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Effects of Phytopesticide on the Fat Body of Adult Male Blister Beetle, *Mylabris indica* (Thungberg) (Coleoptera: Meloidae) in Relation to Reproduction

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Abstract: Histopathological effects of the sublethal concentration of Vijay neem on the fat body of the polyphagous pests *Mylabris indica* was evaluated. The results revealed a characteristic changes in the fat body cells of treated insect than control insect such as the disintegration and lysis of cell membrane, loss of granular nutritive substance in the cytoplasm, appearance of large sized vacuoles, disintegration and disorganization of nucleus were observed in the present study.

Key words: *Mylabris indica* · Fat body · Phytopesticide · Vijay neem

INTRODUCTION

The blister beetle, *M. indica* a polyphagous insect pest on oil seeds, pulse, ornamental and vegetable crops, which make a heavy loss by means of devouring the flowers. Phytopesticides, especially neem derivatives are more reliable, economic, ecofriendly insecticides proved to be environmentally safe and effective against a number of insect pests and do not leave any residue in the environment. The insect fat body is an organ analogous which carries out a variety of different metabolic activities comparable to mammalian liver. It is the place of intense biosynthetic activity throughout the insect life and is the main source for the hemolymph proteins. An equally important function of the fat body is the storage site of food reserves [1-3]. The fat body undergoes growth and development along with the other insect tissues. The fat body cells are united by desmosomes and gap junctions and are often separated from one another by haemolymph spaces. The loose texture and elasticity of the fat body encourage interchanges with the hemolymph [4]. Although regional differences in structure and functions of the fat body have been reported by Wigglesworth [5], Dean *et al.* [6] and Cochran [7], however, these are generally insignificant [4].

This insect fat body tissues are constituting mostly in trophocyte, or adipocytes, of mesodermal with several origin capable of storing proteins, carbohydrates and lipids [2, 6], in addition to synthesizing lipids and proteins [8-10]. The oenocytes are ectodermal origin as well as mycetocyte and urocytes may be meant for excretion. It plays a vital role in reproduction, metabolizes hormones and other essential messenger molecules and also detoxifies wastes or harmful compounds [11-13].

Trophocyte are found in all life stages of insects. Their shape, content and volume vary depending on the developmental stages and nutritional condition of the insect [10]. Chapman [14] has reported that the nuclei of fat body cells are spherical or oval and one or more nuclei with a large nucleoli.

The histological and histopathological features of fat body have been investigated in some insects such as *Odontopus varicornis* [15-16] and *Gryllotalpa africana* [17]. However, the effects of phytopesticide and other known toxic substances on histological changes of fat body have not been well documented as these studies appear to be limited to a few species of insects using, dimethoate [18]; endosulfan [17]; monocrotopus [19]; Neem gold [20] and zoopesticide pygidial secretion [21]. Information on the fat body tissues of the blister beetles are very scanty. Hence, the present study has been aimed to describe the cell types, histomorphology and histopathology of the fat body tissue of male *M. indica* using histological techniques.

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MATERIALS AND METHODS

The insects collected from the fields and gardens were reared in wooden cages, each measuring about 30 × 22 × 28 cm at the laboratory temperature of 28 ± 2°C with a relative humidity of 80 ± 5°C percent. The floor of the cage was covered with fine sand, moderately moistened with water daily in order to maintain the humidity of the cage. The insects were fed daily with fresh host flowers. The insects thrived well on these foods. The insect cages were cleaned properly, every alternative day, by removing the excreta and other waste materials.

Male insects of *Odontopus varicornis* were selected from the cage. The adult insects were dissected using insect Ringer solution. The removed tissues were fixed in aqueous Bouin's fixative. After 24 hours of fixation, the tissues were processed for dehydration using ascending grades of alcohol. The tissue was gross stained in 70% aqueous eosin to facilitate orientation during embedding. The tissues after dehydration in absolute alcohol and acetone were cleared in xylol and finally embedded in paraffin wax (58°C – 62°C). Sections cut at 6ì, thickness were deparafinised using descending grades of alcohol and stained with haematoxylin and counter stained with aqueous eosin for microscopical observations and microphotographs.

RESULTS

The fat body is divided into a peripheral portion (Parietal) which is firmly attached to the overlying epidermis and a central mass (Perivisceral) which exist as a compact mesh work of anastomosing lobes in the space between the gut and body wall (Fig. 1). The fat body exists as a single or double layer of cells, a few small discrete fat droplets may be seen scattered in the cytoplasm and basal lamina that may be or fused when two or more trophocytes were adjacent (Figs. 2 and 3). Histomorphologically, the fat body tissues *M. indica* consists of two types viz., cuboidal cells and round shaped cells. The cuboidal cells are explained as cuboidal trophocytes (CuTr) (Fig. 3) and round cells as round shaped trophocytes (RTr) (Fig. 4), respectively. Cuboidal trophocytes were found in more numbers than that of round-shaped ones.

The histological observations showed that fat body of male insects characterized by their distinct cell boundaries (Figs. 1 and 4). Both CuTr and RTr cells have centrally located spherical or round nucleus, distinct cytoplasm with numerous small vacuoles and fine granular structures may be nutritive substances like lipids, proteins and glycogen. Many of the CuTr cells have a single nucleus but some cells were found to have two nucleus (Fig. 4) whereas, RTr cells have only one round shaped nucleus. Chromatin materials of the both trophocytes were intensively stained with haemotoxylin and granular nutritive secretory materials were observed in the fat body tissues (Figs. 2-4). These cells probably meant for the synthesis of male specific proteins and also play a vital role in immune responsibility.

Histopathological observations on the fat body cells showed hypertorophy, the cell membrane was not distinct and less secretory nutritive materials and the total cell sequence were shrunken to form the large vaculoles in the centre in each lobe or in group in the fat body of the treated insect than that of the control insect (Fig. 5). Increased vacuolization and less amount of granular cytoplasm with swollen nucleus indicated its utilization of stored substances by the insect when exposed to phytopesticide, Vijay neem (Figs. 7 and 8). The effect of phytopesticide showed considerable histopathological damages on the trophocyte cells and their nuclei. The boundaries of the fat body cells were either destroyed or disintegrated and vacuolation were formed within the cytoplasm and nuclei, the nuclei became scattered, unshaped and hypertrophied (Figs. 6-8).

It was observed that occurrence of granular nutritive substance (GrNs) in the treated fat body were greatly reduced due to disintegration of cell membrane and utilization of nutritive substance due to the impact of the toxic effect of phytopesticide. Various characteristic change in the nuclei such as swollen (Fig. 8), highly pycnotic condition and disintegrated nucleated cells were observed, hence these cells may not be able to synthesize the proper quality and quantity of male specific secretory proteins.

DISCUSSION

The current work demonstrated that fat body of the insect is the most important organ that synthesis and stores energy reserve, in addition to regulation the metabolic activities and reproduction.

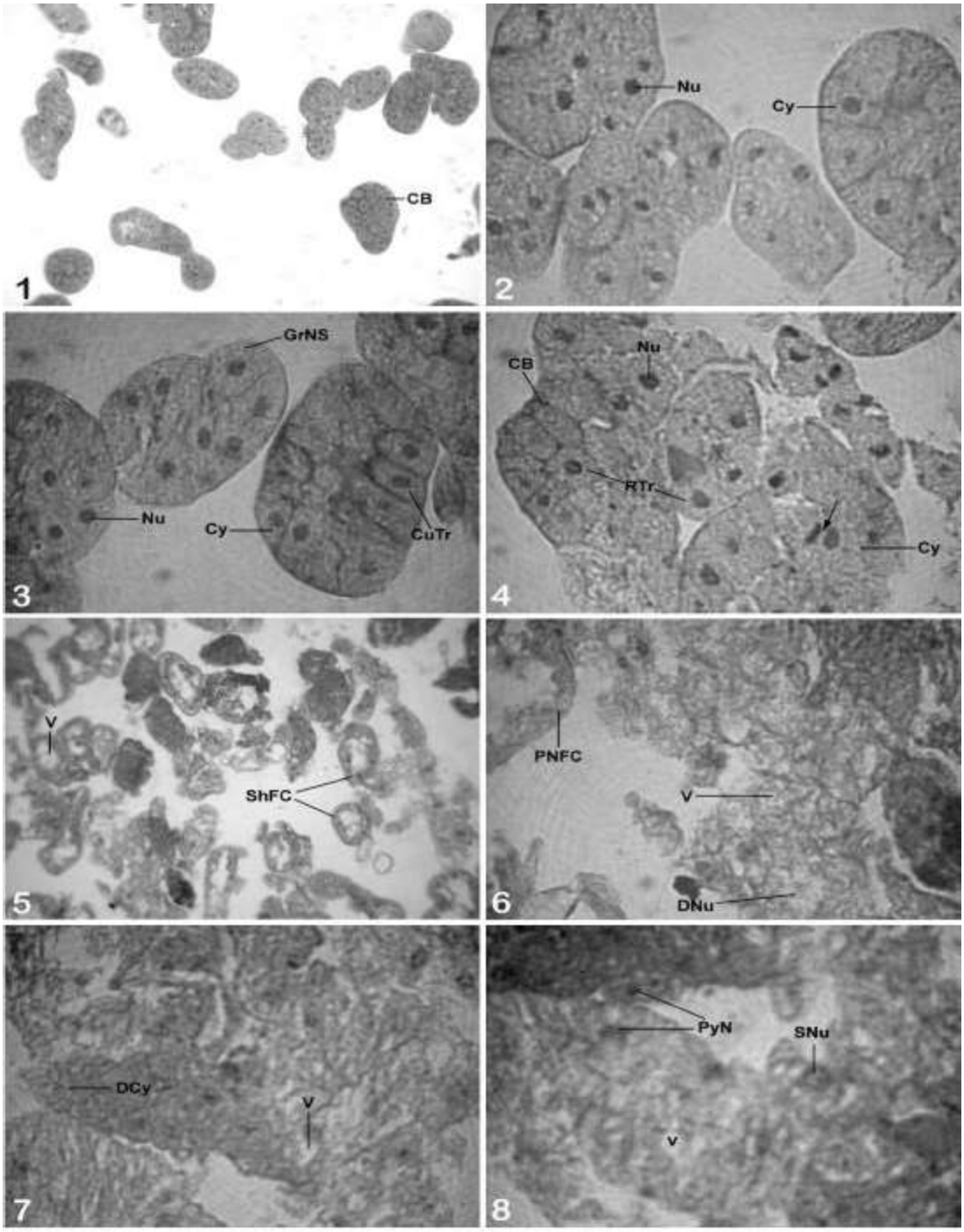

Fig. 1-8: Photographs of control (Figs. 1-4) and treated (Figs. 5-8) fat body tissue of *M. indica,* stained with hematoxylin and eosin (H & E); Fig 1 and 5 X100; Fig. 2 to 4 and 6 to 8 X400
Cell Boundaries (CB), Cuboidal Trophocytes (CuTr), Cytoplasm (Cy), Disintegrated Cytoplasm (DCy), Disintegrated Nucleus (DNu), Granular Nutritive Substance (GrNS), Nucleus (Nu), Pycnotic and Nectrotic Fat body Cells (PNFC), Pycnotic Nucleus (PyN), Round shaped Trophocyte (RTr), Shrunken Fat body Cells (ShFC), Swollen Nucleus (SNu), Vacuolization (V), → indicates Double nucleated cells

In the present study, fat bodies observed nearer to the body wall were expressed as peripheral one and fat bodies around the organs as central one. The structural details revealed that, these are found to be similar to the few earlier reports in other insects like ticks [22], *Rhinocricus padbergi* [23], *Amblyomma cajennense* [13].

Two types of trophocytes observed in male *M. indica*, were designated as cuboidal (CuTr) and round shaped (RTr), based on their morphohistological characteristics. Present findings attest that the earlier reports of Denardi *et al.* [13] on *Amblyomma cajennense* and differs with Roma *et al.* [9,10] and Crossley [24] who reported that the fat body cells comprising the trophocytes and oenocytes in hymenopteran ants and trophocytes and nephrocytes in ticks, respectively. In the present study, it has been observed clear that only trophocytes were present and oenocytes and nephrocytes were not observed in *M. indica*.

Presence of trophocytes which appear like mass of cells arranged as lobular mesh was observed on the fat bodies of *M. indica*. Similar lobular arrangement of fat bodies have been reported by several workers on various insect species, such as *Schistocerca grearia* [25], *Diplotera punctata* [26], *Glossina messilatus* [27], *Pherosphus hilaris* [28], *Bombyx mori* [29] and *Odontopus varicornis* [21]. The trophocytes contained numerous small lipid vacuoles, this type of observation was reported by Cochran *et al.* [30] on other insect orders.

The cytoplasm of trophocytes was finely granulated and it may act as a store house of food reserves. In the present study, it has been observed that the treatment with sublethal concentration of phytopesticide, Vijay neem for 48 h has caused notable changes such as cell lysis and disintegration of cell membrane, swollen, shunken and pycnotic conditions of nucleus and reduction in the occurrence of nutrient with larger cytoplasmic vascolization. The remarkable histopathological changes observed in the fat bodies of *M. indica* may be due to the impact of the phytopesticide. Similar swollen and pycnotic nucleous and cell membrane lysis were reported by Selvisabhanayakam [16]; Guozhong Feng *et al.* [31] and Lousia and Selvisabhanayakam [21] on *Odontopus varicornis*.

The reduction in the occurrence of nutrients with large vacuoles in the cytoplasm may be due to the utilization of nutritive substances, similar findings have been reported for *Odontopus varicornis* [16, 21]. The large sized of vacuolization of trophocytes is an indication of an immediate utilization of food reserves as an immediate energy requirement of cell, when the insect is under stress when exposed to the phytopesticide as reported by Ramanathan [32], Sumathi *et al.* [17], Rajathi [33], Lousia and Selvisabhanayakam [21] who have reported for *Periplaneta americana, Gryllotalpa africana, Sphaerodema rusticum* and *O. varicornis,* respectively.

It was concluded that histomorphological and histological observations on fat body cells of male *M. indica* exhibit two types of trophocytes with more granular secretory materials. Further, the drastic changes in the insect fat body after the treatment with the phytopesticide, it showed disintegration of cell membrane, very less amount of granular substances in the cytoplasm and more pronounced morphological and physiological changes in nucleus, may be due to the toxic nature of phytopesticides.

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