International Journal of Pure and Applied Zoology
Volume 2, Issue 1, pp: 41-45, 2014

ISSN (Print) : 2320-9577
ISSN (Online): 2320-9585
http://www.ijpaz.com

Research Article

SCANNING ELECTRON MICROSCOPIC CHANGES IN THE FAT BODY OF ADULT MALE *ODONTOPUS VARICORNIS* (DIST.) (HEMIPTERA: PYRRCHOCORIDAE) TREATED WITH PHYTOPESTICIDE NIMBECIDINE

T. Ramesh Kumar*, V. Iyyappan, K. Anandaraj and S. Sethuraman
Department of Zoology, Annamalai University,
Annamalai Nagar-608002, Tamil Nadu, India

Article History: Received 2nd January 2014; Accepted 9th February 2014; Published online 17th 2014

ABSTRACT

Neem has a history of use primary against household and storage pests and to a limited extent against crop pests in the Indian subcontinent. Neem pesticides are more reliable, economic, eco-friendly and proved to be environmentally safe and effective against number of insects and pests. Enormous investigations have been carried out the extract obtained from the different parts of the neem plants against different insect and pest on crop plant. In the last decades plant extracts with insecticidal properties have evoked a substantial scientific and practical interest as a natural means of insect and mite pest control in sustainable agriculture. Keeping this in view, the present study is aimed to find out the scanning electron microscopic changes in the fat body of the adult male *Odontopus varicornis* treated with the sub-lethal concentration (LD 50, 3.25%) of Nimbecidine for 96h. The present results revealed that characteristics SEM changes in the fat body of treated insect than control insect such as shrunken ruffled membrane with small pinocytotic pits. The disorganized cytoplasmic inorgination indicates the occurrence of less amount of storage substance may probably be utilized for the insects to avoid the stress during the Nimbecidine intoxication.

Keywords: Male insect, fat body, male insect, Nimbecidine treatment, ultra structure.

INTRODUCTION

India is endowed with rich tropical floras, which are considered store houses of plant protection chemicals. It has been estimated that only 5-15 percent of the existing plant species have been surveyed for biologically active compounds (Balandrin *et al*., 1985). Indian neem tree has been found to be a promising source of natural pesticides. Plant compounds of the neem tree have been successfully used for integrated control of pests in the tropics and in temperate zones (Jacobson, 1987). In the last decades plant extracts with insecticidal properties have evoked a substantial scientific and practical interest as a natural means of insect and mite pest control in sustainable agriculture. The neem tree produces several terpenoid compounds with insect antifeedant and growth regulating properties, showing only low toxicity towards mammals and vertebrates (Buttlerworth and Margan, 1968). Increasing use of various pesticides for arresting the damage caused by pests has brought in its wake certain complications as pesticide pollute the soil, water and atmosphere, and kills the animals (Selvi, 1978). Nowadays, the farmers use a large number of pesticide and insecticides to control the pests, damaging crops and insects. The poor knowledge about the pesticide effect, pesticide dosage for particular pests and pesticide application method among farmers is one of the main reasons for pollution. This revolution in preparing pesticides from plants has reduced the environmental problems; avoid the hazardous effects from chemical pesticides and save the whole universe from the pesticide pollution.

The high biotic potential of insect makes the reproductive process a subject of importance in applied entomology. In recent years the dynamic aspects of insect reproduction has received more attention (Adiyodi and Adiyodi, 1974). The

*Corresponding author e-mail: ktrameshau@gmail.com, Mobile: +91 9489361412

insect fat body consists of loosely arranged (or) compact messes of cells enclosed in a membranous sheath and freely suspended in the haemocoel, so that they are intimately bathed by the blood. The cells are arranged in irregular strands (or) sheets, but the arrangement is relatively constant within a species often there is a parietal layer of fat just beneath the body wall. Less frequently a visceral layer unsheathes the gut (Chapman, 1969). The general appearance of a fat body however varies among different groups of insects, *i.e*. lobes (or) sheets in Hemiptera, fenestrated sheets in Diptera, strands (or) ribbon like structure in Lepidoptera and spheres in Coleoptera. It occurs throughout the haemocoel and usually concentrated around various internal organs, such as the alimentary canal, nervous system, gonads etc. In a large number of insects it roughly forms two layers one outer parietal layer lying beneath the integument and the outer inner visceral layer encircling the gut and various organs. It is reorganized at each moult during the post embryonic period and may retain white, orange, yellow (or) light green colours (Chapman, 1972).

The fat body of *Odontopus varicornis* is composed of different types of cells such as nutritive cells (trophocytes), urocytes and microbial cells similar to Orthopteran insects (Cochran *et al.*, 1979). It is an organ analogous in many functional aspects to mammalian liver. It serves as a main storage organ for accumulation of energy reserves and contains the various enzymes required for deposition and degradation of these reserves (Levenbook, 1985; Sayah *et al.*, 1994; Olivera and Cruz-Landim, 2003, Sarmento *et al.*, 2004). The fat body also plays a role in immune system in that, most of the characterized immune proteins including antibacterial or antifungal peptides included by microbial infection are predominantly synthesized in the fat body and are then released into the hemolymph. Histologically, the fat body of insects could be classified into two groups. In the first group, the cell boundaries are completely lost during early development as in the case of lower Dipteral and the anterior region of some of the species of *Hemiptera* (Karnavar and Nayer, 1973). In the second group, the cell boundaries are maintained at least in part, until the insect is nearly mature or ready for pupation as in the case of Orthoptera, Lepidoptera, Hymenoptera, some coleopteran and the posterior region of some species of Hemiptera (Karnavar and Nayer, 1973). Chapman (1972) has reported that the nuclei of fat body cells are spherical or oval, but later they often become ribbon shaped structure. He has also reported that the cytoplasm is vacuolated and distributed by storage of glycogen, lipid or protein. In *Chironomus* larvae, the fat body may also contain large fluid filled vacuoles which are arising from in folds of plasma membrane (Voiner, 1927). The fat body of insect functions as a food reservoir as well as the site of metabolic activity, especially in support of egg production (Wiggles worth, 1972; Price, 1973; Keeley, 1978). The fat body undergoes growth and development along with the other insect tissues and its functional changes depend upon the developmental stages of insect (Locke, 1980). It has changing metabolic role and integral position in maintaining hormones (Keeley, 1978). The fat body is really a tissue of considerable metabolic activity and is the main source for the Haemolymph, Protein, Lipids and Carbohydrates that serve as precursors for metabolism in other tissues. In the present investigation, the phytopesticide nimbecidine has been selected for the following reasons. Toxicity studies with nimbecidine are scanty. It initiates alternative measures, which are effective, safe and acceptable to mankind. It is broad spectrum insecticide. The study has aimed to understand the grass morphology of scanning electron microscopic changes in the fat body with special reference to male reproductive process in *O. varicornis* when intoxicated with nimbecidine.

MATERIALS AND METHODS

Study design

Adult insects of *O. varicornis*, collected from the Annamalai university campus, were brought to the laboratory and reared in wooden cages, having a dimension 45x32x30cm, at a laboratory temperature of $29^o \pm 1^oC$ with 12 hours dark photoperiod. The sides of the insect cages were fabricated with wire meshes to provide sufficient aeration and light also easy to observation. 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 soaked cotton seed (*bombax ceiba*) as well as seeds of its host plant, *Stericulia foetida* and Gossypium sp. An additional food of the pieces of *Sechium edula* (chow chow) was also given to these insects. The insects were thrived well on these foods. The insects, cages were cleaned properly, every alternative day, by removing the excreta and other waste materials. The eggs laid by them were transferred to another cage and thus, a continuous culture was maintained.

It defended the range of concentration for mortality. The mortality was recorded for *O. varicornis* at 24, 48, 72 and 96 hours of exposure injecting the nimbecidine of various concentrations. The calculated LD_{50} value was 3-0% nimbecidine for 98h. After the exposure period, the fat body of control and treated insects were dissected out and the fat body tissues were fixed in 2.5 Glutaraldehyde in 0.1 m phosphate buffer at pH 7.4 for 24 h, then the samples were washed in 0.1 m phosphate buffer and dehydrated in a graded series of acetone before being subjected to critical point drying after in carbon dioxide, the tissues were mounted on metal stubs and spatter-coated model (JES-1600, coatingtime was 1.5 min with 20Ma) and they were examined with SEM(JEOL-scanning electron microscope, Hithchi-Perkin Elmer S - 450).

RESULT AND DICUSSION

The fat body of the control insect was found to be the occurrence of trophocyte with web like ruffled membrane had several pinocytotic pits. The cytoplasm of the trophocytes seems to be flattened sheet around the nuclei, which could be seen as prominent bulges near the centre of the cells. In some locations, these protuberances to be occurred in sub-peripheral regions, while the outer margin of the membrane was appeared as rope like structure (Figure 1 and 2).

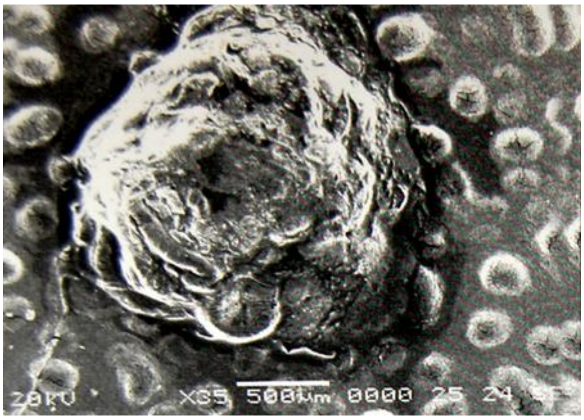

Figure 1. Fat body of control insect showing fat body cells × 500.

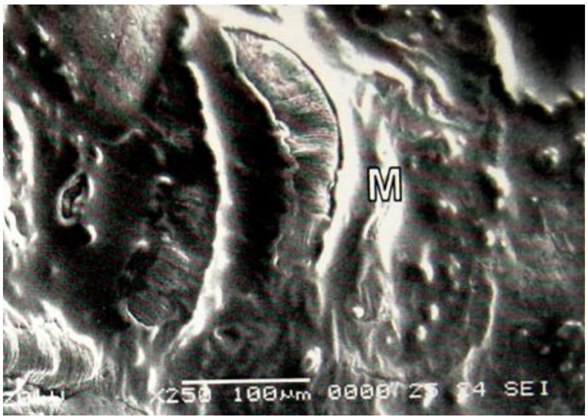

Figure 2. Fat body of control insect showing microvilli (M) × 250.

The invagination resembled like pinocytotic pits was seen inside. Minute blebs associated with cytoplasmic invagination were also seen along the margin of the membrane. Penetration of macrophages and phagocytosis on such trophocytes were visible. The apices of secretory cell seem to be revealed the smooth secretary granules beneath the apical plasma membrane. The trophocytes of treated insects showed remarkable changes in the scanning electron micrograph such as shrunken ruffled membrane with very small pinocytotic pits. The protuberances near the centre of the cells were found to be shrunken (Figure 3 and 4). The outer margin of the rope like structure is disintegrated. Minute blebs associated with cytoplasmic invaginations were found to be disorganized, indicates the lees amount of storage substance probably be utilized for the insects to avoided the stress due to phytopesticide nimbecidine intoxication. In the present study, it has been observed that characteristic SEM changes in the treated insects than the control insects such as shrunken ruffled membrane with small pinocytotic pits. These changes may be attributed due to treatment with sub-lethal concentration of nimbecidine on *O. varicornis*. These results are in consistent with the works of ultra structural study on *Rana esculenta* during starvation. Similar result were observed by Sumathi (2005) and Lousia (2006) for *Gryllotalpa Africana* and *O. varicornis* treated with the pesticide endosulfan and the zoopesticide, pygidial secrection, respectively.

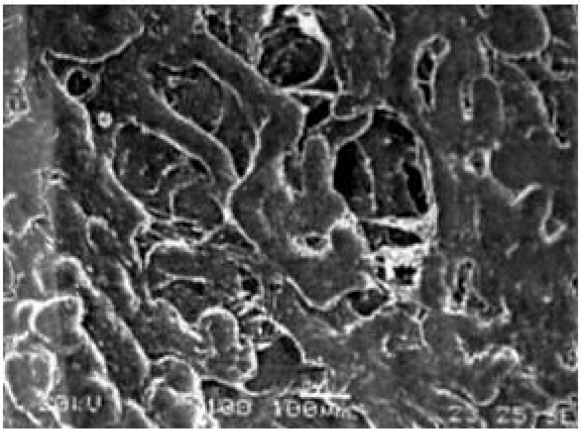

Figure 3. Fat body of treated insect showing disintegrated cytoplasm × 100.

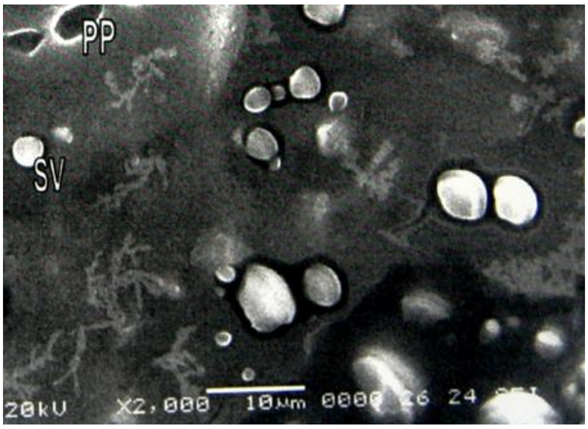

Figure 4. Fat body of treated insect showing shrunken (SV) ruffled membrane with small pinocytotic pits (PP) × 2000.

CONCLUSION

The sub-lethal dose of phytopesticide, nimbecidine caused remarkable changes in the fat body of *Odontopus varicornis* when it exceeds required concentration of nimbecidine for regular biological functions. It reduces the potentiality of reproduction. It reveals that, the phytopesticide, nimbecidine is considered as an effective pesticide, eco-friendly for sustainable in the field of agriculture under Integrated Pest Management (IPM).

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest associated with this article.

ACKNOWLEDGMENT

Authors are very grateful to the authorities of Annamalai University and also to the Head, Department of zoology for providing the facilities to complete this work successfully. And also our special thanks to central instrumentation laboratory, Annamalai University, for the SEM studies.

REFERENCES

Adiyodi, R.G. and Adiyodi, K.C., 1974. Infrastructure of the utriculi majores in the mushroom - shaped male accessory gland of *Periplaneta Americana* (L.). *Z. Zellforsch*, 147: 433-440.

Balandrin, M.F., Klocke, J.A., Wurtele, E.S. and Bollinger, W.H. 1985. Natural plant chemicals: Sources of industrial and medicinal materials. *Sci.*, 228: 1154-60.

Buttlerworth, J.H. and Margan, E.D., 1968. Isolation of substance that suppress feeding in locusts. *J. Chem. Commun.*, 23-24.

Chapman, R.F., 1969**.** The reproductive system. In: the insect structure and function, 3rd Edition, London.

Chapman, R.F., 1972. The Insect Structure and Function. The English Universities Press Ltd., London.

Cochran, D.G., Donald Donald Millins, G. and June Mullnis, K., 1879. Cytology changes in the fat body of the American cockroach, *Periplanta Americana* in relation to dietary nitrogen levels. *Am. Ent. Am.*, 72: 197-205.

Jacobson, M., 1987. Neem research and cultivation in the Westen Hemisphere. In: Natural Pesticides From the Neem Tree and Other Tropical Plants (Eds. Schmutterer, H. and Ascher, K.R.S.), Eschborn, pp. 33-44.

Karnavar, G.K. and Nayar, K.K., 1973. Insect fat body. *J. Anim. Morph. Physiol.*, 20: 64-89.

Keeley, L.L., 1978. Endocrine regulation of fat body development and function . *Ann. Rev. Ent.*, 23: 329-252.

Levenbook, L., 1985. Storage proteins. In: Comprehensive Insect Biochemistry, Physiology and Pharmacology. Pergamon Press, Oxford, pp. 307-346.

Locke, M., 1980. The cell biology of fat body development. In: Insect Biology in the Future (Eds. Locke, M. and Smith, D.S.), Academic Press, New York., pp. 227-252.

Locke, M. and Smith, D.S.), Academic Press, New York, pp. 463-478.

Lousia, M., 2006. Studies on the impact of phygidial secretion on certain selected tissues in the adult male insect *Odontopus varicornis* (Dist.) (Heteroptera: Phyrrhocoridae) in relation to reproduction. Ph.D., Thesis, Annamalai University, Annamalai Nagar, India.

Oliveira, V.T., and Cruz-Landim, C., 2003. Size of fat body trophocytes and ovarian development in workers and queens of *Melipona quadrifasciata anthidioides. Sociobiology,* 41: 701-709.

Price, G.M., 1973. Protein and nucleic acid metabolism in insect fat body. *Biol. Rev*., 48: 333-375.

Sarmento, R.A., H.G. Oliveira, A.M. Holtz, S.M. Silva, J.E. Serrao and A.Pallini. 2004. Fat body morphology of *Eriopis connexa* (Coleoptera: Coccinelidae) in function of two alimentary sources. *Braz. Arch. Biol. Technol.*, 47: 407-411.

Sayah, F., Karlinski A., and Brecizet M., 1994. Hormones regulating structural changes in the adipocytes of the female earwig *Labidura riparia*. *Tissue Cell*, 26: 587-597.

Selvi,T., 1978. Effects of the pesticide sevin on germ cell development and differentiation in *rhysida* species. M.Phil. thesis, Annamalai University, Annamalai Nagar, India.

Sumathi, S., 2005. Studies on the impact of endosulfan on certain selected tissues of the adult male insect *Gryllotalpa Africana* (Palisotde Beaurola) (Orthoptera: Gryllolpidae) in relation to reproduction Ph.D. Thesis, Annamalai University, Annamalai Nagar, India.

Voiner , V., 1927. Sur 1’ Existence d’ un tissue mesenchy matexu vaculacoure dangles larves de chironomous. *C. R. Soc. Biol. Paris*, 96: 1015-1017.

Wigglesworth, V.B., 1972. The principles of insect physiology. 7th Edition, Chapman and Hall, *London*.