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Research Paper

The Metamorphic Changes During Post Embryonic Development and Ultrastructure of Antennae in Red Eye Bug, *Leptocoris augur* (Hemiptera: Rhopalidae)

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Abstract- The *red eye bug Leptocoris auguar is economically important pest as it damage soapberry (rittha) plant, Sapindus saponaria, other vegetables and fruit plants. The post embryonic development of the soapberry bug, L. augur passes through five nymphal instars and adults (male, female). No sexual metamorphic dimorphism found in the adults except their body length. The eggs are rough barreled, bean shaped and red coloured. The bug has four segmented filliform type of antennae, divided into three parts, scape, pedicel and two segmented flagellum. The surface ultrastructural study of antenna showing the presence of three different types of sensilla as sensilla trichoidea (ST-I and ST-II), sensilla trichoidea curvata (STC) and sensilla basiconica (SB). The button like structure present between adjecent segment containing hexagonal and pentagonal plates.*

Keywords: *Leptocoris augur*, metamorphosis, antenna, sensilla etc.

Introduction

Hemiptera are a major group of insects and they are estimated to be from 50000 to 80000 species. (Hamman, 1997). The Hemiptera have modified piercing and sucking type mouth parts. *Leptocoris augur* occurs in almost every state of India throughout the year. The parental care is unique feature of *Leptocoris* and at least one of the adult will remain with the cluster of nymphs. It is commonly called as red eye bug as they have prominent red eyes and soapberry bug because of the common host plant is soapberry *Sapindus saponaria* L. [2, 20, 16]. They deposit eggs into bark crevices and ventral venations of leaves which hatched on nineth day onwards [25, 10]. Adults and nymphs are clustered around the lower portion of tree trunk [23, 12]. The nymph and adult having piercing and sucking type of mouthparts and they damage comman vegetable and fruit plant i.e. cucumber, ladyfinger, tomato, strawberry and grapes etc. They also damage young plants around comman host plants [12]. Therefore, to study the life cycle, surface ultrastructure of antennae and different type of sensillae present on the antenna of red eye bug *Leptocoris augur*, the present work has been undertaken.

Material and Methods

The adult red eye bug, *Leptocoris augur* were collected from soapberry plant, *Sapindus saponaria L.* and rearing was carried out in the insectory of Department of Zoology, RTM Nagpur University, Nagpur. The antennae of adult bug were carefully and gently removed under the dissecting binocular microscope (Zeiss). They were boiled in 5% KOH solution for 5min and washed in distilled water till the contents of KOH removed. The antennae were dehydrated in graded alcohol, cleared in xylene and mounted on slide for whole mount study. For SEM study, boiled and dehydrated antennae were dried at

room temperature and mounted on carbon coated mettalic stubs as per desired view and proceeded for platinum coating in poloron gold coating automatic unit. Finally scanned under Jeol (JSM 6380A) Scanning Electron Microscop (SEM) at desirable magnification at the Instrumentation Centre, Vishveshvaraya National Institute of Technology (VNIT) Nagpur, India.

Results

The development of the red eye bug, *Leptocoris augur* passes through egg, five nymphal instars and adults (male, female). After mating, the female lay eggs within 3-4 days.

Egg: The single female lay about 60-80 eggs in the cluster of 10-15 at a time on the ventral surface of leaf of host plant and crevices of the bark. The colour of eggs changes gradually from dusty white to rusty red within 3-4 days (fig: 1). The incubation period of eggs were about 9 to 10 days.

Post Embryonic Development:

1st Instar: The first instar emerges from the egg on 10th day onwards. After emergence they moved immegiately around the egg shell and spend the entire period near to the egg shells. The first instar nymph were very small, bright red in colour. A pair of antenna were well developed on the head. Eyes are red and ocelli are absent. Wings are absent (not developed) and legs are red in colour. The mouth parts are fused and formed long proboscis which runs backward direction. It is longer than the length of body of 1st instar, might be due to which they didn't suck sap of host plant (fig: 2). After 2 days it undergoes first moulting. It takes 20-25 min. for moulting and transfered into the second instar nymph.

2nd Instar: First moulting gives second instar nymph. The second step of moulting i.e. sclerotization takes 3-4hrs. and started the feeding process within 5-6hrs. The second instar was very small and morphologically similar to the first instar nymph, except length, width and weight (fig: 3). After 3-4 days it undergoes second moulting. It takes 25-35 min. for moulting and transfered into the third instar nymph.

3rd Instar: Second moulting gives third instar nymph. It takes 3-4 hrs. for tanning of cuticle and started to feed within 5-6hrs. The third instar nymph was bright red with long and backwardly directed black proboscis. They have prominent eyes and well developed ocelli. Wing buds are slightly developed from the second thorasic segment (fig:4). After 3-4 days it undergoes third moulting. It takes 35-40 min. for moulting and transfered into the fourth instar nymph.

4th Instar: Third moulting gives fourth instar nymph. It takes 4-5 hrs. for tanning of cuticle and started to feed within 3-4 hrs. The fourth instar was bright red coloured with prominent eyes and well developed ocelli. The wing buds developed on the second thorasic segment (fig: 5). After 3-4 days it undergoes fourth moulting. It takes 35-40 min. for moulting and transfer to the fifth instar.

5th Instar: Fourth moulting gives fifth instar nymph. It takes 4-5 hrs. for tanning of cuticle and started to feed within 2-3 hrs. The fifth instar is morphologically similar to the adult, except partially developed wings (fig-6). After 5-6 days it undergoes fifth moulting and became an adult.

Adult: Fifth moulting gives adult. It takes 3-4 hrs. for sclerotization and started to feed within 2-3 hrs. There was no sextul dimorphism. Male and female were approximately similar in appearance except female was longer than the male. The head and thorax were dull red with the bright red coloured abdomen. The wings were fully developed and their length increased upto the last abdominal segment (fig: 7, 8).

The adult head was dull red in colour containing dorso-lateraly situated large, red compound eyes and one pair of red ocelli present on the middorsal region. Antennae were filiform type, long and four segmented. The proboscis (rostrum) was backwardly directed upto the first or second abdominal segment. The rostrum was made up of mandible and maxillae which were modified into long thin stylets that fits together forming food canals and are enclosed by labium. The labrum covers the upper portion of the rostrum and is inserted under the labial groove at the end of the first segment of proboscis. A flat plate like structure, pronotum, protects the thorax. Another part, the scutellum also covers the parts of thorax. The thorax is three segmented (table-1).

The pair of full developed wings were present on the thorasic segments. The forewing of bug are termed as hemelytra. The hemelytra consist of the leathery corium and membranous tip. The hemelytra fold tightly together over the membranous hind wings on the abdomen. Leathery corium of forewing was dull red in colour while membranous tip was blakish. Hind wings were grey black in colour. The three pairs of legs were originated on ventral side from each of the thorasic segment. The legs were five segmented. The abdomen was six sgemented covered with numerous sensillary hairs. The dull white bandings were present between the two abdominal segments on ventral side.

The bug have four segmented, filiform type of antennae. The antennae were fitted in the socket on the ventro-lateral side of frontal carrinae. The antennae was divided into three parts, the scape, pedicel and flagellum. The flagellum was two segmented while the scape and pedicel were one segmented. The scape was first segment of antenna. It was fixed basaly into antennal socket (fig: 10-11).

SEM Structure of Antenna

Sensilla trichoidea-I (ST-I), sensilla trichoidea-II (ST-II), two types of trichoid sensilla were observed on the surface of the first segment i.e. scape(fig:12-13). Pedicel is the second segment of antenna. It is of uniform in diameter, from anterior to posterior end. Two types of trichoid sensilla were observed on the surface of pedicel, sensilla trichoidea-I (ST-I) and sensilla trichoidea-II (ST-II) (fig: 14). The funiculus is third part of antennae. It is two segmented. The first segment is uniform in diameter from anterior to posterior. while the second segment of funiculus is more in diameter and rounded at the tip. The first segment of funiculus consist of two types of sensilla, sensilla trichoidea-I (ST-I) and sensilla trichoidea-II (ST-II)(fig:15-16). The second segment of funiculus bears three types of sensilla. The sensilla trichoidea (ST) which are long, broad at the base and pointed towards the apex. The sensilla trichoidea curvata (STC) are broad, long and curved towards the apex and the sensilla basiconica (SB) are broad at the base having conical tip with the short length. At the joining of the segment of antenna, there is node (buttan) like structure reveals hexagonal and pentagonal structure (fig: 17) (table-2). The second segment of antenna has conical tip have Sensilla trichoidea-I (ST-I), sensilla trichoidea-II (ST-II) and Sensilla trichoidea curvata (STC) (fig: 18).

Discussion

The life cycle of soapberry bug, *Leptocoris augur* is more or less similar to that of other hemipteran bugs described by earlier workers [13,11,12,2,3, 6 20]. After mating, the adult female lay eggs in cluster on the ventral side of leaves of host plant. The newly laid eggs adhered to the leaf surface similar to other Rhopalidae bugs [20, 16]. The eggs are dusty white in colour at the time of laying which changed into dark brick red after two days. The incubation period of eggs were reported from eight to ten days in various species of bugs. In *Leptocoris augur* the eggs hatched within the period of eight to nine days, depending upon environmental changes. During the present study, it has been notice that after emergence the first instar nymph of *L. augur* do not feed on the plant sap. They spend the entire period near to the egg shells. The similar observation has been noticed on the first instar nymph of Rhopalidae bugs by earlier workers[25,24,20,16] determine the number of larval instars and described as first to fifth nymphal instars in the hemipteran bugs, on the basis of instars body size.

In *L.augur*, first to fifth nymphal instar werw observed and no significant morphological differences has been noticed in their body size, length and weight. The wing pads developed on the third instars and wing developed subsequently. Moreover in first and second nymphal instars, the mouth parts are more longer than the body length while, the length of rostrum decreased in the successive third, fourth and fifth nymphal instars. The colour of the developing nymphal instars are red and remain unchanged upto the adult. There are no significant morphological sexual dimorphism in adult except the length of female which is more than the male. Similar finding were noticed in other hemilpteran bugs by earlier workers [13, 10, 7, 20]. The present study reveals that the presence of five nymphal instars and last instar moulted into the adult, similar to that found in other hemipteran bugs (Chopra, 1966; Hamman, 1997).

The antennae of the Hemiptera are studied and reviewed morphologically and sensilla had been noticed by earlier workers [17, 13, 4]. Richard, (1952) and Imms, (1973) described the antennae of Hemiptera are filliform type. In the present study the antennae of *Leptocoris augur* are observed filliform type, consisting basal scape, pedicel and two segmented flagellum.

By using SEM techniques, Bourgoin and Deiss (1994) reported three basic type of sensilla on the antennae of *Fulgoromorpha* and on the antennae of *Magicaecada*, the homopteran bug [14]. Wcislo, (1994) studied the antennal sensilla of more than 100 hymenopteran species and described the sensilla. SEM study confirms the antennae of *L. augur* consisting three types of sensilla supporting the finding of earlier workers. It is well evident that the base of scape is covered with the film of pipetive sensilla inside the antennal socket in honeybee [15].

Fuente and Catala (2002) reported sensilla trichoidea (ST) on pedicel of *Triatoma infestans* and sensilla trichodea (ST) and sensilla basiconica (SB) on flagellomeres of *Triatoma brasiliensis*, *T.sordida*, *T.pseudomaculata.* Weirauch (2003) reported campaniform sensilla on the pedicel of reduviidea bugs. Diehl *et.al.* (2003) reported the grooved peg on the flagellum of *Triatoma infestans and* sensilla trichoidea (ST) and sensilla trichoidea curvata (STC) on antennal tip of wheat bug *Eurygaster maura* [18]. Silva,*et.al.* (2010) reported trachoid sensilla (ST-I,ST-II), long and short basiconic sensilla (SB-I,SB-II,SB-III), chaetic sensilla (Sch), coelonic sensilla (Sco) on the antennal segments of three sting bug, *Euschistus heros*, *Piezodorus guildinii*, *Edessa meditabunda*.

In *L. augur*, scape of antennae covered with various category of sensilla trichoidea. The pedicel is uniform in diameter consisting only two types of trichoid sensilla. The flagellum contain two flagellomers. The first flagellomere consist of two type of trichoid sensilla (ST-I and ST-II) while the last flagellomere and tip of antenna consist of three types of sensilla, sensilla trichoidea (ST), sensilla trichoidea curvata (STC) and sensilla basiconica (SB) supporting the observations of earlier workers.

Conclusion

The bugs have hemimetabolous metamorphosis, as the development goes through egg,five nymphal instars and adults. The surface ultrastructural study of antenna showing the presence of three different types of sensilla as sensilla trichoidea (ST-I and ST-II), sensilla trichoidea curvata (STC) and sensilla basiconica (SB). Sensilla are the sense organs perceiving the stimuli from external and internal environment. Sensilla trichoidea (ST) are the mechanoreceptor and serving the sense of touch. These are the tactile hair of the antenna which may be perceive earth born vibration as bug is terrestial insect. Sensilla basiconica(SB) are the chemoreceptor, sensitive to chemicals and are primary sensory epithelial cells innervated by neurons. They may be perceiving the stimulus of mating from there next partner.

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Table1: Morphometry of developmental stages of *Leptocoris augur*.

S. N.	Stages		Total Length (mm)	Weight (mg)	Head		Thorax		Abdomen	
					Length(mm)	Width(mm)	Length(mm)	Width(mm)	Length(mm)	Width(mm)
1.	Egg		1.42 ± 0.037	1.48 ± 0.086	-	-	-	-	-	-
2.	1st instar		1.05 ± 0.31	2.04 ± 0.05	0.24 ± 0.01	0.3 ± 0.001	0.3 ± 0.001	0.3 ± 0.01	0.51 ± 0.01	0.5 ± 0.02
3.	2nd instar		3.8 ± 0.41	5.02 ± 0.05	0.75 ± 0.04	0.6 ± 0.001	1.0 ± 0.001	0.9 ± 0.02	2.15 ± 0.04	1.9 ± 0.02
4.	3rd instar		5.22 ± 0.03	8.08 ± 0.05	1.12 ± 0.01	0.9 ± 0.005	1.82 ± 0.01	1.1 ± 0.01	2.38 ± 0.01	1.9 ± 0.02
5.	4th instar		6.99 ± 0.06	10.36 ± 0.15	1.31 ± 0.01	1.1 ± 0.01	2.24 ± 0.005	1.9 ± 0.01	3.45 ± 0.03	2.1 ± 0.01
6.	5th instar		10.28 ± 0.13	11.9 ± 0.07	1.9 ± 0.02	1.3 ± 0.01	2.98 ± 0.03	2.0 ± 0.04	5.16 ± 0.01	3.1 ± 0.05
7	ADULT	Male	9.14 ± 0.026	14.48 ± 0.12	1.8 ± 0.01	1.9 ± 0.02	2.83 ± 0.01	1.9 ± 0.02	4.37 ± 0.01	2.22 ± 0.005
		Female	14.00 ± 0.06	25.66 ± 0.12	2.04 ± 0.05	1.8 ± 0.02	4.93 ± 0.05	2.1 ± 0.05	6.86 ± 0.01	3.2 ± 0.001

Table 2 : Various sensilla present on the antennae of *Leptocoris augur*.

S. No.	Antennae		Unit	Length	Width (µm)	Sensilla	Length (µm)	Width (µm)
1.	Socket		µm	259 ± 1.91	201 ± 1.21	-	-	-
2.	Scape		µm	720 ± 0.11	309 ± 0.21	Sensilla trichoidea-I Sensilla trichoidea-II Sensilla trichoidea-III	60.8 ± 1.01 38.3 ± 1.9 25.6 ± 1.3	5.69 ± 0.2 4.18 ± 0.9 3.38 ± 0.5
3.	Pedicel		mm	2.37 ± 0.01	159 ± 0.91	Sensilla trichoidea-I Sensilla trichoidea-II	79.2 ± 1.1 42.9 ± 0.1	4.81 ± 0.8 3.38 ± 0.5
4.	Funiculus	1st segment	mm	2.28 ± 0.03	157 ± 0.89	Sensilla trichoidea-I Sensilla trichoidea-II	97.1 ± 1.1 38.3 ± 1.9	6.10 ± 0.3 4.18 ± 0.8
		2nd segment	mm	2.74 ± 0.02	301 ± 0.22	Sensilla trichoidea Sensilla basiconica Sensilla trichoidea curvata	52.2 ± 0.91 11.5 ± 0.83 43.5 ± 0.79	5.00 ± 0.01 4.26 ± 0.009 3.40 ± 0.4

Value expressed are mean ± standard error

Fig : Post embryonic developmental stages of *Leptocoris augur*

1: Egg 2: I Instar 3: II Instar 4: III Instar
5: IV Instar 6: V Instar 7: Male 8: Female

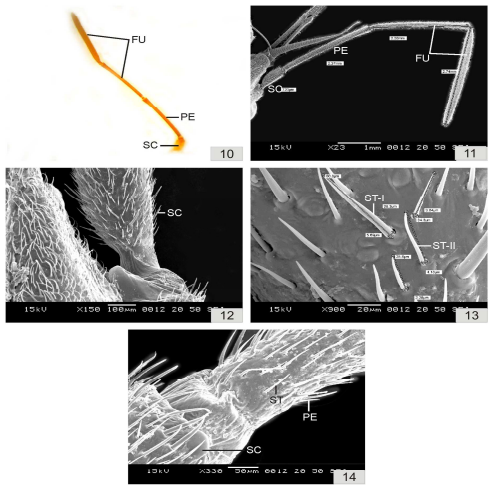

Fig. 10:Antenna whole mount Fig.11: Scanning electron microscopy (SEM) of complete antenna. Fig.12-13: SEM of scape (SC) showing trachoid sensilla (ST-I and ST-II) Fig.14: SEM of pedicel (PE) showing trachoid sensilla (ST).

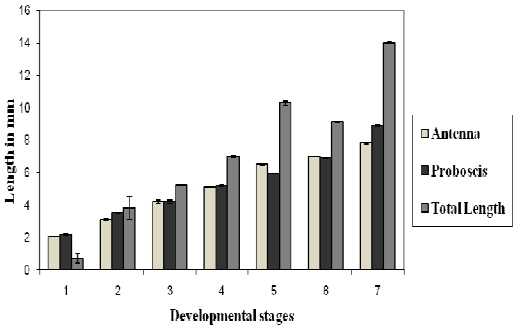

Fig:9- Histogram showing length of antenna, proboscis and total body length of developing stages of *Leptocoris augur*

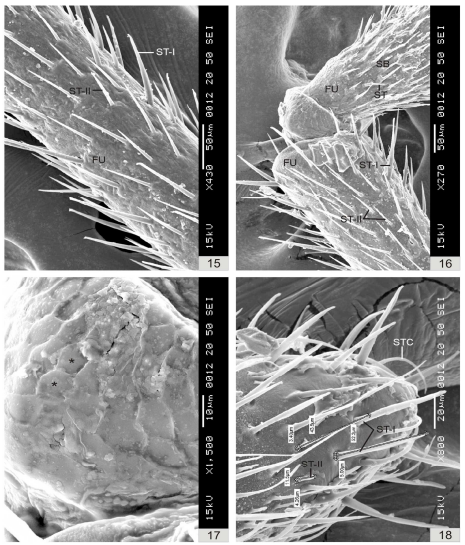

Fig. 15: SEM of first flagellomere showing trachoid sensilla (ST-I, ST-II)
Fig.16-17:Two flagellomeres showing trachoid sensilla(ST-I,ST-II),basiconic sensilla(SB) and node between two segments showing hexagonal and pentagonal plate (*)
Fig.18: Tip of antenna showing trachoid sensilla (ST-I,ST-II) and basiconic sensilla(SB).