EXTERNAL MORPHOLOGY OF ANTENNAL SENSILLA IN RELATION TO ELECTROANTENNOGRAM RESPONSES OF *Apanteles angaleti* MUESEBACK PARASITOIDS (HYMENOPTERA: BRACONIDAE) FOR HOST SEARCHING BEHAVIOUR

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ABSTRACT

Apanteles angaleti (Hymenoptera: Braconidae) is an important solitary endoparasitoid of pink boll worm larvae on cotton crops of many countries. In this study, we described the morphology, and distribution of sensilla on the antennae of both male and female adults parasitoids by using scanning electron microscopy (SEM), the SEM revealed that antennae of A. angaleti were threadlike and the flagella of both male and female antennae indicated that has total number of segments in both male and female were 18 including scape and pedicel. There are three types of sensilla to be presented in both sexes. The types of sensilla present in A. Angaleti could be identified as trichodea sensilla type 1 (TS 1), type 2 (TS2) and type 3 (TS3), Basiconic sensilla (BS) and Placoid sensilla (PS). The Basiconic sensilla presenting in both sexes of A. angaleti were similar. However, their number in male was less than in female. In contrast, the Placoid sensilla (PS) were more numerous in male than in female, also the size of the sensillum in male was longer, and they are most prominent type of sensilla on the antennae and run parallel with the flagella margin. The EAG response of A. angaleti showed significant difference between males and female to both synomonal and kairomonal extracts. The EAG response of both males and females parasitoids of A. angaleti were lesser for synomonal extracts compared to kairomonal extracts possibly due to the highest degree of specificity of the host odour to which receptors in antennae were more responsive. Larval body wash (LBW) and frass of P. gossypiella stimulated highest response at concentration C_3 (100ppm) but response decreased when concentration of extract increased while LBW extract from C. cephalonica was correlation between EAG response and concentrations. These results indicated strongly the potential of semiochemical use as long and short range cues for modulating the host searching behavior of parasitoids in the tritrophic context and therefore aid in the formulation of insect pest management strategies for cotton ecosystems.

Keywords: *Apanteles angaleti, Basiconic sensilla*, electronantennogram, pink boll worm, *Placoidea sensilla*, *Tricodea sensilla*

INTRODUCTION

Pink boll worm (*Pectinophora gossypiella*) is serious pest of cotton and many other crops. The economical damaged due to internal feeding and very difficult to control by conventional insecticides. New control strategy is needed to enhance efficient control in such biological control is to be considered. Specialist larval parasitoids, *Apanteles angaleti* are important parasitoid for controlling pink bollworm successfully in many crops specific on cotton (Sekhon and Varma (1983). Jackson (1980) discusses early biological control introductions for the pink bollworm in the United States. Reviews of biological control of cotton bollworm have been given by Johnson *et al.* (1986), King and Coleman (1989) and King and Jackson (1989). Biological control can enhance if we

understand the relationship in tritrophic interaction between host plant-herbivour-parasitoid. The effectiveness of parasitoid is dependent on their ability to searching the host habitat and host location. They used host-related volatile signals for foraging and location of host. (Dicke & Sabelis, 1988; Turlings *et al.*, 1990, 1991). In recent years, there has been increasing evidence suggesting the role of induced plant defenses serving tritrophic interactions. Parasites have been shown to respond to volatiles emanating from both undamaged plants (Li *et al*., 1992; Turlings and Tumlinson, 1992; Udayagiri and Jones, 1992) and damaged plants (Whitman, 1988; Turlings *et al*., 1990, 1995, 1998, 2000; Mattiaci *et al*., 1994; de Moraes *et al*., 1998; Rose *et al*., 1998; Dicke *et al.,* 1999; Gols *et al*., 1999; Hoballah and Turlings, 1999). Infochemicals offer good prospects as a tool for manipulating parasitoid behavior, particularly in view of possible application to enhance the efficacy of parasitoids in biological control programmes. Hence, present study, we examined the external morphology of antennal chemoreceptors by scanning electron microscopy (SEM) and the peripheral olfactory responses of male and female of *A. angaleti* to infochemicals in interaction of tritrophic level by using the electroantennogram (EAG) technique. Such an approach would allow the elucidation of sensitivity profile of *A. angaleti* to hydrocarbon present in plant and its host by-products. Beside, Gas chromatography helps in identification of hydrocarbon compounds present in synomonal and kairomonal extracts which could be used to increase the efficacy of innundative of *A. angaleti* and they will be trained before releasing against *P. gossypiella.*

MATERIAL AND METHODS

Rearing of the parasitoid *A. angaleti*

The mass culturing of the larval parasitoid, *A. angaleti* was initiated from the *A. angaleti* culture maintained continuously on the laboratory host *Corcyra cephalonica* in the Biological Control Laboratory, Division of Entomology, Indian Agricultural Research Institute, New Delhi - 110012. Male and female adults were kept in glass cages of 20x20x20 cm size and provided with 1-2 day old larvae of *C. cephalonica* for parasitization for 24-36 hrs. The adult parasitoids were fed with opened resin. The parasitized larvae of *C. cephalonica* were transferred to glass jars filled with broken maize grains till the formation of parasitoid pupae in silk cocoons and emergence of parasitoid adults. The cultures of *A. angaleti* were maintained under laboratory conditions of 26±3^{0}C and 65-70% RH.

Preparation of synomones and kairomones

Extraction of synomone

Different plant parts viz., leaves, undamaged buds and bolls damaged bud, damaged buds and damaged bolls by *P. gossypiella* at the flowering stage were collected for extraction of synomones. Thirty grams of each plant part were taken at the active vegetative phases and immersed overnight in 300ml of distilled hexane. The hexane was then filtered through Whatman No. 1 filter paper. Anhydrous sodium sulphate @1 g/10g sample was added to the filtrate for dehydration for 2 hrs and passed through silica gel (60-120 mesh) column. The hexane extract of different plant parts eluted through the column and then distilled at 60-70^{0}C. The left over residue was collected by rinsing the container with a small quantity of HPLC grade hexane (Merck) and stored in separate vials and kept in fridge.

Extraction of kairomone

Field host, *P. gossypiella*: *P. gossypiella* was collected from cotton field for extraction of kairomones. The bodywash of the pink boll worm larvae and its faeces / frass were extracted by the following procedure:

Five g of PBW larvae were collected from the cotton field was dipped in 50 ml distilled hexane for 2 hours and the sample was shaken in the thermostatically controlled water bath (Haake 220 SWB) at 150 rpm initially at 25^0 C for 2 hrs and later at 50^{0}C for 20 min. The hexane extracts were filtered through Whatman No.1 filter paper, eluted through silica gel (60-120 mesh) column and then distilled at 60-70^{0}C. The left over residues were collected by rinsing the container with little HPLC grade

hexane (Merck), stored in separate vials and kept in fridge for further use.

For frass extract, 2 g of PBW frass was dipped in 20 ml of distilled hexane overnight and filtered through Whatman No.1 filter paper and eluted through silica gel (60-120 mesh) column. The hexane extracts then were distilled at 60-70^{0}C and the leftover residues were collected by rinsing the container with little HPLC grade hexane, stored in separate vials and kept in fridge for further use.

Laboratory host, rice moth, *C. cephalonica*

Larval body wash of laboratory host, *C. cephalonica* were extracted by the same procedure with kairomonal extraction of field host in which 10g of larvae of rice moth was used for extraction of kairomone.

Gas-liquid chromatography

The purified hexane extracts of different parts of host plants, larval bodywash of *Corcyra cephalonica*, frass and larval bodywash of *P. gossypiella* were concentrated and injected into Gas Chromatograph (Varian 3900 XL) fitted with Flame Ionization Detector (FID) in a WCOT fused silica 30 m x 0.32 mm ID, CP-SIL 24 LB/MS (# CP5860) Varian Chrompack capillary column at a temperature range programmed between 100-260^{0}C for 56 minutes. The injector and detector temperatures were maintained at 300^{0}C. Nitrogen was used as carrier gas with a flow rate of 20ml/min. The injection volume was 3µl.The hydrocarbon standards for C_{10}-C_{30} were obtained from Sigma Aldrich, USA. GLC of the synomonal and kairomonal extracts were carried out to detect the presence of these saturated hydrocarbons. The resultant chromatographs were analyzed with the help of interactive graphics (Varian Star Chromatography workstation, Version 6.0) software.

EAG recording apparatus.

The EAG apparatus was used in the present studies is the assortment of EAG module IDAC-232, stimus air controller CS-05, Micro manipulator MP-15, developed by Syntech. The EAG was recorded from excised heads of parasitoids with antennae. The tip of antenna was mounted into the capillary electrode positioned at the different electrode holder that was connected with the probe and data acquisition interface. The stimulus from synomonal and kairomonal extracts were applied by using a stimulus air controller designed to deliver controlled air flow towards the antenna. The continuous air flow was maintained at 4-5 m/s with a complementary air supply @ 4m/s connected to the stimulus applicator and mixing tube assembly using a Pasteur pipette/disposable micro pipette tip having the stimulus applied on Whatman No.1 filter paper strip (2.5 x 0.5 cm). All the stimulus sources synomone and kairomone were dissolved in HPLC grade hexane to supply source of stimuli.

The dose of 30µl which was able to elicit better EAG response for *A. angaleti* was selected as optimum doses for all EAG recordings. The antennal preparation was exposed to a series of different stimuli (synomones / kairomones) with intermittent application of reference stimuli (10% honey solution) in recording session with an interval of 40 seconds for recovery. The recording was repeated with at least 5 individual adult parasitoids with 3 replications of stimulus puff through stimulus applicator. The EAG responses for male and female *A. angaleti* were recorded separately. Honey 10% solution was considered as the standard and responses to other compounds are expressed as 100 percent of the amplitude to the standard. In addition, no corrections were made for differences in volatility among the test compounds and as a result, comparisons among responses are relative.

Scanning Electron Microscopy (SEM) studies on antennal sensilla of *A. angaleti*.

The dissected antennae of *C. blackburni* and *A. angaleti* were sputter coated with 22 karat gold solution under vacuum and photographed under a Leica-SEM computerized photo system. Terminologies of Snodgrass (1935), Scheider (1964), Amornsak *et al*., (1998) and Keil (1999) were used to describe the antennal sensilla.

Statistic analysis

Relative EAG response of the parasitoids was

subjected to TWO-WAY ANOVA using SPSS version 13.00. For each group of chemical, relative amplitudes to individual compounds were pooled and averaged and differences between sexes were determined by Student's t-test.

RESULTS

Hydrocarbon profile of infochemicals.

The quality and quantity of saturated hydrocarbon chains varied in both synomones and kairomones. The synomonal extracts of the various plant parts from all the three cotton varieties had hydrocarbons ranging from C_{14} to C_{30}. While nonadecane (C_{19}) was not found in any of the extracts of variety Pusa 8-6, pentadecane (C_{14}) was found in the damaged bud extract and tricosane in the undamaged bud only (Table 1). In variety LD 327, octadecane (C_{18}) and tetracosane (C_{24}) were absent from all the extracts and tricosane (C_{23}) was seen only in leaf, damaged bud and damaged boll.(Table 2) Two hydrocarbons octadecane (C_{18}) and nonadecane (C_{19}) were absent from all the extracts of variety RS 2013. While, tetradecan (C_{14}) was found in leaf, damaged bud and damaged boll extracts, tricosane (C_{23}) was present only in damaged bud and damaged boll extracts and absent from the undamaged bud extracts (Table 3). The length of hydrocarbon chain was different in various kairomones extracts and ranged from C_{10} to C_{30}. Among the identified hydrocarbons, decane, undecane and dodecane (C_{12}) was absent in all the kairomonal extracts while tridecane (C_{13}) was absent in the extracts of, larval body wash of *C. cephalonica* and frass of *P. gossypiella*, nonadecane (C19) was found in frass of P. gossypiella but absent in larval body wash of both *C. cephalonica* and *P. gossypiella.* Tetracosane (C_{24}) was absent from both larval body wash and frass extracts of *P. gossypiella* but present in larval body wash of *C. cephalonica*. Long chain saturated hydrocarbon ranged from C_{25}-C_{30} were present in all kairomonal extracts except C_{27} (heptacosane) was absent in larval body wash of *P. gossypiella* (Table 4).

EAG responses of male and female of *A. angaleti* to infochemicals

EAG responses of male and female of A. angaleti to synomonal extracts

The EAG response of males and females of *A. angaleti* was recorded separately. Among the different synomonal extracts of variety Pusa 8-6, damaged boll extract elicited the maximum mean relative EAG responses in males (146.3%) and in females (154.9%) compared to other extracts. Undamaged bud extract gave the least mean relative EAG response (125.3%) in males. For all the other extracts, response of females was significantly higher than in males**.** In the case of synomonal extracts from cotton variety LD 327, males and females showed maximum mean relative EAG responses (124.1%) and (147.1%) respectively for damaged boll extracts while response was least for both males and female for undamaged boll extract. There was significant difference in the response of the two sexes for the various extracts. The order of mean relative EAG response of males of *A. angaleti* to different synomonal extracts of cotton variety RS 2013 was damaged boll > damaged bud > leaf > undamaged boll > undamaged bud extracts. Similar response was observed for females also (Table 5).

EAG response of males and females of A. angaleti to kairomonal extracts

Different concentrations viz.., C1 (1ppm), C_2 (10ppm), C_3,(100ppm) C_4,(1000ppm) C_5(10,000ppm) of kairomonal extracts were used for both male and female parasitoids to arrive at a concentration which would give maximum mean relative EAG responses. Larval body wash of *C. cephalonica* elicited highest mean relative EAG responses (164.9%) at C_3 concentration in females and (157.4%) at C_5 concentration in males. Both male and female parasitoids showed maximum mean relative EAG response (151.4% and 161.1% respectively) to larval body wash of *P. gossypiella* at C_3 concentration after which responses decreased with increasing concentration. The least mean relative EAG response was always seen at the lowest (C_1) concentration used. Frass extract

from *P. gossypiella* also elicited maximum mean relative EAG responses at C_3 concentration in both males and females of *A. angaleti*. The responses of female parasitoids were seen to increase slowly from lowest concentration C_1 (1ppm) to C_3 (100ppm) after which the response declined at C_4 (1000ppm) and C_5 (10,000ppm) concentrations in contrast to the responses in the male where response increased with increasing concentration of the extracts. Frass extract of *P. gossypiella* showed the highest mean relative responses for both male and female (136.9% and 147.5%) followed by larval body wash of *P. gossypiella* (132.5% in male, 140.5% in female) and larval body wash of *C. cephalonica* (125.3% in male, 130.2% in female) (Table7). In general, males always responded less to all the extracts compared to females. The mean relative EAG responses for both male and female parasitoids were recorded at different concentrations C_1, C_3, C_5 for each kairomonal extract. While males showed maximum mean relative EAG responses to each extract at C_5 concentration the females showed maximum mean relative EAG responses at C_3 concentration for all the extracts (Table 6). Electroantennography studies further confirmed the response of the parasitoids for all the semiochemicals tested. The EAG response was stronger in females compared to males of *A. angaleti* for all the synomonal/kairomonal extracts. Both males and females showed higher response to leaf, damaged bud and damaged boll extracts compared to undamaged bud and undamaged boll extracts.

The basis of the response was further probed by using SEM studies, which indicated that variation in the response of the sexes may be due to the presence in large number of the olfactory receptor, basiconic sensilla which are distributed in large numbers at the tip of the last antennal flagellum of female *A. angaleti* compared to the males. These results indicated strongly the potential of semiochemical use as long and short range cues for modulating the host searching behavior of parasitoids in the tritrophic context and therefore aid in the formulation of insect pest management strategies for cotton ecosystems

SEM studies on antennae of male and female parasitoid *A. angaleti*

SEM of both male and female antennae of *A. angaleti* indicated that the total number of segments in both male and female were 18 including scape and pedicel (fig 1). There was similar in the types of sensilla of both sexes. Male and female antenna showed three types of sensilla, which could be identified as trichodea sensilla (TS). Thus, TS classified as type 1 (TS1) and 2 (TS2) and type 3 (TS3) (fig 3B in male and 4A,B in female), basiconic sensilla (BS) (fig 3C in male and 4C in female) and placoid sensilla (PS) (fig 2C in male and 2D in female). Further based on the shape and size the trichodea sensilla present in males they could be segregated into type 1 (TS1) with a thick wall with deep groove on the surface, relatively long, straight lying in between placodea sensilla and bending at the bottom and type 2 (TS2) sensilla which were relatively short, straight, rounded and tapering, with deep grooves **(**fig 3 B, D). Both TS1 and TS 2 sensilla lie in the same row and alternate each other in their distribution. Additionally, there are one more types of trichodea sensilla present in the females of *A. angaleti*. They are somewhat different in shape compared to TS1 and TS2, which are present in males and also in females. The TS3 sensilla are short, hair-like, curved near the tip and bend near the bottom (fig 4A). The number basiconic sensilla present in both sexes of *A. angaleti* were different, number of BS presented in male was less than in female (fig 5A and B). The placodea sensilla were more numerous in males than in females, also the size of the sensillum in male was longer (fig 2 C, D). They are most prominent type of sensilla on the antennae and run parallel with the flagella margin.

DISCUSSION

Baker (1982) and Jeffree (1986) showed that the long-chain hydrocarbons are commonly present in plant are among the commonest constituents of all plant waxes. These compounds have been found to be of importance in bitrophic herbivore

– plant interactions (Bernays and Chapman, 1994). In insect, the outer surface of an insect cuticle contains some of chemicals as hydrocarbons (Wigglesworth, 1965; Blomquist and Dillwith, 1985; Hadley, 1985; Chapman, 1998), fatty acids and alcohols (Lockey, 1988), the former of which consists of n-alkanes, alkenes, and methyl-branched components (Schal *et al,* 1998.), cuticular hydrocarbons play major roles in chemical communication (Shonouda *et al*,, 1998b). Cuticular hydrocarbons (CHCs) are considered to be stable end products of genetically controlled metabolic pathways (Ross *et al*., 1987; Grunshawn *et al*., 1990). Cuticular hydrocarbons are also known to serve as kairomones in many herbivore – parasitoid associations too (Borges *et al*., 2003; Peri *et al*., 2006). In parasitoid – herbivore interactions long-chain hydrocarbons are used for both habitat and host location. However, it has been observed that these compounds originate from the host or its by-products and not from the plants. In the present studies some of these compounds have been detected from the different cotton varieties; larval body wash (LBW) from *C. cepalonica,* and *P. gossypiella*; and frass extract of *P. gossypiella*. The hydrocarbon profiles of semiochemicals present in each extract were different both in quality and quantity. Plant waxes are known to influence the foraging success of predators or parasitoids in their ability to attach to the plant surface. Also, the wax layer can modulate the detectability of host kairomones (Rostas *et al*., 2008).

Dutton (2002) reported that *n*-C27 (nonacosane) and *n*-C31 (hentriacontane) are the most commonly found hydrocarbons, they were also reported as most abundant in the apple leaves (Hellmann and Stoesser, 1992). This has also been confirmed by our results. These compounds are known to constitute the texture of the epicuticular leaf surface (Baker, 1982). The amount and composition of alkanes in apple leaves change depending on the season, developmental age of the leaves, and on apple tree varieties (Hellmann and Stoesser, 1992). In addition, the quantity of these compounds increases as a result of leaf miner herbivory. This increase might result in a different plant texture, which may contribute to the observed response of the female parasitoids. Indeed, texture has been shown to be an important physical cue for host location by several parasitoid species (Schmidt, 1991; Vinson, 1985).

Insect species usually possess complex mixtures of hydrocarbons including n-alkanes, branched mono-, di-, or trimethylalkanes, and others (Jackson and Blomquist, 1976). Hydrocarbon profiles may serve as fingerprints defining particular species. The composition of CHCs has been extensively studied in social insects, and in termites it is often used in taxonomic discrimination (Watson *et al.,* 1989; Kaib *et al.,* 1991; Bagine *et al.,* 1994; Haverty *et al.,* 1996; Takematsu and Yamaoka, 1997; Haverty and Nelson, 1997) and sibling species recognition (Haverty and Nelson, 1997).

The gas-liquid chromatography analysis of different plant parts from three cotton varieties and kairomones from different hosts were mainly targeted to identify the saturated straight chain hydrocarbons. The GC analysis revealed a wide variation in number and concentration of hydrocarbons in both synomonal and kairomonal extracts. Mostly, kairomonal extracts showed greater number of hydrocarbons compared to synomonal extracts and ranged from C_{11} to C_{30} whereas the number of hydrocarbons of synomonal extracts ranged from C_{14} to C_{30}. Jones *et al.* (1973) found tricosane as the most active compound and elicited high response in *T. evanescences*. This agrees well with our results, as tricosane was seen to be present in all the kairomonal extracts. *A. angaleti* responding more to kairomonal extracts than synomonal extracts in both multi-armed and Y-tube olfactometer probably due to the presence of favorable hydrocarbons like docosane, tricosane, tetracosane and pentacosane (Jones *et al.*, 1973). The Aphidiine wasp *Lysiphlebus cardui* (Marshall) is the dominant parasitoid of the ant-attended aphid, *Aphisfabae cirsiiacanthoidis* and it is successful because the composition of the cuticular hydrocarbons of the wasp is very similar to that of the ant *Lasius niger*,

allowing *L. cardui* to forage in the aphid colonies without being attacked (Liepert and Dettne, 1996).

The hydrocarbons act as chemical mimics. Bakthavatsalam *et al*., (1999) reported that a hexane wash of the body of *Opisina arenosella* (Lepidoptera: Noctuidae), a major pest of coconut in coastal Karnataka in India, elicited positive responses from its parasitoids, *Goniozus nephantidis*, *Brachymeria nephantidis*, and *Elasmus nephantidis* in a Y-tube olfactometer. The hexane wash of the larvae of *O. arenosella* had 20 different alkanes, including the five alkanes identified from cuticle rinses of tea aphids, i.e., undecane, eicosane, heptadecane, nonadecane, and pentadecane. The tea aphid cuticle contained many hydrocarbons and therefore the cuticle rinses of tea aphids acted as short-range cues or contact semiochemicals. Although the hydrocarbon profile of all the three cotton varieties were not much different in terms of the number of hydrocarbon contained but it was different both in nature and quantity in the extracts of the plant parts of the same variety.

The use of electrophysiological techniques has greatly advanced in our understanding of the perception mechanisms. Both electroantennograms and single cell recordings have provided insect neural responses that can be directly related to behavioural responses (Chapman and Blaney, 1979). Many studies have indicated that there is a variation in the EAG responses between different individuals (Park and Hardie, 1998) and the normalization of the EAG responses has been widely used to exclude this individual variation for evaluating the EAG responses (Park and Hardie, 1998). In the present studies, the olfactory perception of male and female parasitoids to the induced odour of synomonal and kairomonal odours was compared to determine key compounds if any mediating host location. The results showed that all of the compounds in the induced odours were perceived by the parasitoids yet there were some differences in the intensity of response to particular compounds, indicating potential differential sensitivity of the parasitoids to the various volatiles. The EAG response of *A. angaleti* showed significant difference between males and female to both synomonal and kairomonal extracts. This is similar to the results of Rojas (1999) who observed responses of females *M. brassicae* was higher to all other green leaf volatiles except 1-hexanol and Z-(3)-hexynyl acetate. The EAG response of both males and females parasitoids of *A. angaleti* were lesser for synomonal extracts compared to kairomonal extracts possibly due to the highest degree of specificity of the host odour to which receptors in antennae were more responsive. Larval body wash and frass of *P. gossypiella* stimulated highest response at concentration C_3 (100ppm) but response decreased when concentration of extract increased while LBW extract from *C. cephalonica* was correlation between EAG response and concentrations.

In contrast to the current results, Jyothi *et al.,* (2002) reported that the EAG response of males and females were not significantly different but they found that the EAG response of female parasitoids increased with increase in length of carbon atom in response to stimulation with alchohol, aldehydes and terpenoids. In the present study it was found that kairomonal extracts with long chain saturated hydrocarbon and favorable hydrocarbon like tricosane which were absent almost in all synomonal extracts, could have contributed to the better EAG responses in females of *A. angaleti* for the kairomonal extracts. Further Jyothi *et al.,* (2002) reported that the EAG response of males of *C. obliquae* was comparatively lower than that of females except for synthetic pheromone blend which elicited similar response in both the sexes. Their comparison of volatiles from host source indicated that the sex pheromone blend evoked larger EAG response than either frass or LBW extracts. During the present studies it was found that frass extract elicited lowest EAG responses in antennae of male *A. angaleti* but the response of female parasitoid was significantly higher from that of males. This may be due to the use of volatiles of host by – product such as host frass, host larvae by

the female parasitoid for short cue while seeking the host (Vinsion, 1998; Steidle and Van Loon, 2002).

The antennae are the primary olfactory organs, but odour receptors can also be found on the mouthparts of several insects (Keil, 1999). Insect antennae have in many cases evolved into sophisticated shapes, such as feather and club shaped structures, to maximize the area carrying odour detecting organs (Keil, 1999). Olfactory receptor neurons (ORNs) are housed within the small sensilla. Apart from the ORNs, the olfactory sensilla consist of a number of auxillary (or enveloping) cells that have supportive functions and are involved in development of the sensilla during ontogeny. Depending on the cuticular structure, the sensilla are classified into different types (Hallberg & Hansson, 1999). The most common are (i) trichoid, long hair-like sensilla, (ii) basiconic sensilla, also hairlike but normally shorter and thicker than the trichoid, (iii) placoid sensilla, platelike and (iv) coeloconic sensilla, which are short peg-like structures situated in pits (Hallberg & Hansson, 1999). Trichoid sensilla are the most dominating sensillum type on the antennae of most insect species. These sensilla in most insects are innervated by 2-3 neurons, but in hymenopterans sensilla trichodea often contain around 10 neurons (Hallberg & Hansson, 1999). Another character typical for hymenopterous insects is a high abundance of sensilla placodea (Hallberg & Hansson, 1999; Bleeker *et al*., 2004). Basiconic sensilla are found in small at the tip of the antenna on the few last flagellar segments of insect antenna. They are roughly parallel with this segment, but point away from it.

In the present studies the terminologies proposed by Snodgrass (1935), Schneider (1964) and Keil (1999) were followed to differentiate and describe the various types of sensilla present in male and female antennae showed the presence of trichodea sensilla of type 1, 2 and 3 covering all the antennomeres. However, Trichodea sensilla, showed sexual dimorphism in *A. angaleti.* Female had three types of trichodea sensilla while males had two. The antennal sensilla have been described in many species belonging to different families of parasitic hymenoptera (van Baaren *et al*., 1996). Their functions have been reported as mechano-sensory in some Braconid was described by Navasero and Elzen (1991), Ochieng *et al*., (2000). In *A. angaleti*, the length of antennae differs between males and females. The male antennae are longer than those of the females as seen in the SEM studies. This is consistent with the findings in many other parasitic such as two *Microplitis* species (Navasero and Elzen, 1991); Ochieng *et al*., 2000; Gao *et al*., 2007), *Aphidius rhopalosiphi* (Navasero and Elzen, 1991); Ochieng *et al*., 2000; Gao *et al*., 2007) two A*naphes* species (van Baaren *et al*., 1999). The significance to this may be to enlarge the surface area for sensory receptors (Schneider, 1964). Studies have reported that male placoid sensilla is shorter than female placoid sensilla as in *Aphidius smithi* (Borden *et al*., 1978b) and two *Trichogrammae* species (Olson and Andow, 1993; Amornsak *et al*., 1998). The number of placoid sensilla in males of *A. angaleti* was found to be higher than in females. This is a common phenomenon in many hymenoptera males (Borden et al, 1978a, b; Navasero and Elzen, 1991; Bourdais *et al*., 2006; Gao *et al*., 2007).The large number of placoid olfactory sensilla in males were found to be associated with sex pheromone searching behaviour by which males are attracted to females (Chapman, 1982). The males of some braconid species may use placoid sensilla to detect sex pheromones in conjunction with host plant odour (Bleeker *et al*., 2004). This kind of sensillum may be used by females for host recognition at long distances (Bourdais *et al*., 2006). Bin *et al*., (1989) suggested different functions for placoid sensilla in male and females. In males they could be receptors for sexual pheromones while in females they could be receptors for kairomones and other odours in the host environment. Basiconic sensilla present in the distal area of all a few flagella segments of both males and females of *A. angaleti* may be for contact chemoreception or gustatory function. Keil (1999) cites that the function of basiconic sensilla is usually olfactory as was observed for species *A.*

angaleti. This function can be understood from their behaviour also. They are probably involved in host location especially in the detection of short range cues emitted from the host as demonstrated by the electroantenogram results. This was found to be in agreement with those of Weseloh (1972), who suggested that the antennal tip are the principle location for organs involved in initiating host acceptance behaviour in the parasitoid species, *Cheiloneurus noxius.* Also found to be in agreement with Roux *et al*., (2005) who postulated that a gustatory stimulus following antennal contact is probably the key stimulus including the oviposition behaviour in the braconid *Cotesia plutellae.*.

CONCLUSION

The result of SEM on the antennae of *A. angaleti* are differentiated and described to the various types of sensilla present in male and female. The presence of trichodea sensilla of type 1, 2 and 3 covering all the antennomeres. However, trichodea sensilla, shows sexual dimorphism in *A. angaleti.* Female has three types of trichodea sensilla while male has two. In *A. angaleti*, the length of antennae differs between males and females, male antennae are longer than those of the female as seen in the SEM studies. This is consistent with the findings in many other parasitic. The large number of placoid olfactory sensilla in males is found to be associated with sex pheromone searching behaviour by which males are attracted to females. It is suggested that males of some braconid species may use placoid sensilla to detect sex pheromones in conjunction with host plant odour. Also, this kind of sensillum may be used by females for host recognition at long distances. There are some of different functions for placoid sensilla in male and females may be concerned, in males they could be receptors for sexual pheromones while in females they could be receptors for kairomones and other odours in the host environment. Basiconic sensilla present in the distal area of all a few flagella segments of both males and females of *A. angaleti* may be for contact chemoreception or gustatory function. The function of basiconic sensilla is usually olfactory as was observed for species *A. angaleti* and this function can be understood from their behaviour also. They are probably involved in host location especially in the detection of short range cues emitted from the host as demonstrated by the electroantenogram results. The results show that all of the compounds in the induced odours are perceived by the parasitoids. Nevertheless, there are some differences in the intensity of response to particular compounds, indicating potential differential sensitivity of the parasitoids to the various volatiles. The EAG response of *A. angaleti* shows significant difference between males and female to both synomonal and kairomonal extracts. The EAG response of both males and females parasitoids of *A. angaleti* were lesser for synomonal extracts compared to kairomonal extracts possibly due to the highest degree of specificity of the host odour to which receptors in antennae are more responsive.

Table 1. Hydrocarbon profile of synomonal extracts of Pusa 8-6 cotton variety (quantity in ppm)

Carbon No.	Hydrocarbon	Leaf	Undamaged bud	Damaged bud	Undamaged boll	Damaged boll
C_{10}	Decane	ND	ND	ND	ND	ND
C_{11}	Undecane	ND	ND	ND	ND	ND
C_{12}	Dodecane	ND	ND	ND	ND	ND
C_{13}	Tridecane	ND	ND	ND	ND	ND
C_{14}	Tetradecane	1672.77	2462.95	ND	ND	1469.61
C_{15}	Pentadecane	ND	ND	389.03	ND	ND
C_{16}	Hexadecane	7051.75	7767.54	2306.14	1664.91	4014.04
C_{17}	Heptadecane	4231.66	2577.30	851.17	ND	695.89
C_{18}	Octadecane	3554.20	ND	ND	ND	719.13
C_{19}	Nonadecane	ND	ND	ND	ND	ND
C_{20}	Eicosane	12775.57	3708.07	1487.13	ND	7446.32
C_{21}	Heniecosane	24676.27	9228.40	593.84	ND	5313.80
C_{22}	Docosane	9150.79	4189.34	446.71	418.93	2117.91
C_{23}	Tricosane	ND	2299.40	ND	ND	ND
C_{24}	Tetracosane	ND	ND	ND	ND	ND
C_{25}	Pentacosane	ND	3398.87	ND	418.84	ND
C_{26}	Hexacosane	10892.69	10886.33	59794.12	1463.43	11754.37
C_{27}	Heptacosane	164295.91	8575.55	66058.99	2492.86	111841.01
C_{28}	Octacosane	5287.37	12709.79	406.10	1271.52	4676.92
C_{29}	Nonacosane	20461.25	7141.58	143233.55	12444.11	47532.04
C_{30}	Triacotane	37322.83	10277.50	358.21	562.74	5339.41

ND- Not detected

Table 2. Hydrocarbon profile of synomonal extracts of LD 327 cotton variety

Carbon No.	Hydrocarbon	Leaf	Undamaged bud	Damaged bud	Undamaged boll	Damaged boll
C_{10}	Decane	ND	ND	ND	ND	ND
C_{11}	Undecane	ND	ND	ND	ND	ND
C_{12}	Dodecane	ND	ND	ND	ND	ND
C_{13}	Tridecane	ND	ND	ND	ND	ND
C_{14}	Tetradecane	ND	331.99	1122.40	ND	998.33
C_{15}	Pentadecane	ND	174.34	730.47	ND	ND
C_{16}	Hexadecane	6257.89	1022.81	4955.26	9771.93	2077.19
C_{17}	Heptadecane	1448.55	ND	ND	ND	1010.15
C_{18}	Octadecane	ND	ND	ND	ND	ND
C_{19}	Nonadecane	ND	ND	2780.26	ND	ND
C_{20}	Eicosane	5381.54	ND	2587.40	11549.25	1558.12
C_{21}	Heniecosane	1921.55	73.49	1116.19	9395.23	2783.88
C_{22}	Docosane	1288.55	ND	715.42	3607.71	1171.77
C_{23}	Tricosane	915.16	ND	2273.13	ND	868.37
C_{24}	Tetracosane	ND	ND	ND	ND	ND
C_{25}	Pentacosane	825.91	192.01	1596.52	ND	3932.92
C_{26}	Hexacosane	6003.18	1866.45	124756.76	5784.58	5458.66
C_{27}	Heptacosane	3318.74	683.16	5444.34	77336.82	15670.79
C_{28}	Octacosane	3383.87	286.90	3252.37	1575.69	4267.27
C_{29}	Nonacosane	2453.80	8227.27	3169.90	31265.28	37666.92
C_{30}	Triacotane	5919.29	236.59	4656.16	7319.51	10760.09

ND- Not detected

Table 3. Hydrocarbon profile of synomonal extracts of RS 2013 cotton variety

Carbon No.	Hydrocarbon	Leaf	Undamaged bud	Damaged bud	Undamaged boll	Damaged boll
C_{10}	Decane	ND	ND	ND	ND	ND
C_{11}	Undecane	ND	ND	ND	ND	ND
C_{12}	Dodecane	ND	ND	ND	ND	ND
C_{13}	Tridecane	ND	ND	ND	ND	ND
C_{14}	Tetradecane	1877.60	ND	6601.17	ND	11824.30
C_{15}	Pentadecane	ND	ND	3169.17	ND	2490.43
C_{16}	Hexadecane	6037.72	4128.95	22712.28	1412.28	18311.40
C_{17}	Heptadecane	1015.23	1126.44	2459.16	419.01	2843.56
C_{18}	Octadecane	ND	ND	ND	ND	ND
C_{19}	Nonadecane	ND	ND	ND	ND	ND
C_{20}	Eicosane	6302.57	3245.79	8031.94	2196.10	16881.10
C_{21}	Heniecosane	3048.66	2145.98	2950.35	829.20	10624.63
C_{22}	Docosane	1172.34	890.02	1722.79	431.97	4479.02
C_{23}	Tricosane	ND	ND	1454.30	ND	3609.74
C_{24}	Tetracosane	ND	ND	ND	ND	2151.22
C_{25}	Pentacosane	ND	2476.70	1587.81	ND	2529.44
C_{26}	Hexacosane	8523.85	2667.73	173224.17	8334.66	158538.95
C_{27}	Heptacosane	119257.85	3544.24	160667.94	29807.80	42352.05
C_{28}	Octacosane	3398.77	1302.98	1224.46	3782.17	6128.90
C_{29}	Nonacosane	14200.45	12874.07	52763.04	17263.04	109560.36
C_{30}	Triacotane	14191.27	ND	4597.57	5075.73	21559.47

ND- Not detected

Table 4. Hydrocarbon profile of kairomonal extracts of *P. gossypiella* and *C. cephalonica*

C.No	Hydrocarbon	*C.cephalonica* Larval body wash	*P.gossypiella* Larval body wash	*P. gossypiella* Frass
C_{10}	Decane	ND	ND	ND
C_{11}	Undecane	ND	ND	ND
C_{12}	Dodecane	ND	ND	ND
C_{13}	Tridecane	ND	1323.03	ND
C_{14}	Tetradecane	99.92	5940.05	7348.04
C_{15}	Pentadecane	50.18	1947.75	4529.23
C_{16}	Hexadecane	69.30	4282.46	15066.67
C_{17}	Heptadecane	53.53	964.93	7789.57
C_{18}	Octadecane	70.46	534.78	2192.41
C_{19}	Nonadecane	ND	ND	4983.63
C_{20}	Eicosane	3699.20	4359.36	36970.59
C_{21}	Heniecosane	6585.90	8849.06	11461.77
C_{22}	Docosane	2157.03	2004.54	5967.12
C_{23}	Tricosane	505.20	1614.12	938.70
C_{24}	Tetracosane	223.73	ND	ND
C_{25}	Pentacosane	15790.58	11718.89	24939.34
C_{26}	Hexacosane	21162.16	59414.94	56767.89
C_{27}	Heptacosane	12315.03	ND	107411.99
C_{28}	Octacosane	12840.35	23134.58	12906.58
C_{29}	Nonacosane	46513.41	96332.34	42441.88
C_{30}	Triacotane	2516.80	51491.98	99451.63

ND- Not detected

Table 5. EAG response of males and females of *A. angaleti* to different plant part extracts of cotton varieties

Variety	Mean relative EAG response (%) ± SD					
	Leaf		Undamaged bud		Damaged bud	
	M	F	M	F	M	F
Pusa 8-6	130.8±41.32	154.9±37.54	125.3±42.71	145.8±29.72	128.7±28.33	151.57±27.43
LD 327	93.33±30.76	134.8±17.31	113.13±37.26	113.07±15.03	93.67±30.1	113.0±22.3
RS 2013	120.8±21.64	142.6±29.18	112.8±26.3	135.9±23.16	142.3±19.34	161.1±22.13

Variety	Mean relative EAG response (%) ± SD			
	Undamaged boll		Damaged boll	
Pusa 8-6	**M**	**F**	**M**	**F**
LD 327	141.7±43.95	147.2±37.98	146.3±58.95	160.2±17.54
RS 2013	92.0±30.9	98.6±24.14	124.13±40.0	147.13±24.82
	117.7±19.34	135.3±11.76	148.10±13.55	167.0±16.01

	FACTORS	SE (±)	CD (0.01)
	Treatment (T)	2.496	4.17**
PS8-6	Gender (G)	1.248	2.08**
	Interaction (TxG)	3..530	5..89**
	Treatment (T)	2..578	4.28**
LD327	Gender (G)	1.488	2.14**
	Interaction (TxG)	3.465	6.05**
	Treatment (T)	2.326	5.56**
RS2013	Gender (G)	1.163	1.94**
	Interaction (TxG)	3.289	5.49**

** Significant at 1%

Tabe 6. Electroantennogram responses of males and females of *A. angaleti* to different concentrations of various kairomonal extracts

	Mean relative EAG response (%)± SD									
	PgL1	**CcL1**	**PgF1**	**PgL3**	**CcL3**	**PgF3**	**PgL5**	**CcL5**	**PgL5**	**Mean**
M	121.8±20.9	118.1±10.4	127.6±15.4	144.1±16.4	139.3±13.2	139.7±14.7	155.8±14.5	140.2±12.1	152.3±18.6	125.6±13.2
F	144.9±13.4	138.4±15.8	145.3±14.3	164.1±17.9	146.3±15.3	155.3±6.8	132.7±18.0	134.7±11.2	137.1±17.8	131.9±11.4
Mean	133.4±19.4	128.2±16.5	136.4±17.1	154.0±21.5	142.8±18.5	147.5±7.4	144.3±15.2	137.4±14.0	144.7±16.5	

	SE (±)	CD (0.01)
TREATMENT (T)	3.971	6.532**
GENDER	1.621	2.660**
INTERACTION (TxG)	5.616	9.234**

PgL1*: P. gossypiella* larval body wash (conc. 1ppm)

PgL3: *P. gossypiella* larval body wash (conc. 100ppm)

PgL5: *P. gossypiella* larval body wash (conc. 10000ppm

CcL1: *C. cephalonica* larval body wash (conc. 1ppm)

CcL3: *C. cephalonica* larval body wash (conc. 100ppm)

CcL5: *C. cephalonica* larval body wash (conc. 10000ppm)

PgF1:*: P. gossypiella* feces extract (conc. 1ppm)

PgF3*: P. gossypiella* feces extract (conc. 100ppm)

PgF5*: P. gossypiella* feces extract (conc. 10000ppm)

** Significant at 1%

Table 7. Electroantennogram response of males and females of *A.angaleti* to different concentrations of kairomonal extracts

CONC.	Mean relative EAG response (%)								
	***P. gossypiella larval* body wash (Pg LBW)**			***C. cephalonica* Larval body wash (Cc LBW)**			***P. gossypiella* FRASS (Pg frass)**		
	M	**F**	MEAN	**M**	**F**	MEAN	**M**	**F**	MEAN
C1	117.6±17.3	131.9±14.8	**124.7±13.7**	108.2±6.9	131.6±14.6	**119.9±11.7**	137.1±13.3	148.1±18.5	**142.6±18.4**
C2	128.5±11.9	135.1±13.7	**131.5±16.7**	119.7±13.1	144.4±16.1	**132.0±9.1**	145.2±18.7	144.3±19.6	**144.7±18.7**
C3	151.4±22.3	161.1±19.2	**156.6±17.5**	127.3±9.4	161.9±20.1	**144.6±15.8**	160.3±23.7	167.8±19.3	**164.0±24.0**
C4	148.1±15.7	158.8±17.2	**153.5±19.2**	138.0±14.2	125.2±13.2	**131.3±13.1**	140.3±18.9	161.5±20.2	**150.8±20.6**
C5	149.4±22.5	157.7±12.4	**153.6±18.4**	157.4±18.6	117.5±14.7	**137.4±14.6**	138.7±19.8	163.4±23.4	**151.0±14.5**
Mean	**132.5±18.2**	**140..5±16.6**		**125.3±12.8**	**130.2±15.5**		**136.9±17.4**	**147.5±21.5**	

Factor	SE (±)			CD (P<0.05)		
	PgLBW	**CcLBW**	**PgFRASS**	**PgLBW**	**CcLBW**	**PgFRASS**
Treatment (T)	3.701	2.276	3.292	7.328**	4.506**	6.518**
Genger (G)	2.137	1.314	1.464	4.231**	2.602**	2.898**
T x G	5.234	3.219	4.655	10.363 ^{ns}	6.374**	9.217*

** Significant at 1%

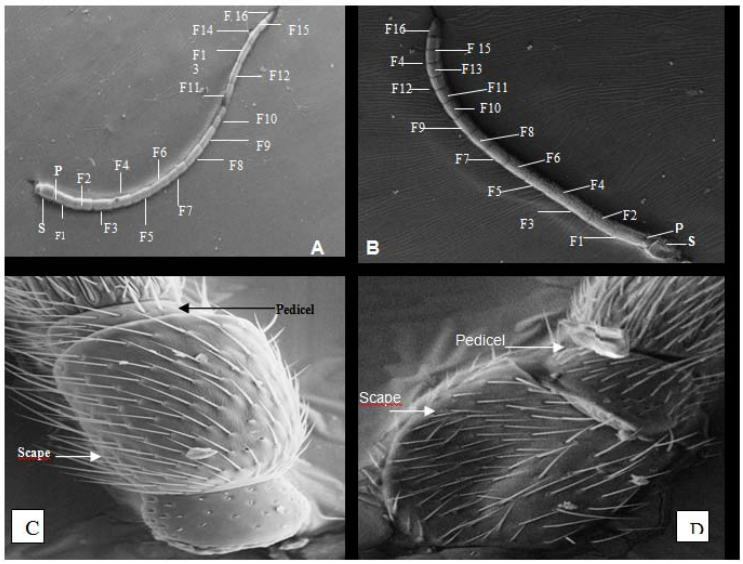

Figure 1. SEM photomicrograph of male and female of *A. angaleti* antenna. A. Antenna of male. B. Antenna of female. S = scape; P = pedicel; F1= flagellum1; F2 = flagellum2; F3 = flagellum3; F4 = flagellum4; F5 = flagellum5; F6 = flagellum6; F7 = flagellum7; F8 = flagellum8; F9 = flagellum9; F10= flagellum 10; F11= flagellum11; F12 = flagellum12; F13 = flagellum13; F114 = flagellum14; F15 = flagellum15; F16 = flagellum16. bar = 200µm. C, D. Closed-up view of scape and pedicel with distribution of sensilla of male (C) and female (D).

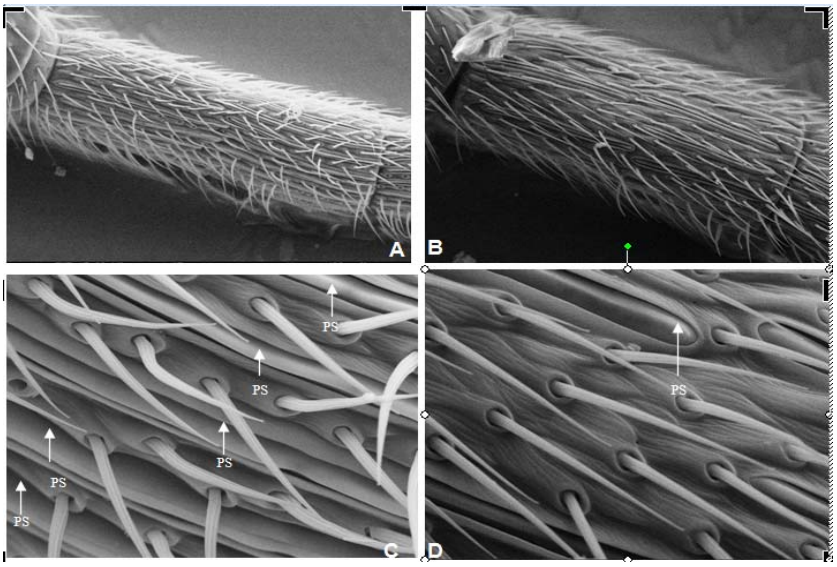

Figure 2. SEM photomicrograph of male and female *A. angaleti* antenna. A and B. The first flagella of male (A) and female (B). C. The distribution of placoid sensila on male antenna. D. The distribution of placoid sensilla on female antenna of segment number 1

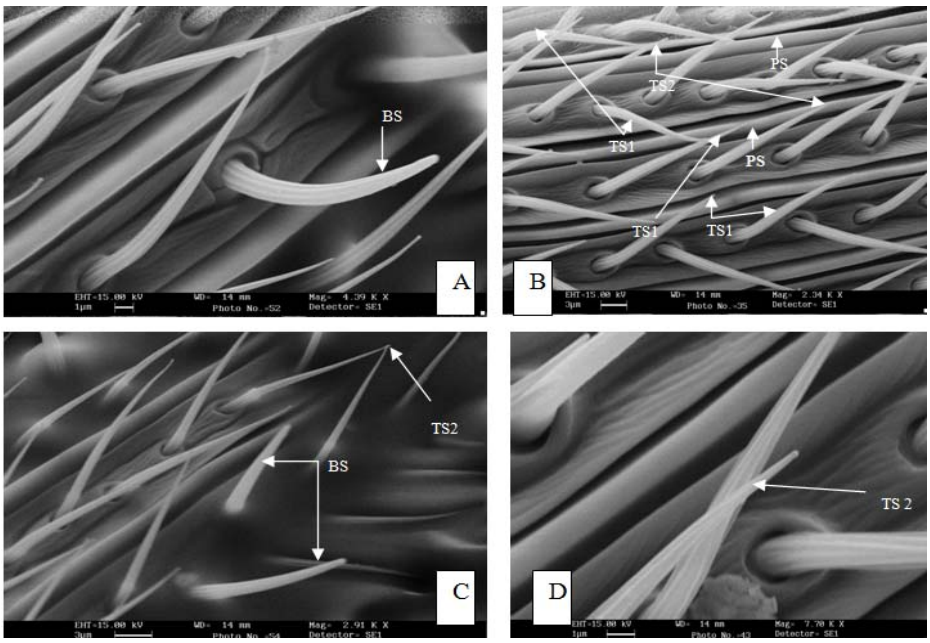

Figure 3. SEM photomicrograph of male *A. angaleti* antenna. A.BS= Closed –up view of basiconic sensilla. B. TS = Trichodea sensilla type 1 and 2 (TS1 and TS2), PS=placoid sensilla. C. Distribution of BS in between trichodea sensilla. D. Closed-up view of trichodea type 2

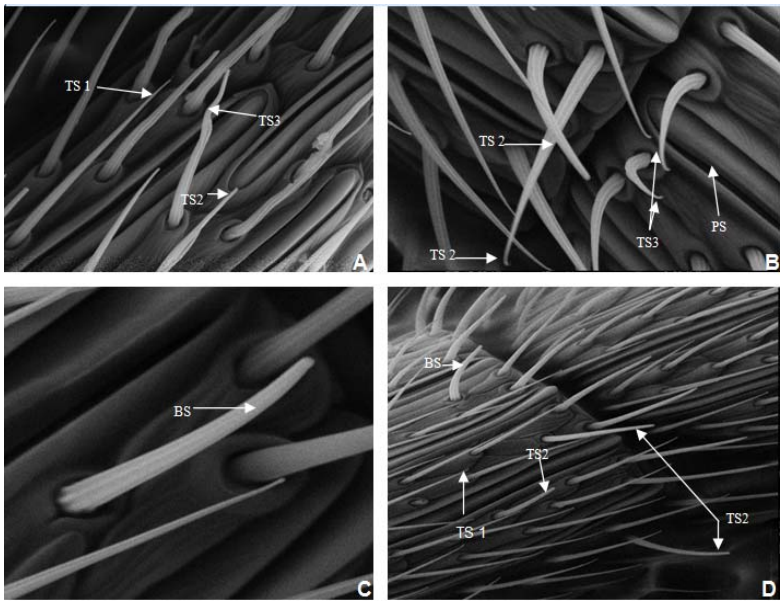

Figure 4. SEM photomicrograph of female *A. angaleti* antena. A, B. trichodea sensilla type 1, 2 and 3 (TS1, TS2, TS3). C. Closed - up view of *Bassiconic sensilla* (BS). D. rrangement of Basiconic and trichodea sensilla on flagella segment.

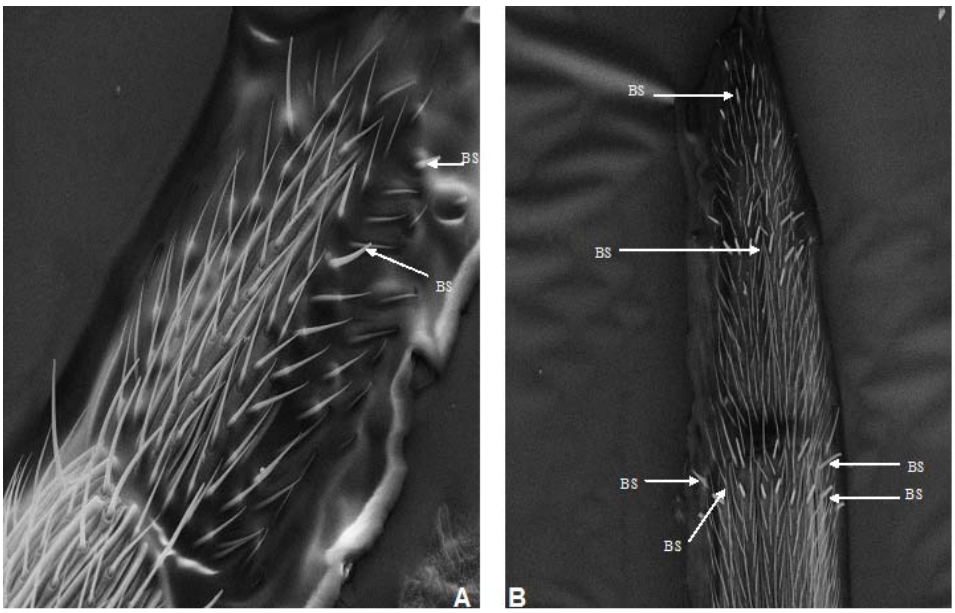

Figure 5. SEM photomicrograph of male and female *A. angaleti* antenna. A. Basiconic sensilla distribution in the last segment of antenna in male. B. Basiconic sensilla distribution in the last segment of antenna in female

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NGHIÊN CỨU MỐI QUAN HỆ GIỮA CẤU TRÚC HÌNH THÁI ANTENNA CỦA *Apanteles angaleti* Mueseback ĐỐI VỚI THUỘC TÍNH TÌM KIẾM VÀ ĐỊNH VỊ KÝ CHỦ

Apanteles angaleti (Hymenoptera: Braconidae) là loài ong ký sinh có tiềm năng trong phòng trừ sinh học rất quan trọng trên ấu trùng của *Pectinophora gossypiella* cây bông. Kết quả nghiên cứu SEM về antenna trên cả con đực và con cái của *A. angaleti* cho thấy cấu trúc của antennae bao gồm 18 đốt với 3 loại cấu trúc của sensilla như trichodea sensilla type 1 (TS 1), type 2 (TS2) and type 3 (TS3), basiconic sensilla (BS) and placoid sensilla (PS). Basiconic sensilla giống nhau trên cả con đực và cái, tuy nhiên số lượng BS trên con đực ít hơn so với con cái nhưng ngược lại, placoid sensilla (PS) trên con đực nhiều hơn con cái. Nhiều kết quả nghiên cứu cho thấy con đực của vài loài braconid sử dụng placoid sensilla để phát hiện sex pheromones và con cái dùng để nhận biết ký chủ ở khoảng cách xa (cơ quan cảm nhận kairomone). Basiconic sensilla của *A. angaleti* có thể sử dụng như cơ quan cảm nhận hóa học hoặc có chức năng vị giác. Một số nghiên cứu cho rằng basiconic sensilla của *A. angaleti* cũng có vai trò như cơ quan khứu giác. Điều này được chứng minh bởi kết quả của EAG, cả con cái và con đực đều có phản ứng EAG đối với synomone trích từ những phần cây bị xâm hại bởi ấu trùng hơn là những phần không bị xâm hại. Đối với kairomone, trích từ cơ thể lớp cutin của ấu trùng và chất thải của *P. gossypiella* và *C. cephalonica* cho thấy con cái có khả năng phản ứng EAG cao với kairomone ở nông độ thấp (100ppm) và con đực gia tăng phản ứng khi nồng độ kairomone cao. Phản ứng EAG của con cái cao hơn con đực trên tất cả các hợp chất infochemicals. Kết quả chứng minh rằng cấu trúc, số lượng của các sensilla đóng vai trò trong sự hình thành thuộc tính hoạt động, khả năng tìm kiếm và định vị ký chủ của hầu hết các loài ong ký sinh.