

Anatomical studies of the female reproductive system of *Halys dentatus* (Hemiptera: Pentatomidae) and histopathological effects of dichlorovos on ovaries

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Abstract

Anatomical and histological investigation of the female reproductive system of adult *Halys dentatus* has been done using light microscopy. Female reproductive system found to be consisted of pair of ovaries with seven meroistic telothrophic ovarioles, a pair of lateral oviducts, common oviduct, spermatheca, accessory glands and genital chamber. Adult females were treated with dichlorovos were dissected for histopathological effect on structures of ovary. Histological alteration viz. fragmented epithelial sheath of ovaries; compaction of germinal cells with altered shape; distortion of egg tube of ovarioles; separation of follicles of early stages from subsequent follicle; compaction of epithelium of follicle with homogenous secretion has been investigated.

Keywords: *Halys dentatus*, anatomy, histology, histopathology, female reproductive system, dichlorovos

Introduction

The pentatomid bug *Halys dentatus* Fabricius is belongs to one of the largest family Pentatomidae of order Hemiptera commonly known as stink bug. It is polyphagous pest known to infests the variety of trees viz. *Acacia arabica*, *Acacia moniliformis*, *Azadirachta indica*, *Bauhenia*, *Brauzonansia papaunifera*, *Casia javanica*, *Casuarina equisitifolia*, *Dalonix rogia*, *Eugenia*, *Ficus religiosa*, *Graphalium*, *Gravelia robusta*, *Mangifera indica*, *Maurus aiba*, *Millingtonia hortensis*, *Pinus longifera*, *Psidium guajava*, *Putranjiva roxbergii*, *Pyrus communis*, *Samania saman*, *Sesbania sesban*, *Spathodea companulata*, *Zizyphus jujuba* etc. (Dhiman and Yadav, 2003; Gangurde, 2017) causing considerable loss in severe infestation result in fall of leaves, lowering vigor of plant and shortening life span of plant. The *H. dentatus* Fabr. is a medium-sized, dull brown, coarsely and darkly punctate insect. Its head and pronotum have obscure, oblique black bands, and its apical margins are brownish black and pale, respectively. The connexivum is ochraceous and dark brown, and its membrane is greyish brown. The body beneath its legs is ochraceous, with brown spots, and its antennae are dark brown. The base of the fifth joint and the apices of the second and third joints are yellowish. The *H. dentatus* Fabr. has partial metamorphosis. The phases of the life cycle are nymph, adult, and egg. The female lays 14–20 eggs on the bark or leaves of plants in two batches. The spherical eggs have protrusion called micropyles on them. When they first hatch, they have a grey-white appearance, but their color eventually turns brown. The bug is univoltine, and its winter diapauses are required. The nymphs are sexually immature but otherwise comparable to adults. Only in the mature stage are the wings functional. The nymphal instar has five stages. The nymph sheds its old cuticle and moults at every nymphal instar. As female has good fecundity result into increase in population

causing severe damage to host plants, thus present study undertaken to study anatomy of female reproductive tract and to observe histopathological effects dichlorovos pesticide on ovaries.

Material and Method

The adult females were collected from host plant viz. *Millingtonia hortensis*, *Spathodea companulata*, *Acacia moniliformis*, *Pithocelebium dulce*, *Acacia arabca* and *Samania saman* from K.T.H.M. College campus (20°00'26.46" North latitude and 73°46'42.24' East longitude), Nashik, Maharashtra by hand picking method. Laboratory culture was maintained at 28±1°C temperature, 65% relative humidity and 14:10 daily light to acclimatize the insects and were fed on pumpkin and potato.

The healthy adult of same size was chosen for anatomy and histological investigations. Insects were anesthetized using chloroform and mounted in dissecting tray using paraffin wax to dissect under stereoscope microscope. Reproductive system was exposed by removing the tergal plates from dorsal side; the anatomy was studied by isolating system in petri dish and measurement have been done using micrometer scale. Further system was fixed in Bouin's fluid for histological studies and embedded in paraffin wax. Sections were obtained using Leica microtome, stained with HE staining and photographed using microphotographic unit.

Result

Anatomy: Female reproductive system found to be consisted of pair of elongated ovaries with seven meroistic telothrophic ovarioles, a pair of whitish lateral oviducts, common oviduct, spermatheca, accessory glands and genital chamber (Fig 1).

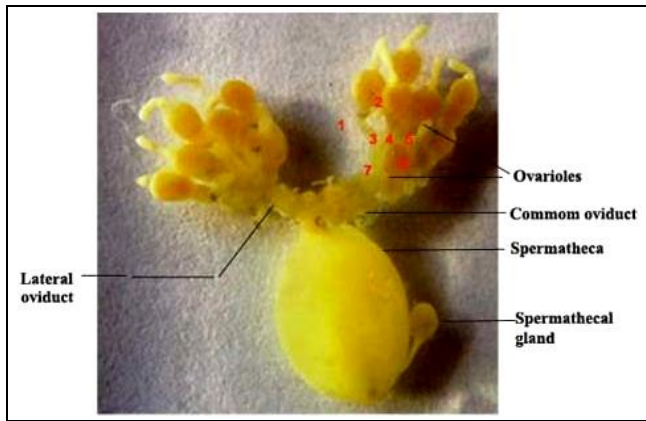


Fig 1: Female Reproductive system of *Halys dentatus* fixed in Bouin's fluid

Ovaries: Ovaries were white coloured, measures 6-7 mm long and composed of seven ovarioles connected by numerous tracheoles. The anterior portion of the each ovarioles joined together by thread like suspensory ligament. The ovarioles were distinguish into three parts viz. the terminal filament, an egg tube, and the pedicel. The terminal filament was found to be slender and arises from the anterior end of the egg tube. An egg tube was prominent part containing germinal cells and their derivatives. The

pedicel was short duct connecting ovariole and lateral oviducts.

Oviduct: Lateral oviducts emerge posteriorly from the ovaries, which appear as paired whitish elongated tubes measuring 4 to 5 mm in length. The lateral oviduct inflated to form a receptacle called the calyx. Lateral oviducts united to form short tube-like common oviduct known as oviductus communis, measuring 3 mm. The common oviduct posteriorly continuous in the genital chamber and the spermathecal duct.

Spermatheca: Is whitish sac-like structure measures 7 to 8 mm long and associated with its accessory gland.

Histology: Histology of ovary, lateral oviduct, common oviduct and spermatheca has been studied by HE preparation and obtained following results.

Ovary: The L.S. of ovariole showed three distinct zone viz. terminal filament (Tropharium), an egg tube (germarium, vitellarium) and a pedicel can be recognized (Fig 2-A). The filaments were surrounded by an epithelial sheath with intermittent oval nuclei. The epithelial cells were resting on a thin basement membrane followed by longitudinal and circular muscles (Fig 2-a).

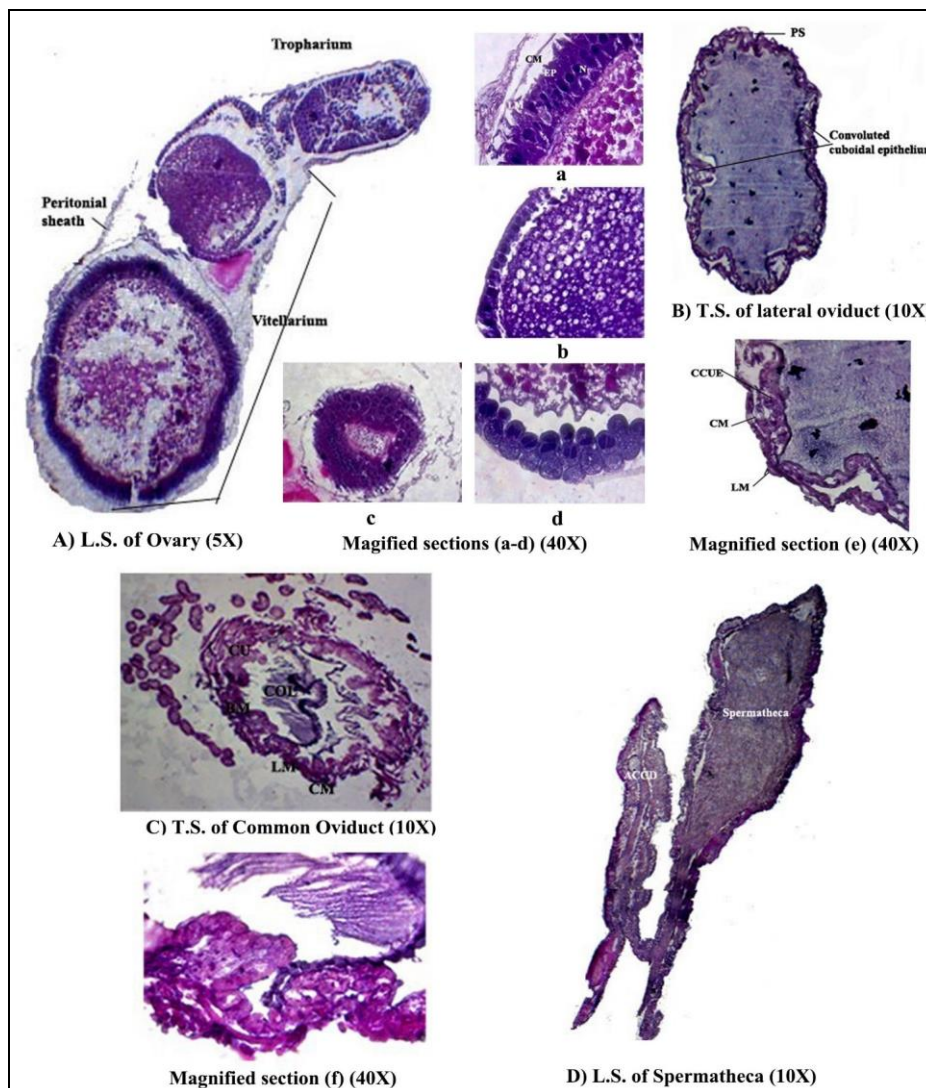


Fig 2: Histology of female reproductive system of *Halys dentatus*

A) L.S. of Ovary: a-magnified section showing LM-Longitudinal Muscle, CM-Circular Muscle, E-Epithelium, N-Nucleus; b – Matured oocyte; c – immature oocyte; d - oocytes shows the double layer of epithelium with binucleus; B) T.S. of lateral Oviduct: PS-Peritoneal Sheath; e- magnified section showing CCUE-Convolutd Cuboidal Epithelium, CM- Circular Muscle, LM-Longitudinal Muscle; C) T.S. of Common oviduct: BM-Basement Membrane, LM-Longitudinal Muscle, CM-Circular Muscle, CU-Cuboidal Epithelium, COL-Columnar Epithelium; f-magnified section showing cuboidal epithelium; D) L.S. of spermatheca: ACCD- Accessory duct.

In the egg tube, the germ cells and accessory tissue develop to form the germarium and vitellarium. Germarium consisted of trophocytes, oogonia, young oocytes, and pre-follicular tissue. Trophocyte clusters lie at the front of egg tubes containing nuclei and cytoplasm. A nutritive cord that connects developing oocytes that receive nutrition from trophocytes. After the trophocytes have developed into oocytes, the later of the trophocytes, the germarium, showed intermittent discs of germ cells. Toward the posterior end of germarium, the pre-follicular tissue that continues into vitellarium. There were no clear boundaries between pre-follicular cells, and follicular epithelium but it forms the walls of the vitellarium. After maturation, the mature oocytes reach the vitellarium, surrounded by pre-follicular cells called epithelial layers. There was a single layer of squamous epithelium in the older follicles (Fig 2-b) while immature and younger follicles have several layers of squamous cells with bean-shaped nuclei respectively (Fig 2-c & d). The egg showed the full of yolk granules and presence of irregular ooplasm. The binuclei observed round in shape.

Lateral oviduct: Transverse section of the lateral oviduct showed an oval-shaped Structure (Fig 2-B) with folds in the cuboidal epithelium surrounded by circular and longitudinal muscles. The cytoplasm contains fluid material. The calyx

connects the lateral oviduct with the common oviduct at the posterior. A longitudinal section of the calyx showed the presence of a thin layer of cuboidal epithelium followed by circular and longitudinal muscles.

Common oviduct: In transverse section, the common oviduct appeared oval shape structure, the epithelium lies independently on the basement membrane surrounded by circular and longitudinal muscle layers followed by intima. Several folds were visible inside the cuboidal-columnar epithelium with round nuclei which were continuous at the center of the duct on which numerous fiber-like projections were positioned (Fig 2-C).

The spermatheca: The longitudinal section of the spermatheca showed an elongated sac-like structure along with a short accessory duct. A bulb-like structure appeared at the anterior end of the accessory duct. Both the spermathecal sac and accessory duct bears a serrated chitinous layer at their interior. There were cuboidal epithelium cells with round to oval nuclei and indistinct cell boundaries. These cells were in the active state of secretion, with empty spaces below. A layer of circular muscle surrounds the cells on the outside (Fig 2-D).

Histopathology of ovary: After exposure of dichlorovos the females of *H. dentatus*, showed alteration of histological pattern of ovary. The ovarioles exhibit distortion of the egg tube; the terminal part and follicle of the early stage separate from the later follicle; the epithelial sheath fragments or breaks due to cellular disruption. After treated with dichlorovos disappearance of peritoneal sheath can be observed. Germarium cells were compacted and stained darkly as they lose their shape. The squamous epithelium of follicle becomes compacted; the cytoplasm shows complete homogenous secretion. The muscles are disrupted in the follicle (Fig 3).

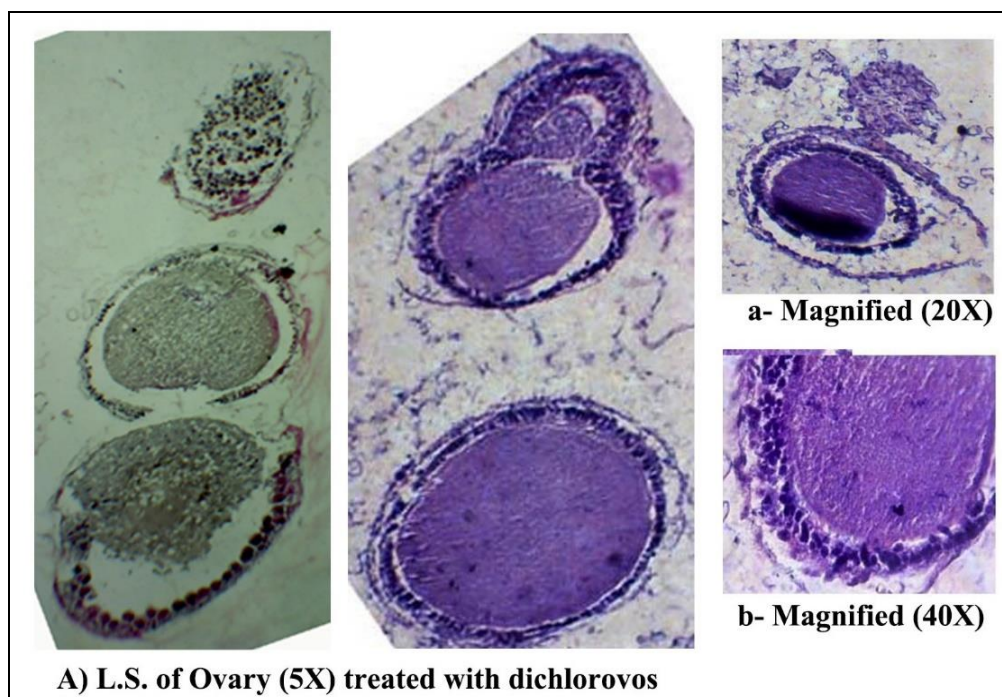


Fig 3: Histopathology of Ovary of *Halys dentatus* treated with Dichlorovos

A) L.S. of Ovary treated with dichlorovos showing cellular disruption and disappearance of peritoneal sheath; a- cells of germarium lose shape and compacted; b- muscle disruption in follicle and compaction of squamous epithelium.

Discussion

The seven types of ovariole were found among heteropteran [20]. The meroistic telotrophic type of ovarioles reported in *T. limbativentris* [1]; *Nazeeriana sindellus* [10] and *Scotinophora limosa* [15]. The terminal filament of the meroistic telotrophic ovariole, arising from each ovariole, joins at apex of the ovaries, which were attached to the body wall. Distally, each ovariole opens in its respective pedicel and is connected to a lateral oviduct, which eventually joins in a common oviduct. A swollen bulb-like structure like a spermatheca appears between the lateral oviducts. The similar structure of ovary was also confirmed by many entomologists [1, 10, 15, 20]. A terminal filament was reported to be absent in *Orthezia urticae* [24]. In the hemipteran insects the number of ovarioles in ovary varies greatly viz. 8-12 in *Parapiesma quadratum* (Piesmididae) [6]; 10 in *Abedus ovatus* (Belastomatidae) [11]; and *Cosmolepius nigroannulatus* (Reduviidae) [8]; *Leptoglossus occidentalis* (Coreidae) [2] and *Coreus marginatus* (Coreidae) [17]. There are total 30 ovarioles reported in *Orthezia urticae* (Ortheziidae) [24], whereas 48 ovarioles in *Elasmucha grisea* (Acanthosomatidae) [18]. Furthermore, Szklarzewicz reported wide variation in number of ovarioles that ranges from 100 to 200 in few hemipteran species [23] viz. *Dysmicoccus newsteadi* (Pseudococcidae); *Kermes quercus* (Kermesidae), *Eriococcus buxi* (Eriococcidae), *G. spuria*, *Cryptococcus fagisuga* (Cryptococcidae), *Pseudochermes fraxini* (Cryptococcidae). The highest number of ovarioles are reported is 400 in *D. coccus* [21].

Female reproductive system of *Abedus ovatus* is consist of pair of ovaries composed of five telotrophic ovarioles united anteriorly by filaments attached to the abdomen; these ovarioles leads to lateral oviduct and unite posteriorly forming common oviduct [11]. Female reproductive tract of *Podisus nigrispinus*, known to have telotrophic meroistic type of yellowish ovaries covered by epithelial sheath; ovary composed of seven ovarioles joined by a terminal filament forming cluster; ovarioles were further leads into lateral oviducts which joins further forming common oviduct [25]. In SEM studies of female reproductive system of *Graphosoma lineatum* showed the presence of seven meroistic telotrophic ovarioles distinguished in four regions viz. terminal filament, tropharium, vitellarium and pedicel [19].

In case of *G. lineatum* the a telotrophic meroistic ovary enclosed by peritoneal sheath [9, 12, 13]. Each ovariole was divided into a terminal filament, tropharium, vitellarium, and pedicel. The terminal filament was formed by spindle-shaped cells, while the tropharium was the anterior most portion created by germinal cells and nurse cells. Previtellogenesis, vitellogenesis, and choriogenesis are the three differentiation stages that form the vitellarium. Previtellogenesis occurs when the oocytes move towards the anterior of the vitellarium to increase their size, nourishment is provided by nutritive cords, and the oocyte is surrounded by prefollicular epithelium. The oocyte is surrounded by the follicular epithelium during vitellogenesis, while the vitelline membrane is formed during choriogenesis.

In the present study of *Halys dentatus* the ovarioles are telotrophic meroistic types with an egg tube (germarium, vitellarium) and pedicel as well as a terminal filament. The terminal filament is surrounded by epithelial sheath and consists of intermittent oval nuclei. Egg tubules contain germ cells and accessory tissue which form the germarium and vitellarium of the ovary. The germarium comprises the trophocytes, oogonia, young oocytes and pre-follicular tissue. Trophocytes are clusters of nuclei lined with cytoplasm, located at the termination of the egg tube. The trophocytes that provide nutrition to developing oocytes through the nutritive cords. Later on, the trophocytes in the germarium show a disc of germ cells that produce oogonia, which develop into oocytes. Prefollicular cells have indefinite boundary lines, whereas follicular epithelium forms the wall of vitellarium. The matured oocytes are transported to the vitellarium where the pre-follicular cells surround the oocytes as epithelial layers. The developing young follicles usually encircled by multiple layers of squamous cells whose nuclei are bean shaped, but the mature follicles have a single layer of squamous epithelium. Egg contains yolk granules and patches of irregular empty spaces. The nuclei were observed to be round.

Ramaya *et al.* has shown the ovaries developed a pale black colour due to exposure to pesticide nimbecidine [22] also ovarioles of treated insects were become shorter and shrunk than control insects [22]. Campion (1973) showed inhibition of development of ovary when exposed to chemo-sterilant. Some entomologists have reported changes on histological pattern of *Odontopus varicornis* after Nimbecidine treatment [3, 7, 16]. Effects on germarium the cytoplasm of the germarium cell exhibits vacuoles, and cytological abnormalities are visible in zone I. Cells were improperly arranged and their shapes and sizes were reduced. The study by Coehoun demonstrated that Nimbecidine alters the histology of the vitellarium [3]. The size, shape, and number of the primary oocytes are reduced. The cytoplasm was vacuolized, and empty spaces were observed at the periphery. Germinal vesicles changed from spherical to elliptic, and there was a reduction in yolk material in the ooplasm. The mature oocyte appears deformed and shrieked.

In response to dichlorovos treatment in present study, primary follicles of *H. dentatus* are separated from secondary follicles; membrane of ovarioles has been disintegrated. Germarium cells lose their shapes, become compacted and darkly stained, while the nutritive cord disappears. A squamous epithelium forms on the follicle; its cytoplasm shows homogenous secretion.

References

1. Aner U. Pentatomídeos em hibernação em touceiras de gramíneas no município de Eldorado do Sul, RS (Insecta: Heteroptera: Pentatomidae). Dissertação (Ecologia), UFRGS. Porto Alegre, RS, 1991:116.
2. Chiang RG. Functional anatomy of the vagina muscles in the adult western conifer seed bug, *Leptoglossus occidentalis* (Heteroptera: Coreidae), and its implication for the egg laying behaviour in insects. *Arthr. Struct. Dev.* 2010;39:261-267.
3. Coehoun EH. Some pharmacological effects of chlorinated hydrocarbon and organo phosphorus poisoning. *Proc. N. Cent. Br. Entomol. Soc. Amer.* 1959;14:35-37.

4. Dhiman SC, YK Yadav. Host plants and defence behaviour of *Hylas dentatus* Fabr. (Heteroptera: Pentatomidae). *Journal of Experimental Zoology India*,2003;6(1):205-209.
5. Gangurde JH. Studies on morphology, anatomy and histopathology of *Halys dentatus* Fabr. (Hemiptera: Pentatomidae). Ph.D. thesis submitted to Savitribai Phule Pune University:2017. Accessed January 2024. <http://hdl.handle.net/10603/194572>
6. Grozeva S & Kuznetsova VG. The reproductive system of some bug families (Heteroptera, Pentatomorpha), in: *Bennetova B., Gelbic, I., Soldan, T.* Advances in regulation of insect reproduction. Institute of Entomology, Czech Academy of Sciences,1992:97-102.
7. Hughes PR & Penton MA. Effect of Dienochlor on oogenesis of the Maxican bean beetle (Coleoptera: Coccinellidae). *J. Econ. Ent*,1983;76(5):1156 – 1158.
8. Jahnke SM, Redaelli LR, Diefenbach & LMG. Internal reproductive organs of *Cosmoclopius nigroannulatus* (Hemiptera: Reduviidae). *Braz. J. Biol*,2006;66 (2A):509-512.
9. Jawale SM & Ranade DR. Morphology of the ovaries of *Sphaerodema* (Diplonychus) *rusticum* (Heteroptera, Belostomatidae). *J. Morphol*,1990:205:183-192.
10. Kamaluddin S & Ahmad I. Internal anatomy and histology of alimentary organs, salivary apparatus and reproductive organs of *Megarrhamphini*, *Phyllocephalini* and *Tetrodini* (Hemiptera: Pentatomidae: Phyllocephalinae) and their bearing on classification. *Philipp. J. Sci*,1992:121:129-156.
11. Lalitha TG Shyamasundari K & Hanumantha Rao K. Morphology and histology of the female reproductive system of *Abedus ovatus* Stal (Belostomatidae: Hemiptera: Insecta). *Mem. Inst. Oswaldo Cruz*,1997:92 (1):129-135.
12. Lemos WP Serrao, JE Ramlho, FS Cola Zanuncio JC & Lacerda M. Effect of diet on male reproductive tract of *podisusu nigrispinus* (Dallus) (Heteroptera: Pentatomidae). *C. Braz. J. Biol*,2005;65 (1):91-96.
13. Ma WK & Ramaswamy SB. Histological changes during ovarian maturation in the tarnished plant bug, *Lycus lineolaris* (Hemiptera: Miridae). *Int. J. Insect Morphol. Embryol*,1987:16 (5/6):309-322.
14. Misra DS. Histopathology of insect tissue exposed to pesticides. *Indian Journal Entomology*,1981:43:348-360
15. Mohammad FA, ahmad I & naqvi SNH. Some aspects of internal anatomy of the rice black bug *Scotinophara limosa* (Walker) (Hemiptera: Pentatomidae) with reference to its cladistic relationships. *Pakistan J. Zool*,1994:26:257-260
16. Moriarty F. The sub lethal effect of synthetic insecticides on insect. *Biological review* 1969:44:321-357.
17. Ogorzalek A. Structural and functional diversification of follicular epithelium in *Coreus marginatus* (Coreidae: Heteroptera). *Arth. Struct. Dev*,2007:36:209-219.
18. Ogorzalek A & Trochimczuk A. Ovary structure in a presocial insect, *Elasmucha grisea* (Heteroptera, Acanthosomatidae). *Arth. Struct. Dev*,2009:38(6):509-519.
19. Ozyurt N, Candan S, Suludere Z & Amutkan D. Reproductive system in *Graphosoma lineatum* (Heteroptera: Pentatomidae) based on optical and scanning electron microscopy. *Journal of Entomology and Zoology Studies*,2013:1(4):40-46
20. Pendergrast JG. Studies on the reproductive organs of the Heteroptera with a consideration of their bearing on classification. *Trans. R. Ent. Soc. London*,1957:109:1-63.
21. Ramírez Cruz A, Llanderal-Cázares C & Racotta R. Ovariole structure of the cochineal scale insect, *Dactylopius coccus*. *J. insect sci*,2008:8:1-5.
22. Ramya P, Sathishkumar G, Mahesh K, Loganathan M & Sridharan G. Effects of the phytopesticide nimbecidine on the ovary, brain and Corpus allatum of adult female insect *odontopus varicornis*. *Int. J. Modn. Res. Revs*,2014:2(8):241-45.
23. Szklarzewicz T. Structure of ovaries of scale insects. I. Pseudococcidae, Kermesidae, Eriococcidae, and Cryptococcidae (Insecta, Hemiptera, Coccinea). *Int. J. Insect Morphol. Embryol*,1998:27:162–172.
24. Vogelgesang M, Szklarzewicz T. Formation and structure of egg capsules in scale insects (Hemiptera, Coccinea) I. Ortheziidae. *Arthropod Structure & Development* 2001:30:63–68.
25. Walkymário de PL, Francisco de SR, Jose ES & Jose CZ. Morphology of female reproductive tract of the predator *Podisus nigrispinus* (Dallas) (Heteroptera: Pentatomidae) fed on different diets. *Braz. arch. biol. Technol*,2005:48(1):129-138.