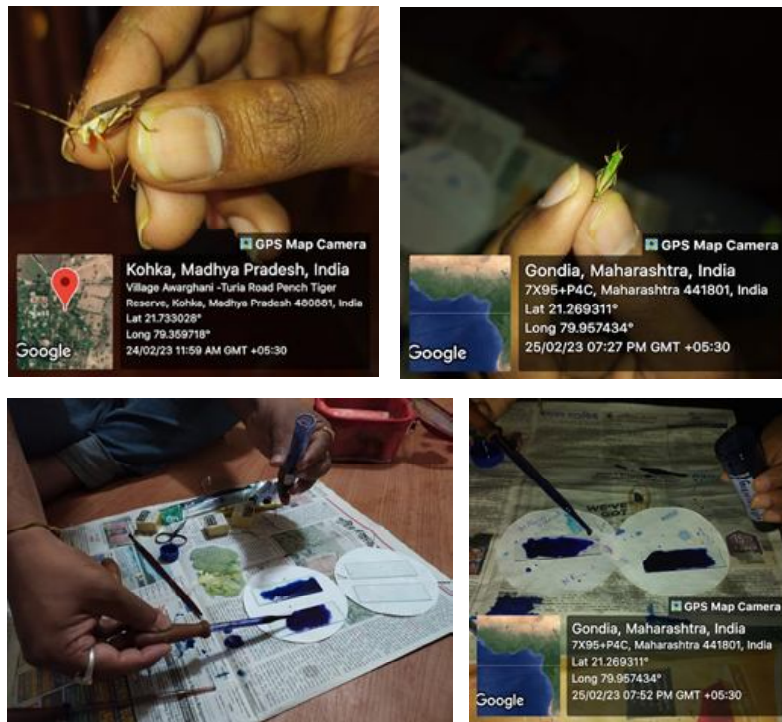


Fig. 2. Sample analysis

Prohemocytes, plasmatocytes, granulocytes were observed in all examined slides. The presence of vermicytes and podocytes has rarely been found.

Prohemocytes:

In Pench and Nagzira group, prohe-

mocytes have been found dominated in all hemocytes. They are thought to be the stem cells of insect hemocytes that differentiate into other cells types. They were round or oval cells with variable sizes (Fig. 3).

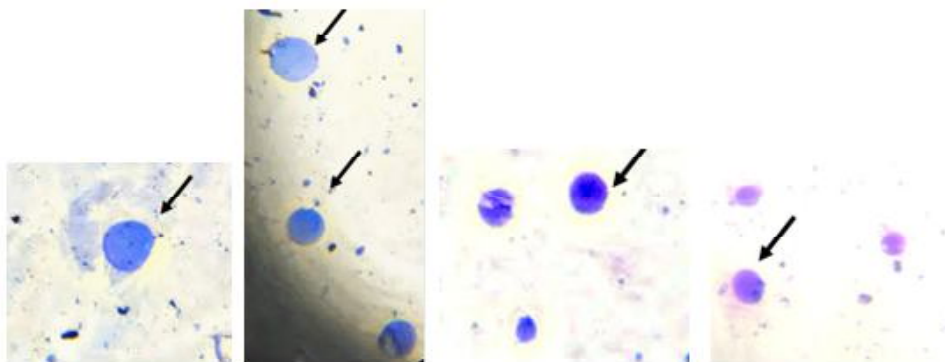


Fig. 3. Prohemocytes evaluated by light microscopy (indicated by arrow) (x400)

Granulocytes :

The cytoplasm was characteristically granular and the cell membrane was usually articulated. Giemsa stained granulocytes showed short pseudopodial growth, binding of

charcoal particles, food cup formation (Fig. 4). Phagosomes or pinocytotic vesicles were observed in the granulocytes. Cells showed neutral red positive response indicating lysosomal compartments (Fig. 5).

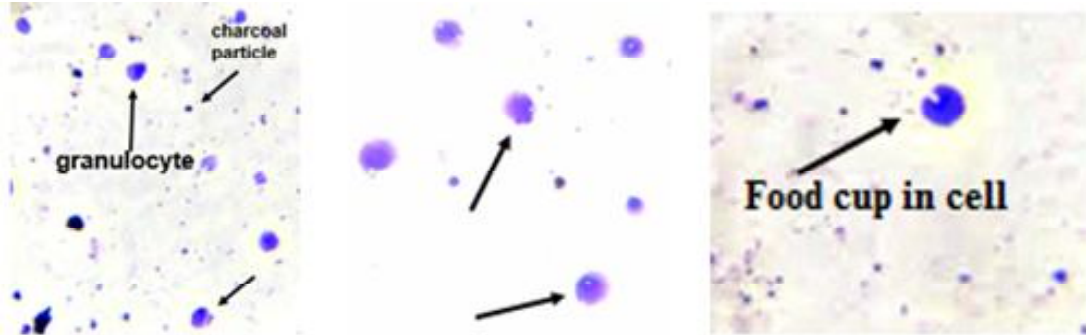


Fig. 4. Granulocytes evaluated by light microscopy. Binding of charcoal particles, food cup formation were noted (x400)

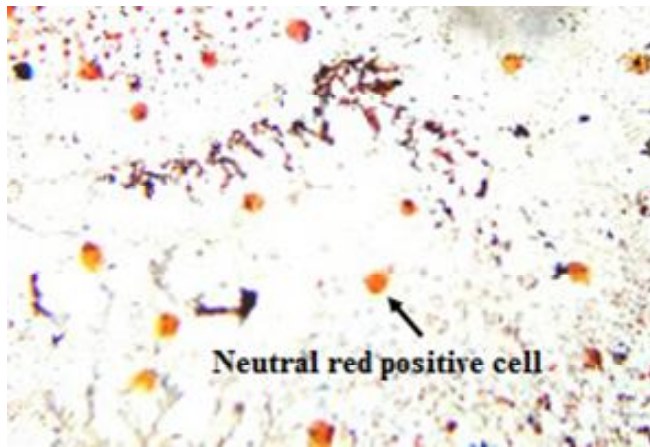


Fig. 5. Cells showed Neutral Red positive response (indicated by arrow) (x100)

Plasmatocytes:

They varied in size and shape, mostly because of the divergence of their pseudopodia and other projections (Fig. 6). The size and

shape of plasmatocytes can differ markedly even within the same individual. They were round, oval, spindle and irregularly shaped. Attachments of foreign objects were found in both plasmatocytes and granulocytes.

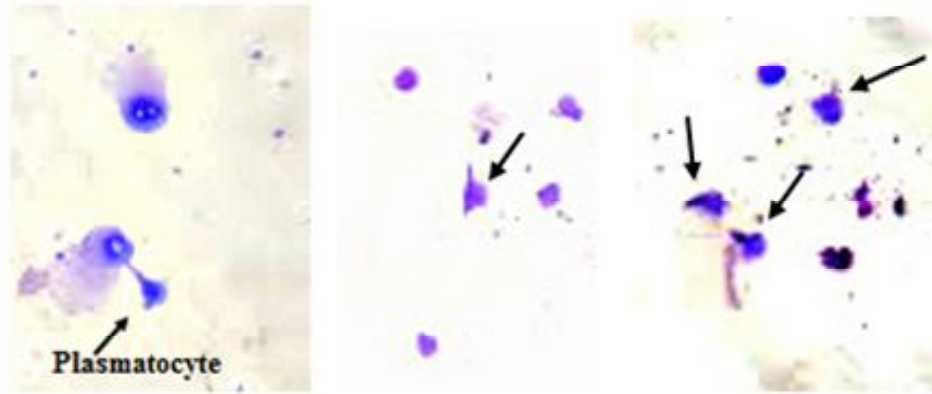


Fig. 6. Plasmatocytes evaluated by light microscopy (indicated by arrow) (x400)

Cell aggregation :

The granulocytes aggregated on slides but rarely formed large clusters (Fig. 7A and 7B).

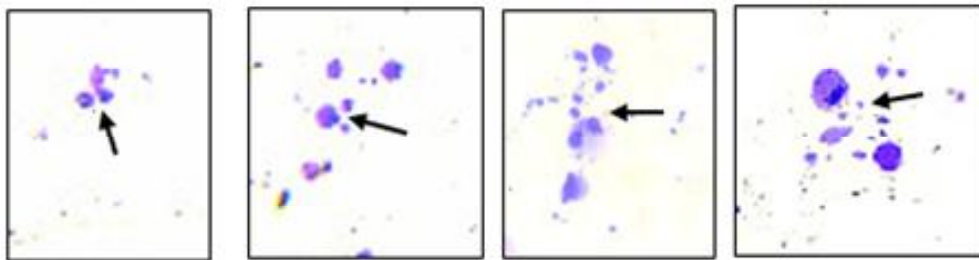


Fig. 7A. Cell aggregation evaluated by light microscopy (indicated by arrow) (x100)

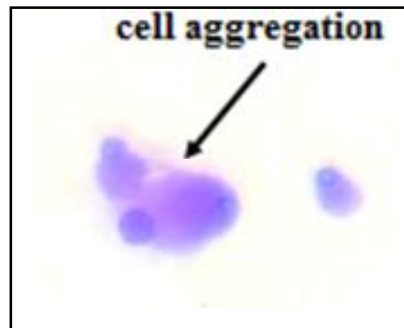


Fig. 7B. Cell aggregation evaluated by light microscopy (indicated by arrow) (x400)

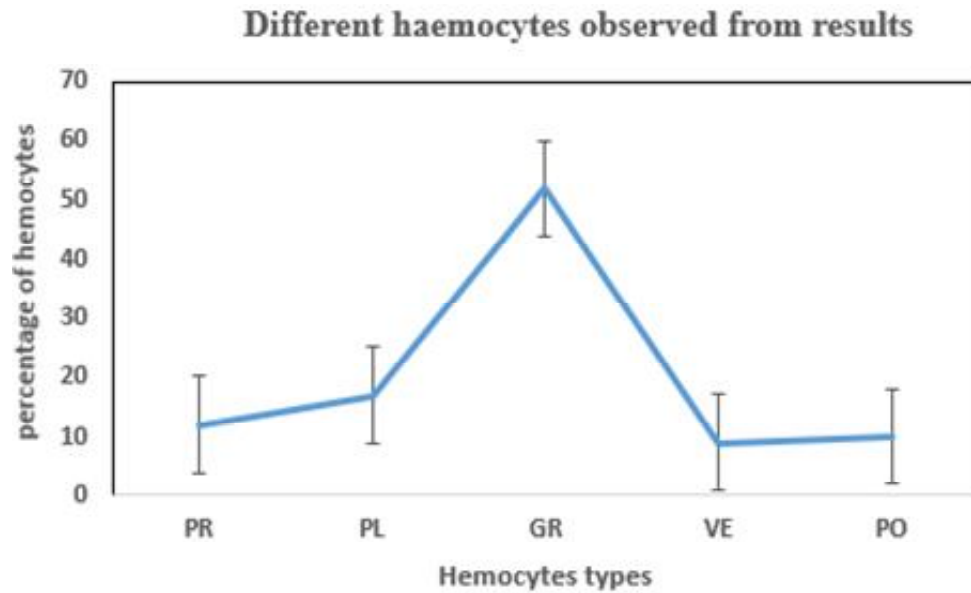


Fig. 8. The prevalence of different hemocytes. PR: prohemocyte; PL: plasmatocyte; GR: granulocyte; VE: vermicyte; PO: podocyte. Values are expressed as Mean \pm SEM

Prohemocytes, plasmatocytes, granulocytes, vermicytes, podocytes were found in the examined species. The high abundance of granulocytes and plasmatocytes was found (Fig. 8) which was similar to previously reported studies⁵. Prohemocytes have been found in all reported hemocytes of orthoptera⁶. They are thought to be progenitor cells that differentiate into other hemocytes¹³. Several studies have supported this hypothesis³. Prohemocytes were found to increase in number during recovery following blood loss¹. Because of the low abundance of prohemocytes in many species, their importance in insect immunity has been questioned.

Guria and Chatterjee¹⁰ showed analysis of hemocyte types in orthoptera collected from surrounding areas of Navegaon

and Tadoba forest¹⁰. The phagocytic response of insect hemocytes, neutral red response and cell aggregation was reported¹⁰. Previous studies showed normal cell shape and behavioural activities like phagocytosis in insects collected from Murti, and adjoining areas^{8,9}. The present result corroborated previous studies.

“Phagocytosis”, *i.e.* engulfment of foreign particles of the blood is one of the important functions of the hemocytes which has been experimentally demonstrated by many researchers using ink or fungi injected into the haemocoel. Metalnikov and Chorine (1929) were the first to demonstrate phagocytosis by hemocytes (using *Galleria mellonella*)¹⁶. Researchers showed injection of *Aspergillus flavus* and *Aspergillus niger* into the

haemolymph of *Cecropia* sp. was followed by an increase in the total counts of hemocyte¹⁹. Sussman (1952) recorded phagocytosis of *Aspergillus flavus* by the hemocytes in *Hyalophora* sp.¹⁹. Insect hemocytes are also known for a defense mechanism in which foreign bodies that are generally larger than those engulfed by phagocytosis are encapsulated²⁰. According to Gupta¹², the granulocytes are normally involved in the encapsulation¹².

Studies with *Galleria mellonella* (Lepidoptera) suggested during encapsulation, Granular cells and plasmatocytes form the capsule¹⁸. In contrast, Brehelin *et al.*,² reported that only a single class of cells, that is granular haemocytes, mediates encapsulation in *Locusta migratoria* (Orthoptera)². Present result exhibited the granulocytes aggregated on glass slides after charcoal particles injection but rarely formed large clusters (Fig. 7). Both granulocytes and plasmatocytes were gathered together (Fig. 7). The granulocytes appeared to attach not only to other granulocytes, but also to different types of hemocytes (data not shown). Cho Youngwoo and Cho Saeyoull (2019) investigated granulocyte activation by carboxylate-modified polystyrene latex beads and observed hemocytes participated in the various steps of encapsulation and nodulation⁴.

The coming years of research into insect hemocytes will lead to effective knowledge for invertebrate immunity.

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