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RESEARCH ARTICLE

IMPACT OF PYGIDIAL SECRETION (ZOOPECTICIDE) ON THE HISTOLOGICAL CHANGES IN THE SEMINAL VESICLE OF THE ADULT MALE SPHAERODEMA RUSTICUM (HETEROPTERA: BELOSTOMATIDAE).

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The male reproductive system of *Sphaerodema rusticum* consists of a pair of testis, vas deference, a pair of seminal vesicle and a median ejaculatory duct without the male accessory reproductive gland. The seminal vesicle is a white oval shaped body which was richly supplied with tracheoles. The seminal vesicle has dual function that is secretory and storage of sperms. In the present study, the seminal vesicle showed some marked histological changes in the insects treated with zoopesticide, pygidial secretion of *Pheropsophus hilaris* (25ppm median lethal concentration). It shows the highly disintegrated epithelial cells with weakly stained cytoplasm and nuclei with many vacuoles.

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INTRODUCTION.

Among invertebrates, especially in arthropods, insects have received much attention in the study of reproductive biology. Insect reproduction is an essential physiological process from the point of view of propagation. The reproductive system of the insect is found to be a complicated one. General views of insect reproduction are given by Engelmann⁽¹⁾ and Wigglesworth⁽²⁾. The structure of reproductive organ is described by Miall and Denny⁽³⁾ and Musgrave⁽⁴⁾. Reproduction always does not imply the bisexual methods, but other reproductive process like parthenogenesis, polyembryonic, etc., may also involved Nayar⁽⁵⁾. Insects are not always sexually mature when they have completed the final moult to the adult stage and in species with an adult diapause there may be a considerable delay before mature sex cells are produced Chapman⁽⁶⁾. However, sexual reproduction is by far the common and sexual differentiation on the external appearance.

The seminal vesicle of *Sphaerodema rusticum* appears to be short, stout, slender tube bearing the posterior region of the vas deferens Osanai et al⁽⁷⁾. In many groups, including Coleoptera, there are regions along the ducts such as the seminal vesicles are the secretory accessory glands, where spermatozoa are stored until mating Chapman⁽⁸⁾. Some insects, such as stingless bees Colletto⁽⁹⁾ and sand flies have no accessory glands, while in others the epithelial cells of the seminal vesicle are associated with the secretory function. In *Sphaerodema rusticum* the seminal vesicles is found to be a single layer of columnar epithelial layer surrounding the narrow lumen Numata and Hidaka⁽¹⁰⁾. The seminal vesicle has an outer connective tissue sheath and an inner circular muscle fiber Joseph⁽¹¹⁾ Jayakumar⁽¹²⁾ and Premavathi⁽¹³⁾. The secretion of these cells are found to be in the form of fine eosinophilic foamy secretory substance which are released into the lumen by the rupture of the apical region of the cell membrane and thus, it represents an apocrine mode of secretion Mernitt and Cummins⁽¹⁴⁾. The products, of these epithelial cells have same function as those of accessory glands Cantacuzene⁽¹⁵⁾ Riemann and Thorson⁽¹⁶⁾ Couche and Gillotte⁽¹⁷⁾ Fausto et al⁽¹⁸⁾. In other insects, the seminal vesicle apparently produces no secretion. Odhiambo⁽¹⁹⁾ Cruz-Landium and Dallacqua⁽²⁰⁾. Pesticide research and development has brought a large number of chemicals in protecting the crop against insect pest. Pesticides are one of the major xenobiotic substances that have been used in India for longer period. Increasing use of various pesticides for arresting the damage caused by pest has brought certain complications as pesticides pollute the soil, water, atmosphere which kills the animals Singh et al⁽²¹⁾. Hence biologists all over the world concerned over the application of pesticides, as it is likely to pose problems of

indiscriminate annihilation of useful organisms as well. Ding and Wu ⁽²²⁾. Nowadays the farmers use a large number of pesticides to control the pests, damaging crops and insects respectively. Selvi ⁽²³⁾. The poor knowledge about the pesticide effect, pesticide dosage for particular pest and pesticide application method among farmers is one of the main reasons for pollution. The complete reliance on chemical interaction for the pest control, however, has only led to resurgence and resistant development in insect pest besides eliminating their natural enemies and thereby upsetting the eco-biological balance at the end Glass and Thurston ⁽²⁴⁾. The use of insecticides has increased tremendously during the last 50 years because of the need of producing more food to meet the growing demands of the population by contaminating the amount of pesticides; biological control is also advocated and attempted on Affrent Sexvels Selvi ⁽²⁵⁾. In recent years, in addition to the bio-control, bio pesticides come into existence for integrated pest management (IPM).

The Coleopteron suborder contains eight families, several activities of glandular substances, all families possess paired pygidial glands are relatively uniform in structure but vary in chemical constituent. Gland opening in the pygidium or posterior tip of the abdomen of certain insects are pygidial glands Eisner ⁽²⁶⁾. The pygidial secretions are considered as the zoopesticides because of its chemical nature and function. Foresyth ⁽²⁷⁾. These pygidial secretions are used by many insects for their defensive mechanisms Schnepf et.al ⁽²⁸⁾. The structure of the defence system in the *Pheropsophus hilaris*, is in complex in nature consist of two sets of secretory lobes, collecting ducts collecting reservoirs, one way valves, sphincter muscles and exit nozzles Anon ⁽²⁹⁾. The most common pygidial gland compounds (Zoopesticides) are aromatic aldehydes (e.g; Hydroxyl benzaldehyde), esters, (i.e.; Methyl P-hydroxy benzoate) and Acids (e.g. Benzoic acids), Scrimshaw and Kerfoot ⁽³⁰⁾. The secretion are pungent volatile and highly suffocative when spilled on the human skin, it causes a burning secretion followed by the formation of blisters and leaves a dark yellow stain on the skin Eisner et al ⁽³¹⁾. Studies on the zoopesticide on insects are scanty. This consideration led to investigate the effect of zoopesticide, pygidial secretion on the seminal vesicle of male *Sphaerodema rusticum*.

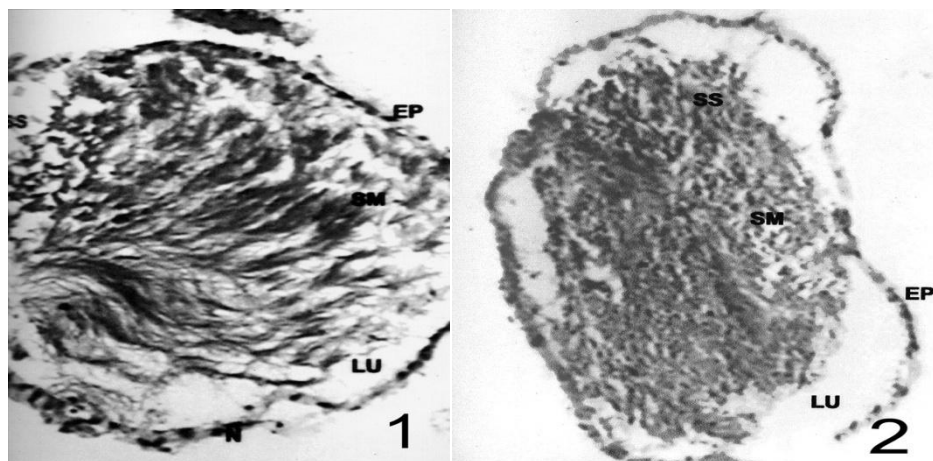
MATERIALS AND METHODS.

The adult control and treated *Sphaerodema rusticum* was kept separately, after 48hrs they were dissected under binocular microscope by using ringer solution Ephrussi and Beadle ⁽³²⁾. The ringer is subsequently removed and the tissue was fixed in bourns fluid for 24hour. Later the tissue was processed by adopting the standard histological techniques Gurr ⁽³³⁾.

The nuclear volume and diameter of the seminal vesicle were calculated using a compound microscope fixed with a range of stage micrometer and ocular micrometer statistical calculations were done and students T- test were applied.

RESULT AND DISCUSSION.

Histologically the seminal vesicle of *Sphaerodema rusticum* consists of an outer thin delicate membrane folded with epithelium, internally with numerous nuclei, the columnar epithelium which encloses a central lumen. The lumen is surrounded by a layer folded columnar epithelium and contains rich secretory substance and sperm mass. These substances have given activation and nourishment to the stored sperm mass. The cytoplasm contains a prominent spherical nucleus without any vacuoles. (Fig. 1, 2, and 3).



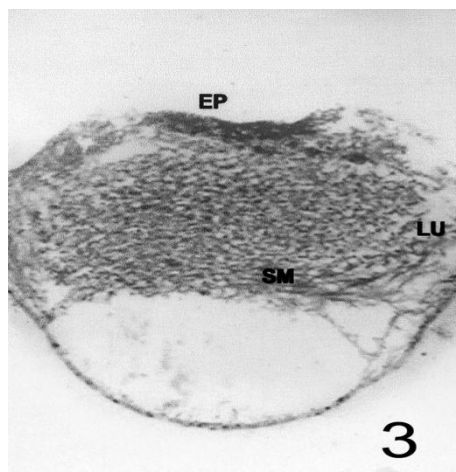


Fig.1, 2 and 3- Transverse section of seminal vesicle of control insect. EP- Epithelium, LU- Lumen, SM- Sperm mass, SS- Secretary substance.

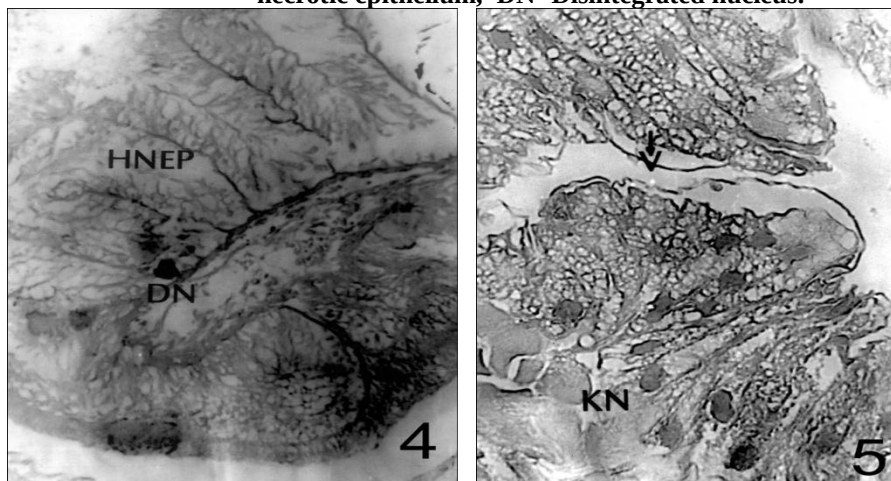
These nucleus are deeply stained with haemotoxylin. The nuclear diameter and its volume are measured about $58.55 \pm 1.2 \text{ mm}$ and $1860.12 \pm 1.4^3 \text{ mm}$ respectively.

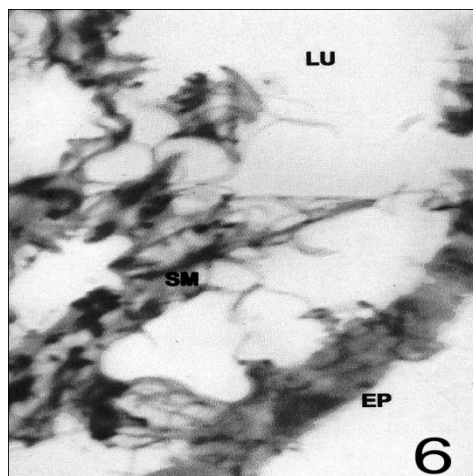
Tissue	GROUP OF INSECTS			
	Aspects	Control	Treated	Inference
Seminal Vesicle	Nuclear diameter (mm)	$58.55 \pm 1.2 \text{ m}$	$32.75 \pm 1.6 \text{ m}$	Change in the nuclear diameter after treatment
	Nuclear volume (mm^3)	$1860.12 \pm 1.4^3 \text{ mm}$	$680.20 \pm 1.22 \text{ m}^3$	Change in the nuclear volume after treatment

* Cytometric data of the seminal vesicle of the adult male *Sphaerodema rusticum*.

The seminal vesicle of zoopesticide treated insects exhibits remarkable histological changes in its architecture such as highly disintegrated folded epithelium with feebly stained cytoplasm and nuclei. The size of the nucleus and its volume are reduced and measuring about $32-75 \pm 1.6 \text{ m}$ & $680-20 \pm 1.22 \text{ m}^3$ respectively. The lumen contains a negligible quantity of secretary substance which are weakly stained (Fig. 4, 5 and 6).

Fig. 4, 5, and 6- Transverse section of seminal vesicle of treated insect. KN- Kariolytic nucleus DEP Disintegrated epithelium, LU- Lumen, NSM- Necrotic sperm mass, HNEP- Highly necrotic epithelium, DN- Disintegrated nucleus.





It has been reported earlier that the seminal vesicle of control insect showed the presence of an outer thin, delicate membrane and internally it has folded epithelium with numerous nuclei. The columnar epithelial cells surrounding the lumen and their spherical nuclei were intensively stained with haematoxylin. The cytoplasm contains a prominent nucleus without any vacuoles these findings appear to suggest that the secretory nature of seminal vesicle and its secretory substance may be used for the nourishment of stored sperms. On the other hand, the seminal vesicle of *Sphaerodema rusticum* treated with zoopesticide, pygidial secretion showed a highly disintegrated epithelium with many vacuoles in the cytoplasm. These histological observations indicate that the secretory nature of the seminal vesicle has been affected, therefore, inadequate nourishment to the developing and stored sperms. Thus, similar changes have been reported in *Odontopus varicornis* when exposed to dimethoate, enlargement of epithelial cell wall and nuclear pyknosis in the seminal vesicle Jayakumar⁽³⁴⁾ and in *Laccotrephes ruber* when exposed to mercuric chloride Palanichamy⁽³⁵⁾. From the above findings, it may be inferred that the zoopesticide pygidial secretion produces serve histopathological changes in the seminal vesicle so that the seminal vesicle has lost its secretory nature, therefore no nourishment to the developing sperms in *Sphaerodema rusticum*.

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