Documentation and validation of chemosensory structures in antennae of *Spodoptera litura* (Fabricius) through Scanning Electron Microscope (SEM)

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**ABSTRACT**

The studies with SEM clearly depicted that the antenna of female was longer than male antenna. Five types of sensilla namely trichodea, chaetica, styloconica, coeloconica and auricillica, and scales were observed on the flagellum of both sexes. Sensilla trichodea were distributed randomly on all segments and were the most frequent type. Six sensilla chaetica were observed on each flagellar segment in both sexes, except in the apical segment. A sensillum styloconicum was always found at the upper-middle region of each flagellar subsegment. This sensillum had a smooth petiole and a conic extremity with one to three apical structures. Sensilla coeloconica were situated from middle to the distal portion of segment. Sensilla auricillica were found among the scales and had the typical shape of rabbit’s ear. Sensilla squamiformia were present on dorsal part of the antenna among scales. They were shorter and finer than scales and were embedded in a socket and point distally. Sensilla basiconica were smaller and least abundant of all sensilla.

**Key words:** Chaetica, Coeloconica, Styloconica, Trichodea.

**INTRODUCTION**

The tropical armyworm, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) is a generalist herbivore and an important pest in many agricultural cropping systems. Wu *et al.* (2004) reported that *S. litura* infested more than 290 species of plants belonging to 99 families. Most organisms rely on their olfactory system to detect and analyze olfactory cues in the environment, cues that are subsequently utilized in the context of behaviour. Response to volatile environmental chemosensory cues is essential for insect survival. Olfaction, the sensing of airborne chemicals from the environment, is a critical process for insects, allowing detection of food, danger and mates.

Antennal sensilla are important sensory receptors (Ochieng *et al.*, 2000) which were proved to be involved in the perception of various kinds of stimuli in different insect orders (Altner and Loftus, 1985; Keil, 1999; Kristoffersen *et al.*, 2008). Insect sensilla consist of an exocuticular structure through which stimuli are conveyed to one or more sensory cell processes within the sensilla. Shape and size of the exocuticular structures vary, but most sensilla appear as hairs or pegs (Altner and Frillinger, 1980). Besides antenna, few chemosensillums are also present on mouthparts, wings, legs and ovipositors. Most of the sensilla are generally distributed on the flagellum part of antennae (Chapman, 1998). Various functional types of antennal olfactory sensilla have been characterized (Anderson *et al.*, 1995; Ljunberg *et al.*, 1993). As most olfactory sensilla are located on the antennae of insects (Gullan and Cranston, 2000; Hallberg and Hansson, 1999), a detailed study on antennal sensilla is necessary for better understanding of the host and mate location mechanisms. Hence, a study was undertaken to document the sensilla present in the antenna of larvae and male and female *S. litura* adults using Scanning Electron Microscope.

**MATERIALS AND METHODS**

**Culturing of *S. litura***: Mass culturing of *S. litura* was carried out at the Insectary Unit of Department of Agricultural Entomology, Tamil Nadu Agricultural University (TNAU), Coimbatore by following the detailed procedure (Subramanian 1998). Healthy adults of *S. litura* were obtained from a healthy colony and sexed based on dimorphic characters (Mochida, 1973). Five pairs of adults were released into plastic buckets (7 litre capacity) for oviposition. Fresh nerium (*Nerium odoratum* L.) shoots were provided in the oviposition chamber as ovipositional substrate. Ten per cent sugar solution fortified with 0.03 per cent multivitamins was provided in cotton wicks as adult feed. The eggs were collected and then the larvae were fed with castor leaves till pupation.

**Preparation of antennal samples for Scanning Electron Microscopy**: The antennal samples were prepared by following the procedure described (Sukontason, 2003). Twenty larvae from each instar of *S. litura* were observed for the antennal growth and development during larval period with Scanning Electron Microscope (SEM). The larvae of...
S. litura were anesthetized using CO₂ for about 10 min. and head was dissected from the larvae. Ten individual adults of male and female S. litura were used for the documentation of the chemosensory structures. In the adult insects, antennae from the head capsule of individuals were excised under a stereomicroscope at 40X prior to pre-fixation. Then, the antennae were pre-fixed in 2.5 per cent glutaraldehyde mixed with phosphate buffer solution (PBS) at a pH of 7.4 at 4°C for 24 h followed by post fixation in one per cent osmium tetroxide for 24 h. Specimens were then rinsed with PBS and dehydrated in a graded ethanol series of 30, 50, 70, 80, 90 and 95 per cent and absolute ethanol, in each case for 12 h. The samples were then dehydrated through increasing concentrations of alcohol, dried and mounted on stubs (copper grids) with double-side adhesive tape. Micrographs of the antennae and sensilla were taken from five individual S. litura and dimensions of the sensilla were measured with a FEI QUANTA 250 (Netherland) at the Department of Nanoscience and Technology, Tamil Nadu Agricultural University, Coimbatore. Abundance and distribution of antennal sensilla types were compared between male and female of S. litura adults.

RESULTS AND DISCUSSION
Larval antennae and antennal sensilla of S. litura: SEM images of the larval antennae revealed that the head of S. litura larvae was oval, smooth and hairy (Plate 1). It had many simple hair-like setae including long tactile setae and short tactile setae. The antenna of larvae was located on the side of the head and inserted in between stemmata and mandibles. The antennal development progressed from first instar to fifth instar and the segments were clearly visible from fourth and fifth instars (Plate 1a to 1e). In the fifth instar, the antenna was short and composed of three segments viz., a basal segment of 34.18±0.6 µm long and 59.71±0.4 µm thick, a medial segment of 147.43±0.5 µm long and 83.65±0.3 µm thick, and a distal short segment of 21.37±0.4 µm long (Plate 1e). The sensilla were not present in the first segment whereas, the latter two segments had sensilla. Observations on the SEM images showed that in the fifth instar, the antennae were composed of three segments: a basal segment, a medial segment and a distal short segment. The types, numbers and distribution of sensilla on the antennae of S. litura larva were similar to those of other lepidopterous larvae (Xie, 2006). In the Lepidopteran insects, the antennal sensilla basiconica play an indispensible role in olfactory discrimination among different host plants (Xun et al., 2008).

The second segment had two sensilla chaetica and three sensilla basiconica. The sensilla chaetica were smooth with a socket at the base and were of various lengths. One sensillum chaetica was located externally, while the other was located ventrally and close to the sensillum basiconicum. The sensilla basiconicum were slender and short pegs, which had enlarged basal portions and were located ventrally. The third segment possessed one sensillum styloconicum and

Plate 1a: First instar of S. litura larvae.
Plate 1b: Second instar of S. litura larvae.
Plate 1c: Third instar of S. litura larvae.
Plate 1d: Fourth instar of S. litura larvae.
Plate 1e: Fifth instar of S. litura larvae.

Plate 1: SEM photograph of Antennal growth in the larvae of S. litura.
three sensillum basiconicum. The sensillum styloconicum was located ventrally and was aportous. All sensillum basiconicum were thick, two-walled and multiporous. Xun et al., (2008) observed that the antenna of S. exigua was found to bear two sensilla chaetica, one sensillum styloconicum, three large sensilla basiconica and three small sensilla basiconica. The sensilla chaetica were smooth with a socket at the base and were of various lengths. These sensilla were innervated by one mechanosensory cell and were adapted for the reception of tactile stimuli including air currents, vibrations of the substrate, and shocks sensed by the sensilla from exploratory movements of the antennae (Kent and Hildebrand, 1987). The sensilla basiconicum were slender and short pegs, which had enlarged basal portions. The third segment possessed one aportous sensillum styloconicum and three sensillum basiconicum. Three sensilla basiconica of the antennae were reported to be olfactory receptors (Dethier, 1973; Schoonhoven and Dethier, 1966).

**Adult antennae and antennal sensilla of S. litura:** The studies with the SEM clearly showed that each antenna consisted of two basal segments, the scape and pedicel, and a distal part, the flagellum. The antenna of female was longer than male. The number of subsegments in the flagellum with 49±4 in males and 51±5 in females. Five types of sensilla viz., sensilla trichodea, chaetica, styloconica, auricillica and coeloconica were found on the ventral surface of the flagellum of both sexes (Plates 2 and 3), which was referred as the “sensory area”. The dorsal side of flagellum was covered with scales and was referred as “scale area” (Plate 4). Sensilla chaetica were observed on both the sensory and the scale areas. There was no difference in their abundance and distribution between the sexes. More attention was focused on the identified mechano and olfactory sensilla owing to their importance in mating and host finding (Steinbrecht, 1984). Typically, porous sensillar walls suggested olfactory function (Hallberg et al., 2003; Kristoffersen et al., 2006; Onagbola and Fadamiro, 2008). Odorant substances including sex pheromones and host plant volatiles diffused through the wall pores of antennal sensilla into the sensillar lymph and were transferred to olfactory receptors on the dendrites of olfactory neurons by odorant binding proteins (Leal, 2005). Similar observations were recorded in different species of Spodoptera by various research workers (Monti et al., 1995).

**Sensilla trichodea:** Sensilla trichodea were distributed randomly on all segments of the antennae and were the most frequent type. The total number of trichodea was greater in males than in females (Plates 2a and 3a). The studies by Mochizuki and Shibuya (1991) showed that trichodea were sensitive to pheromone even at low concentration.

**Sensilla chaetica:** Sensilla chaetica were straight, wide at the basal part and curved apically with a corncob-like structure. The base of chaetica was inserted into a
membranous socket, which consisted of a cuticular structure. The sensilla chaetica were present on each flagellar segment in both sexes, except in the apical segment. In the scale area, one chaeticum was present on each subsegment, while in the sensory area, the chaetica generally appeared first on second subsegment (Plates 2b and 3b). It has been suggested that the chaetica rose from a socket and had pores in the hair tips (Vanderpers et al., 1980). The sensilla chaetica observed in *S. litura* antennae were also commonly seen in other insects (Gao et al., 2007; Onagbola and Fadamiro, 2008). The sensilla chaetica were present on each flagellar segment in both sexes, except in the apical segment. Based on the location and structure of this type of sensilla, it was believed to have a dual function of mechanoreception and contact chemoreception (Isidoro and Solinas, 1992; Jourdan et al., 1995).

**Sensilla styloconica**: Sensilla styloconica were cone-shaped with apical cuticular pegs and had wrinkles on the surface. This sensillum had a smooth petiole and conic, extremity with one to three apical structures but had neither socket nor pores. A sensillum styloconicum was always found at the upper-middle region of each flagellar subsegment in the centre of the sensory area from fifth to the most distal subsegment (Plates 2c and 3c). Morphological and electrophysiological results indicated that sensilla styloconica in Lepidoptera were hygro- and thermosensitive (Steinbrecht, 1984).

Plate 3: Sensilla present on the female antennae of *S. litura.*

Plate 4: SEM images of antennal scales of adult *S. litura.*
Sensilla coeloconica: Sensilla coeloconica were small and commonly called as pit pegs. They were situated mainly from middle to the distal portion of segment. Two types of sensilla coeloconica were found in the antennae of S. litura. One type had a cuticular fringe around a central peg and was present on each subsegment. The sensilla, without fringe was present only one to two per antenna. Each sensillum consisted of a depression surrounded by 12-14 cuticular “spines” and a porous peg. They were found to arise from centre of the depression and had longitudinal striations on its surface (Plates 2d and 3d). Similar types have been reported in the European corn borer, Ostrinia nubilalis (Cornford et al., 1973). The sensilla coeloconica have been reported to have a chemo, thermo or hygrosensory function (Zacharuk, 1985).

Sensilla auricillica: These sensilla were usually found among the scales. They were simply dorsoventrally flattened in structure. Sensilla auricillica were concave with numerous pores arranged vertically on the surface. One to three auricillica were located on distal end of the subsegment at the border of sensory and scale areas (Plates 2e and 3e). In Adoxophyes orana, sensilla auricillica were shown to have the potential for the detection of apple odour (Denotter et al., 1978). These sensilla were considered to be involved in insect-plant interactions.

SEM dimensions of adult S. litura antennae: The length and breadth of the antennae of male and female S. litura were measured with SEM FEI QUANTA 250. The length of antennae in male was 5.42±1.2 mm whereas in female it was 5.96±1.6 mm. The breadth of antennae in male was 105±0.9 µm whereas in female it was 121.2±1.1 µm. The female adults of S. litura were found to have different types of sensilla in greater length and breadth than the male. Among the different sensilla observed, chaetica had the highest length of 58.65±1.9 µm and 53.50±2.7a µm respectively in female and male and was followed by trichodea. The shortest sensilla was coeloconica, which recorded the length of 4.18±0.6d µm and 4.96±1.8b µm in female and male respectively. In the case of breadth, the sensilla styloconica were found to have maximum of 3.68±0.3a µm and 3.55±0.4a µm respectively in female and male and was followed by chaetica (Table 1).

### Table 1: SEM dimensions of antennal sensilla of adult male and female S. litura.

| Type of sensilla      | Length (µm)* | Breadth (µm)* |
|-----------------------|--------------|---------------|
|                       | Male         | Female       | Male         | Female       |
| Trichodea             | 36.41±1.5    | 38.76±1.7    | 2.50±0.7     | 2.93±0.6     |
| Chaetica              | 53.50±2.7    | 58.65±1.9    | 3.26±0.5     | 3.60±0.3     |
| Styloconica           | 16.42±2.3    | 18.68±1.8    | 3.55±0.4     | 3.68±0.3     |
| Coeloconica           | 4.96±1.8     | 4.18±0.6     | 2.15±0.2     | 2.80±0.3     |

* Mean of five replications

**CONCLUSION**

From the studies, it is concluded that the antennae of S. litura is covered with abundant types of sensilla, each have an important role in the perception of odorant. However, physiological study is needed to confirm the role and behaviour of the insects with respect to the response of proteins with the sense structure especially present in the antennae.

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