



MORPHOFUNCTIONAL ANALYSIS OF HEMOCYTES IN INSECTS (ORTHOPTERA) COLLECTED FROM MURTI AND ADJOINING AREAS (DOOARS) AND DIFFERENT AGRICULTURAL FIELDS OF NORTH 24 PARGANAS, WEST BENGAL

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ABSTRACT

A small village named Murti is situated along the bank of river Murti in Dooars. The adjacent area of murti is rich in insect biodiversity. Knowledge on the diversity and the role of Orthopteran insects in tea ecosystem of West Bengal including Dooars and Terai is very scanty. Hemocytes are the chief immune cells of insects. The number and shape of hemocytes can change rapidly in response to environmental stress and pollution. Grasshopper (Orthoptera) species were collected from Murti and adjoining areas and from different agricultural fields of North 24 Parganas (West Bengal). Hemolymph was stained by Giemsa, and Neutral Red. Cellular morphology and phagocytosis was examined. Normal cell shape and behavioural activities like phagocytosis, cell-cell fusion was observed in insects collected from Murti, and adjoining areas of Dooars. But in case of insect hemocytes isolated from different agricultural fields of North 24 Parganas and its adjacent sites showed altered shape and different phases of cell death like degeneration of nuclei, formation of membrane blebs and rupture of plasma membrane. Toxic environmental pollutants may be responsible for alteration of hemocytes size and function which may effect on biodiversity. Hemocytes can be used as an effective bio-indicator by which the health of the ecosystem can be screened.

Keywords: Hemocytes, Cytomorphology, Cell Death, Bio-indicator.

1. INTRODUCTION

Murti is the name of a river that originates close to Bengal-Sikkim-Bhutan border tri-junction and soon comes down from the hills into the Dooars. Into Dooars the eastern side of the river is mostly forested with Chapramari Wildlife Sanctuary whereas in its western side are some of the best tea gardens of Dooars. A small village named Murti is situated along the bank of river Murti. The adjacent area of murti is rich in insect biodiversity.

Knowledge on the diversity and the role of Orthopteran insects in tea ecosystem of West Bengal is very scanty. The insects under order Orthoptera are known as grasshoppers, crickets etc. They are found in open grasslands, agro-fields etc. Previous reports stated 06 species under 06 genera belonging to 03 families of Orthoptera from the tea gardens of North Bengal [1]. Worldwide Orthopterans are considered of immense economic and ecological importance, because many species are pests of crops [1].

Tea with perennial foliage is infested by about 167 insect species in the North-Eastern tea growing regions

of India [2] including the Dooars and Terai. However, Orthopterans may feed on leaves, flowers, fruits, pollen, etc. [3, 4].

Hemocytes are the chief components and immune cells of haemolymph in the open circulatory system of insects (hexapods) as well as in other arthropods and invertebrates [5, 6]. The number of hemocytes in circulation can change rapidly in response to environmental stress, pollution, wounding or infection [7]. The toxic exposure may impair immune response of hemocyte of the animal that may lead to decline of biodiversity.

2. MATERIAL AND METHODS

2.1. Sample collection

Grasshopper (Orthoptera) species were collected from Murti and adjoining areas (Dooars West Bengal, total no. of grasshopper 7) during educational visit. Grasshoppers (total no. 50) were also collected from different agricultural fields of North 24 Parganas (West Bengal).

2.2. Hemocytes isolation and staining

Hemolymph samples were withdrawn from the insects by means of incision made near the 3rd coxae. Hemolymph 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 study. Cellular morphology was examined [8,9].

3. RESULTS AND DISCUSSION

3.1. Cytomorphological profile of hemocyte

Normal cell morphology was noticed in insects collected from Murti, and adjoining areas (Dooars, West Bengal) (Fig.1). Normal cytomorphological profile like pseudopodial growth (a, b) and behavioural activities like cell fusion (c) were also observed in insects collected from Murti (Fig.1).

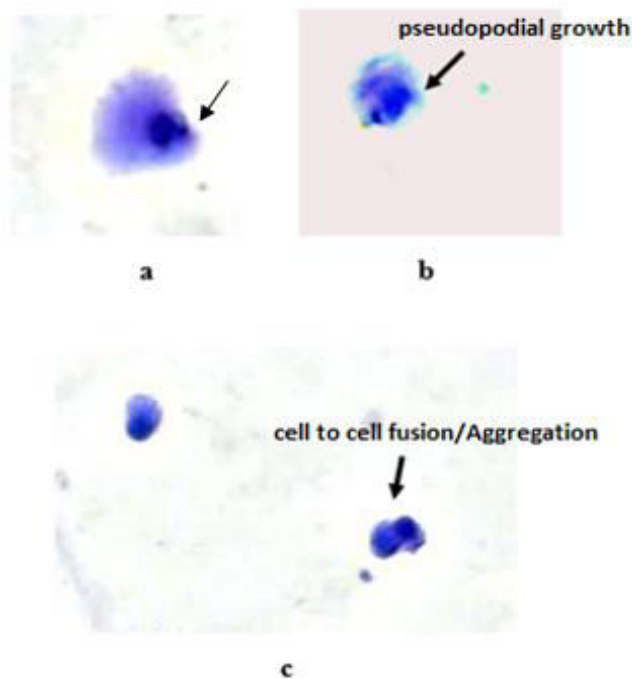


Fig. 1: (a) Normal cell shape with distinct nucleus, (b) Pseudopodial growth, (c) Cell-cell fusion (indicated by arrow) (x400)

3.2. Phagocytic response

The result indicated phagocytic response of hemocytes (observed in insects from Murti) under the challenge of charcoal particles like pseudopodia formation (a, b) and surface binding of charcoal particle (c). The result showed neutral red positive response (d) (Fig. 2).

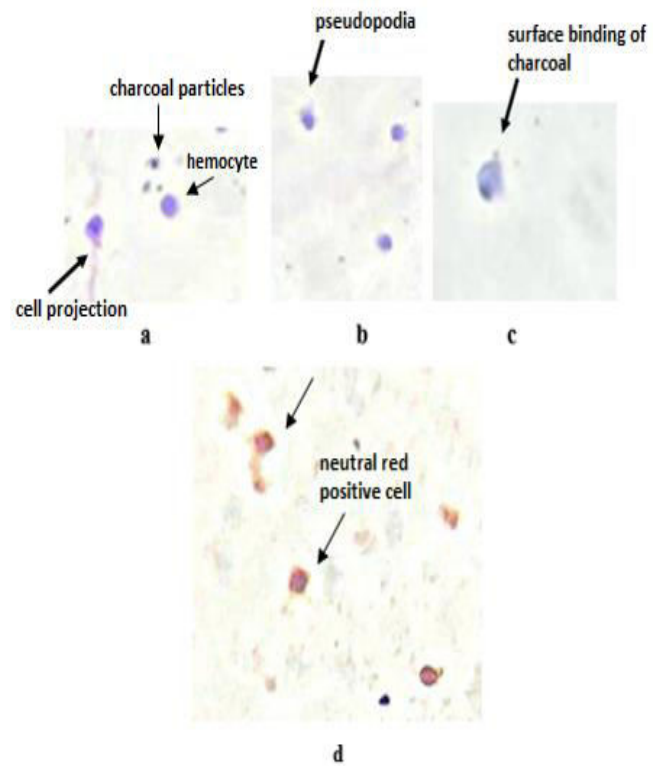


Fig. 2: (a, b) Pseudopodia formation, (c) Surface binding of charcoal particle and (d) Neutral Red positive response of hemocytes (indicated by arrow) (x400)

3.3. Alteration of cell shape

Significant changes were observed in the cytomorphology of hemocytes in North 24 Parganas group (Fig.3). Significant number of cells showed different phases of death like degeneration of nuclei (a), formation of membrane blebs (b), alteration (c, d) and rupture of plasma membrane (e) (Fig.3).

Guria and Chatterjee showed morphological characterization (Prohemocytes, plasmatocytes, granulocytes, vermicytes, oenocytoids and podocytes) of hemocytes in Orthoptera collected from Navegaon and Tadoba National Park and its adjoining areas [8]. Normal cell shape (Fig.1) and behavioural activities like phagocytosis were observed in insects collected from Murti, and adjoining areas of Dooars, West Bengal [9].

Phagocytosis is considered a classical immune response of the invertebrate phyla. It is an established immunological response and is considered as a biomarker of any pollution [10, 11]. 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 [12]. Present result reported

the phagocytic response of hemocytes under the challenge of charcoal particles and showed Neutral Red positive response (Fig. 2). Cell-cell fusion or aggregation may be considered as indication of “encapsulation response” [11, 13].

Murti, and adjoining areas of Dooars, West Bengal is biodiversity rich zone as well as there are less chance of exposure of pollutants like insecticides, pesticides and others chemicals. So, the hemocytes were normal in cytomorphology and showed normal activities [8, 9], but in case of insect hemocytes isolated from different agricultural fields of North 24 Parganas and its adjacent sites showed altered cytomorphological and behavioural activities (Fig.3). Environmental toxicants may be the responsible factors for this alteration of hemocytes size and behaviour which may effect on ecological food chain as well as biodiversity [9].

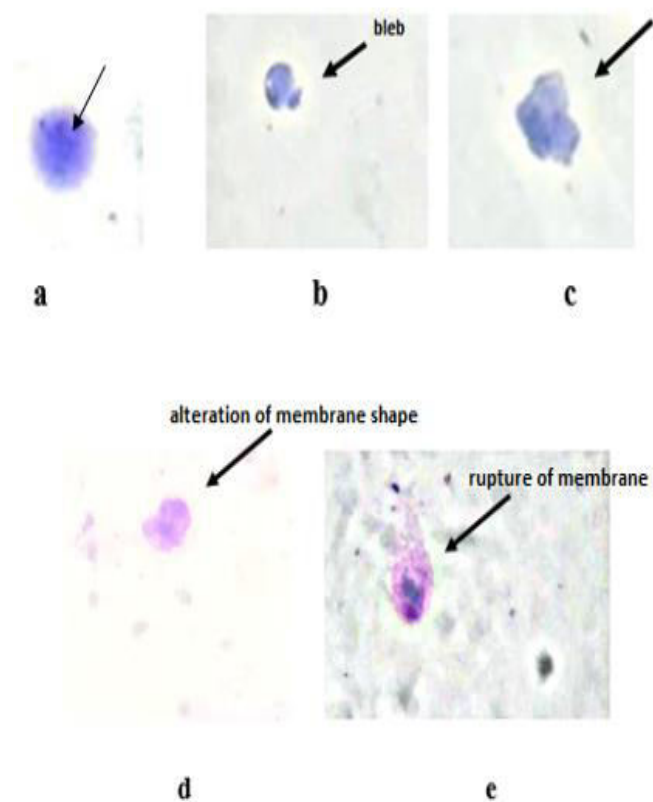


Fig. 3: (a) Degeneration of nuclei, (b) Formation of membrane blebs, (c and d) Alteration of membrane shape and (e) rupture of plasma membrane(indicated by arrow) (x400)

4. CONCLUSION

By analysing the morphology of hemocytes and behaviour, one can determine how much the concerned ecosystem is polluted [9]. Present study may help to establish an effective bio-indicator by which the health of the grassland ecosystem can rapidly and accurately be screened to protect its important bio resource.

5. ACKNOWLEDGEMENT

Author is thankful to Head, Post Graduate Department of Zoology and Principal, Barasat Govt. College for necessary support.

Conflict of Interests

There is no conflict of interests regarding the publication of this paper.

6. REFERENCES

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