Effect of *Varroa destructor* Infestation on Antennal Sense Organs of The Worker and Drone Honey Bees and Some Physiological Activities in Egypt

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ABSTRACT

Honey bees *Apis mellifera* (Hymenoptera: Apidae) as all hymenopteran insects have specialized antennal sensilla to facilitate their biological behaviors, such as habitat searching, food recognition, selection, and acceptance, courtship, mating and oviposition. In the present work, scanning electron microscopy (SEM) was used to study the external morphology of the antennal sensilla of workers and drones of the craniolian hybrid which infested with varroa mites (*Varroa destructor* Oud.), Based on the morphology of the sensilla in each sex, seven types of sensilla were identified, i.e. sensilla trichodea (Tr.a, Tr.b Tr,c.) sensilla basiconica (Ba.a, Ba.b), sensilla placodea (Pl), sensilla coeloconica (Co), sensilla chaetica (Ch), sensellae ampulecium (Am) and sensilla campaniformia (Ca). Placodea, trichodea and basiconica sensilla were the most common sensilla. They are more in the antenna of drones than workers.

Mean number of sensilla placodea, sensilla trichodea, sensilla campaniformia, sensilla coeloconica and sensilla basiconic (type a and b) on flagellomeres no.2, 4,6,8 and 10 were calculated. They were significantly decreased with the infestation by varroa mites especially in the deformed newly emerged honey bee workers and drones compared with the healthy ones while the surface area of the placoid sensilla increased in the infested bees. The obtained results provide a basis of further studies on the searching behavior of bees in relation to food source by workers or mating by drones by using electrophysiology studies**.**

Key words: ***Apis mellifera*****, antennal sensilla,** ***Varroa destructor*****, electrophysiology.**

INTRODUCTION

Honey bee *Apis mellifera* L.(Hymenoptera: Apidae), is an important social insect for the humans utilization of its products, such as honey, bees wax, royal jelly … etc. In addition, it is maintaining natural vegetation since they are a mean of transferring pollen between flowers.

Varroatosis is a honeybee (*A. mellifera* L) disease caused by the mite *Varroa destructor* Oud. (Acarina: Varroidae) (De Jong 1997 and Anderson & Trueman 2000). Varroa infestation causes serious health disorders in honeybees. *V. destructor* mites feed on honey bee haemolymph causing morphological abnormalities, transmitting virus, bacterial and fungal infections to the recipient host, changes in the physiology and biochemistry of the infested bees (Weinberg and Madel, 1985; Glinski and Jarosz , 1984,1988 and 1992; Allen and Ball, 1996 and Zóltowska *et al*., 2005). The infested colonies produced smaller adult and brood populations (Downey and Winston, 2001). Finally, it causes the death and losses of several colonies of honey bees in Egypt and other countries of the world (De Jong *et al*., 1982; Abd El- Wahab, 1996 & 2001 and Salem *et al*., 2001).

Insects have sense organs responsible to receive mechanical and chemical stimuli, they are scattered along insect body (Gaaboub, 1990, 2000 and 2010). Insect antennae, provided with various kinds of sensillum (Schneider 1964 & 1987). Hymenopteran insects have specialized sensory organs, such as antennal sensilla, to facilitate their biological behaviors, such as habitat searching, host localization, recognition, selection, and acceptance, courtship, mating and oviposition (Das *et al*., 2011; Zhou *et al*., 2015).

Honey bee as any social insect have complex social interactions within their colonies casts (Wilson and Holldobler, 2005). To regulate the honeybee interactions many organs evolved in an intricate system of chemical communication that includes numerous glands that produce complex blends of pheromones (Slessor *et al.*, 2005) and sense organs which scattered on antennae (Gupta, 1992 and Graham Joe, 1999).

To understand the communication behavior of honey bee, the antennal morphology, types and distribution of sensilla, in both drones and workers adult were observed using a scanning electron microscope (SEM). Therefore, this study aimed to clarify the effect of varroa mite infestation on the numbers, distribution and length of the workers and drone antennal sensilla. Also, electrophysiological parameters were studied. This study provides a basis for future electrophysiological and behavioral studies.

MATERIALS AND METHODS

Insect collecting:

Experiments were conducted during autumn 2014, at apiary of faculty of Agriculture, Alexandria University, Abis district. Ten colonies of carniolian hybrid honey bees were chosen for this study, five of them were infested with varroa and the other five were healthy. These colonies did not receive any chemical control against varroa infestation during the experimental period. Samples of newly emerged honey bee workers and drones infested with 4 mites/ bee as well as infested deformed bees and samples of healthy worker bee (as control) were collected randomly for the antennal slide and for Scanning Electron Microscopy preparations.

Preparation of slide mounting:

Infested and healthy honey bee heads were soaked in 10 % NaoH solution for 2 days then rinsed in distilled water several times. The specimens passed through series of ethyl alcohol from 60 – 95 % then to absolute alcohol one hour for each concentration. Then they were cleared in clove oil for one hour. Antennae were separated and mounted on slides using Canada balsam medium. The slides were dried at 50 ºC for one week. Then examined under stereoscopic microscope and photographed by digital camera.

Preparation of material for scanning electron microscopy (SEM):

For scanning electron microscopy examination (SEM) antennae were removed carefully from the freshly collected workers and drones bees then dried to the critical point by passing in a series of ethanol. They were mounted in a stub by means of double side adhesive tape under binocular microscope. The specimens were coated with gold in (JOELFC-1100E) high resolution sputter coater for 25 minutes to a thickness of 12 nm. Preparations were examined in JOEL (JSM 5300) Scanning Electron Microscopy at the Faculty of Science, Alexandria University. Antennal sensilla were photographed and described briefly.

Measurements:

Lengths of different types of sensilla were measured by using micrometer eye piece. The mean surface area of sensilla placodea was measured (μm^2/organ) according to the following formula of (Maurizio, 1954)

placodea surface area = π x ab / 2

(π = 3.14, a = maximum length; b = maximum width).

Electrophysiological measurements

Responses of individual sensilla (trichodium or basiconic on the antennae) to chemical stimuli were recorded using the tip recording technique described by Hodgson *et al*., (1955) and Gaaboub, (1990 & 2000). The potentials were amplified and filtered using AC amplifiers (Fig.1). A blunt glass microelectrode filled with different solutions was placed over the shaft of the sensilum. Electrodes containing salt (NaCl 100 mM), sugar as glucose (0.01 M to 3.0 M), acids as citric acid (0.01M, 0.1M and 1.0 M), 100 mM of NaCl mixed with the glucose, were used to stimulate the chemosensory afferents. Controlled movements of this electrode were used to deflect the sensillum so as to elicit spikes in the mechanosensory afferents. The same electrode was therefore used simultaneously to evoke and record the spikes of the afferents. The displacement of a sensilum did not deform its short and stout shaft. Each stimulus was repeated 8-10 times for each stimulant chemical. For testing the specific response of stimulants all basic classes of stimulating chemicals (salts, acids, sugars) diluted in water with electrolyte (100 mM NaCl) were applied consecutively with interspersed pauses of several minutes in each experiment. To identify the sensory receptors on the surface of antennae of *A. mellifera* L., scanning electron micrographs of the cuticle surface were taken.

Statistical analysis:

Mean number and measurements were calculated and analyzed by using F test, standard deviation were recorded for each value at probability 0.05%. Analysis of variance (ANOVA) was carried out for the obtained data according to the method of Waller and Duncan, (1969).

RESULTS AND DISCUSSION

Morphological studies:

The antenna of the honey bee has 3 distinct regions, scape, pedicel and the flagellum in addition to the basal radical. Antennae are considered to be a sensory center (Olfactory organ) so it is provided with various types of sensilla scattered on each segments. The results of the SEM analyses show that seven different types of sensilla were identified in both sexes, i. e. sensilla trichoide (Tr a, b & c), sensilla basiconica (Ba a &b), sensilla placodium (Pl), sensilla coeloconica (Co), sensilla chaetica (Ch), sensilla ampulecium(Am) and campaniformia (Ca). Trichoide, basiconica and sensilla placodium are the most abundant of antennal sensilla types.

The sensilla Trichodea (Tr) are characteristically hair like and appeared to arise from a pit. They have multiple, one, or no pores on the wall. There were three types of these sensilla, one type was short and straig (Tra), the 2nd type short and curved (Trb) and the 3rd type was long with plumose terminal part (Trc). They spread all over the antennal regions except the last type which are found only on the scape (Fig. 2 A). The basiconica sensilla are peglike with pointed tip. There were two types of these sensilla, one type was short and straight (Ba a), the 2nd type somewhat longer and curved (Ba b) (Fig. 3B).

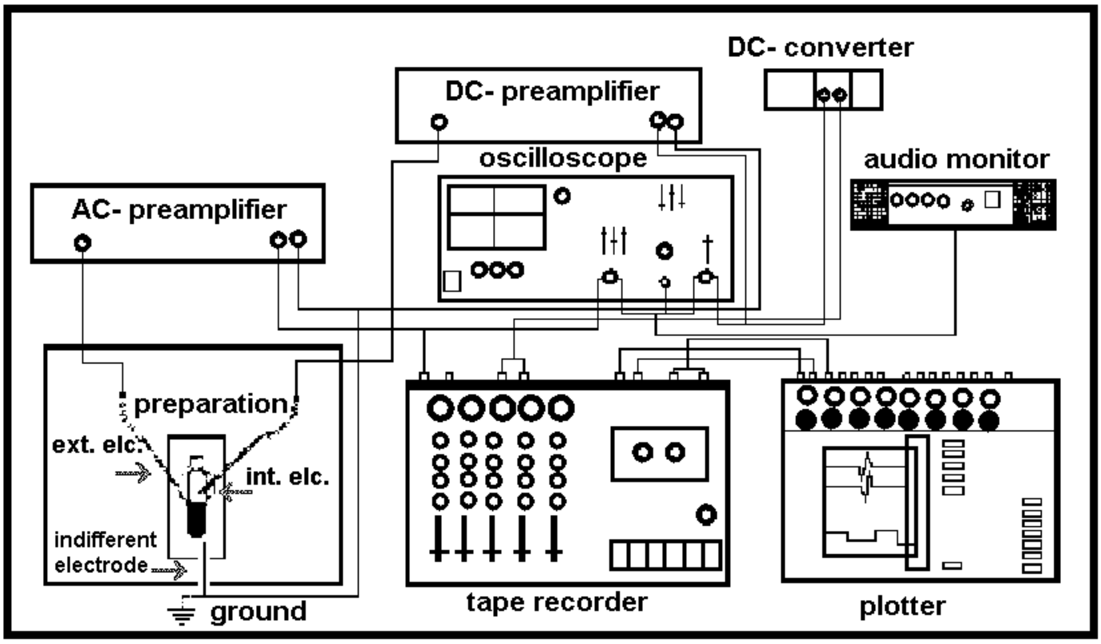


Fig.1: Diagram of the recording arrangements

They covered the first flagellomer (Fig. 2A). Sensilla placodea (Pl) are plate like (Fig., 2A, B, and D)and (Fig., 3 B, C). They scattered all over the flagellar segments except the first one. The sensilla chaetica (Ch) is erected bristle like hair (Figures. 2C & 3E). Also, sensilla ampulecea (Am) are sunken in comparatively deep pit (Fig., 2C). They have been described in *Apis*, although many of the studies used different nomenclature to describe them.

Concerning to the infestation by varroa mite there were abnormality in the workers and drone antennae. Table (1) indicated that the mean numbers of all types of sensilla decreased due to the infestation in both worker and drone in the flagellar segments 2nd, 4th, 6th, 8th and 10th. Basiconic sensilla (type a) ranged from 1.3 - 4.2 and 1.2 – 3.8 in healthy and infested workers, respectively. While in drone ranged from 1.6 – 3.6 and from 1.45 – 3.2 in healthy and infested drones, respectively. Basiconic sensilla (type b) showed the same trend. It is higher in numbers in healthy than infested workers and drone and also, it increased in numbers than type a.

Sensilla trichodea types (a&b) showed the highest numbers of sensilla in both sexes and also affected with infestation. Mean numbers of type a ranged from 21.12– 36.21 and from 13.14 –34.21 for healthy workers and drones, respectively. They ranged from 16.8 –29.2 and from 14.8 –28.12 for infested workers and drones, respectively. Type b of trichode sensillae showed same trend where it decreased in numbers in both workers and drones due to infestation.

Mean number of placodae sensillae on flagellomeres 2, 4, 6, 8 and 10 were (5.82 & 5.22), (14.2 & 11.1), (11.37 & 10.54), (14.16 & 12.18) and (15.61 & 10.65) for (healthy and infested) workers, respectively. While in (healthy & infested) drones they were (3.2 & 2.1), (12.3 & 10.4), (23.3 & 26.43), (23.43 & 25.2) and (38.32 & 45.21), respectively. It is increased in number in drone than in workers especially in the terminal segment of infested drone.

Mean number of campaniform sensilla ranged from (1.2 –3.66 & 1.1–3.34) and from (1.1 –3.11 & 0.94–2.81) for (healthy & infested workers) and (healthy & infested drone), respectively.

Mean number of coeloconic sensilla on flagellomeres 2, 4, 6, 8 and 10 were (2.9 & 1.88), (6.72 & 4.61), (4.3 & 3.21), (4.8 & 3.98) and (3.8 & 3.56) for (healthy and infested) workers, respectively. While in (healthy & infested) drones they were (1.8 & 0.77), (5.8 & 4.53), (3.98 & 2.86), (2.67 & 2.2) and (2.78 & 2.43), respectively.

Data represented in Table (2) indicated the measurements (µm ± SD) of different types of sensilla on 2nd, 4th, 6th, 8th and 10th flagellar segments of healthy and infested of both worker and drone. The length of sensilla basiconica type a ranged from 6.5 ± 0.33 µm to 8.37 ±0.44 µm and from 6.46 ±0.33 µm to 8.88 ±0.33 µm for healthy and infested workers, respectively. While in drone it ranged from 4.33 ± 0.17 µm to 7.71 ±0.34 µm and from 4.29 ± 0.31 µm to 6.56 ± 0.27 for healthy and infested drones, respectively. Type b showed the same trend, it recorded (8.21±0.67 and 7.98 ± 0.71 µm in healthy worker and drone) in length then decreased to (7.75±0.38 and 7.21 ±0.49 µm in infested workers and drone).

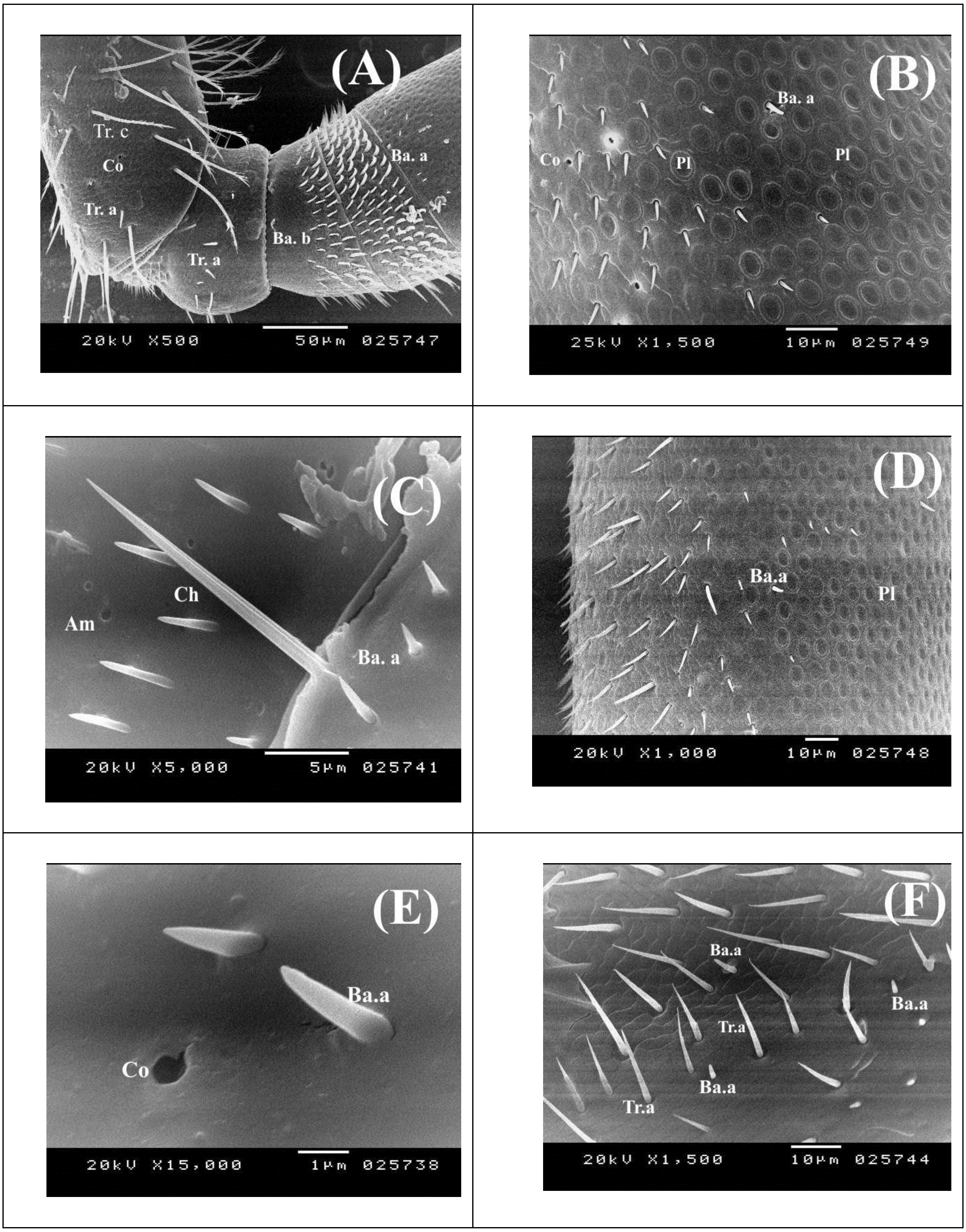


Fig. 2: flagellar segments of *A. mellifera* (A, B & D)healthy drone and (C, E and F) healthy workers. (Am.) ampulecium (Ba. a) Basiconic sensilla type a, (Ba. b) Basiconic sensilla type b, (Ch) chaetica (Co.) coeloconic sensilla, (Pl)Placoid sensilla, (Tr. a) trichoid sensilla type a, (Tr. b) trichoid sensilla type b.

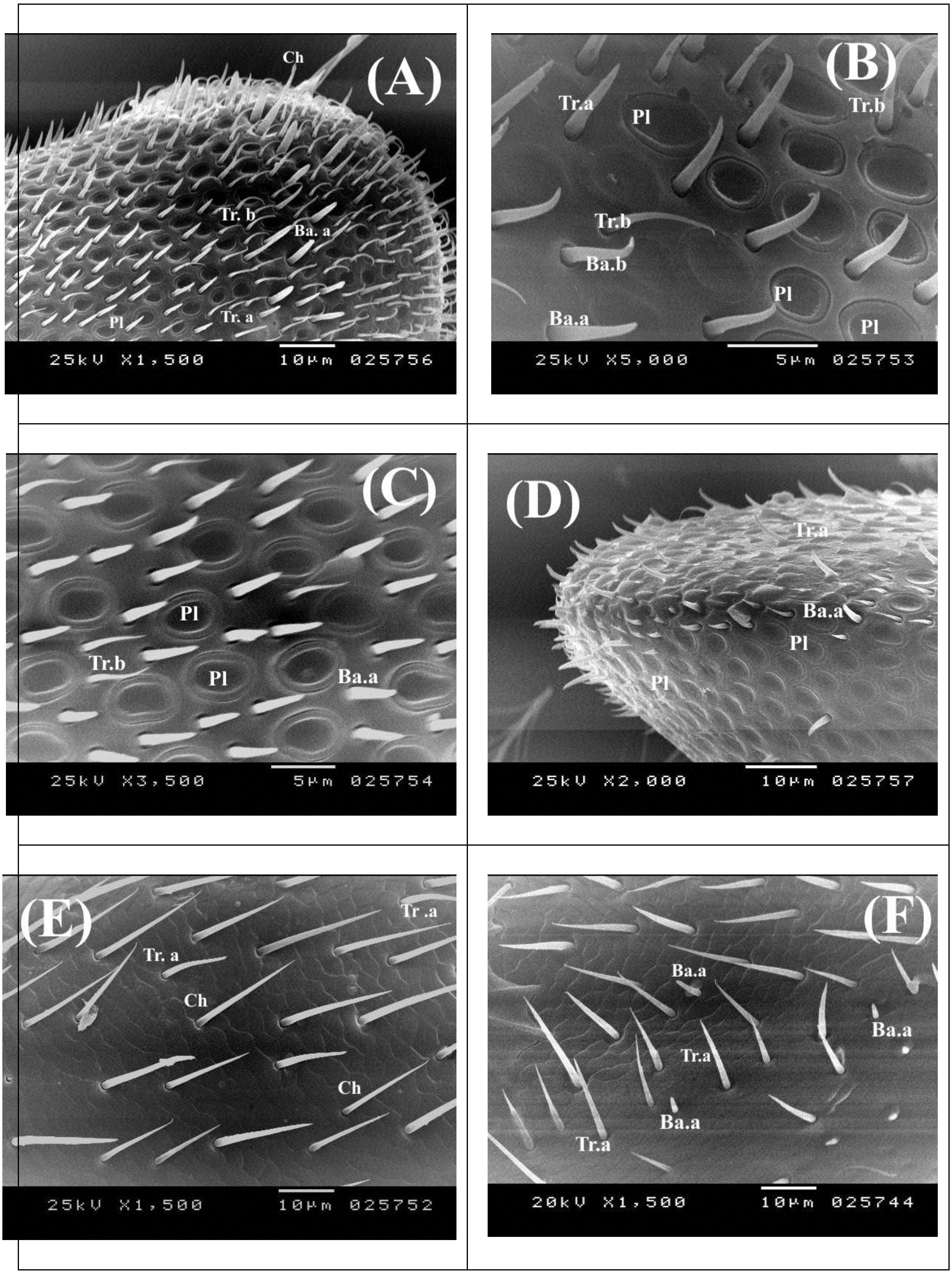
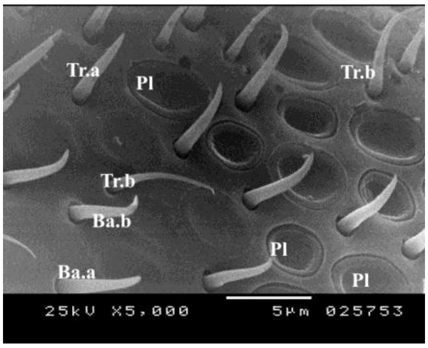
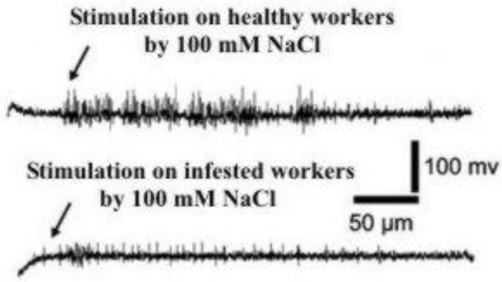


Fig. 3: flagellar segments of infested *A. mellifera* workers. (A – E and healthy on (F)(Ba. a) Basiconic sensilla type a, (Ba. b) Basiconic sensilla type b, (Ch.) chaetica sensilla, (Pl) Placoid sensilla, (Tr. a) trichoid sensilla type a, (Tr. b) trichoid sensilla type b.





A B

Fig. 4: (A) Recording membrane potential from antennal sensilla trichodea (a) in healthy and infested workers to 100 mM NaCl was used to stimulate the chemosensory afferents. (B) A scanning electron micrograph of the 10th segment of *A. mellifera* worker antenna showing the distribution of sensilla types. Sensilla basiconica (Ba a & b); sensilla placodae (Pl) and trichod sensillla (Tr a & b)

Sensilla trichodea also affected by infestation, type a measured 12.34 ±0.34 and 12.32 ±0.3 µm in healthy workers and drones. While they were 10.56±0.45 and 10.76±0.58 in infested workers and drones. Also, type b ranged from 12.13 ±0.69 to 10.21 ±0.28 µm and from 11.03 ±0.28 to 9.87 ±0.43 µm for healthy and infested workers. Drone trichodea type b ranged from 12.01 ± 0.64 to 10.21 ±0.57 and from 10.89 ± 0.66 to 8.98 ±0.43 µm for healthy and infested drone.

Mean surface area (μm^2) of placoidea sensilla were ranged from 91.94 ±5.83 to 202.6 ±13.85 and from 92.41 ±5.43 to 237.6 ±12.98 (μm^2) for healthy and infested workers. Placoid sensila in drones lower than workers and the mean surface area have the same trend where they ranged from 101.06 ±6.13 to 129.38 ±3.74 and from 67.13 ± 3.10 to 111.21 ± 8.33 (μm^2) for healthy and infested drone, respectively.

Diameter of campaniform sensilla measured (µm) to the healthy and infested workers and drones. Workers recorded 4.96 ±0.5 to 7.21 ±0.22 and 4.01 ± 0.5 to 6.75 ±0.38 for healthy and infested, respectively. While in healthy drones it recorded 4.43 ±0.15 to 6.99 ± 0.2 and infested drones recorded 4.01 ±0.34 to 6.21 ± 0.6. Ceoloconic sensilla showed same trend of campaniform where the diameter is slightly reduced in infested workers and drones.

The tolerant worker bees of different races and hybrids of honey bees (Carniolian Manzala, Egyptian race, Carniolian and Italian hybrids) to varroa mite infestation recorded higher number of sensilla organs on the antennae than the non-tolerant ones (Abd El-Wahab, 2001 and Abd El- Wahab *et al.* 2006). In *Apis* sp., two types of Tr were found on drones and workers. Tr. (b) was much longer and thicker than Tr. (a) but both were straight, whereas Tr. was curved. In general, nonporous sensilla Tr have been described as having putative mechanoreceptive functions (Alm and Kurczewski 1982; Stort and Rebustini, 1998; Das *et al.*, 2011). In this study, the absence of pores on sensilla Tr in *Apis* sp. suggests that they function as mechanoreceptors in both sexes. Sensilla Ba mainly have a thicker wall than that of Tr, with multiple pores on the sensilla tips or around the walls (Amornsak *et al.*, 1998; Coensoli *et al.*, 1999; van Baaren *et al.*, 1999; Amornsak *et al.* 2000;Suwannapong and Wongsiri, 2004; Gao *et al.*, 2007; Das *et al.*, 2011; Li *et al.*, 2011). However, Ba is nonporous in some cases (Gao *et al.*, 2007). In our study of *Apis* sp., there were two types of Ba on the antennae. Ba. (a) had a thumb-like shape, which was similar to the long sensilla Ba with tip and wall pores of the ant-like bethylid wasp *Sclerodenma. guani* (Li *et al.*, 2011). Sensilla Ba type b are presumed to function as olfactory receptors in many insects (Steinbrecht 1987; Hansson *et al.*, 1991; Steinbrecht 1997; Bleeker *et al.*, 2004;Das *et al.*, 2011). Thus, we suggest that Ba.(b) in *Apis* sp. is putative olfactory sensilla.

Sensilla Placodea (Pl) are the most common sensilla on the antennae of Hymenoptera species, although they occur in various sizes and shapes (Coensoli *et al.*, 1999; van Baaren *et al.*, 1999; Bleeker *et al.*, 2004; Roux *et al.*, 2005; Abd El-Wahab *et al.*, 2006; Gao *et al.*, 2007; Onagbola and Fadamiro 2008; Wang *et al.*, 2010; Das *et al.*, 2011; Li *et al.*, 2011). In most *Apis* spp., Pl are arranged in alternate rings around the antennae. In this study, both drone and worker *Apis* sp. had Pl. These sensilla had an oval structure with their long axis being parallel to the long axis of the flagellar segments. However, the external structure of Pl was different in infested insects from that observed in healthy insects. The closest resemblance of this type of Pl has been found in honeybees, *A. mellifera adansonii* (Dietz and Humphreys 1971) and *A. mellifera ligustica* (Gramacho *et al*., 2003). The multiple wall pores on Pl suggest an olfactory function (Ochieng *et al*., 2000; Bleeker *et al*., 2004,; Roux *et al*., 2005; Marques-Silva *et al*., 2006; Gao *et al*., 2007). Sensilla Coeloconica (Co) are recessed in deep pits (Ryan 2002), and the least abundant sensilla in *Apis* sp. (Li *et al*., 2011). This sensillum type is found in many Hymenoptera species (van Baaren *et al*., 1999; Bleeker *et al*., 2004; Roux *et al*., 2005; Gao *et al* ., 2007; Onagbola and Fadamiro 2008; Das *et al*., 2011; Li *et al*., 2011). In *Apis* sp., Co were also one of the least abundant sensilla types, and only located on flagellomeres with only one Co for each segment in both sexes. These nonporous sensilla are generally presumed to be associated with thermo- or hygroperception (Altner *et al*., 1983;Bleeker *et al*., 2004; Onagbola and Fadamiro 2008). The Co in *Apis* sp. might have a similar role owing to the few sexual differences in their abundance and the absence of pores.

Electrophysiology response

All living organisms, including bacteria, protozoans, fungi, plants, and animals, detect chemicals in their environment. The sensitivity and chemical range of animal olfactory systems is remarkable, enabling *A. mellifera* to detect and discriminate between thousands of different odor molecules. Although there is a striking evolutionary convergence towards a conserved organization of signaling pathways in vertebrate and invertebrate olfactory systems (Suwannapong, *et al.,* 2011). Afferent responses from gustatory receptors of antennae *A. mellifera* were tested with the stimulation/recording electrode containing a minimum content of salt (100 mM NaCl) for the conduction in water between the inner surfaces of the receptor. So at least the two potential stimulants water and salt are present and that can be coded by different receptor neurons of a single basiconic (a) or trichoide sensillum (a) at contact with the electrode solution. Therefore, we could not test directly the afferent responses to pure water but rather at the postsynaptic level of afferents: from higher order interneurons of the terminal ganglion.

The changes in membrane potential of basiconic and trichoide sensilla on infested workers and drones antennae to concentrations (100 mM NaCl mixed with 50 or 100 mM glucose) were significantly different from that recording from healthy antennale sensilla of workers and drones Fig., (4). The sensitivity was decreased with increasing of infestation by varroa. The changes in membrane potentials of both healthy and infested to these two chemicals suggest that antennal sensilla of this species have different concentration threshold sensitivities to each chemical. It seems likely that variable pheromone concentrations could lead to differences of membrane potential changes during depolarization that are essential for honeybee responses to stimuli (Suzuki and Tateda, 1974; Homberge, 1984). Also the reduction of number of the main olfactory sensilla is sensilla placodae which are abundant over the last segment of the antenna (Table 1). This sensilla type is innervated by 15 to 30 neurons which respond to flower odors and honeybee pheromones (Claudia *et al*., 2002). 2-heptanone, the major component of the mandibular glands of honey bees, is an alarm pheromone and has repellent properties affecting foraging bees (Shearer and Boch, 1965; Reith *et al*., 1986; Yokoi and Fujisaki, 2007).

Due to the function of 2-heptanone, which may be a repellent at high concentrations, but an attractant at low concentrations (Maschwitz, 1964; Shearer and Boch, 1965; Boch and Shearer, 1971; Vallet *et al.,* 1991), we assume that low concentration leads to a passage for molecules of 2-heptanone to a specific type of sensory receptor that represents as an attractant. In contrast, at high concentrations it might lead to a passage for molecule of 2-heptanone to other types of antennal sensilla distributed over the tip of the flagellum, resulting in acting as a repellent. However, the effect of varrao infestation on honey bees has not yet been verified until now.

The antennae are the first to encounter fresh substrates both when landing and searching the substrate. Their contact chemoreceptors can record chemical compounds of the substrate before contact and collect their food. When a chemical component of the substrate elicits responses in interneurons via contact chemoreceptors it can be considered as perceived by the CNS. Primary sensory responses of insect contact chemoreceptors are usually tested by stimulating and recording from the terminal pore of a gustatory hair (Hodgson *et al.,* 1955) since extracellular recording directly from the afferent axons of their very small neurons is impossible. We could study both the type of chemicals recorded by the contact chemoreceptors and their perception due to integration by higher order interneurones extra- or intracellularly from the brain. In this way, taste sensilla can be stimulated by just one chemical diluted in pure water (without the salts added for electrical conduction) or possibly even gaseous chemicals: smells (for acids: Gaaboub and Hustert, 1998; Gaaboub, *et al.,* 2005; Newland *et al.,* 2000; Gaaboub and Tousson, 2010).

It could be concluded that Scanning Electron Microscopy of the antenna of the deformed infested honey bees with varroa mites showed increase in mean number of Sensilla placodea on the antennal flagellomeres no 6,8 and 10 of honey bee drones. Heavily infested and deformed worker and drone bees showed decrease in mean number of Sensilla trichodea type a and b than the healthy ones. Future studies on the functional morphology of antennal sensilla using transmission electron microscopy coupled with electrophysiological recordings are likely to confirm the functions of the different antennal sensilla identified in this study.

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الملخص العربى

تأثير الاصابة بالفاروا على اعضاء الحس على قرن استشعار ذكر وشغالة نحل العسل وبعض الانشطة الفسسيولوجية فى مصر

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يحتوى نحل العسل كبقية حشرات غشائية الأجنحة على اعضاء حس خاصة على قرون الاستشعار تساعدها فى أنشطتها البيولوجية كالبحث عن العوائل وتميزها وسلوك التزاوج ووضع البيض. استعمل فى هذا البحث الميكرسكوب اللإلكترونى الماسح لدراسة اعضاء الحس لكل من الشغالات والذكور المصابة بطفيل الفاروا لمقارنتها بمثيلتها فى الأفراد السليمة. وقد سجلت ٧ أنواع من اعضاء الحس وهى Trichoid و Basiconic و Placoidea و Coeliconica و Chaetica و Ampulicum و Campaniformia. وقد أظهرت النتائج أن النوع Placoid أكثر وجودا فى الذكور عن الشغالات. وبتسجيل أعداد ومساحات هذا النوع على العقل٢، ٤، ٦، ٨ و ١٠ وأعداد كلا من أنواع و Campaniformia و Coeliconica و Trichoid , Basiconic وجد أنها انخفضت معنويا فى الأفراد المصابة مقارنة بالسليمة بينما ازدادت مساحة الأطباق الحسية فى الافراد المصابة مقارنة بالسليمة لذا فان هذا البحث يقدم اساسا للدراسات الالكتروفسيولوجية المستقبلية المختصة بسلوك نحل العسل.