International Journal of Pharmacy and Biological Sciences-IJPBS™ (2019) 9 (3): 789-794
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Biological Sciences | Open Access | MCI Approved
UGC Approved Journal

Study on Different Types of Haemocytes and Their Behaviour in Insects (Orthoptera) Collected from Navegaon and Tadoba National Park and Its Adjoining Areas

Srikanta Guria[1]* and Susraba Chatterjee[1]
[1]Post Graduate Department of Zoology, Barasat Govt. College, Barasat, Kolkata - 700124, West Bengal, India.

Received: 16 Mar 2019 / Accepted: 18 Apr 2019 / Published online: 1 Jul 2019
***Corresponding Author Email:** srikantarna@yahoo.com

Abstract
Insect immunity consists of cellular and humoral reactions. Cellular reactions comprise haemocytes which include phagocytosis, nodulation (haemocyte aggregation) and encapsulation. Morphologically, haemocytes are distinct variety of cells comparable to the vertebrate leukocytes and macrophages. Many researchers categorised the haemocytes of insects (Orthoptera) as prohaemocytes (PRs), plasmatocytes (PLs), coagulocytes (COs), oenocytoids (OEs), podocytes (POs), granulocytes (GRs) and reticular cells (RTs). The aim of present study was morphological characterization of circulating haemocytes in Orthoptera. Grasshoppers were collected from Navegaon and Tadoba National Park and its adjoining areas of Maharashtra, India during educational visit. Prohaemocytes (PRs), plasmatocytes (PLs), granulocytes (GRs), oenocytoids (OEs), podocytes (POs) were categoriged. Haemocyte aggregation and phagocytic behaviour were also noticed. But fundamental studies on insect haemocytes demand more investigations and researches.

Keywords
Haemocytes, Insects, Orthoptera, Phagocytosis, Aggregation.

*****

INTRODUCTION
Tadoba Andhari Tiger Reserve is located in Chandrapur district, Maharashtra, India. It is Maharashtra's oldest and largest national park. Navegaon National Park is located in the Arjuni Morgaon subdivision of Gondia district in the state of Maharashtra, India. Insect diversity in this area is very high. The Orthoptera fauna of India comprise about 1033 species belonging to 398 genera (Sunil *et al*., 2018; Chandra *et al*., 2010), whereas from Maharashtra 143 species of Orthoptera belongs to 98

genera in eight families were reported (Sunil *et al*., 2018; Chandra and Gupta, 2012).

In general, insect immunity consists of cellular and humoral reactions (Ravindranath, 1977). Cellular reactions involve haemocytes which include phagocytosis, nodulation (haemocyte aggregation) and encapsulation. Morphologically, haemocytes are distinct variety of cells (Price and Ratcliffe, 1974; Mead *et al*., 1986), comparable to the vertebrate leukocytes and macrophages (Jones, 1950), which constitute the chief and inevitable components of haemolymph in the open circulatory system of insects (hexapods) as well as in other arthropods and invertebrates (Wigglesworth, 1939, 1955 and 1979).

Akai and Sato (1979) categorised the haemocytes of *Locusta migratoria* (Orthoptera) as prohaemocytes (PRs), plasmatocytes (PLs) coagulocytes (COs), oenocytoids (OEs), and reticular cells (RTs). However, Sharma and Dutta (1979) recorded the presence of granulocytes (GRs) and vermicytes (VEs) in orthoptera. The aim of present research is morphological characterization of circulating haemocytes. However, type of haemocytes and their role in insects are debatable and need more information on the subject for clarity. However, to-date, there exist lacunae, pitfalls and controversies on overall knowledge about insect haemocytes. Therefore, the scope for fundamental studies on insect haemocytes demands more investigations and researches. In view of this, in the present research attempt has been made to unify the haemocytes classification. In Drosophila, plasmatocytes are responsible for the disposal of both microorganisms and apoptotic cells, and to date several candidate receptors have been described on plasmatocyte (Frank *et al.*, 1996, 1999). Aggregation and encapsulation are reactions achieved by specialised haemocytes reported in Drosophila (Carton *et al*., 1986).

MATERIALS AND METHODS

Grasshoppers (total no.10) were collected from Navegaon and Tadoba National Park and its adjoining areas during educational visit. Haemolymph samples were withdrawn from the insects by means of incision made near the 3rd coxae. Haemolymph was smeared directly on sterilized glass slides and stained by Giemsa, and neutral red. For phagocytosis study activated charcoal particles suspended in normal saline (0·67% NaCl) was injected into insect leg and the aspirate was taken for haemocytes study. Cellular morphology was examined.

RESULT

Following haemocyte categories were recognized

Prohaemocytes (PRs) and Granulocytes (GRs)

PRs are usually the smallest haemocytes found in the haemolymph, round or oval cells with variable sizes. The nucleus is large, round, usually centrally located and almost filling the most part of cytoplasm. A thin peripheral layer of cytoplasm surrounds the nucleus (Fig 1A).

GRs are small to large, spherical or oval cells of variable sizes. The nucleus is rounded and is centrally located. The cytoplasm is characteristically granular and the cell membrane is usually articulated (Fig 1B).

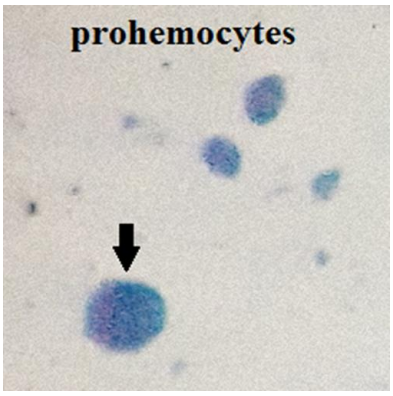


Fig 1A

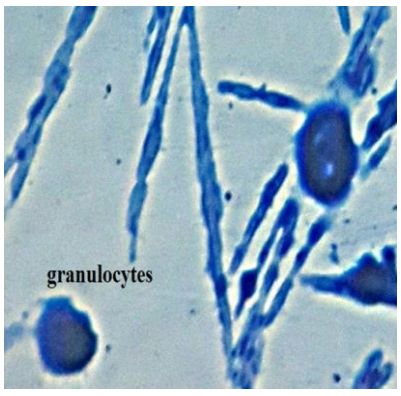
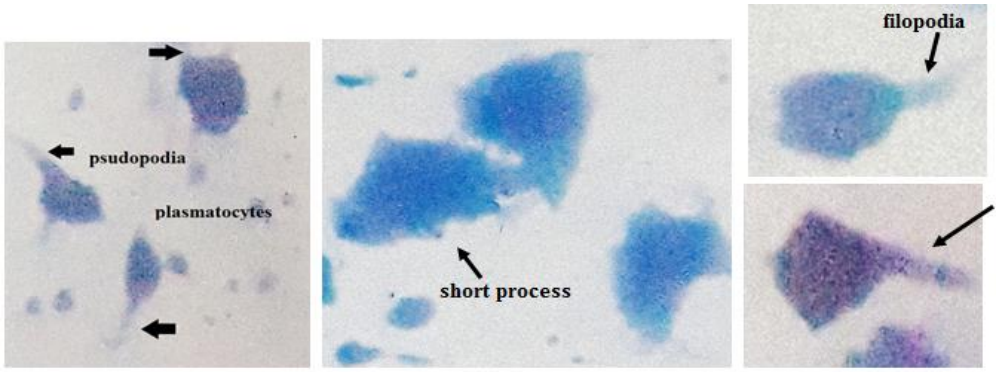


Fig 1B

Fig1A and B: Giemsa stained prohaemocytes (Fig 1A) and granulocytes (Fig 1B) (x400)

Plasmatocytes (PLs)

PLs are small to large, polymorphic cells. The cytoplasm is abundant. The cytoplasmic membranes have filopodia. The nucleus may be centrally placed or eccentric (Fig 1C).

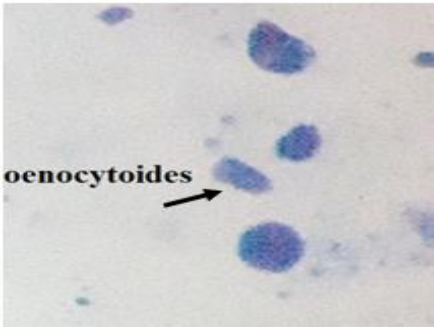


(Fig 1C)

Fig1C: Giemsa stained plasmatocytes with pseudopodia and filopodia (x400)

Oenocytoids (OEs)

OEs are small to large, oval or spherical cells. The plasma membrane is generally without processes (Fig 1D).

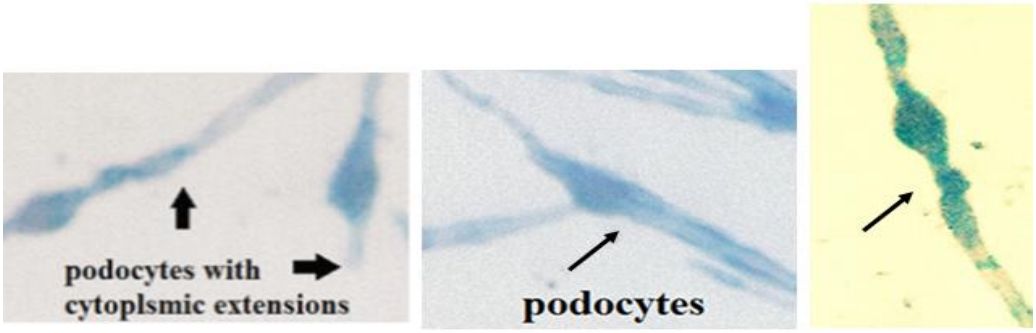


(Fig 1D)

Fig1D: Giemsa stained oenocytoids (indicated by arrow) (x400)

Vermicytes (VEs) and Podocytes (POs)

VEs are extremely elongated cells with slightly granular or agranular cytoplasm. The nucleus may be located centrally or eccentrically (picture not shown). POs are very large in size, extremely flattened with several cytoplasmic extensions (Fig 1E).



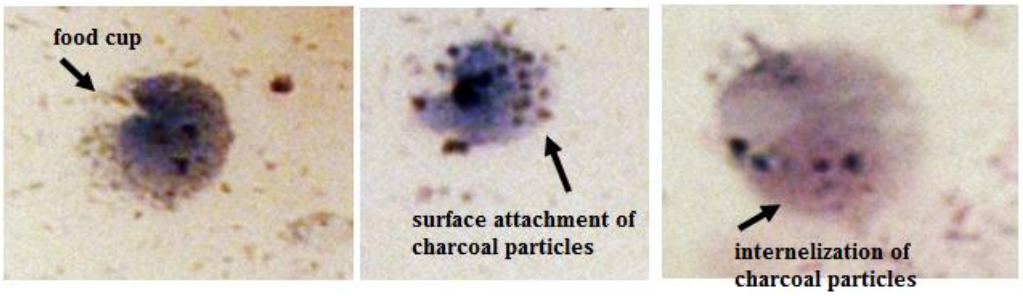
(Fig 1E)

Fig1E: Giemsa stained podocytes (indicated by arrow) (x400)

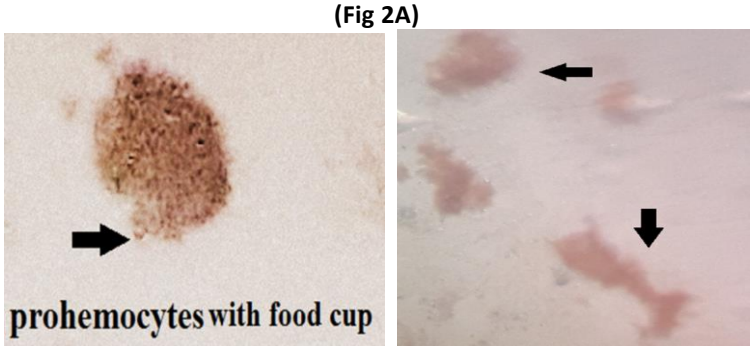
Phagocytic behaviour of haemocytes

Haemocytes showed different stages of phagocytosis like attachment of charcoal particle on cell surface, phagocytosis of charcoal by cell or internalization of charcoal by cell. Formation of small cytoplasmic process, food cup and filopodia were noticed (Fig 2A).

Haemocytes showed neutral red positive reaction indicating the presence of lysosomal compartments (Fig 2B).



(Fig 2A)

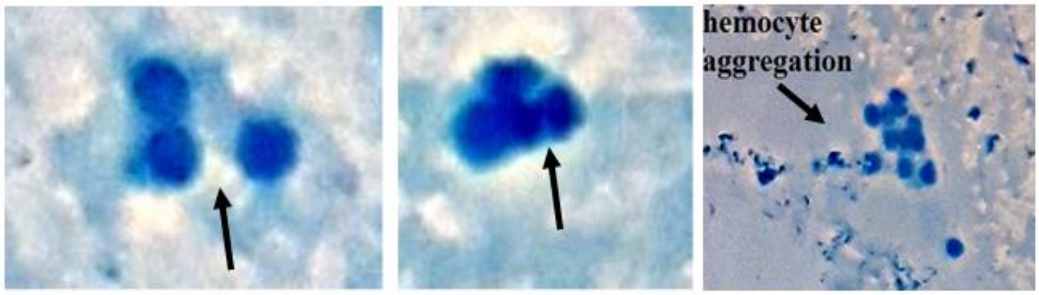


(Fig 2B)

Fig2: Haemocytes showed different stages of phagocytosis (Fig 2A) and neutral red positive reaction (Fig 2B) (x400)

Cellular aggregation

Haemocyte aggregation was noticed (Fig 3).



(Fig 3)

Fig3: Different stages of aggregation response of haemocytes (giemsa stained) of grasshopper

DISCUSSION

Blood cells or haemocytes are reported as chief immune effector cells of invertebrates and are capable of performing multiple immunological functions including aggregation, phagocytosis, encapsulation, nodulation and generation of cytotoxic agents. Invertebrates including arthropods rely on innate immune defences (Galloway and Depledge, 2001). Neutrophils, macrophages and dendritic cells of vertebrate are professional phagocytes and have a regulatory role in adaptive immunity by producing co-stimulatory molecules and immune-modulatory cytokines (Aderem and Underhill, 1999). Phagocytosis is a primordial aspect of innate immunity and is conserved in all arthropoda: in Drosophila, plasmatocyte resembles the mammalian monocyte/macrophage lineage exerts this function (Girardin *et al.*, 2002). Our result revealed different types of haemocytes that corroborated previous studies (Fig 1) (Akai and Sato,

1979; Sharma and Dutta, 1979; Guria *et al*., 2016). Phagocytosis is considered a classical innate immune response reported in the majority of the invertebrate phyla. It is an established immunological response and is considered as a biomarker of any pollution (Oliver and Fisher, 1999; Guria *et al*., 2016). Phagocytic cells are known to be enriched with lysosomal vesicles and the degree of lysosomal membrane fragility can be quantitated by neutral red retention assay (Guria, 2018).

We reported the phagocytic response of insect haemocytes under the challenge of charcoal particle and our result showed neutral red positive response (Fig 2). Cell–cell aggregation is considered as an immunological response for host defence. Aggregation of haemocytes around invaded microorganisms is termed as "encapsulation response" and is considered as an important immunological reaction (Nappi and Christensen, 2005; Guria *et al*., 2016). Our result also revealed haemocyte aggregation (Fig 3).

Most recent studies of both the humoral and cellular components of the invertebrate immune system have revealed that invertebrates share many of the fundamental immunological mechanisms with vertebrates, including humans. This article includes brief speculation on how the insect immune system may have evolved (Gupta, 2001).

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this paper.

ACKNOWLEDGEMENT

We thank Head, Post Graduate Department of Zoology and Principal, Barasat Govt. College for necessary support.

REFERENCES

1. Aderem A and Underhill DM. (1999). Mechanisms of phagocytosis in macrophages. *Annu Rev Immunol*. 17: 593–623.
2. Akai H and Sato S. (1979). Surface and internal Ultrastructure of hemocytes of some insects. In: Insect hemocytes (ed. A.P. Gupta), Cambridge University Press, New York, London: 129-154.
3. Carton Y, Bouletreau M, Van Lenteren JC and Van Alphen JCM. (1986). The Drosophila parasitic wasps. In The Genetics and Biology of Drosophila. Edited by Thompson JN. Academic Press: 347-394.
4. Chandra K and Gupta SK. (2012). Insecta: Orthoptera. Fauna of Maharashtra. State Fauna Series. 20 (2): 429-436.
5. Chandra K, Shishodia MS and Gupta SK. (2010). Diversity of Orthoptera (Insecta) In India: State of Our Knowledge. *In Advancement in Invertebrate Taxonomy and Biodiversity*. 43-80.
6. Franc NC, Dimarcq JL, Lagueux M, Hoffmann J and Ezekowitz RA. (1996). Croquemort, a novel Drosophila haemocyte/macrophage receptor that recognizes apoptotic cells. *Immunity*. 4: 431-443.
7. Franc NC, Heitzler P, Ezekowitz RA and White K. (1999). Requirement for croquemort in phagocytosis of apoptotic cells in Drosophila. *Science*. 284: 1991-1994.
8. Galloway TS and Depledge MH. (2001). Immunotoxicity in invertebrates: measurement and ecotoxicological relevance. *Ecotoxicol*. 10 (1): 5-23.
9. Girardin SE, Sansonetti PJ and Philpott DJ. (2002). Intracellular vs. extracellular recognition of pathogens -common concepts in mammals and flies. *Trends Microbiol*. 10 (4): 193–199.
10. Gupta AP. (2001). Humoral Immunology of Invertebrates. *Encyclopedia of Life Sciences. Nature Publishing Group* / www.els.net
11. Guria S. (2018). Alteration of Cytomorphology of Peritoneal Macrophages of Albino Rat Exposed to Mercuric Compound. *World Scientific News*. 92(2): 392-399.
12. Guria S, Parveen S and Goswami DS. (2016). Alteration of Morphology, Phagocytic Behaviour and Aggregation of Insect Haemocytes Exposed to Contaminated Food with Arsenic and Lead. *International Journal of PharmTech Research*. 9(5): 177-186.
13. Jones JC. (1950). The normal hemocyte picture of the yellow mealworm *Tenebrio molitor* Linnaeus. *Iowa State Coll. J. Sci*. 24: 356-361.
14. Mead GP, Ratcliffe NA and Renwrantz LR. (1986). The separation of insect hemocyte types on percoll gradients, methodology and problems. *J. Insect Physiol*. 32: 167-177.
15. Nappi AJ and Christensen BM. (2005). Melanogenesis and associated cytotoxic reactions: Applications to insect innate immunity. *Insect Biochem Mol Biol*. 35(5): 443-459.
16. Oliver LM and Fisher WS. (1999). Appraisal of prospective bivalve immune markers. *Biomarkers*. 4: 510-530.
17. Price CD and Ratcliffe NA. (1974). A reappraisal of insect haemocyte classification by the examination of blood from fifteen insect orders. Z. Zellforsch. mikrosk. *Anat*. 147: 537-549.
18. Ravindranath MH. (1977). A comparative study of the morphology and behaviour of granular haemocytes of arthropods. *Cytologia*. 42: 743-751.
19. Sharma VN and Dutta SK. (1979). Studies on the haemocytes of *Chrotogonus trachypterus* Blach and Acrida exaltata. *Res. Bull: Sci*. 29: 1-9.
20. Sunil MG, Yogesh JK, Gopal AR and Ganesh PB. (2018). Diversity of Short-Horned Grasshoppers (Orthoptera: Caelifera) from Forested Region of Kolhapur District, Maharashtra, India of Northern Western Ghats. *World Academy of Science, Engineering and Technology.*

International Journal of Bioengineering and Life Sciences. 12 (10). 358-367.
21. Wigglesworth VB. (1939). The principles of insect physiology, 1st ed. Methuen, London.
22. Wigglesworth VB. (1955). The role of haemocytes in the growth and molting of an insect, *Rhodnius prolixus* (Hemiptera). *J. Exp. Biol*. 32: 649-663.
23. Wigglesworth VB. (1979). Hemocytes and growth in insects. In: Insect hemocytes (ed. A.P. Gupta). Cambridge University Press, New York. 303-318.