ABSTRACT

**Aim:** Revamp studies on the morphology and histology of plant bug *Halys dentatus* F. (*Hemiptera: Pentatomidae*).

**Study Design:** *Halys dentatus* Fab. is acting as pest of many plants, multivoltine in nature, therefore their reproductive cycle observed throughout the year. Hence, the number of reproductive cycles increased the population of *H. dentatus* to cause harm to plants, therefore there is need to study the male reproductive system of *H. dentatus* as a part of fundamental studies.

**Place and Duration of Study:** Entomology Research Laboratory; P.G. Department of zoology, K.T.H.M. College, Nashik (MS, India).

**Methodology:** Adults of *H. dentatus* (Fab.) were collected, anesthetized with anesthetic ether & embedded in dissecting paraffin wax plate, dissected in insect saline solution (Lum, [13]) using stereoscopic research binocular microscope. The male reproductive system exposed and isolated, fixed in Debocqui’s Bouin’s fixative for 18 hrs., dehydrated (acetone grades), Cleared (xylene; acetone), blocks were prepared, sections were cut on Leica microtome, stained & micro
1. INTRODUCTION

The *Pentatomidae* is one of the largest family of suborder *Heteroptera*, represented by over 760 genera & 4,112 species [1,2]. The *Pentatomidae* includes subfamilies viz: *Asopinae, Cyrtocorinae, Discocephalinae, Edessinae, Pentatominae, Phyllocephalinae, Podopinae & Serbaninae* [2].

The male reproductive system in typical heteropteran insect consist of pair of testis, vas deferens, seminal vesicles, accessory glands (ectodermal & endodermal) & ejaculatory duct (bulbus & ductus).

**Results and Observation:**
- **Morphology:** The male reproductive system of *H. dentatus* (Fab.) constitute a pair of testis, pair of vas deferens, seminal vesicles, accessory glands (ectodermal & endodermal) & ejaculatory duct (bulbus & ductus).
- **Histology:** Histology of testis of *H. dentatus* showed the six numbers of testicular follicles with different development zones; the growth zone, the maturation zone and the zone of differentiation. The inner layer of vas deferens and seminal vesicle was composed of cuboidal epithelial cells. The accessory glands are both ectodermal and endodermal in origin. The ectodermal accessory glands are triplate, milky white in colour while mesodermal accessory glands are convoluted bunch of fine tubular structure.

**Conclusion:** The male reproductive system was studied with reference to revamp morpho-histology; during the year 2009-2011. The anatomy resemble with other pentatomid bug with little difference in vas deference, investing sac and major difference is the testicular follicles are six in number not seven.

Keywords: Halys dentatus; male reproductive system; morpho-histology.

2. MATERIALS AND METHODS

Adults of *H. dentatus* (Fab.) were collected from Samnia saman, Millingtonia hortensis, Spathodea companulata, Acacia moniliformis and Griseb sp. in the present locality. As the *Halys dentatus* is polyphagus in habit, they found under loose bark of trees or on surface of stem, leaves, etc. They are multivoltine in nature, therefore their reproductive cycle observed throughout the year. Hence, the number of reproductive cycles increased the population of *H. dentatus* to cause harm to plants, cited above, therefore there is need to study the male reproductive system of *H. dentatus* as a part of fundamental studies.

Adults of *H. dentatus* (Fab.) were collected from Samnia saman, Millingtonia hortensis, Spathodea companulata, Acacia moniliformis & Griseb sp. plants of K.T.H.M. College campus Nashik (India) by hand picking method. The collected adults segregated according to gender & the males were anesthetized with anesthetic ether & embedded in dissecting paraffin wax plate. Specimens were dissected in insect saline solution 0.65% [13] using stereoscopic research binocular microscope. Pleural sclerites removed by giving lateral cut & tergal plates were removed using fine forceps. The male reproductive system exposed and isolated. Different parts of male reproductive system of *H. dentatus* was fixed in Debrocqui’s Bouin’s fixative for 18 hrs. The tissues were washed in 70% alcohol to remove the fixative and dehydrated in series of acetone grades. Cleared in xylene; acetone, infiltrated in paraffin wax (52-54°C) & the blocks were prepared, 5-6 µm thickness sections were cut on Leica microtome (RM 2235, Germany). Serial sections were stained with Delafield
3. OBSERVATION

3.1 Morphology

The male reproductive system of _H. dentatus_ (Fab.) constitute a pair of testis, pair of vas deferens, seminal vesicles, accessory glands (ectodermal & endodermal) & ejaculatory duct (bulbus & ductus). The testes are red colored, kidney shaped structure lie in genital abdominal segment (Fig. 1). The vas deferens is long tubular duct. It is red pigmented and acts as a passage for sperms. The seminal vesicles are not clearly differentiated from the tubular vas deferens. At the distal portion of vas deference there lies folding of accessory glands. The accessory glands are both ectodermal and endodermal in origin. The ectodermal accessory glands are triplate, milky white in color while mesodermal accessory glands are convoluted bunch of fine tubular structure. The bulbous ejaculatorious emerges on accessory glands and opens in ductus ejaculatorious. At the joining of bulbus & ductus ejaculatorious there is presence of a large ballon shaped transferent sac known as investing sac. The ductus ejaculatorious is continuous with cuticular aedeagus.

3.2 Histology

The testis of _H. dentatus_ Fab. composed of six numbers of testicular follicles (Figs. 2-4). Each testis is kidney shaped and shows different zone of development viz: the growth zone, the maturation zone & the zone of differentiation (Figs. 5-7). These follicles show germarium at apical region (Fig. 5). Each follicular tube is lined from outside to inside is peritoneal sheath & tunica propria. The spermatogonia separate from the germarium to form spherical sac known as cyst (Figs. 5.1 and 5.2). These spermatogonia further divides meiotically to form spermatocytes. Later the spermatocytes develop into spermatid at the zone of differentiation where which then further transform into spermatooza by spermiogenesis (Figs. 6,7). These spermatooza are grouped in bundles known as sperm bundle (Fig. 8). The sperm bundle travel from vas deferens to seminal vesicle where it stored temporarily and liberate during copulation (Figs. 9 and 10). Histologically the vas deferens & seminal vesicle are similar in structure with presence of inner layer of cuboidal epithelium lie on basement membrane, which is surrounded by strong muscular coat of circular fibers. The accessory glands are like a triplate structure which shows complete secretion inside (Figs. 11 and 12). The bulbus ejaculatorious shows folds of epithelium probably the columnar & cuboidal cells (Figs. 3 and 14). Both the columnar & cuboidal cells secrete the intima where as the columnar cells secrete the secretion which come up to the lumen (Fig. 15).

4. DISCUSSION

Number of researchers studied on pentatomid bug like _Carbula scutellata_, _Tropicoris punctipes_, _Erthesina fullo_, _Dolydoris baccarum_, _Graphosom lineatum_, _Apodiphus amygdalis_ where they observed and reported that there are five testicular lobes in _C. scutellata_ but the number is not evident from outside in _E. fullo_ and _T. punctipes_ [14-17]. In _C. scutellata_, _T. punctipes_, _E. fullo_ the testicular sheath are enclosed in red sheath whereas in _D. baccarum_, _G. lineatum_, _A. amygdalis_ the testes, vas deferens & seminal vesicle are red. At the base of each testis arises red colored vas deferens which is slightly swollen at the apical region but not demarcated into a distinct seminal vesicle. The paired testes in _D. baccarum_ [10], _G. lineatum_ [18], _A. amygdalis_ [19] are connected to the seminal vesicles by the vas deferentia. In most of the pentatomids, distinct seminal vesicle is absent with exceptions of _Nezara viridula_, _Brachymena cincta_ and _Dolycoris indicus_ [14,15,20,16]. Santos et al. [16] also studied on accessory glands of these three plant bug species and compared with other pentatomides and reported that accessory glands are tubular & wrap each vas deferens at its distal region before its junction with bulbous ejaculatorious in _Nezara viridula_, _Brachymena cincta_ and _Dolycoris indicus_ species. The bulbus ejaculatorious is pear shaped in _Carbula scutellata_ & is enclosed in an investing sac. The sac is bulbus apically & tapers down basally in _Tropicoris punctipes_ while it is elongated in _Erthesina fullo_ [14]. In case of _Tropicoris punctipes_ & _Erthesina fullo_ the investing sacs are absent but in _Nezara viridula_ & _Piezodoros lituratis_ they are present [15]. Kumar [6] reported investing sacs in _Dalpada versicolor_ & Abbasi [14] observed sacs in _Piezodoros rubrofascitatus_, _Aeliomorpha lineaticollis_ & _Eysarcoris inconspicuous_. Similar sacs reported by Santos et al. [16] & Lemos et al. [8] in _Oebalus poecilus_ & _Podisus nigrispinus_.

Haematoxyline & Eosin (HE). The stained slides were observed, screened & micro photographed under trinacular microscope (40X) using camera ultrascope 9.1(Bioarch).
Fig. 1. *Halys dentatus*: Male reproductive system (8X MP)- T-testis, VD-vas deference, AG-accessory gland, AD-aedeagus

Fig. 2. *Halys dentatus*: Longitudinal section of testis (10X) showing different zones of development viz: GZ- zone of growth, MZ- zone of Maturation, DZ- zone of differentiation

Fig. 3. *Halys dentatus*: Transverse section of testis showing six seminiferous tubules (10X)

Fig. 4. The 6th seminiferous tubule at high magnification (40X)
Figs. 5, 5.1 and 5.2. *Halys dentatus*: Longitudinal and transverse section of testis showing germarium at apical region with cysts. CY- cyst, ST- spermatid

Fig. 5. Germarium at apical region (10X)
Fig. 5.1. Showing cysts in clusters (40X)
Fig. 5.2. Cell are in meiotic stage (40X)

Figs. 6, 7. Shows the formation of spermatoze from spermatids. SZ- spermatozoa (10X)
Fig. 8. Show the sperm bundles (40X)
Figs. 9 and 10. *Halys dentatus*: The longitudinal and transverse section of vas deferens showing epithelium and peritrophic membrane (10X)

Fig. 11. *Halys dentatus*: Longitudinal section across the vas deferens and accessory glands VD- vas deference, AG- accessory gland (10X)

Fig. 12. *Halys dentatus*: Accessory gland showing complete secretion inside (40X)

Fig. 13. *Halys dentatus*: Longitudinal section of bulbus ejaculatorious shows the folds of Columnar Cells (10X)

Figs. 14 and 15. *Halys dentatus*: Longitudinal section of bulbus ejaculatorious shows the secretory columnar cells, CU- cuboidal cells, SR- secretion, COL- columnar cells
Studies of Santos et al. [16] on Oebalus poecilus reported that the testis & vas deference are orange in colour with seven testicular follicles which is dissimilar with H. dentatus but other structures are resemble with other pentatomid bug. The testes in A. amygdales & Perillus bioculatus in which consists of seven number of sperm tubes but in Nezara viridula [15,20], Podisus nigrispinuis [8], D. baccarum [10] & in G. lineatum [18] the number of sperm tube are variable.

The specimen of Halys dentatus Fab. are noted that the testes are kidney shaped with seven testicular follicles covered with red pigmentation, indistinct seminal vesicles, vas deference very long ‘W’ shaped with red pigmentation [14] but the present study showed that, there are only six testicular follicles and the vas deference are wavy in appearance in H. dentatus, other parts of reproductive organ are similar as described in morphology and authors are agreed.

4. CONCLUSION

The male reproductive system of Halys dentatus Fab was studied with reference to revive morpho-histology; during the year 2009-2011. The anatomy of some male reproductive organs resemble with other pentatomid bug with little differences in vas deference, investing sac and major difference is the testicular follicles are six in number not seven which is reported by earlier workers.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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