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HEMOCYTES PROFILE OF STINK BUG, *CANTHECONA FURCELLUTA* Wolff: A SEVERE PEST OF *ANTHERAEA MYLITTA* DRURY

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ABSTRACT:

The five types of hemocytes were documented in Stink bug, *Canthecona furcelluta* Wolff, they are prohaemocytes (PRs), plasmatocytes (PLs), granulocytes (GRs), adipohaemocytes (ADs) and oenocytoids (OEs). PRs are small spherical cells with a centrally located large rounded nucleus filling the entire cytoplasmic area. Mitotic divisions are frequently seen in these cells. PLs are spindle shaped biramous, triramous and possess relatively smaller central nucleus with cytoplasmic prolongations. GRs are spherical with centrally located nucleus. Their cytoplasm is normally crammed with thick granules of diverse sizes. ADs are large, roughly rounded to ovoid in shape with a comparatively small eccentric nucleus. OEs are also large sphere-shaped with a small rounded eccentric nucleus and their cytoplasm is generally thick and uniform. In addition great deal of variations was recorded in the differential hemocyte count (DHC) of nymhal and adult stage.

Key words: Hemocyte, differential hemocyte count, C*anthecona furcelluta, Antheraea mylitta*,

INTRODUCTION

Man has faced the nuisance of insect pests quite early in the history of his existence. They cause not only heavy loss to agricultural crops and silk industry but also, fruits, vegetables, clothes, various houses hold commodities *etc*. Stink bug *Canthecona furcelluta* Wolff is the blood sucking pest of the tropical Tasar insect of India, *Antheraea mylitta* Drury and cause serious damage to Tasar industry every year. Haemolymph cells (hemocytes) are key component of the insect defense system with multiple functions such as nodule formation, phagocytosis, encapsulation, synthesis and transport of nutrients, hormone carrier, wound healing *etc*. Hemocytes are also reported as a source of storage protein. Hemocytes categories and their counts were studied by several researchers (Wigglesworth 1959; Jones 1962; Gupta, 1979; Lackie 1988; Jalali *et al*., 2008). The various aspects of hemocytes have been studied in hemipterous insects by a number of workers (Sonawane and More, 1993; Sharma *et al*., 1998; George and Ambrose, 2004*).* In *A. mylitta* five types of hemolymph cells were recorded viz. prohaemocytes (PRs), plasmatocytes (PLs) granulocytes (GRs), spherulocyte (SPs), adipohaemocytes (ADs), oenocytoids (OEs) (Sharan *et al*., 2005). In our knowledge best hemocyte profile of Tasar silkworm *A. mylitta* serious pest *C. furcelluta* are totally ignored by researchers. Considering all these aspects the present study was, therefore, conducted to investigate the hemocytes categories, their structure and profile of Stink bug*, C. furcelluta***.**

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MATERIALS AND METHODS

Insect culture conditions: Stink bug, *C. furcelluta* Wolff **(**Hemiptera: Pentatomidae) insects were obtained from field premises *i.e.* silkworm rearing site, Central Tasar Research and Training Institute, P.O. Piska Nagri, Ranchi, India.

Blood smear slide preparation: Blood samples were obtained from desired stages and age groups in live insects. A drop of haemolymph obtained near the edge of a slide by cutting antenna was drawn into a thin uniform film by pulling the edge of an inclined slide backward. The film was air-dried and stained.

Giemsa Hemocyte Staining Technique: Stock solution of Giemsa stain was prepared by employing the method of Yeager (1945) and a portion of it were diluted to 10 times with distilled water. The air-dried smear was stained with the diluted stain for 20 minutes. The slide was rinsed in distilled water and mounted in DPX. Observations on hemocyte categories based on morphology, function and staining reaction were made by light and phase contrast microscopy.

Differential hemocyte count (DHC): 200 cells of different categories were selected and from random areas of stained smears of 10 insects from both the experimental and control groups made it separately and the percentage of different cell types was calculated.

RESULTS

Based on morphology and staining reaction, five types of hemocytes viz. prohaemocytes (PRs), plasmatocytes (PLs), granulocytes (GRs), adipohaemocytes (ADs) and oenocytoids (OEs) have been documented in *C. furcelluta* Wolff (Fig.1).

PRs: These cells were spherical with scanty cytoplasm and a large rounded nucleus both the nucleus and cytoplasm are basophilic. Mitotic divisions were often seen in PRs.

PLs: The PLs were fusiform, spindle shaped, biramous or triramous. The somewhat smaller central nucleus either rounded or oval in shape. These cells possessed a spherical or a triangular central body with small or long cytoplasmic prolongations. The cytoplasm of these cells appears finely granulated and the nucleus with discrete chromatin granules.

GRs: The GRs were spherical and their rounded nucleus was centrally located. The nuclear chromatin was homogeneous and the cytoplasm was granular. The nucleus occupies 25 to 35 per cent of the cell space. The cytoplasm was characteristically filled with dense granules of different size. The granules due to their large numbers every now and then hide the nucleus.

ADs: ADs were large roughly rounded with the spherical nucleus was eccentric occupying 12-18 per cent of the cellular space. The nucleus was relatively small rounded or slightly elongated. A number of globules possibly of fat were observed in the cytoplasm.

OEs: In these were large spherical cells with widely variable sizes small round eccentric nucleus occupying 9-21 per cent of cellular space. The cytoplasm was generally thick and homogeneous. These cells were small to large and rounded in shape with a smooth cell boundary. Infrequently, two nuclei may be present.

DHC: The relative percentages of different types of hemocytes vary greatly in 24hr old V instar nymphs and adult bugs. The count of PRs and ADs were more in 24 hr old nymph and more PLs and OEs were recorded in 24 hr old adult bugs. The count of GRs was very less and pattern of change in profile of PLs and ADs looks interrelated in nymph and adult insects (Fig. 2).

DISCUSSION

In present study, five types of hemocytes; PRs, PLs, GRs, ADs and OEs have been recognized on the basis of their distinctive morphological features and staning reaction. These cell types have been reported by several researchers in different insects (Wigglesworth

1959; Jones 1962; Gupta, 1979; Lackie 1988; Jalali *et al*., 2008). The hemocytes in hemipteran insects have been studied by several workers (Sonawane and More, 1993; Sharma *et al*., 1998; George and Ambrose, 2004). PRs are reported in almost all the insect orders except Collembola, Thysanura and Odonata (Gupta, 1985a) and have been variously named for instance, proleucocytes (Hollande, 1911). Hollande's proleucocytes were later changed as PRs by Jones (1965) on the basis of their different origin and relationships among insects. Except Collembola, PLs have been reported in all insect orders as leucocytes, micronucleocytes, giant fusiform cells, lamellocytes and immunocytes (Gupta, 1985a). Because of the typical features of their own, PLs and PLs have been given separate identities in present investigation. GRs are plesiomorphic and are the only hemocyte types that have been reported in all arthropod groups and Onychophora (Gupta, 1985a) as amoebocytes with granular cytoplasm, pycnoleucocytes (Wille and Vecchi, 1966). ADs were reported only in seven higher insect orders as adipospherules, adipoleucocytes, spheroidocytes and adipocytes.

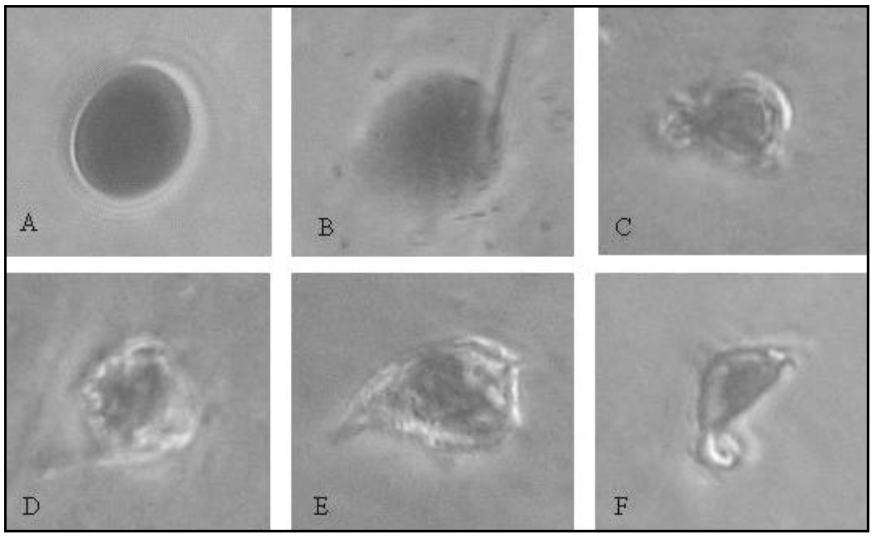

Fig.1. Types of hemocyte in *Canthecona furcelluta* A- prohaemocytes, B- granulocytes C- adipohaemocytes, D- oenocytoids and E-F- plasmatocytes.

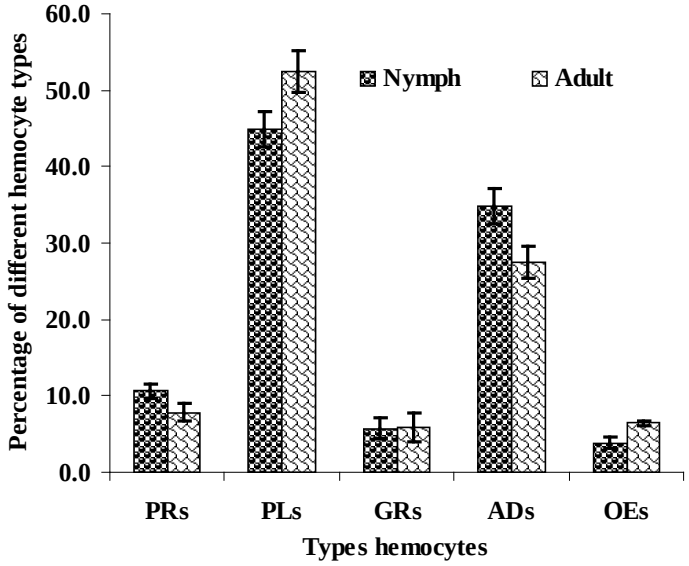

Fig 2. DHC of 24 hr old V instar nymph and adult of Stink bug, *Canthecona furcelluta* (*n*=10).

While Ashhurst and Richards (1964) and Shapiro (1968) reported ADs in *G. mellonella.* Likewise in present investigation, ADs were regarded as a distinct cell type. OEs were not detected in Collembola, Thysanura, Odonata, Orthoptera, Dermaptera, Mantodea, Plecoptera and Neuroptera and thus have been reported in ten insect orders (Gupta, 1985a). These cells (OEs) were variously named as oenocytes, non-granular spindle cells and the crystalloid cells. While these cells have been attributed various shapes viz. rounded to ovoid, rod-shaped, elongated, fusiform or even triramous in different species of Noctuidae (Arnold, 1982), Gupta (1985b) found them crescent shaped in the cockroach, *Gromphadorhina portentosa*. Like other researchers a variation in DHC was observed in present insect also. More PRs count was noticed in our study corroborate the report of Yeager (1945) and Jalali and Salehi (2008).They found the PRs have their peak in early instar nymph/larvae and this gives weight to the normally established view that PRs are the stem-cells which give rise to other cell types (Jones, 1962; Gupta, 1979). More PLs and and OEs number in adult *C. furcelluta* in comparison to nymph suggest their involvement in egg production. Very less number of GRs was recorded in present insect probably due to i) other cell types are doing analogous function similar to GRs ii) *C. furcelluta* is utilizing the haemolymph of other insects (such as *A. mylitta*) as equivalent function of GRs. Further confirmation need to be done to prove why the count of GRs is very less in present insect.

Conclusions: A hemocyte profile of Stink bug, *C. furcelluta* was studied probably first time and five types of hemocytes were identified they were PRs, PLs, GRs, ADs and OEs. Obvious changes in DHC of 24 hr old nymph and adult were observed. Since hemocytes are key component of the insect defense system with multiple functions such as nodule formation, phagocytosis, encapsulation, synthesis and transport of nutrients, hormone carrier, wound healing *etc.* Present study at hemocytic level will open the door for researchers in the area of *A. mylitta* pest control at cellular level.

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