SCANNING ELECTRON MICROSCOPIC STUDIES ON ANTENNAL SENSILLA ORGANS OF ADULT HONEY BEE WORKERS IN GENUS *APIS* (HYMENOPTERA: APIDAE)

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INTRODUCTION

Antennae are the main sites of the olfactory receptors in most insects. The flagellum is covered with several kinds of small innervate setae and other minute sensory structures. Sensilla organs thought responsible for hygienic behavior particularly inside bee colony. They are capable of identifying dead, injured or infested brood inside capped brood cells (Gramacho and Goncalves, 2003). Stort and Rebustini (1998) found relationship between the defensive behavior and the number of sensilla Placodea, Campaniformea and Coeloconica in the Africanized honey bees. Antennal sensilla organs of adult *Apis florea* (F.) workers are Placodea, Basiconica, Trichodea, Ampullacea, Coeloconica and Campaniformia (Gupta,1992). *Apis cerana* are able to perceive a stimulus from a mite infested worker brood cells (Rath and Drescher, 1990). The number of the sensilla organs in the antenna of tolerant worker bee against varroa mites was higher than non tolerant worker bees (Salem *et. al.*, 2001). This study describes the types, measurements and distribution of the sensilla organs on the different segments of the antennae of *Apis florea* (F.) and *Apis mellifera* (L.) workers by SEM and compare the results obtained by the other studies. The major objective of this work was to examine the sensilla organs of some races of genus *Apis*, particularly the native bees; *Apis mellifera yemenitica* and aggressive Africanized bees.

MATERIAL AND METHODS

Fifteen honey bee colonies represented; Yemenitica race (*Apis mellifera yemenitica*), Carniolian race (*A. m. carnica*), Italian race (*A. m. ligustica)*, Africanized bees (*A.m. scutellata)* and Yemenitica hybrid were used in this study. Samples of *Apis florae* Fab. workers were collected randomly from different nests at

Riyadh Region, Kingdom Saudi Arabia (KSA), whereas the Africanized bee samples were obtained from Ribeirao Preto, Brazil. Ten samples of each tested worker bees were used for the antennal Scanning Electron Microscopy as follows; The antennae were dissected out of the tested bees and dried to critical point using Co_2 as the transition fluid. The antennae were mounted and coated with gold. The coated specimens were examined and photographed by the Scanning Electron Microscope (SEM) (JOEL-JXA -840 A).

Six types of the sensilla organs; Trichodea type (A&B), Placodea, Basiconica, Coeloconica and Campaniformia were determined in the flagellomeres No. 2,4,6,8 &10 of the worker antennae. The numbers of the each sensilla organs were counted / flagellomeres, calculated and expressed as the mean numbers (60 x 95µm) and mean measurements (Gupta, 1992, Naik *et. al.*, 1995 and Stort & Rebustini 1998).The mean surface area(μm^2) were determined according to formula of Maurizio,(1954).

Statistical analysis

L.S.D. tests were carried out for the obtained results according to the method of Gomez and Gomez (1984).

RESULTS AND DISCUSSION

The mean number and type of the studied sensilla organs of flagellomeres No, 2, 4, 6, 8 & 10 of the examined antennae of worker bees represented *Apis mellifera* (Yemenitica race, Carniolian race, Italian race, Africanized bees and Yemenitica hybrid) and *Apis florae* workers were illustrated in Table (1) and Figs. (1, 2, 3, & 4). The total numbers of the counted sensilla organs were higher in *Apis mellifera* races and hybrid in comparison with those of *Apis florea* workers. Yemenitica hybrid recorded the highest number of the sensilla organs followed by Carnica and Yemenitica races. The total numbers of the counted sensilla organs were higher in flagellomere 8 in *Apis florea* workers among the other tested sensilla organs, while the lowest numbers were recoded in flagellomere 4 in same workers. Flagellomere 6 in Yemenitica hybrid and Carnica race recorded the higher numbers, whereas the lowest value was detected in flagellomere 4 in Carnica race (Table 1). Significant differences in the mean numbers of the sensilla organs; Trichodea type (A), Basiconica, Placodea and Campaniformia were detected between the antennae of *Apis mellifera* and *Apis florea* workers.

TABLE (I)

Mean numbers of the sensilla organs of different flagellomeres of honey bee workers antennae of *Apis florea* and different bee races and hybrids of *Apis mellifera*.

Bee Species /race		Segment No.	Type of sensilla organs						
			Trichodea		asico-nica	Placod-ea	Campani-formia	Coelo-conica	Total
			A	B					
Apis florae Fab.		2	19	43	1	1	-	6	70
		4	4	7.6	11.6	17.3	2.3	3.6	46.4
		6	9.6	9	5.4	18.3	1	19	62.3
		8	12	32.3	9.3	11	1.66	10	76.26
		10	18.3	24.3	11	0.6	0.6	4.6	59.4
		Average	12.58c	23.24	7.66ab	9.64ab	1.11bc	8.64	62.87
Apis mellifera L.	Yemenitica race	2	25.33	7.6	13.7	8.33	4	10.7	69.66
		4	21	5.33	12	18.6	6	4.33	67.26
		6	26.7	6	11	15.33	3.66	6.33	69.02
		8	12.7	9.33	14.66	10.7	4.7	1.7	53.79
		10	46	12.33	3	16.66	2.66	2	82.65
		Average	26.34bc	8.11	10.87a	13.92a	4.204a	5.012	68.47
	Y. hybrid	2	26.3	25	2.7	0.0	0.0	4	58
		4	39.7	5.7	2	2.3	0.0	10	59.7
		6	45	22	0.0	5.3	0.66	73	145.96
		8	39.3	30.3	2.3	3	0.6	8	83.5
		10	23.7	74.3	1.7	1.7	1.3	5.7	108.4
		Average	34.8ab	31.46	1.74c	2.46b	0.512c	20.14	91.11
	Italy race	2	35	23.3	0.66	0.0	0.33	0.0	59.29
		4	32.3	10.77	4.6	6	1	1	55.67
		6	14	19.6	11.6	4.3	2.3	1	52.8
		8	41.3	10.6	2	1.6	4.6	0.0	60.1
		10	37.3	46.6	1.6	0.0	2.3	0.33	88.13
		Average	31.98ab	22.17	4.092bc	2.38b	2.106b	0.466	63.19
	Carnica race	2	56.7	3.3	1.33	0.0	0.66	0.33	62.32
		4	39	4.6	1	0.0	0.0	6.3	50.9
		6	12.3	4.3	7.6	7	1.6	3.7	104.9
		8	34.7	25	4	3	0.0	1.7	68.4
		10	34.3	25	1	6.7	1	1.3	69.3
		Average	35.4ab	12.44	2.98c	3.34b	0.652c	2.666	71.16
	Africa race	2	37.3	7.3	1.7	11.6	0.33	2	60.23
		4	42.7	5	0.66	13	1	5	67.36
		6	41.7	11	0.33	7.33	2	0.0	62.36
		8	54	0.6	1.3	6.75	0.6	1.3	64.55
		10	40.7	9.7	1.3	0.33	2.7	1	55.73
		Average	43.28a	6.72	1.058c	7.8ab	1.326bc	1.86	62.04
$LSD_{0.05}$			13.87	F=2.279	4.473	6.273	1.387	F=1.69	F=1.64

LSD: Least significant differences between the means.

Data presented in Table (2) showed significant differences in the mean numbers of the sensilla organs in different flagellomeres of worker honey bee antennae between tested honey bee races and hybrid of *Apis mellifera* and *Apis florea* workers particularly, Trichodea type (A) with segment No.2, Trichodea type

(B) with segments No. 2 & 10, Basiconica with segments No. 2, 8 and 10, Campaniformia with segment No. 4 and Coeloconica with segments No. 2 and 6. As shown in Table (1&2) Trichodea sensilla organs type (A) showed the higher mean numbers with Africa race followed with Carnica, Yemenitica hybrid and Italian races. Mean numbers of Trichodea sensilla type (B) showed the higher values with Yemenitica hybrid of *Apis mellifera* workers followed with them of *Apis florea*, while Africanized bees recorded the lowest values. Basiconica, Placodea & Campaniformia sensilla organs which are responsible for tactile and chemoreceptor of worker bees showed higher numbers with Yemenitica race than those of the other tested honey bee races and hybrid of *Apis mellifera* and *Apis florea* workers. Coeloconica sensilla organs recorded the higher mean numbers with *Apis florea* followed with Yemenitica hybrid of *Apis mellifera* workers.

The results obtained as illustrated in Table (3) showed clear significant differences in the mean measurements of the examined sensilla organs between tested honey bee races and hybrid of *Apis mellifera* and *Apis florea* workers particularly with Basiconica, Placodea and Coeloconica. Trichodea type (A) recorded the highest mean length (μ) with Africanized bees and Italian race. Trichodea type (B) recorded higher mean measurements with Africa and Italian races followed by *A. florea* workers. Basiconica sensilla organs in the antennae of *Apis florea* workers recorded the higher mean length followed by Carnica and Italian races, while the lowest value was detected with Yemenitica hybrid. Placodea sensilla organ showed the highest mean area (μm^2) with *Apis florea* worker followed with Yemenitica and Africa races. Campaniformia and Coeloconica sensilla organs recorded the higher diameter with *Apis florea* workers among tested honey bees.

It could be summarized that Yemenitica race be distinguished by presence higher mean numbers of the sensilla organs; Basiconica, Placodea & Campaniformia which responsible for chemoreceptor, while Africa race specialized in presence higher number and mean length (μ) of the Trichodea type (A), whereas *Apis florea* workers recorded highest mean area (μm^2) of the Placodea sensilla organ followed with Yemenitica and Africa races.

It can be concluded that there are significant differences in the mean numbers, meas irements and distribution of the sensilla organs between and within different tested antenna segments of Apis florea and races and hybrids of Apis mellifera workers in Genus Apis particularly the native bees; Apis mellifera yemenitica and aggressive Africanized bees. These differences may be because of

TABLE (II)

Differences between sensilla organs in the different flagellomeres of honey bee workers.

Type of the sensilla	Bee species/ race		Segment No. 2	4	6	8	10	Total Numbers	Means numbers
Trichodea A	*Apis florea* F.		19 a	15.66	9.6	12	18.3	62.9	12.58 c
	A. mellifera L.	Yemenitica	25.33a	21	26.7	21.6	46	131.73	26.34 cb
		Y. hybrid	26.3 a	39.7	45	39.3	23.7	174	34.8 ab
		Italy	35 ab	32.3	14	41.3	37.3	159.9	31.98a b
		Carnica	56.7 bc	39	12.3	34.7	34.3	177	35.4 ab
		Africa	37.3 ac	42.7	41.7	54	40.7	216.4	43.28a
L $SD_{0.05}$			24.29	F=2.06	F=0.498	F=1.78	F= 1..31		14.25
Trichodea B	*A. florea* F.		43 a	7.6	9	32.3	24.3 a	116.2	23.24 ab
	A. mellifera L.	Yemenitica	7.6 b	5.33	6	9.33	12.33 ab	40.59	8.11 b
		Y. hybrid	25 c	5.7	22	30.3	74.3 c	157.3	31.46 a
		Italy	23.3 c	10.77	19.6	10.6	46.6 d	110.87	12.98 ab
		Carnica	3.3 b	4.6	4.3	25	25 a	62.2	12.44 ab
		Africa	7.3 b	5	11	0.6	9.7 b	33.6	6.72 b
L $SD_{0.05}$			7.85	F=0.66	F=0.925	F=0.96	12.22		22.74
Basiconica	*A florea* F.		1a	11.6 a	5.4	9.3ab	11 a	38.3	7.66 a
	A. mellifera L.	Yemenitica	13.7 b	12.0 ab	11	14.66a	3 b	54.36	10.87 ab
		Y. hybrid	2.7 a	2 c	0.0	2.3 b	1.7 b	8.7	1.74 c
		Italy	0.66 a	4.6 abc	11.6	2 b	1.6 b	20.98	4.09 ac
		Carnica	1.33 a	1 c	7.6	4 b	1.0 b	14.93	2.98 c
		Africa	1.7 a	0.66 c	0.33	1.3b	1.3 b	5.29	1.05 c
L $SD_{0.05}$			9.49	F=9.24	F=1.217	9.01	4.58		4.33
Placodea	*A. florea* F.		1	17.3	18.3	11	0.6	48.2	9.64 ab
	A. mellifera L.	Yemenitica	8.33	18.6	15.33	10.7	16.66	69.62	13.92 a
		Y. hybrid	0.0	2.3	5.3	3.0	1.7	12.3	2.46 c
		Italy	0.0	6.0	4.3	1.6	0.0	11.9	2.38 c
		Carnica	0.0	0.0	7	3	6.7	16.7	3.34 bc
		Africa	11.6	13	7.33	6.75	0.33	39.01	7.8 abc
L $SD_{0.05}$			F=2.18	F=2.14	F=1.08	F=1.07	F=2.91		6.44
Campaniformia	*A. florea* F.		0.0	2.3 a	1	1.66	0.66	5.56	1.11 a
	A. mellifera L.	Yemenitica	4	6.0 b	3.66	4.7	2.66	21.02	4.2 b
		Y. hybrid	0.0	0.0 c	0.66	0.6	1.3	2.56	0.51 a
		Italy	0.33	1 ac	2.3	4.6	2.3	10.53	2.1 a
		Carnica	0.66	0.0 c	1.6	0.0	1	3.26	0.65 a
		Africa	0.33	1 ac	2	0.6	2.7	6.63	1.32 a
L $SD_{0.05}$			F=0.54	2.25	F=0.25	F=1.34	F=0.57		1.34
Coeloconica	*A. florea* F.		6 ab	3.6	19 a	10	4.6	43.2	8.64 a
	A. mellifera L.	Yemenitica	10.7 a	4.33	6.33 b	1.7	2	25.06	5.01 abc
		Y. hybrid	4b	10	7.3 b	8	5.7	35	7 ab
		Italy	0.0 b	1	1 b	0.0	0.33	2.33	0.46 d
		Carnica	0.33 b	6.3	3.7 b	1.7	1.33	13.36	2.67 bcd
		Africa	2 b	5	0.0 b	1.3	1	9.3	1.86cd
$LSD_{0.05}$			6.31	F=0.07	10.36	F=2.68	F=2.07		4.5

LSD: Least significant differences between the mean numbers.

TABLE (III)

Mean measurements (µm) of the sensilla organs at different flagellomeres of honey bee worker antennae.

Bee Species /race		Segment No.	Type of the sensilla organs							
			Trichodea (length)		Basiconica length	Placodea			Campaniformia	Coeloconica
						Length	Width	Area (μm^2)		
			Type A	Type B					Diameter	Diameter
Apis florea Fab.		2	11.33	16.43	6.50	16.5	10.0	259.05	4.0	3.12
		4	10.84	9.92	9.17	12	7.6	143.18	10.75	5.04
		6	9.00	8.59	7.83	12	7.66	144.3	5.87	5.07
		8	8.51	8.83	8.0	11.5	6.83	123.3	5.82	5.77
		10	9.53	9.11	7.16	14	10..33	227.0	5.24	4.99
		Average	9.84	10.37	7.73a	13.2	8.48	179.36a	6.33	4.79a
Apis mellifera L.	Yemenitica rarace	2	11.5	11.72	3.66	10	7.5	117.75	3.24	4.91
		4	9.68	8.93	4.71	11.64	7.50	137.06	5.29	3.70
		6	9.05	8.66	6.11	9.75	5.37	82.20	5.70	3.28
		8	7.92	7.53	4.66	16.50	5.37	139.10	6.0	2.41
		10	7.56	10.25	5.6	10.25	8.50	136.78	4.5	2.58
		Average	9.14	9.41	94bc	11.62	6.84	.57ab	4.94	3.37bc
	Y. hybrid	2	11.55	9.13	3.55	3	2	9.42	4.10	3.76
		4	9.53	8.90	4	9.6	7.20	108.51	4.62	3.43
		6	11.68	8.29	3	11.83	8.33	154.71	3.74	2.95
		8	8.33	8.72	2	8	5	62.8	6.37	2.68
		10	7.35	6.28	4.25	8	5	62.8	3.79	2.68
		Average	9.68	8.26	3.36c	8.08	5.50	79.64b	4.52	3.10bc
	Italy	2	10.70	10.70	8.0	6	4	37.68	3.0	3.0
		4	10.42	13.62	6.91	12	7.14	134.51	5.24	4.5
		6	11.27	9.84	6.64	11.62	7.5	136.82	7.50	3.0
		8	10.70	10.46	6.0	9.66	7.33	111.16	4.0	3.24
		10	9.51	9.13	3.0	6.0	4.20	39.56	7.91	3.12
		Average	10.52	10.75	6.11b	9.05	6.03	91.94 b	5.53	3.37bc
	Carnica	2	9.34	9.82	6.71	5	4	31.40	3.62	4.45
		4	9.34	9.82	6.71	11..62	7.50	136.82	4.50	3.52
		6	10.77	11.13	4.33	10..9	7.23	123.72	3.50	4.30
		8	8.93	9.23	6.45	8.87	6.37	88.70	2.37	4.15
		10	9.04	8.32	6.45	9.53	5.86	87.67	3.37	3.59
		Average	9.48	9.66	6.13 ab	9.18	6.19	93.66 b	3.47	4.0ab
	Africa	2	12.96	11.95	6.0	11.11	6.02	105	2.19	2.49
		4	10.54	10.35	8.0	12.38	5.92	115.06	4.0	2.89
		6	11.60	12.03	5.0	11.90	6.72	125.54	9.50	2.5
		8	8.82	9.80	5.80	12.11	6.88	130.80	4.74	3.24
		10	10.80	10.06	4.5	10.09	7.63	120.86	5.12	3.12
		Average	10.94	10.83	5.86 b	11.51	6.63	119.45b	5.11	2.85c
$LSD_{0.05}$			F=1.24	F =.505	1.604	=2.56	F=1.96	56.89	F=1.29	0.911

LSD: Least significant differences between the means.

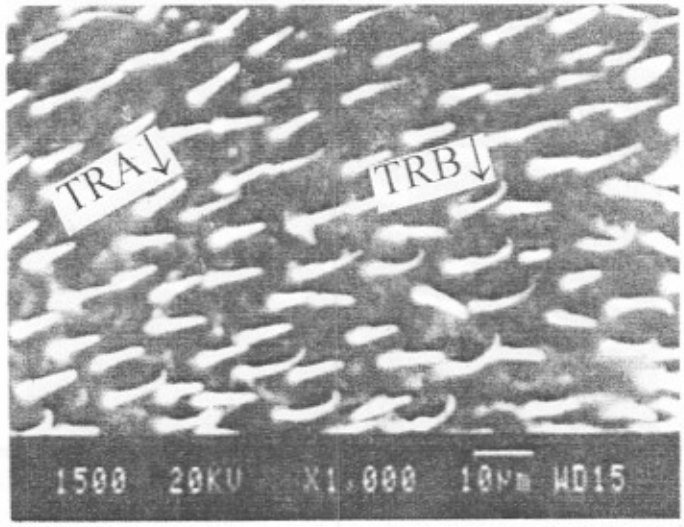

Fig.(1): Trichodea sensilla organs type A (TRA) & type B (TRB) of honey bee worker antenna. (X- 1200)

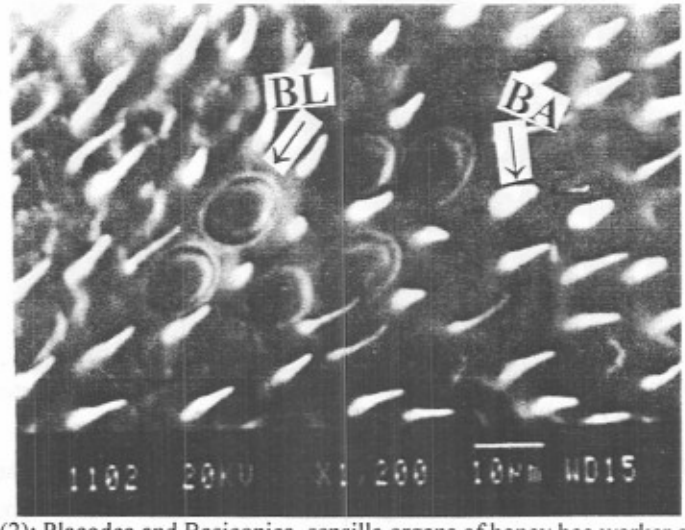

Fig.(2): Placodea and Basiconica sensilla organs of honey bee worker antenna. (X- 1200). BL: Placodea; BA: Basiconica.

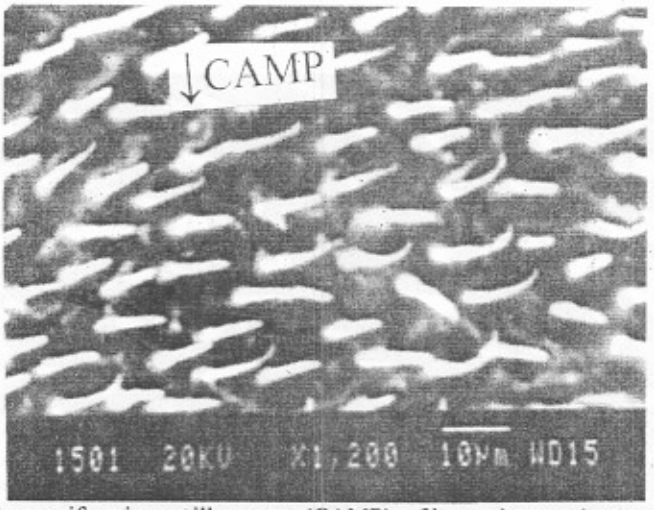

Fig.(3): Campaniformia sensilla organs (CAMP) of honey bee worker antenna. (X- 1200)

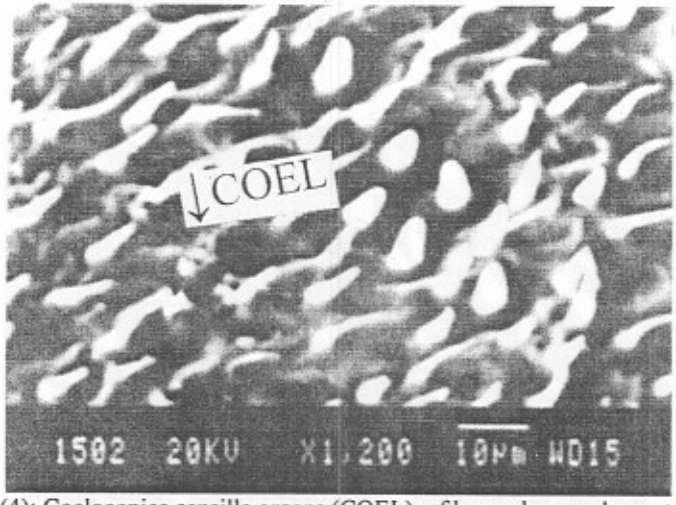

Fig. (4): Coeloconica sensilla organs (COEL) of honey bee worker antenna. (X- 1200).

differences in bee races and hybrids as well as the ecological factors. The results obtained are go in line with the finding of Gupta (1992), who found six types of the sensilla organs on the antennal of honey bee *Apis florea* were; Placodea, Basiconica, Trichodea, Ampullacea, Coeloconica and Campaniformia. In addition the distribution of various types of the sensillae along the antenna was similar to that found in the other *Apis* species and the population of the types of olfactory sensilla

was comparatively much less on the antenna of *A. florea*. In the other hand sensilla Trichodea type (A) are the most common structures on the antenna. While Amornsak *et. al.*, (2000) identified ten types of the sensilla organs in the antenna of the dwarf honey bee worker *Apis florea* (F.) as Basiconic Peg, Campaniform, Chaetica (type 1, 2, 3 & 4), Coeloconica, Falcate, Placodea and Trichodea. Suwannapong & Wongsiri (2004) reported that eight types of the sensilla organs; Ampullaceaous, Basiconica, Campaniforme, Placodae and Trichodae (type A, B, C & D) were most abundant at the distal ends of the flagella of the *Apis dorsata*. Stort and Rebustini (1998) that found correlation between number of the Campaniformia and Coeloconica sensillae with defense behavior in Africanized honey bees. Rath and Drescher (1990) and Rosenkranz *et al.*, (1993) found relationship between the hygienic behavior and the infestation with mites as well as between dead brood within sealed worker cell. Buchler *et. al.*, (1992) and Flottum (1997) stated that in case of allogrooming behavior (between bee and bee) the nestmates inspect the whole body of the mite infested bees by their antennae, paying greater attention to the petioles region and the wing bases. Salem *et. al.*, (2001) concluded that the high counts of the sensilla organs in the flagellomeres of the antennae in tolerant worker bees to varroa mites may be due to occurrence of one or more natural defense mechanisms towards the mites particularly grooming and brood removal behavior (Hygienic behavior).

SUMMARY

Samples of *Apis florea* (F.) and *Apis mellifera* (L.) workers represented; Yemenitica race, Carniolian race, Italian race and Africanized bees as well as Yemenitica hybrid were examined to evaluate their antennal sensilla organs. Numbers of the sensilla organs; Trichodea (Type A&B), Placodea, Basiconica, Coeloconica and Campaniformia of the flagellomeres No. 2, 4, 6, 8 & 10 of all examined bee workers antenna were counted, measured and photographed by Scanning Electron Microscope (SEM). The results showed significant differences between and within tested sensilla organs in different segments of the antennae of the tested bees particularly with Yemenitica & Africa races.

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