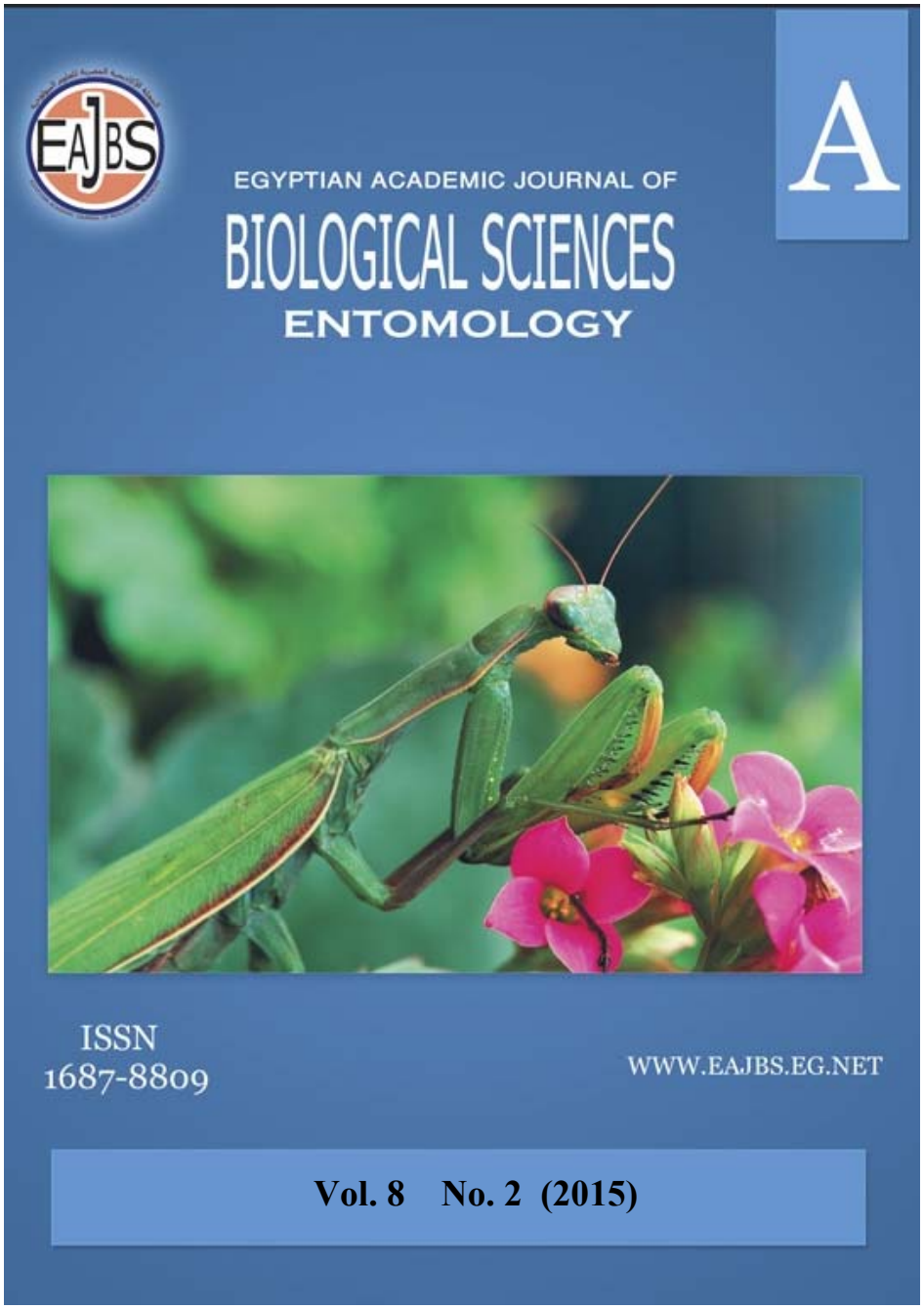

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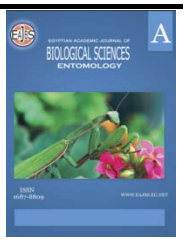

Comparative Studies on the Antennal Sense Organs of Queen and Worker Honey Bees, *Apis mellifera* L.

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ABSTRACT

The antennal forms and sensilla of queen and workers honeybees *Apis mellifera* L. (Hymenoptera: Apidae) were examined using scanning electron microscopy (SEM) to describe the types, and distribution of sensory structures to ascertain the relationship between sensory structures and behavior. Differences were found in sensilla types' diversity and distribution on the antennae of queens grafted from 12 and 48 hrs and workers honey bees. The scape with long trichoid were found in workers but with special very long in size for the queens honey bee. The pedicle with trichoid (short and long) were found in both worker and queen honeybee. There is a significant association between type, size and density of sensilla on flagellum segments. The segments of flagellum from 3rd to 10th are bearing placoid different in shape and size (oval, circle and square) and different in number on queen and worker. Basiconicum (large and small) with apical one pore were present with strong hook (curved shape) on second segment of flagellum in the worker but absent in the queen honey bees. The sensilla amplluicica and coeloconica were found on the same flagellum segments. There is a great variety of sense organs on the apical segment of flagellum for both workers and queen honeybees. The numbers of sensilla trichoid, placodea, campaniform, Basiconicum, amplluicica, and coeloconica varied most among bee casts and also antennal segments (scape, pedical and flagella).

INTRODUCTION

The antennae of insects contain several sensory units known as sensilla (Saïd *et al.,* 2003; and Yan *et al.,* 2010), and the type, abundance, and distribution of which vary among species (Faucheux *et al.,* 2006). The distribution and type of sensilla might also vary according to insect behavior, sex, and geographical distribution (Chen *et al.,* 2003). Sensilla can be distinguished based on their morphology and function. Morphologically, sensilla are categorized as trichodea, basiconica, placodea, campaniformia, coeloconica, chaetica, or ampullacea. Functionally, sensilla are categorized as mechano - chemo - thermo - or hygro-receptors and are CO2-sensitive (Nakanishi *et al.,* 2009). The sensory plate on the antennal tip plays a key role in the perception of mechanosensory and gustatory information (Scheiner *et al*., 2005). In eusocial insects, antennal sensilla are crucial for mating, foraging, recognition, and

communication between colony members (Lambin *et al.,* 2005).

Furthermore, in social insects, the type and number of sensilla vary according to sex and caste. For instance, male *Apis mellifera* exhibit more sensilla placodea than workers. These sensilla are believed to help males to encounter females during nuptial flights because increased numbers of sensilla placodea enhance the olfactory acuity of the males (Stort and Moraes-Alves 1999).

Despite scanning electron microscopic (SEM) studies of worker honey bees, *Apis mellifera*, have been carried out earlier (Dietz and Humphreys, 1971) and also on many other Hymenopterans. (Agren, 1977, 1978), differences in their morphology and distribution according to sex and caste remain poorly understood. This study describes the types and distribution of sensilla on the antennae of *Apis mellifera* F queen and workers honey bees and compared both qualitatively and quantitatively and also, with other studies in the literature. The major objective of this work was to examine the external various sensilla and different sense organs on the antennae of queen and workers honey bees as well as their distribution pattern. This information will be useful in conducting physiological studies associated with their behavior.

MATERIALS AND METHODS

Insect culture

The samples used in the present study were the workers and virgin queens from colonies of African honey bees (*Apis mellifera*) maintained at the apiaries of Agriculture college, Ain Shams University, Cairo, Egypt. Sense organs on antennae of newly virgin queens grafted from worker larvae (12 and 48 hrs old) and those of adult workers.

Scanning electron microscopy

The antennae of the two different casts, queen and daughter worker of the honey bee *Apis mellifera* L were taken out live, anesthetized and then fixed in Khal's solution. These preparations were dehydrated in ethanol series, followed by critical point drying and mounted on SEM stub. For antennal study, specimens were dissected under the microscope with a shard of razor blade. To avoid losing the sectioned parts, dissections were made on double-stick tape. Samples were gold coated in a layer of approximately 300 A^0 using a fine gold coating device, ion sputtering device (JEOL-JFC-1100 E).

The coated specimens with gold were examined through JOEL scanning electron microscope (JSM- T330 A) linked with image recording and processing system (semaphore), at voltage between 10 and 15 kV, at higher magnifications for identifications of sensillar types. in the Central laboratory of the Faculty of Agriculture, Ain Shams University. Five specimens for each cast are photographed directly from the SEM video monitor and then sensilla are identified and compared. The antenna was designated as scape, pedicle and 10 segmented flagella. The scanning electron micrographs of this study are arranged in plates.

RESULTS AND DISCUSSION

SEM analysis showed that the antenna of *A. mellifera* queen and workers was geniculate and it was composed of three basic segments, i.e., the scape, pedicel, and flagellum. The flagellum is formed of ten flagellomeres (Fig.1).

A considerable varieties and types of sensilla were revealed as follow:

The scape:

The scape appears as an elongated segment, which carries the following sense organs; Mechanosensilla and Chemosensilla. Investigations by Scanning Electron Microscope (SEM), the mechansensilla showed two types only; Sensilla trichodea and Sensilla companiform. Sensilla trichodea are long and have different number according to the honey bee casts. In the workers and queens grafted from 12 and 48 hrs old worker larvae, the mean number of these sensella was 1140, 1380 and 1215, respectively. The mean number of sensilla trichodea examined on the queen scape increased significantly compared with newly emerged honey bee workers (Table 1 and Fig.1). The difference between these means proved to be highly significant (F=258.5), and the L.S.D. value (28.81) emphasizes the obtained results.

Table 1: Sense organs on antennae of worker and newly virgin queens produced from worker larvae grafted at different age (12 and 48 hr) (Means ± S.E.).

Antennal segments	Type of sensilla	Size and shape	Number			
			Worker	Queen (12hr)	Queen (48hr)	"F" value L.S.D.
Scape	Trichoid	Long	1140 ±10.1c	1380 ±18.2a	1215 ±16.9 b	258.5** 28.81
		Very long	0 c	285 ±13.3 a	192±10.1 b	906.6** 18.21
	Campaniform		1	0	0	
	Coeloconica		1	1	0	
	Amplluicica		0	3	0	
Pedicle	Trichoid	Short	168 ±17.8 a	18 ±4.1 c	90±9.0 b	162.3** 22.21
		Long	87 ±7.8 a	24 ±4.1 c	51±5.9 b	106.3** 11.57
Flagellum 1	Trichoid	Short	450 ±12.2 b	540 ±11.8a	390±8.1 c	197.1** 20.49
2	Trichoid	Short	630 ± 14.5a	600 ± 10.6b	450±14.2 c	213.7** 24.88
	Basiconicum	Large (curved)	123 ±10.6 b	0c	192±12.8 a	411.9** 18.08
	Coeloconica		0	1	0	
3-9	Basiconicum	Small	1050 ±18.0a	810 ±9.3b	660±10.9c	870.9** 25.15
	Coeloconica		11{1+2+4+1 +3+2}	5 {2+2}on seg 3,4	10{2+5+3}on seg 5,6,7	
	Amplluicica		21{2+2+2+2 +6+7}	0	0	
	Placoid		690 ±11.9 a	396 ± 11.8 b	360±11.9 c	934.6** 22.34
10	Trichoid	Short	663 ±11.9 b	600±7.1 c	690±8.0 a	99.9** 17.43
	Campaniform	Right side	32	0	7	
		Left side	5	10	5	
	Basiconicum	Small	1123 ±17.0a	750 ± 9.2 c	830±9.6 b	990.5** 23.54
		Large in right side	3	5	9	
		Large in left side	10	8	2	
	Placoid		715 ±18.9a	650 ±7.3 b	669±16.8 b	19.29** 28.71
	Coeloconicum	Right side	5	0	18	
		Left side	0	1	0	

Moreover, very long trichoidea were found in scape segment of the queen but absent in that of the honeybee workers. The very long trichoidea number in the queen scape varied according to the age of grafted worker larvae. It was 285 and 192 on the scape of the honeybee queen produced from 12 and 48 hrs grafted larvae,

respectively.

The second type of mechanosensilla found in the scape segment was the campaniform sensellum which appear as one organ in worker and completely disappeared in queen.

Chemosensilla has two types' sensilla, coeloconica (small pore) and amplluicica (large pore). The first was found as one organ only in both worker and queen produced from 12 hrs old worker larva, while the second type, i.e., amplluicica was found in the scape of queen grafted from 12 hrs old larva only (Table, 1).

The pedicle:

SEM analysis showed one type of sense organ on the pedicle segment, namely trichoidae. The sensilla trichoidae has two shapes; short and long sensilla (Figs.2). The number of each shape varies according to the bee casts and also the queen grafted from the age of worker larvae. Generally, the number of short trichoidae was found to be higher than that of the long ones (Table, 1). On the other hand, the highest number of short trichoides (168 sensilla) was recorded in the pedicle of the honeybee workers followed by that in the pedicle of the honeybee queen grafted from larvae aged 12 and 48 hrs (18 and 90 sensilla, respectively); the difference between these numbers proved to be statistically significant. The same trend could be applied for the long trichoides. The mean number of the long trichoidae sensillum was 87, 24 and 51 long sensilla on the pedicle of the honeybee workers and queen grafted from 12 and 48 hrs old larvae, respectively. These results showed highly significant difference of the mean numbers of trichoidae sensillum on the honeybee workers and the queen (Table, 1).

The flagellum:

It flagellum consists of 10 segments with a great varieties of sense organs.

a) The first segment has mechanosensilla; short trichoid sensilla only. The numbers of the short trichoid sensilla was very according to the honey bee casts. The mean number of trichodea sensilla was 450, 540 and 390 sensilla in the honeybee worker and queen produced from 12 and 48 hrs old worker larvae, respectively (Table,1). The results showed that the difference between these numbers was statistically highly significant.

b) The second segment has two kinds of sense organs; mechanosensilla and chemosensilla. The mechanosensilla is short trichoid sensilla. The highest number (630 sensilla) was found in the honeybee worker, followed by that recorded in the queen produced from 12 and 48 hrs worker larvae was 600 and 450 sensilla, respectively (Table, 1). The results showed that the difference between these numbers was highly significant. The chemosensilla organ has two types of sensilla; the basiconical and coeloconical. Basiconicum is large and curved shape with one apical pore. It is found in high number in the honeybee worker and completely absent in the queen grafted from both 12 hrs worker larvae. The second type of sensilla; coeloconica presented only in the honeybee queen grafted from 12 hrs larva (Fig, 3).

c) The following seven segments (from 3rd to 9th): these segments have four types of chemosensilla as follow:

The numbers of small basiconicum vary according to the bee casts and the age of grafting larvae. The mean number of sensilla was1050, 810 and 660 sensilla in the workers and queen grafted from 12 and 48 hrs old larvae, respectively. The difference between these numbers was highly significant.

The placoid sensilla have different sizes and shapes (circle, oval and square

shapes) (Fig, 4). The mean numbers of placoid sensilla was 690, 396 and 360 sensilla in the workers and queen grafted from 12 and 48 hrs larvae, respectively. The results indicated highly significant difference between them (Table, 1).

The numbers of the coeloconica sensilla vary depending on the different segments of the bee casts. Table,1 showed the mean number of coeloconica sensilla in the honey bee workers ; 13 sensilla are distributed on the successive segments from the 3rd to the 9th i.e, 0, 1, 2, 4, 1, 3 and 2 sensilla, respectively. In the queen grafted from12 hrs old larva, two sensilla on each of the 3rd and 4th segments were found; while in the queen grafted from 48 hrs larva, the number of coeloconica sesilla was 2, 5 and 3 sensilla on the 5th , 6th and 7th segments, respectively (Table,1 and Fig, 4).

The sensilla amplluicica was not be distinguished in the honeybee queen grafted from larvae at any tested age but it was found in the honeybee workers and it was 0, 2, 2, 2, 6 and 7 sensilla on the successive segments from the 3rd to the 9th (Table, 1 and Fig, 4).

d)The apical segment of flagellum:

This apical segment (flagellumere) carries the following sense organs; mechanosensilla and Chemosensilla (Fig, 5).

The apical segement contained two types of mechanosensilla. The first is short trichoids, which presented in high number in the queen grafted from 12 hrs old larva (690 sensilla), followed by worker (663 sensilla) and then the queen grafted from 48 hrs old larva (600 sensilla). The second type, companiform sensilla found in higher number in the right side of the segment than in left side. These numbers vary according to the bee casts and the age of grafted from worker larva. In the right side, the highest (32 sensilla) was found in the honeybee worker and the lowest number was, however, recorded in the queen grafted from 48 hrs old larva (Table, 1 and Fig, 5). In the queen obtained from 12 hrs old larva, this type of sensilla is completely absent. In the left side, means of 5, 10 and 5 sensilla are presented in workers and queen of the honey bee A.mellifera L.grafted from 12 and 48 hrs old larvae, respectively (Table,1). Chemosensilla has three types of sensilla are found namely, basiconicum, placoid and coeloconicum. Basiconicum sensillum is found in large and small shapes. The number of small shape is higher than that of the large shape especially in the apical flagellum the honeybee workers. (Table, 1) clearly showed the difference between the small and large types of basiconicum numbers was significantly high. The second type; placoid vary with different numbers in the different casts of the honey be A. mellifera. The mean number was 715, 650 and 669 in the workers and queen grafted from 12 and 48 hrs old larvae, respectively (Table, 1). The third type; coeloconicum is found in right and left side. The number of sensilla in right side is different numbers in the different individuals. Means of 5, 0 and 18 were found in worker and queen obtained from 12 and 48 hrs old larvae, respectively. The sensilla coeloconicum was not presented in the honeybee workers and the queen grafted from 48 hrs old larvae but it was found only one sensilla in the queen grafted from 12 hrs old larvae.

Finally, it could be summarized that *A. mellifera* queen distinguished by the presence of higher mean numbers of sensilla organs; Basiconica, placodea and campaniformia an its antenna, which are responsible for chemoreceptors. The high number and length of trichodea type was recorded an antenna of worker bees.

Generally, the present results are in accordance with those recorded by Gupta (1992) and Al-Ghamdi (2006), who found six types of the sensilla organs on the antenna of worker honeybee, *A. florae*. Such sensilla organs are placodea, Basiconica, Coeloconica and Campaniform. In addition, the distribution of various types of

sensillae along the antenna varied according to the types of female bee (worker and queen) and the age of worker larvae at grafting to produce queens. In Amornsak *et al*., (2000) identified ten types of the sensilla organs in the antenna of the dwarf honeybee workers, *A. florea* as Basiconica peg, Campaniform, Chaetica (sevsral types), Coeloconica, Falcate, Placodea and Trichodea. However, Suwannapong and Wongsiri (2004**)** reported that eight types of sensilla organs; Ampullaceous, Basiconica, Campaniform, Placodea and Trichodea (four types) were most abundant at the distal ends of the flagella of *A. dorsata* antenna. Stort and Rebustinia (1998) found correlation between number of Campaniformia and Coeloconica sensilla with defense behavior in Africanized honeybees.

According to Al- Ghamdi (2006), the total numbers of sensilla organs of *A. mellifera* worker bee flagellomeres were higher than those of *A. florae* workers. This means that the bee race plays an important role in this respect. Generally, the same auther found relatively similar findings for the number of different types of sense organs distributed on the different flagellomeres of the worker antennae of *A. mellifera* races.

The ultrastructure has been previously carried out by many researchers, e.g. Dietz and Humphreys, (1971); Gupta, (1992); Yokohari *et al*. (1992); Amornsak, *et al*. (2000); Suwannapong and Wongsiri (2004); Al- Ghamdi, (2006) as they all described and recorded the different sensilla and their distribution on the antenna of the honeybee worker. The number of sensilla varied in the different bee races and was therefore was used as a feature for the classification of *Apis* sp.(Al- Ghamdi, 2006). Although sensilla coeloconic were known to act as chemoreceptors, Yokohari *et al*. (1992) reported that they may act as temperature and humidity receptors in the honeybee worker. Any abnormality in the antennal structure and their associated sensilla may affect the behavior of the honeybee whether feeding or foraging. Results of the present work showed that the antennal sensilla were not affected by 20, 40 or 50 rads of gamma irradiation as their structure was comparable to the untreated. However, with the application of 100, 150 or 200 rads, some deformalities were induced only in sensilla placodea but not in any other sensilla.

In conclusion, bee wolves possess a large number of different sensilla and a high density. Five of the nine sensillar types possibly have an olfactory function. With regard to our initial question about differences between the sexes in the equipment with sensilla there was a qualitative difference in that males lack the large basiconica. This suggests that the basiconica have a function in location or identification of the prey of the females, honeybee workers. It has also to be taken into account that the functional diversity of sensilla is much greater than the number of morphological types (Zacharuk, 1980).

Morphologically similar sensilla may have different numbers of sensory cells, different specificities and different response characteristics, not only in different insect species, but also between the sexes of the same species (Davies, 1977; and Städler, 1978). Hence, olfactory abilities of males and females might be more pronounced than suggested differences in by differences in morphology of the sensilla. (Sawires *et al.,* 2012) distinguished on the flagella : trichoid semsilla, campaniformia sensilla, placodea sensilla and ampulacea sensilla. The sensilla acampaniformia are probable tactile mechano sensill. Function for basiconica , placodea and ampulacae sensilla include hygro/ thermoreception and chemoreception. The type of sensilla aplacodea was located at the apical part of 3rd to 10th segments of the flagellum. (Barsagade *et al.,* 2013) The pedicel bears sensilla trichodea densely in all castes, and sensilla basiconica in the female only. The flagellum contains

sensilla trichodea curvata, sensilla trichodea, sensilla basiconica and sensilla chaetica in females, sensilla basiconica in workers and sensilla chaetica in males. Investigations by Scanning Electron Microscope (SEM) showed that the mean number and mean surface area of sensilla placodea on flagellomeres no. 6, 8, 10 of the examined bee antennae increased significantly with the infestation by varroa mites especially in the deformed newly emerged honey bee workers compared with the healthy ones. Also, heavily infested and deformed newly emerged drone bees shows higher significant differences of the mean number and mean surface area of sensilla placodea than the healthy drone bees.

Numbers and types of sense organs vary in Queens who was raised from larvae of different ages (Mahbobi *et al.* 2014; and Sawsan, 2015**)** found that the most efficient colonies were headed by queens reared from 1 day-old larvae which were superior to queens reared from 3 day-old larvae in brood production, bee population and in honey production. It is well known that newly emerged queens from the younger larvae are heavier than those emerging from older ones. Gengerr *et al.* (2000) found that the queens reared from 1-day-old larvae were significantly ($P< 0.01$) heavier than those from 2-day-old larvae.

Members of the family Anthomyiidae are a large and taxonomically difficult group of flies because of unstable taxonomy and nomenclature (Michelsem, 2014). The family was poorly studied from the Arabian regions except Deeming and Van Harten, 2014, who recorded four genera with ten species from Arabian Peninsula. However, Buttiker *et al.* (1979), Dabbour (1979 a, b and 1981), Martin (1972) Abu Thuraya (1982) Abu-Zoherah *et al.* (1993) and Al-Ahmadi and Salem (1999 a, b) reported, listed and noted on the dipterous flies of Saudi Arabia, including anthomiid flies. The present study threw light on the members of this family in Saudi Arabia with additional new records from Saudi Arabia and assist for more studies on this important family in Saudi Arabia.

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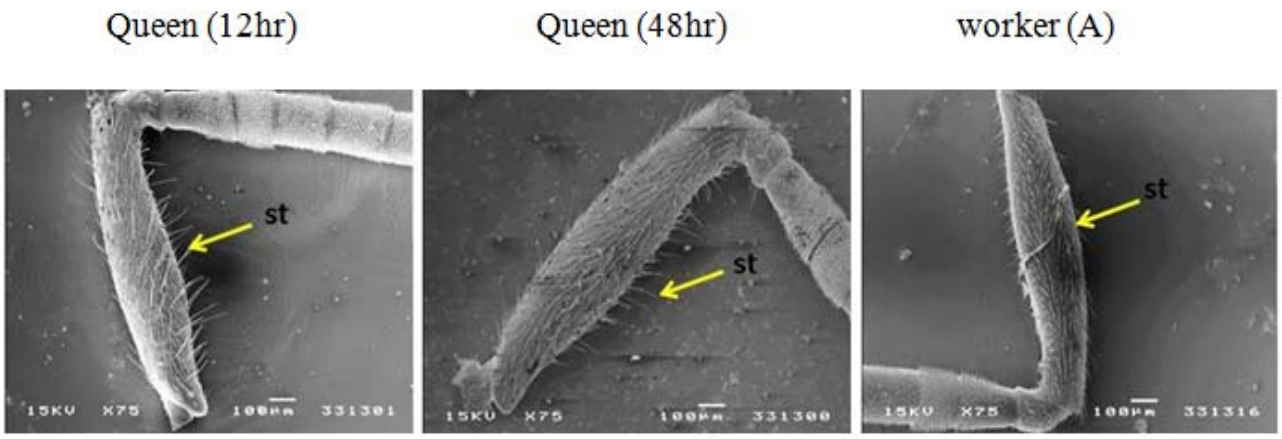

Fig. 1: SEM of antennal segments of scape for both the honeybee queens (12hr and 48hr) and worker (75 x) showing sensilla trichodea different lengths.

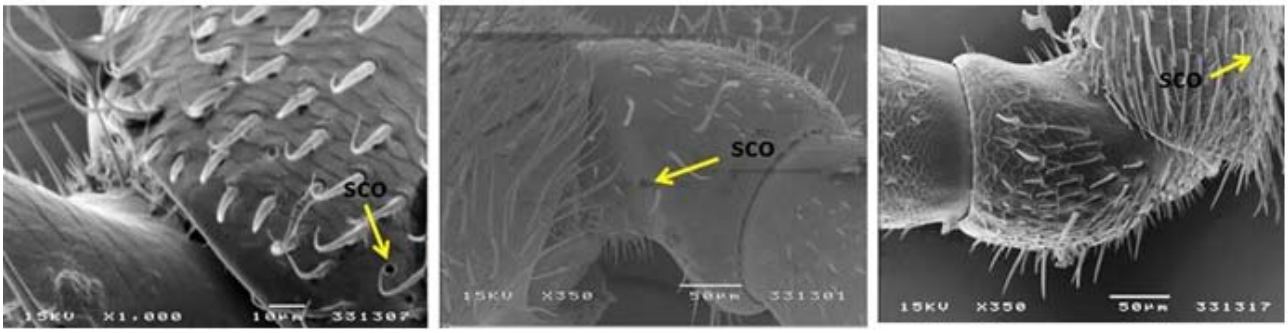

Fig. 2: SEM of antennal segments of pedicle for both honey bee queens (12hr and 48hr) and workers (350 and 1000x).

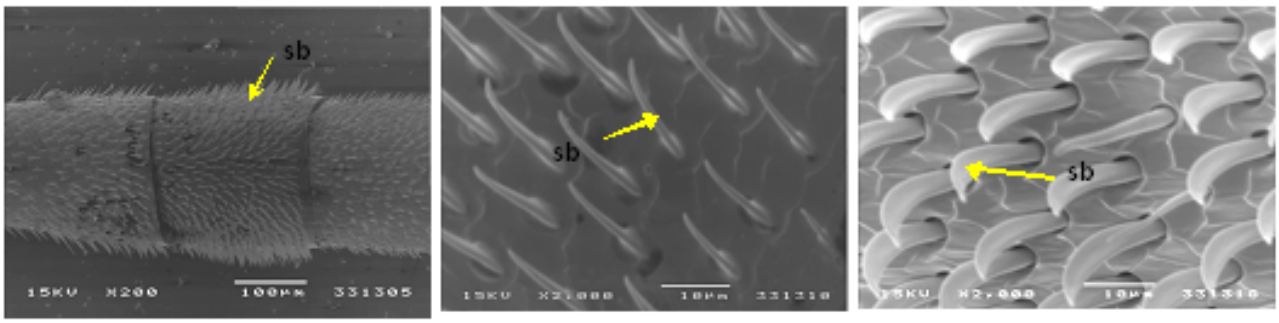

Fig. 3: SEM of second segments of flagellum in honey bee queens (12hr and 48hr) and worker (350 and 1000x) showing sensilla basiconicum of different in size and shap.

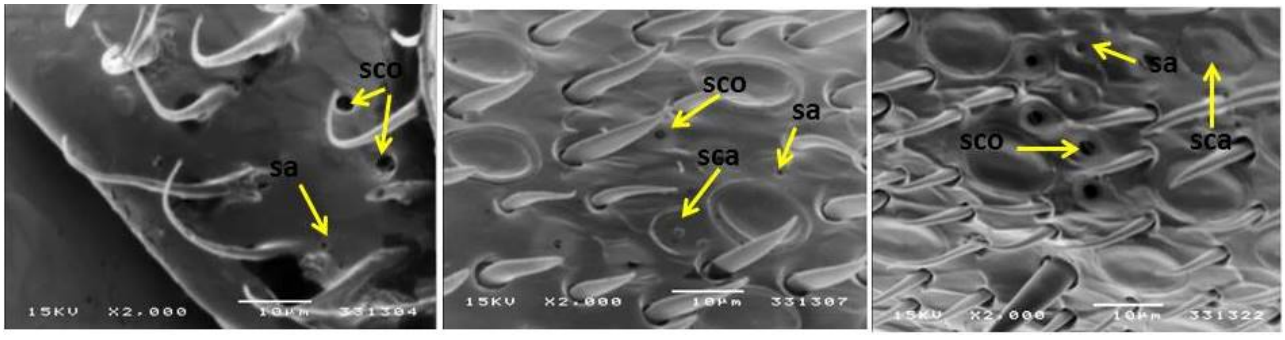

Fig. 4: SEM of antennal segments (from 3rd to 9th) of honey bee queen (12hr and 48hr) and workers (2000 x) showing sensilla placoid, sensilla coeloconica, sensilla ampullicica and campaniforum sensilla.

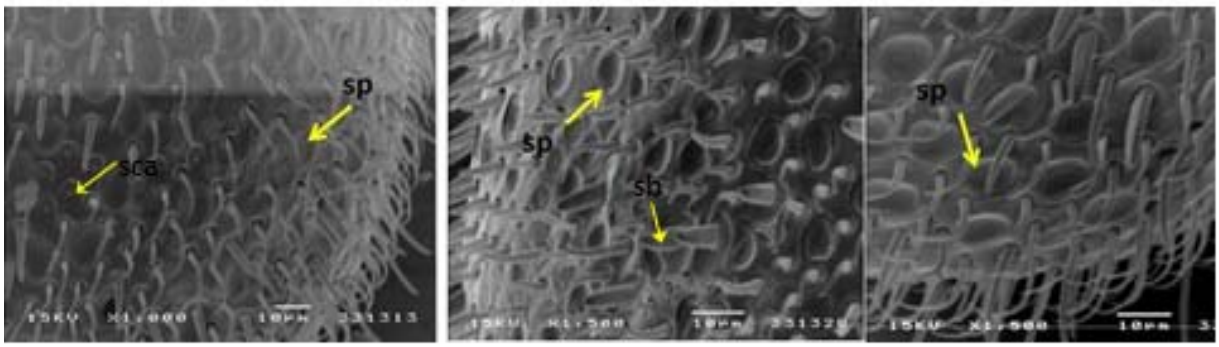

Fig. 5: SEM of apical segments of flagellum in honey bee queens (12hr and 48hr) and workers (500) showing sensilla of trichod, campaniform, basiconicum, placoid and coeloconicum.

Abbreviations

st = sensilla trichodea
sca = sensilla campaniformia
sb = sensilla basiconica
sco = sensilla coeloconica
sp = sensilla placodea
sa = sensilla ampulacea

ARABIC SUMMERY

دراسات مقارنة على أعضاء الحس على قرن استشعار الملكات الناتجة من يرقات الشغالات المختلفة فى العمر.

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تم فحص قرن استشعار الملكات والشغالات فى نحل العسل باستخدام المجهر الإلكتروني الماسح (SEM) لوصف أنواع وتوزيع أعضاء الحس للتأكد من العلاقة بين التراكيب الحسية والسلوك:

تم العثور على اختلافات في أنواع الشعيرات 'وتوزيعها على قرن استشعار الملكة المرباه من يرقات عمر 12 و48 ساعة والشغالات. وجد على عقلة الأصل شعرات حسية خاصة باللمس طويلة فى الشغالات ولكن تكون طويلة جدا في ملكة نحل العسل. ووجد أيضا على عقلة العذق شعرات حسية (قصيرة وطويلة) في كل من الشغالات وملكة نحل العسل.

هناك ارتباط كبير بين نوع وحجم وكثافة الشعيرات على عقل السوط. تحمل عقل السوط من 3 إلى 10 صفائح حسية مختلفة في الشكل والحجم (بيضاوي، دائرة ومربع) وتختلف في أعدادها بالنسبة للملكة والشغالات. وتحمل أيضا أوتاد حسية ذات ثقب واحد فى القمة مختلفة فى الحجم (الكبيرة والصغيرة) وتكون منحنية الشكل (منجلية) على العقلة الثانية من السوط في الشغالات ولكن تغيب في ملكة نحل العسل. تم العثور على ثقوب مختلفة فى قطرها على عقل السوط. وهناك مجموعة كبيرة ومتنوعة من أعضاء الحس على العقلة الأخيرة من السوط لكل من الشغالات وملكة نحل العسل. أعداد (Trichoid، placodea، campaniform، Basiconicum، amplluicica وcoeloconica) تفاوتت بين الشغالات والملكات وأيضا على عقل قرن الاستشعار (الأصل، العذق والسوط).