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Samar Mahmood
Department of Zoology,
Aligarh Muslim University,
Aligarh, Uttar Pradesh, India

Mohammad Amir
Department of Zoology,
Aligarh Muslim University,
Aligarh, Uttar Pradesh, India

Ritika Gupta
Department of Zoology,
Aligarh Muslim University,
Aligarh, Uttar Pradesh, India

Shagufta Yasmeen
Department of Zoology,
Aligarh Muslim University,
Aligarh, Uttar Pradesh, India

Correspondence
Mohammad Amir
Department of Zoology,
Aligarh Muslim University,
Aligarh, Uttar Pradesh, India

Histological effects of chlorpyrifos on the reproductive organs of red cotton stainer, *Dysdercus koenigii* (Fabricius) (Hemiptera: Pyrrhocoridae)

Samar Mahmood, Mohammad Amir, Ritika Gupta and Shagufta Yasmeen

Abstract
Red cotton stainer is a serious pest of cotton crops. It is found damaging crops in Pakistan, South-eastern Asia and various states of India-Gujarat, Uttar Pradesh, Bihar, Madhya Pradesh and Tamil Nadu. It occurs on malvaceous (e.g. okra) as well as non-malvaceous (e.g. sweet potato) plants as well. It damages cotton crops either by feeding on the seeds and staining them through their excreta or by transmitting cotton staining fungi. In present study, the effect of chlorpyrifos was studied on the reproductive organs of both the male and female of *Dysdercus koenigii* to gain information for control of their population. Chlorpyrifos in the concentration 0.005% and 0.01% was given in the feed of *D. koenigii* and observations were made after 24 hours treatment with insecticide. The ovaries of *D. koenigii* are of the telotrophic meroistic type in which, a large amount of yolk in the oocytes is localized in the vitellarium. The treated ovaries showed a considerable reduction in the amount of yolk resulting in the vacuolization and resorption of yolk which show greater damage at higher concentration. In testes major changes were found in germ cells especially spermatozoa, which were either assembled in irregular groups or adhered to each other. At high concentration (0·01%) the spermatozoa were found damaged altogether showing irregular clumping and disrupted structure. The degeneration observed in these organs were directly proportional to the concentration of chlorpyrifos used.

Keywords: Histology, chlorpyrifos, *Dysdercus koenigii*, ovaries, testes

Introduction
Dysdercus koenigii is a species of true bug in the family Pyrrhocoridae, commonly known as red cotton stainer. It is a serious pest of cotton crops. The adults and older nymphs feeds on the emerging bolls and the cotton seeds as they mature and transmit cotton staining fungi Nematospora gossypii that develops on immature lint and seeds, these are probably transmitted from contaminated boll to uncontaminated boll mechanically, on the mouthparts of the bug[1].
The red cotton stainer is called by various names in different parts of India such as Chainpa in Punjab, Kappa poka in Orissa, Lal chingum in Uttar Pradesh and Lal chusiya in Gujarat [2]. The nymphs and adults suck up the juices from the seed of either green or ripe cotton bolls. Both are voracious feeders and cause extensive damage to the crops. The damaged bolls remain shrivelled and vitality of plants and the quality of the lint is also severely affected in case of heavy infestation. As each host plant ages and becomes unsuitable, the winged adults migrate to new host plants of the same or different species. While migrating they often feed on nectar from non-host plants (usually citrus plants) and probe fruits with their rostra (beak like mouthparts). Since these insects are damaging such wide variety of plants, it is important to control them. The most efficient and easy way to control a pest is to apply insecticide in the fields. But their application poses other damages too which could be harmful for non-target species. Other non-conventional methods to control pests are there but they are not so much practically in use at farmer's level. In order to control these insects an effective and less hazardous insecticide should be used. Chlorpyrifos is a crystalline organophosphate insecticide. It acts on the nervous system of insects by inhibiting acetyl cholinesterase. It is produced via a multi-step synthesis from 3-methylpyridine. Primarily chlorpyrifos interfere with the signalling from the neurotransmitter acetylcholine. Once chlorpyrifos metabolite, chlorpyrifosoxon, binds permanently to the enzyme acetylcholinesterase, preventing this

enzyme from deactivating acetylcholine in the synapse. By irreversibly inhibiting acetylcholinesterase, chlorpyrifos leads to a build-up of acetylcholine between neurons and a stronger, longer lasting signal to the next neuron. Only when new molecules of acetylcholinesterase have been synthesized can normal function return.

Materials and Methods

The adults and nymphs of *Dysdercus koenigii* were collected from the okra field located near the Aligarh Muslim University campus and brought to the laboratory for the present work. These insects were kept in glass rearing jars containing a thick layer of damp sterilized sand at the bottom and were maintained at 28±2 °C temperature and 70±5% relative humidity. The insects were fed on overnight soaked cotton seeds, which were changed on alternate days. Over-crowding was avoided for proper culture of the insect. 10 replicates per batch for a concentration were studied at a time.

Application of Insecticide: Cotton bugs were kept in a jar containing 2 cm thick layer of sterilized sandy soil at the bottom of the jar. This soil layer was kept moist to provide the suitable environment for cotton bugs. Cotton bugs were fed with cotton seeds infused with insecticide placed in a petridish within the glass jar. Glass jars was covered with muslin cloth.

10 cotton bugs were exposed to insecticide (Chlorpyrifos) in two setups each i.e. one setup containing feed with 0.005% concentration and second setup with 0.01% concentration. In addition to this one controlled setup was also performed. The cotton bugs were examined and dissected after 24 hours.

Preservation and histological preparation: The cotton bugs were dissected after 24 hours of examination. The ovaries and testes were dissected out from male and female cotton bugs respectively and were immediately fixed in Bouin's solution for 18-20 hours. After this dehydration proceeded in ascending grades of alcohol i.e. 30%, 50%, 70%, 80%, 90% for 15 minutes each while in 96% and 100% for half an hour each followed by mixture of 100% and xylene solution (1:1) for 10 minutes then incubating the tissue in xylene and paraffin (1:1) at 60 °C for 15 minutes. The tissue was then incubated in pure wax for 2 hours. The testes and the strand of ovarioles were embedded in paraffin wax. 5µm sections were cut using microtome. The ribbons were then placed on the glass slide lubricated with a solution of albumin and glycerine (1:1). The slides containing section were warmed slightly to straighten the creases.

The slides were processed in 2 changes of xylene and then descending grades of alcohol series 100%, 96%, 90%, 80%, 70%, 50%, 30% for 5 minutes each, then in distilled water for 5 minutes. Slides were stained in Delafield's haematoxylin for 10 seconds and counterstained with Eosin for 20 minutes followed by upgrade dehydration in alcohol and then 2 changes of xylene for 5 minutes each.

After air drying, slides were mounted in DPX and observed under microscope. Photomicrographs were taken using LEICA DFC 295 microscope.

Results and Discussion

1) Ovary

a) Normal histology of ovary (Figs. 1, 2, 3 &4): The female reproductive system of *D. koenigii* consists of a pair of telotrophic-meroistic ovaries having 5-7 strands of ovarioles (Ov) which are joined together with the help of terminal filament (TF). Similar results were found in *D. cingulatus* by Sukumar and Naidu [3]. These ovarioles opens in a duct called as lateral oviduct (LO) which joins to open in median oviduct (MO). The median oviduct then opens into the genital chamber which develops to form the bursa copulatrix. A single ovariole is divided into 4 distinct regions i.e. Terminal filament (TF), Germarium (Gr), Vitellarium (Vt) and Pedicel (Pd). The terminal filament is a thread-like apical prolongation of the peritoneal layer. Germarium forms the apex of an ovariole, where anteriorly the nurse cells and posteriorly the young oocytes are located. Vitellarium constitutes the major portion of an ovariole and composed of a series of oocytes in their follicular sheaths, which become progressively large towards the posterior end. In pedicel, the mature eggs are lodged in before passing into the lateral oviduct.

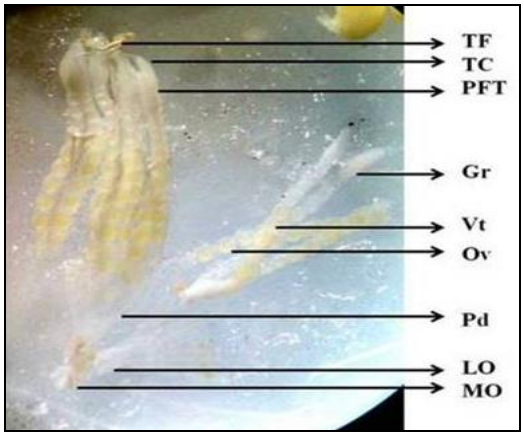

Fig 1: Ovarries of *D. koenigii* Control

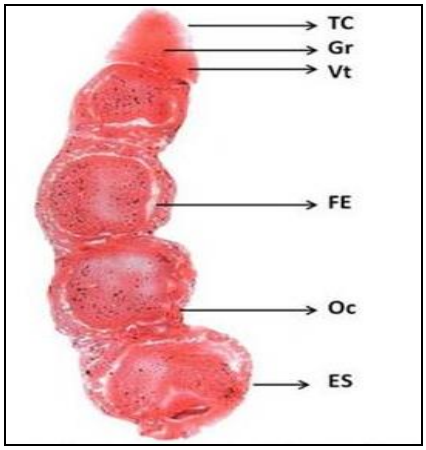

Fig 2: L.S. of an ovariole of *D. koenigii* Control (4x)

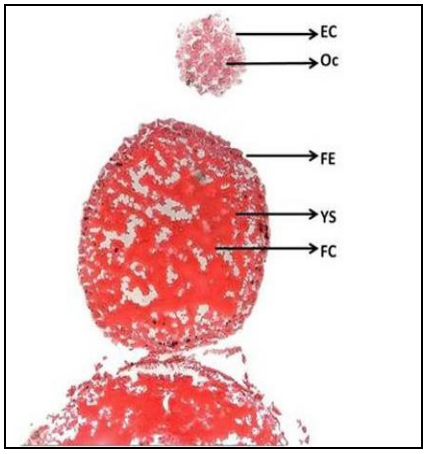

Fig 3: L.S. of an ovariole of *D. koenigii* Control (10x)

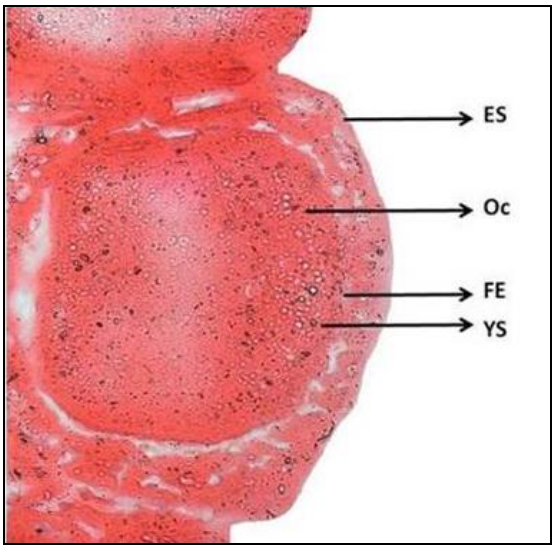

Fig 4: L.S. of an ovariole of *D. koenigii* Control (40x)

b) Effects of Chlorpyrifos on ovaries (Figs. 5, 6, 7, 8, 9 &10): The histological longitudinal section of ovariole treated with Chlorpyrifos (0.005%) showed a significant change in the epithelium sheath and yolk spherules. There is a major disruption in epithelium sheath of the ovariole which is almost reduced to thin membrane. Similar results were obtained in *Chrysomya megacephala* treated with deltamethrin by Gupta and Amir [4]. The densely packed follicular epithelium loses its columnar nature and shows occasional breakage. Similar results were obtained in *D. cingulatus* treated with Tepa by Sukumar and Naidu [3]. Follicular epithelium shows some intercellular spaces. Aberrant deposition of yolk in oocyte is seen which is due to the clumping of yolk spherules. Shrinkage in the oocyte yolk was observed and thus acquired several vacuoles indicating resorption of yolk at several places. At high concentration (0·01%), the epithelial sheath reduced to thin lining. Similar results were obtained in *Sarcophaga ruficornis* treated with dieldrin, cypermethrin and malathion by Amir [5]. Damage to ovarioles increases proportionally with the increase in the concentration of the insecticide. Clumping of yolk spherules is prominent and there is a significant amount of resorption of yolk at 0.01% concentration in comparison with low concentration (0.005%). Identical results were found in *D. koenigii* topically treated with andalin by Khan and Qamar [6] and in *Chrotogonus trachypterus* treated with deltamethrin by Meena and Singh [7]. Loss of yolk could hamper the normal development and may result in deformed insect with low potential activity. The significant damage in the ovarioles accounts for the positive signs towards control in the population of *D. koenigii*.

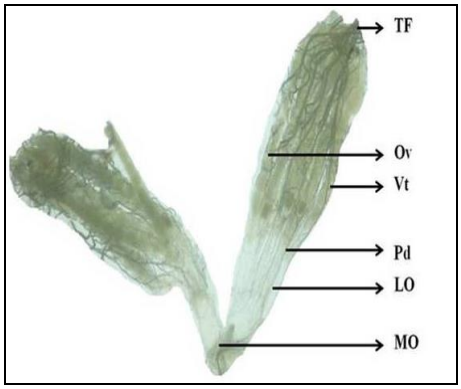

Fig 5: Ovariole of *D. koenigii* treated with 0.005% chlorpyrifos.

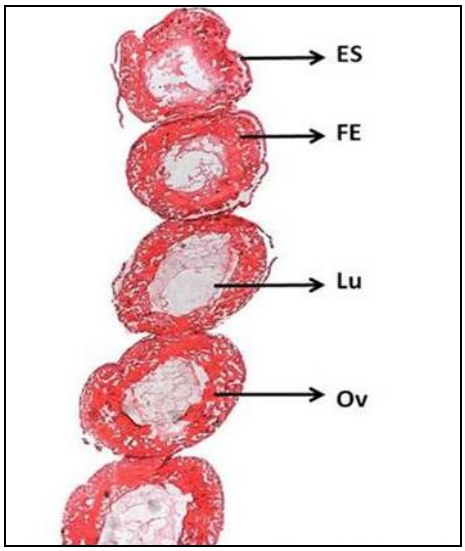

Fig 6: L.S. of an ovariole of *D. koenigii* treated with 0.005% chlorpyrifos (4x)

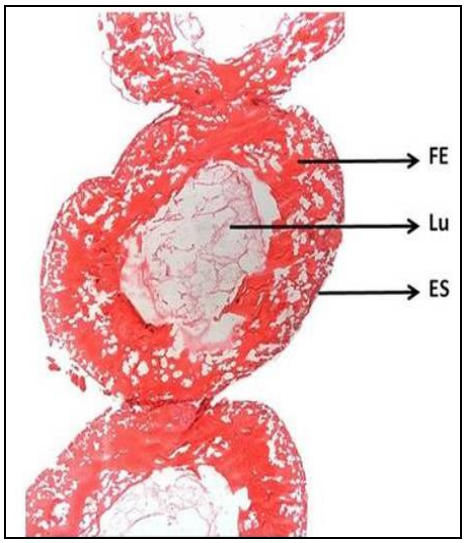

Fig 7: L.S. of an ovariole of *D. koenigii* treated with 0.005% chlorpyrifos (10x)

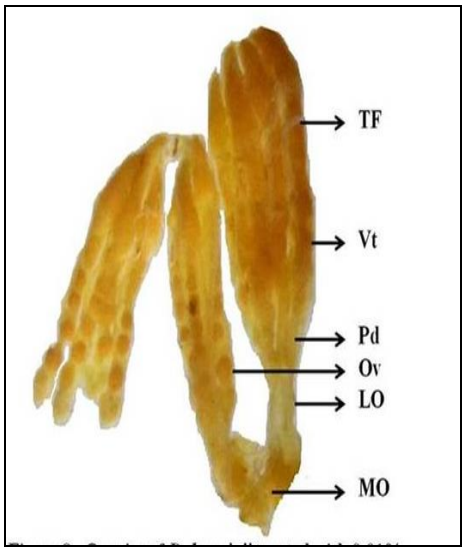

Fig 8: Ovariole of *D. koenigii* treated with 0.01% chlorpyrifos.

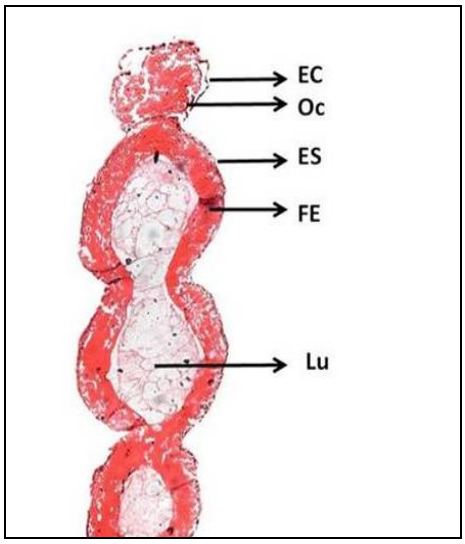

Fig 9: L.S. of an ovariole of *D. koenigii* treated with 0.01% chlorpyrifos (4x)

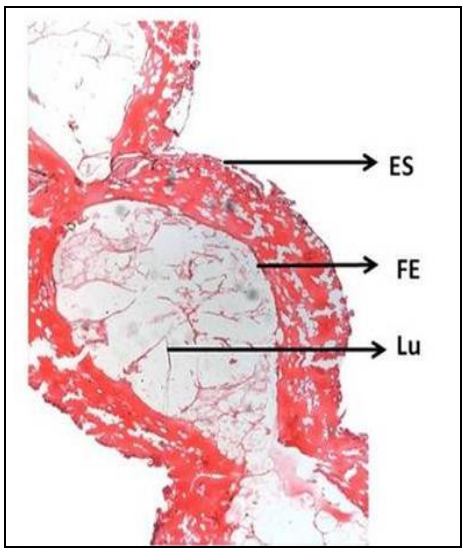

Fig 10: L.S. of an ovariole of *D. koenigii* treated with 0.01% chlorpyrifos (10x)

2) Testes

a) Normal histology of testes (Figs. 11&12): The histological longitudinal section of testes of male species of *D. koenigii* reveals a complete process of spermiogenesis. A large apical cell (AC) is located in the upper most regions of testes which act as stem cell for the sperm development. The testes show a classical arrangement of tubules which acquire the different stages of spermatocyte. The tunica of the tubules consists of irregularly stranded cells. The tunica is nucleated and the space between these cells is filled with spongy cells (SC). A heavy population of interstitial cells (IC) located proximal to the developing spermatogonia is uniformly present in the testes. They are filled with secretory granule. Bunches of spongy tissue (ST) separates the tubules from each other. Some large sized granules were found scattered in the tissue. A layer of flattened cells (FC) was present in between the testes and vas deferens. The spermatocyte differentiation begins with the formation of primary spermatogonia (PSg) which undergoes mitotic division and develops into slightly large sized secondary spermatogonia (SSg). These cells were found scattered in the apical region. The SSg develops into primary spermatocyte (PSc) which undergoes meiotic division to form a haploid secondary spermatocyte (SSc). Meiotic anaphase was observed in some PSc in a number of longitudinal sections. PSc undergoes chromatin condensation to form almost round shaped spermatids (St). Bundles of sperms were found restricted to the lower extremity of the spermatic tubules.

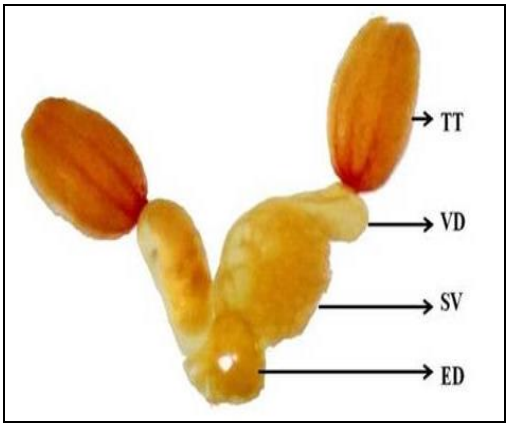

Fig 11: Testes *D. koenigii:* Control

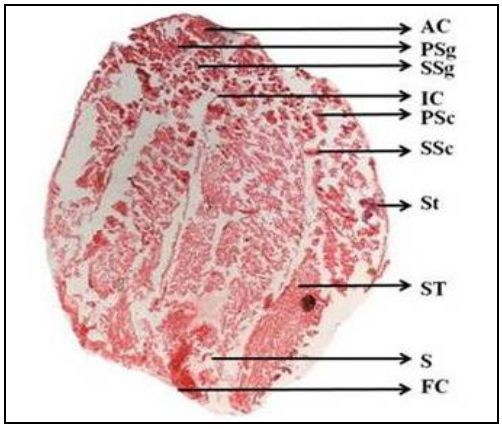

Fig 12: L.S. of a Testes *D. koenigii:* Control (4X)

b) Effects of Chlorpyrifos on the testes (Figs. 13, 14, 15 &16): The treated males with 0.005% Chlorpyrifos showed slight change in the shape of the testes. The testes became narrower and tapered towards apical region. Similar results found in *D. koenigii* treated with *Acorus calamus* vapours by Koul *et al.* [8]. The histological longitudinal section shows considerably no effect on spermatogonial division. At lower concentration, the spermatocytes (PSc and SSc) were normal but on higher concentration spermatocytes show an inhibition at intervals. Major effect observed in case of spermatozoa(S), they were found either assembled in irregular groups or adhered to each other. Sperms that descend towards the efferential passage were found damaged or compact or irregular. At lower concentration (0.005%), spermatozoa were clumped and disrupted. Similar results were obtained in *D. koenigii* treated with *Acorus calamus* oil vapours Koul *et al.* [8]. At higher concentration (0.01%), the spermatozoa were found damaged altogether showing irregular clumping and disrupted structure. At this concentration spermatids (St) were fully mature but most of them appeared in irregular structure or with deformed shapes and incapable of developing into motile spermatozoa which could be possible in inducing infertility in males. Similar results were found in *Mylabris indica* treated with neem extract by Vivekananthan *et al.* [9]. The testicular debris increased at higher concentration of insecticidal treatment. Similar results were obtained in *Chrotogonus trachypterus* treated with monocrotophos by Shakeet and Bakshi [10]. This elucidates the use of

chlorpyrifos which limits the motility of spermatozoa and hence could result in infertility of male. Other than this, there was a decrease in population of interstitial cells (IC) as well as spongy tissue. Spongy tissues were reduced or disorganized. Flattened cells (FC) were grouped at multiple intervals.

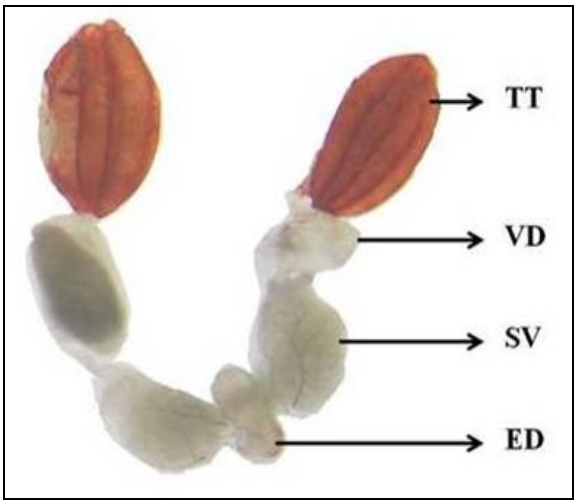

Fig 13: Testes of *D. koenigii* treated with 0.005% chlorpyrifos

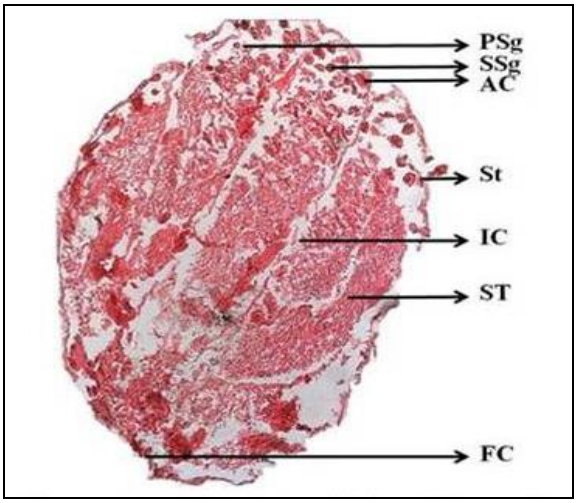

Fig 14: L.S. of a Testes of *D. koenigii* treated with 0.005% chlorpyrifos (4x)

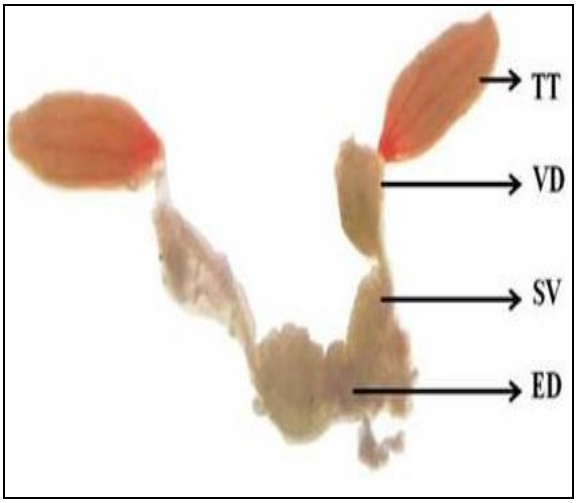

Fig 15: Testes of *D. koenigii* treated with 0.01% chlorpyrifos

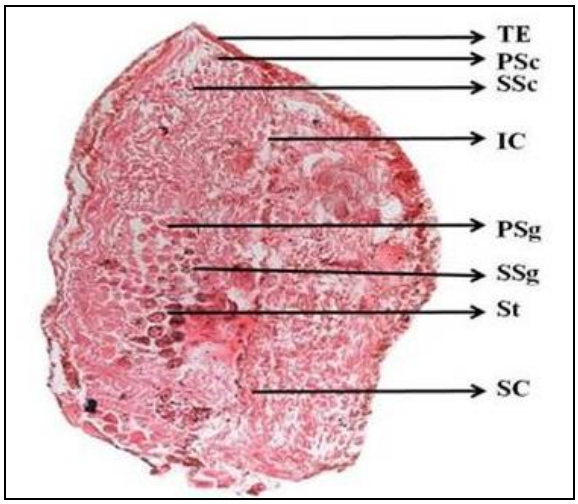

Fig 16: L.S. of a Testes of *D. koenigii* treated with 0.01% chlorpyrifos (4x)

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