Effects of Latex (Phytopesticide) on Scanning Electron Microscopic Changes in the Male Accessory Reproductive Glands of Adult Male Insect *Odontopus varicornis* in Relation to Reproduction

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ABSTRACT

Man and insect have been at war for the same food and same places to live in insect attack man and his domestic animals by causing diseases. Man has succeeded in bringing down the insect populations to level at which both of them can-exist to materialize this ecological balance. Insecticides are introduced in modern methods of pest control. The term pesticide is used for those chemicals which kills and control the pest population. Pesticides are one of the major xenobiotic substances that have been used in India for a longer period of management for pest in agriculture fields and control of vectors in public health operations. The toxicity of the pesticides used against pest found to be damaging not only to the pest but also to other animals in the ecosystem. The study was undertaken to find out the effect of latex (phytopesticides) on *Odontopus varicornis*. They were exposed to latex for 24, 48, 72 and 96 hrs and its sublethal concentration was found to be about 4.1% for 48hrs. When the insects were injected with sublethal concentration 4.1% for 48hrs, the study revealed certain remarkable changes in the scanning electron microscopic of the male accessory reproductive glands (MARGs) such as folded cuiticle, secretory granules contain less secretory substances. It suggested that phytopesticide causes several SEM changes in the MARGs of *O.varicornis* and affect the reproductive potentiality of *O.varicornis*.

Key words*: Odontopus varicornis*, SEM changes, MARG, Latex

INTRODUCTION

The use of chemical pesticides and fertilizers in Indian agriculture has seen a sharp increase in recent years. The heavy use of these chemicals has already caused grave damage to health and ecosystems. It is therefore increasingly urgent that environmentally friendly methods of improving soil fertility and pests and disease control are used. About 80,000 tons of pesticides are used in agriculture in India annually (Srinivasan, 1997). The intensive use of pesticides in agriculture is a cause of serious concern. The problem is especially serious because of the development of resistance to pesticides in important pests and the presence of pesticide residue in agricultural. This article investigates the potential and constraints in the use of biopesticides. Pesticide resistance has mainly been caused by excessive and indiscriminate use of pesticides (Jayaraj, 1989).

Pesticides of spurious quality, which are commonly sold in small towns and villages, have also contributed to resistance in many areas. It is clear that the excessive use of chemical pesticides in agriculture is a serious cause of concern (Schmutterer, 1995). It is, therefore, important that alternative, environmentally friendly methods of plant protection are adopted, such as integrated pest management (IPM) techniques, including the use of biopesticides. This has necessitated the use of ecofriendly products. Ecofriendly biopesticide agents are available abundantly in nature (Swaminathan, 1992).we select *Calotropis gigantea* latex for pest control agent.

Reproductive physiology of male insect is a complicated phenomenon, and it deals with the structure and functions of various tissue components of the system. Male accessory gland products have attained great importance in insect reproduction as they are a means of transport for sperm and can form a mating plug. They have specific compounds that can modify the behavior and physiology of mated females (David *et al*., 1998).

The principal secretory products of these glands in male insects are proteins, carbohydraes, and lipids.(Selvisabhanayakam *et al*., 1995; Gillott ,2003)

Calotropis gigantia (L) R.Br. (Ascelpiadaceae) is widely growing and native plant of India. (Lindley, 1985). It is a 3-4 m tall shrub with milky latex. Traditionally the milky juice of *Calotropis gigantia* has been used as a violent purgative, gastrointestinal irritant and abortion inducer (Chopra *et al*., 1956). Hence, the present study was undertaken to find out the impact of latex on the test insect, *Odontopus varicornis*.

MATERIALS AND METHODS

The insects collected from the fields and gardens were reared in wooden cages, each measuring about 30 cm x 22 cm x 28 cm at the laboratory temperature of 28±2°C with a relative humidity of 80±5 per cent. 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 seeds *(Bomba ceiba)* as well as seeds of its host plant, *Stericulia foetida* and *Gossypium* sp. An additional food of the pieces of chow-chow *(Sechium edule)* was also given to these insects. The insects were thrived well on these foods. The insect cages were cleaned properly every alternative days 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. An adult male is smaller in size than the female with pointed aedeagus, female with broader abdomen.

Collection of plant latex

Calotropis gigantia plant latex was collected between January and March 2010 from the surroundings area of our university campus.

Extraction of latex

The fresh latex of *Calotropis gigantia* was collected from the aerial parts of the healthy plants. The collected latex were subjected over drying at 60^0 C for 12 h. the dried matter (100 mg) of latex was extracted by using 1.0 ml of organic solvents (each of chloroform, distilled water, dimethyl solfoxide, ethyl acetate, hexane and methanol). The resultant mixture was vortexes and centrifuged at 3000 rpm for 10 minutes. The supernatant was used for insect control agent.

Scanning electron microscopic study

Male accessory reproductive gland of control and treated samples were dried in vaccum for getting good moisture free specimen was needed. Then the samples were mounted in stereo scan 440- model electron microscope UK. For Scanning Electron Microscopy (SEM), the male accessory reproductive glands of anesthetized specimens were removed and fixed in the karnovsky solution(2% paraformaldehyde and 2.5 glutaraldehyde in 0.2m sodium). The tissues were then dehydrated in a graded ethanol series (70%-100%) and in 100% ethonal acetone(1:1) solution, followed by four washes in 100%acetone. After drying, the samples were assembled on aluminium stubs, coated with gold examined and photographed with Joel JSM-P15. Scanning Electron Microscope (Wood Ward, 1972).

The ascertaining voltage given was 20kw and the beam current used was in between 18-25pa (Pica amperes) notching distance was between 39 mm to 1 mm. The secondary electron images were taken for all the samples with varied magnifications from 50 x to 10,000 x.

RESULT AND DISCUSSION

The accessory glands appeared as transparent, short, pear-shaped body, pale white in color, and richly supplied with tracheole tubes. They lie at the posterior median end of the abdominal cavity just below the junction of vasdeferentia, and the pear-shaped structures situated on either side of the reservoir which led to the common ejaculatory duct . These glands were mesodermal origin in *O. varicornis*, hence they are called "mesadene glands." The glands showed periodic cyclicity, i.e., the size varied depending upon the activity of the gland and age of the insect. These glands constitute a continuous tube, curved and coiled without any order to form a globular mass of tube, ensheathed by thick cuboidal cells of single layer. Superficially, they look like a wrinkled ball and were colorless. These glands were also called mucus glands or cement glands. The secretions of these glands were poured into the posterior end of the ampulla.

The MARGs of the control insects exhibited tubular structure with myoepithelial cells which were covered with tracheoles. The tubules externally were covered with thick folded cuticle. The secretory epithelial cells consisted of numerous pores and secretory vesicles with short microvilli were responsible for the specific

mode of secretion by these cells. The lumen contains secretory substances and numerous secretory vesicles. The nucleus was found to be larger hole in centre

The myoepithelial cells that cover the tubular glands were disintegrated with thick muscular layer. The secretory epithelial cell was covered with very thick rope like structure with basement membrane consists of less amount of secretion by these affected cells, when insects are intoxicated with the phytopesticide, latex.

Several SEM changes have been observed in the male reproductive organs of *Odontopus varicornis* when exposed to latex for 48h. Such as the tubular testis follicle became highly pycnotic with folded cuticle, disorganized and disintegrated secretory granules. The spermatids of the treated insect have shrunken with numerous vacuoles, which interfere with the reproductive potentiality of the treated insects than the control insects. Further, the present study has revealed that there were numerous changes have been noticed in the rest of the reproductive tissue of MARGs. The following SEM changes were observed in the treated insects like shrunken tubule with thick myoepithelial cells, the rope like structure of the plasma membrane, which was observed to be highly pycnotic and disintegrated cubical epithelial cells. The muscular layer was also found to be thickened and highly pycnotic. The pinocytotic vesicles pits were found to be disintegrated with less organized microvilli. These SEM changes perhaps attributed to interaction with the phytopesticide, latex.

In the present study, the pesticide intoxication led to disturbance in the specific mode of secretion by these pseudostratified epithelial cells than the control insects. In treated insects, it has further been revealed that the secretory vesicle and granules with more vacuoles and pits were not observed in the control insects, suggested that the mechanism of secretion may become impaired in the treated insects.

The SEM changes in the MARGs of the treated insects have shown numerous folded cuticle and the epithelial cells were not distinct. The secretory granules were found to be less which may be attributed due to fewer amounts of secretory substances in the lumen consists of less packed sperms. These changes might be disturbed the reproductive physiology of *Odontopus varicornis*, similar results have been also reported by Verma and Raji (2000) and (Sumathi,2002) exposed to dichlorvos and endosulfan for *Oreochromis mossambicu* ; *Gryllotalpa Africana*, respectively.

Jayakumar,(1988) has observed the same changes in the *O.varicornis* treated with dimethoate. Glandular layers of accessory glands varied hisstructure and secretion were found to be nonhomogenous in the dimilin-affected *Dysdercus similis* by Arunakumari, 1984. Similarly, Sita(1984) has reported in steroid treated *D.similis* that accessory glands were deformed with reduced lumen and reduced secretions. Balakrishnan,(1990) has noticed several SEM changes in pherosphus lissoderus treated with dimethoate, which are similar to the results of other workers namely Nirmaladevi,(1990) for *Catacanthus incarnates* exposed to phosphomidon. Rajathi (2004) exposed to heavy metal mercury for *Spherodema rusticum* and also Rameshkumar (2004) for *Laccotrephes ruber* exposed to heavy metal zinc.

In the present study, it has been observed that the reduction in the size, structure of accessory gland were attributed due to the latex (phytopesticide) that brought about reproduction disturbances and sterility in the test insect, *O.varicornis* that the control insect.

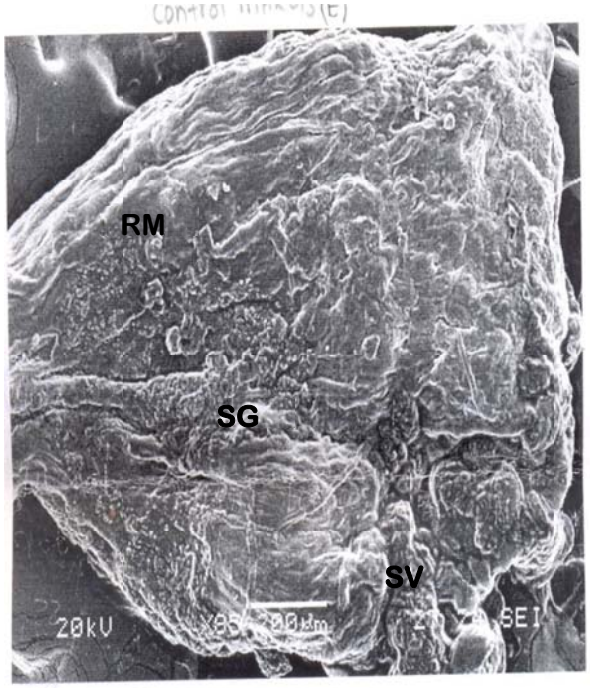

Fig.I Scanning electron macroscopic view of MARG in the control insect exposed x 200
RM-Ruffled Membarane
SV-Secretary vesicle
SG-Secretory granules

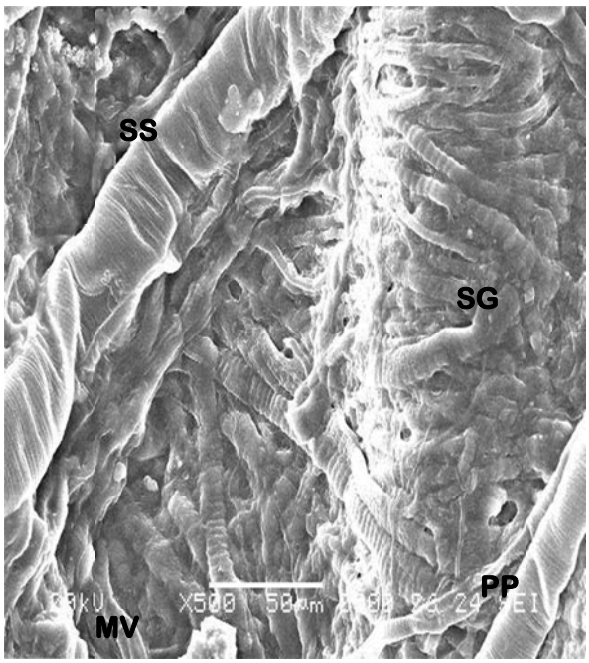

Fig.II Scanning electron macroscopic view of MARG in the control insect exposed x 500
SS – secretory substance
PP-pinocytotic pits
MV-microvillai
SG-secretory granules

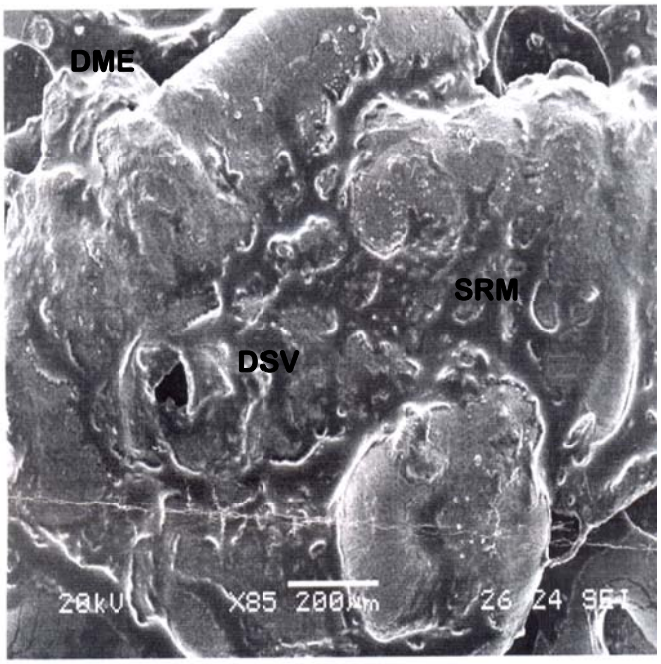

Fig.III Scanning electron macroscopic view of MARG in the treated insect exposed x 200
DME-disintegrated myo epithelium
DSV-disintegratedsecretory vesicle
SRM-shrunken ruffled membrane

Conclusion

It may be concluded in the present study that the SEM changes in the male accessory reproductive gland. The *Calotropis gigantean* latex treated insects showed less amount of secretory substances in the secretory granules and shrunken tubule with thick myoepithelial cells, the rope like structure of the plasma membrane, which was observed to be highly pycnotic and disintegrated cubical epithelial cells comparatively shrunken than control insect. These are all physiological changes which alter the function and motility of sperms which intern affects the process of reproduction.

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REFERENCE

[1] Arunakumari. Control by sexual sterility and related histological and histochemical studies in Red cotton bug *Dysdercus similis* (F) with Dimilin (Diflubenzurone) Ph.D., Thesis. Osmania University. 1984
[2] Balakrishnan S. Studies on the effect of roger on the histology and histochemistry of the testis and accessory reproductive gland of the adult male *Pherosophus lissoderus*. M.Phil., Thesis. Annamalai University,1990
[3] Chopra,R.N., Nayar,S.L., Choprs,I.C. In:glossary india medicinal plants ,vol.46.CSIR, new delhi. 1956
[4] David VB, Kumaraswami K. Madras: Popular Book Depot, Elements of Economic Entomology, 1998.
[5] Gillott C. Male accessory gland secretions: Modulators of female reproductive physiology and behavior. *Ann Rev Entomol.* 2003; 48:163–84.
[6] Jayakumar C.. Effect of dimethoate on accessory reproductive gland, testis, fatboby, haemolymph neurosecretary cells and corpusallatum in *Odontopus varicornis* Ph.D Thesis. Annamalai University,1988.
[7] Jayaraj S. Advances in biological means of pest control. *The Hindu Survey of, Indian Agriculture*. Hindu Newspaper Group, India. 1989
[8] Lindley J. Flora medica.Ajay book Services, New Delhi, 1985.
[9] Nirmaladevi P. Effect of Phosphomedon on histology and histochemistry of the testis and accessory glands of adult male *catacanthes incarnates* (Spin) (Hemiptera: pentatomidae), M.Phil, Thesis. Annamalai University, 1990.
[10] Rajathi V. Impact of heavy metal mercury in adult male reproductive physiology of *Sphaerodema rusticum* (Heteroptera: Belostomatidae) Ph.D., Thesis. Annamalai University; 2004.
[11] Ramesh Kumar T. Studies on the impact of the heavy metal zinc on certain selected tissues in the adult male insect *Laccotrephes ruber* (Linn) (Heteroptera:Nepidae) 2004.
[12] Schmutterer, H., (Ed). The Neem Tree:Source of Unique Natural Products for Integrated Pest Management, Medicine, Industry and other Purposes. Weinheim. 1995.
[13] Selvisabhanayakam. Studies on reproductive physiology with special reference to mating in the adult male insect *Odontopus varicornis* (Dist.) (Heteroptera: Pyrrhoccoridae) Ph.D., Thesis. Annamalai University; 1995.
[14] Sita V. Studies on certain steroids as development inhibitor and chemosterilant on some insects of agricultural and medical importance, Ph.D., Thesis. Osmania University. 1984
[15] Srinivasan G. Panel for reduced use of chemical pesticides. *Hindu Business Line* November 5, 1997.
[16] Sumathi. Studies on the impact of endosulfan on certain selected tissues of adult male *Gryllotalpa Africana* (Orthoptera:Gryllotalpidae) in relation to reproduction. Ph.D Thesis. Annamalai University. 2002.
[17] Swaminathan, M.S.. Cultivating food for a devel-opment world. Environmental Science and Technol-ogy, 1992,26, 1104-1107
[18] Verma G,P and A.V.Raji. Scannaing electron microscopical study of gastric and intestinal mucosa of a dichlorvas exposed fresh water teleost,*Oreochromis mossambica*(Peters). Proc.Nat.Acad.sci, India. 2000,70(B):1
[19] Wood Ward L.A. Introduction to the theory of molecular vibrations and vibration spectroscopy. Oxford University Press, London, 1972.