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Morphological changes induced in the antenna of cowpea beetle, *Callosobruchus maculatus* (Fabricius) (Coleoptera : Bruchidae) after treatment with lufenuron

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ABSTRACT

The effect of a chitin synthesis inhibitor (Lufenuron) on the antennal segements and their sensilla of cowpea beetle, *Callosobruchus maculatus* (Fabricius) was studied by using scanning electron microscope.

Seven types of sensilla were recognized on both untreated and treated antennae with 9 ppm of lufenuron, including Böhm bristles (BB), two types of sensilla trichodea (ST_1, ST_2), sensilla chaetica (SC), two types of sensilla basiconica (SB_1, SB_2) and grooved peg (GP). The functions of these sensilla were discussed in relation to their position on the antenna.

Significant difference in lengths of most segments of the antennae between untreated and treated beetles was observed. A significant difference in lengths and diameters of nearly all types of sensilla was recorded. The highest increase in length of sensilla was noticed for the sensilla chaetica followed by sensilla trichodea. These types are used by insects as mechanosensory, sex pheromone and gustatory receptors that help them to make a decision to select their food and oviposition sites.

Keywords: Cowpea beetle, *Callosobruchus maculatus*, antennal sensilla, insect growth regulator, scanning electron microscope (SEM).

INTRODUCTION

Callosobruchus maculatus is a cosmopolitan pest of stored grain legumes (Fabaceae) particularly of the genus *Vigna* (Cope and Fox, 2003). As a consequence of infestation, seed lots become warm resulting in quality loss and mould growth (Rees, 2004). Damaged seeds are unsuitable for human consumption, and cannot be used for agricultural and commercial purposes.

Searching for new effective pest control agents, much attention has been focused on compounds which disrupt the normal process of insect development. Insect growth inhibitors, in contrast to traditional insecticides are generally non to slightly toxic to higher animals. They inhibit the synthesis of chitin of insects by causing abnormal endocuticular deposition and absorptive moulting (Post and Vincent, 1973). Lufenuron (chitin synthesis inhibitor) is one of the most newly introduced synthetic insect growth regulators. It is used for controlling lepidopterous and Coleopterous larvae on cotton, maize, vegetables, citrus white fly and rust mites on citrus fruits.

Insects antennae are mobile, segmented, paired head appendages and are found in nearly all insects groups (Gullan and Granston, 1994). Numerous sensory-organs or sensilla occur on antennae in the form of hairs, pegs, pits or cones.

Several studies have characterized the antennal sensilla of various species of beetles using electron microscopic techniques (Jourdan *et al.*, 1995 and Said *et al.*, 2003). However, very few of such studies have focused on Bruchidae. Thus, the aim of this work is to describe the sensilla and to study the effect of Lufenuron on the different types of antennal sensilla of *Callosobruchus maculatus.*

MATERIALS AND METHODS

The cowpea beetle, *Callosobruchus maculatus* (Fabricius) used in the present investigation was obtained from the Plant Protection Center, at Dokki, Giza. Insects were reared in 500ml glass jars which contained a suitable number of cowpea grains. The jars were covered with muslin secured by rubber bands and were introduced in an incubator for rearing at 27°C and 75 ± 2% RH.

The morphology and distribution of various types of antennal sensilla in untreated and F_1 adults of *Callosobruchus maculatus* resulting from adults treated with 9 ppm Lufenuron were studied by using scanning electron microscope.

The antennae of adults of both untreated and treated groups were separately fixed in a solution of 2.5% glutaraldehyde, and kept over night at 4C .Then they were rinsed in a phosphate buffer pH (7.2) three times for 15 min, subjected to postfixation in 1% osmium tetraoxide for 2 hr, bathed three times for 15 min in phosphate buffer. Finally, They were dehydrated in 30, 50, 70, 90 and 100% ethanol (three times) and each for 15 min. Afterwards, they were dried and finally coated with gold using sputter coating for examination by using scanning electron microscope (SEM) JEOL, JSM 5300.

Statistical analysis:

In classifying sensilla, the terminology of Schneider (1964) and Zacharuck (1985) was applied, measurements (µm) were obtained from photomicrographs of at least 5 individuals. The data obtained were analyzed using t-test with SPSS 10.0 for windows to determine any significant difference ($p < 0.05$).

RESULTS

The antennae of *Callosobruchus maculatus* is serrate in shape. It consists of a scape, pedicel and nine-segmented flagellum as shown in Fig. (1). All flagellomeres, except for the first one, have acute-angled wedge-shaped ventral extensions, where most types of the sensilla are located. However, the antennomeres vary markedly in size and length (Table 1).

Treatment with 9 ppm Lufenuron led to losing of the above mentioned serrate shape of antenna. In addition, the lengths of scape, pedicel, first, second and fifth segments of flagellum became significantly shorter than those for the control ones. The highest decrease was recorded for the first flagellar segment where the length of segment was 0.04 ± 0.002 mm compared with 0.10 ± 0.002 mm for the control ones. On the other hand, sixth, eighth and ninth segments of flagellum became longer than those of the untreated ones. Their mean lengths were 0.14 ± 0.002, 0.14 ± 0.001 and 0.18 ± 0.002 mm compared to 0.12 ± 0.002, 0.12 ± 0.001 and 0.16 ± 0.002 for the control ones.

Table (1): Mean lengths (mm) of antennal segments of untreated and treated adult of *Callosobruchus maculatus* with Lufenuron.

Segments	**Untreated**	**Treated**
	Mean length ± SE (mm)	**Mean length ± SE (mm)**
Scape	0.14 ± 0.002	0.10 ± 0.002
Pedicel	0.08 ± 0.002	0.06 ± 0.002
F_1	0.10 ± 0.002	0.04 ± 0.002
F_2	0.14 ± 0.004	0.12 ± 0.002
F_3	0.14 ± 0.002	0.14 ± 0.002
F_4	0.14 ± 0.002	0.14 ± 0.002
F_5	0.14 ± 0.001	0.12 ± 0.002
F_6	0.12 ± 0.002	0.14 ± 0.002
F_7	0.12 ± 0.003	0.12 ± 0.002
F_8	0.12 ± 0.001	0.14 ± 0.001
F_9	0.16 ± 0.002	0.18 ± 0.002
Total	1.4	1.3

(n = 5).

Table (2) : Mean lengths of the measured sensilla of untreated and treated adult of *Callosobruchus maculatus* with Lufenuron

Sensilla types	**Untreated antenna**		**Treated antenna**	
	Mean length (µm) ± SE	**Diameter ± SE**	**Mean length (µm) ± SE**	**Diameter ± SE**
BB Böhmbristle	1.9 ± 0.1	0.3 ± 0.08	1.7 ± 0.1	0.3 ± 0.1
St_1 sensilla trichodea 1	10.1 ± 0.8	0.5 ± 0.2	15.2 ± 2.5	0.4 ± 0.1
St_2 sensilla trichodea 2	7.1 ± 1.0	0.5 ± 0.2	7.3 ± 1.0	0.9 ± 0.3
Sb_1 sensilla basiconica 1	5.9 ± 1.2	0.9 ± 0.4	6.1 ± 1.2	0.7 ± 0.1
Sb_2 sensilla basiconica 2	1.7 ± 0.5	0.6 ± 0.1	3 ± 0.4	0.9 ± 0.1
Sc sensilla chaetica	10 ± 2.6	1.0 ± 0.4	16.4 ± 0.9	1.0 ± 0.1

(n = 5)

Antennal sensilla types:

Based on size, shape, distribution and cuticular attachment. Seven types of sensilla were recognized on both untreated and treated adult antennae of *Callosobruchus maculatus.* The sensilla are one type of Böhm bristles (BB), two types of sensilla trichodea (ST_1, ST_2), one type of sensilla chaetica (SC), two types of sensilla basiconica (SB_1, SB_2) and one type of grooved peg (GP). The characteristic morpholgocial features of the antennal sensilla in untreated and treated *Callosobruchus maculatus* are shown in figs. (1-10) and Table (2).

1- Böhm bristles (BB):

Each sensillum is a triangular peg- like structure that tapers to a blunt apex. They are inserted into a wide sockets (Figs 5, 6). The BB is short having a mean length and diameter of 1.9 ± 0.1 and 0.3 ± 0.08 µm, respectively compared with 1.7 ± 0.1 and 0.3 ± 0.1 µm, respectively for the treated ones (Table 2). The BB sensilla occur on the base of the scape and pedicel at the joints between the scape and the head and between the scape and the pedicel.

2- Sensilla trichodea 1 (ST_1):

The ST_1 sensilla are sharp-tipped hairs and are nearly straight or slightly curved.The hair bases inserted tightly into a small cuticular socket (Figs.3,4,5,6,7,8,9 and 10a,b). They have a mean length and diameter of 10.1 ± 0.8 and 0.5 ± 0.2 µm, respectively compared with 15.2 ± 2.5 and 0.4 ± 0.1 µm, respectively for the treated ones (Table 2). Fusion of some sensilla was noticed in treated ones (Fig. 6). The ST_1 is the most abundant sensilla type on the whole antenna.

3- Sensilla Trichodea 2 (ST_2):

The ST_2 sensilla are blunt-tipped straight hairs. They are located tightly in a small socket among ST_1 (Figs. 3, 7). They have a mean length and diameter of

7.1 ± 1.0 and 0.5 ± 0.2 µm, respectively compared with 7.3 ± 1.0 and 0.9 ± 0.3 µm, respectivelyfor the treated ones (Table 2).

4- Sensilla basiconica 1 (SB_1):

The SB_1 are characterized by a straightly blunt Tip. They are set into a tight socket (Figs. 7,8,10a,b). They have a mean length and diameter of 5.9 ± 1.2 and 0.9 ± 0.4µm, respectively compared with 6.1 ± 1.2 and 0.7 ± 0.1 µm, respectively for the treated ones (Table 2). Most of the sensilla of this type are located on the lateral side of the flagellar segments except the first and second segments.

5- Sensilla basiconica 2(SB_2):

SB_2 are characterized by a blunt tip which curved at the distal end. They are inserted into wide sockets (figs.7,8,10a,b). They are short having a mean length and width of 1.7 ± 0.5 and 0.6 ± 0.1 µm, respectively compared with 3 ± 0.4 and 0.9 ± 0.1 µm, respectively for the treated ones (Table 2).

In addition, some pores appeared in treated sensilla (fig.10 b). The distribution of this type of sensilla likes that of the above mentioned type.

6- Sensilla Chaetica (SC):

SC are straight hairs with blunt tips. They are inserted into a wide socket (fig. 10a & b). The sensilla had a mean length and width of 10 ± 2.6 and 1.0 ± 0.4 µm, respectively compared with 16.4 ± 0.9 and 1.0 ± 0.1 µm, respectively for treated ones. They occur on each antennomere of the antennae.

7- Grooved pegs (GP):

This type of sensilla is characterized by grooved surface and straight pegs with blunt tip (Figs.6,7). GP are bulb-like structures projecting from a depression in the center of raised area of cuticle. An accurate measurement of sensillum length could not be made.

From the above mention types, table (2) showed a significant difference between the lengths and diameter of most types of sensilla on the untreated and treated antennae in this study.

DISCUSSION

The general structure of the antenna of *Callosobruchus maculatus* is similar to that in other beetle species (Barlin and Vinson, 1981; Ritcey and Mclver, 1990; Skilbeck and Anderson, 1996; Merivee *et al.*, 1999, 2001; Said *et al.*, 2003 and Hu *et al.*, 2009).

Observations of SEM illustrate that the antennal morphology and sensilla of untreated *Callosobruchus maculatus* and Lufenuron treated have seven different types of antennal sensilla.

Three functional types of insect sensilla have been identified in immature insects, aporous, that are mostly mechanosensilla, but include many thermo-hygrosensilla; uniporous that may be gustatory chemosensilla or chemomechano-sensilla and mutliporous, olfactory chemosensilla (Altner, 1977 and Zacharuk, 1980). The sensilla of insects consists of a specialized sensory cuticle innervated by the dendrites of one or more sensory neurons and usually three or four accessory cells that ensheath the neurons and associated sinuses (Zacharuk, 1985).

The Böhm bristles sensilla (BB) described in the current study are similar to the sensilla (Hair plate sensilla) on the antennae of the cabbage stem flea beetle *Psylliodes chrysocephala* (Bartler *et al.,* 1999); the click beetles *Limonius aeruginosus* (Merivee *et al.,* 1998); *Bembidion properans* (Merivee *et al.*, 2002) and (Hu *et al.,* 2009) on bruchid beetles *Callosobruchus chinensis* and *Callosobruchus maculatus* the presence of the BB on the scape and pedicel suggests that these might be mechanoreceptors (Schneider, 1964 and Zacharuk, 1985). Also, their presence at the intersegemental joints between the scape and head and pedical in other insects, indicates that these sensilla probably perceive the antennal position and movements (Merivee *et al.,* 2002).

Sensilla trichodea 1 (ST_1) has been described in different insects as having putative mechanoreceptive functions, such as in the perception of mechanosensory stimuli (Pettersson *et al.,* 2001; Roux *et al.*, 2005 and Onagbola and Fadamiro, 2008).

Keil (1999) cited that trichoid sensilla may be olfactory, but sensilla found on the pedicel are usually mainly mechanoreceptive. Schneider (1964) suggests that trichoid sensilla may be dye-permeable and so may posses chemoreceptivity.

Sensilla Trichodea 2 (ST_2) are common on the antennal flagellum of insects. Merivee *et al.,* (1999), suggested that they probably function as sex pheromone receptors. In the ground beetle, *Platynus dorsalis* (Merivee *et al.,* 2001) it might indicate that these sensilla probably respond to aggregation pheromone.

In the present study, the sensilla chaetica (SC) are found in all parts of the flagella. These observations have been recorded by many authors in other insects (Merivee *et al.,* 1998, 1999, 2002; Said *et al.,* 2003; Roux *et al.,* 2005; Faucheux *et al.,* 2006; Gao *et al.,* 2007 and Onagbola and Fadamiro, 2008). The SC sensilla believed to have a dual function of mechanoreception and contact chemoreception. The lack of wall pores and the presence of a tubular body suggests a combined mechanosensory and gustatory function (Zacharuk, 1980, 1985). Similar gustatory sensilla have been reported in the antennae of several species of beetles including the pine weevil, *Hylobius abietis* (Mustapara, 1973) and in the saw toothed grain beetle , *Oryzaephilus surinamensis* (White and Luke, 1986). Antennal tapping of leaf surfaces prior to feeding probably exposes these gustatory sensilla to tactile and chemical stimuli.

The sensilla basiconica 1, 2 (SB_1) and (SB_2) resemble the sensilla basiconica type I and type II in the eucalyptus woodborer, *Phoracantha semipunctata* (Lopes *et al.,* 2002) and to their homologous species which described by Hu *et al*. 2009 in *Callosobruchus maculatus* and *Callosobruchus Chinensis.* These sensilla may have a sex-pheromone receptor role and olfactory function.

The grooved pegs in *Callosobruchus maculatus* are similar in shape and small number to the following "sensilla styloconicum in the cigarette beetle *Lasioderma serricorne* (Okada *et al.,* 1992); sensilla basiconica type IV in *Limonius aeruginosus* (Merivee *et al.,* 1998) and Basiconica capitate peg sensillum (B.C.P.S) in *Liporrhopalum tentacularis* (Timothy, 2005). The probable function of these sensilla is chemo-or thermoreception (Zacharuk, 1985).

In thermo-hygrosensilla, the sensory is an aporous peg. The tip of the dendrites of the humidity sensitive neurons are presumed to be the ones that are tightly encased within the peg (Altner and Loftus, 1985). These pegs are usually also thermosensitive.

The present study showed that treatment of adult beetles with 9 ppm of Lufenuron caused abnormalities in the shape of the antennae and the length of sensilla were longer than the untreated one except (BB). Fusion of some sensillae was also observed in treated sensilla trichodea. Besides, some pores appeared in sensilla basiconica .These abnormalities may be attributed to the effect of insect growth regulator indirectly on the release of ecdysteroids, through interfering with the neuroendocrine sites responsible for the release of this hormone. In agreement with Zohry (2008) who revealed that LC_{50} of flufenoxuron caused the formation of abnormal antennae and affect the length and distribution of the sensilla on *Spodoptera* *littoralis.* Similarly, Schmutterer *et al.,* (1993) observed morphogenetic defects on antennae due to treatments of nymphs of desert locust, *Schistocerca gregaria* and the red locust, *Nomadacris septemfasciata* with neem oil. Also, Akyurtlakli and Karacali (2000) and Hazarika and Baishya (1997), observed malformed antennae on *Melanogryllus desertus and Dicla dispa armigera* as affected by Juvenile hormone analogue methoprene.

In conclusion, the present study has identified and characterized the different sensillum types on the antenna of *Callosobruchus maculatus.* At least, two olfactory sensilla, two sex-pheromone receptors, and two chemoreceptors are described on the antennae of untreated and treated beetles with CSI (Lufenuron). Future studies on the functional morphology of the antennal sensilla using (TEM) transmission electron microscopy coupled with electrophysiological recordings must be performed to confirm the function of the different sensilla identified in this study.

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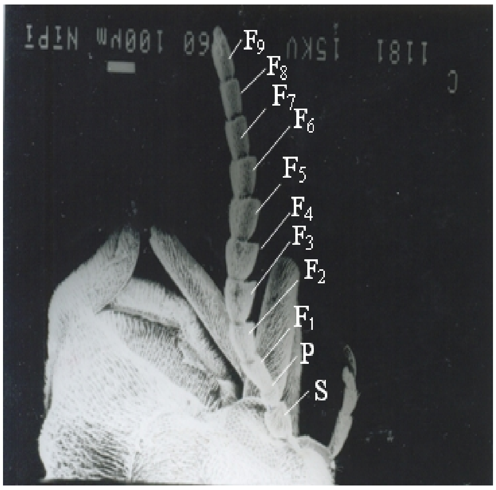

Fig. (1): SEM photomicrograph of untreated adult, *Callosobruchus maculatus* showing parts of the antenna: Scape (S), Pedicel (P) and nine flagellar segments (F_1- F_9). Bar 100 µm.

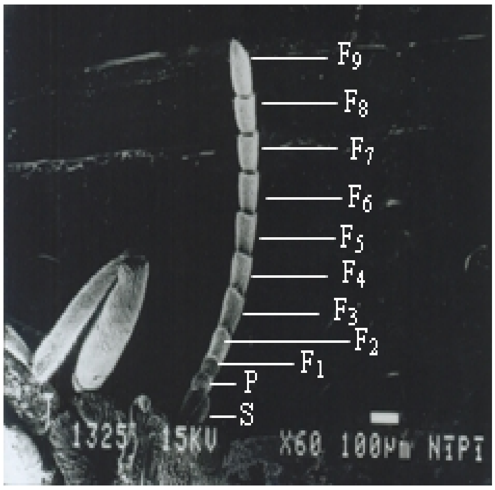

Fig. (2): SEM photomicrograph of treated adult of *Callosobruchus maculatus* with Lufenuron showing parts of the antenna: Scape (S), Pedicel (P) nine flagellar segments (F_1- F_9). Bar 100 µm.

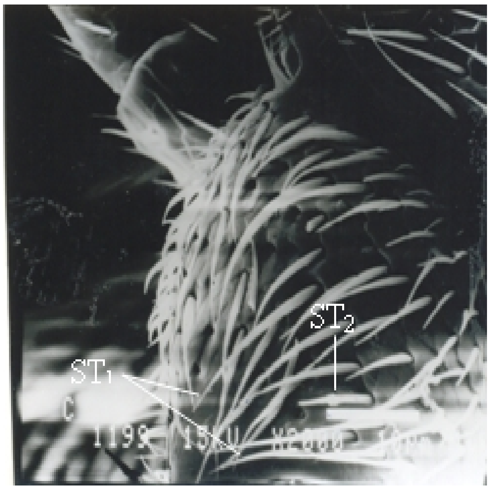

Fig. (3): SEM photomicrograph of untreated adult of *Callosobruchus maculatus* in scape region showing sensilla trichodea (ST_1 and ST_2). Bar 100 µm.

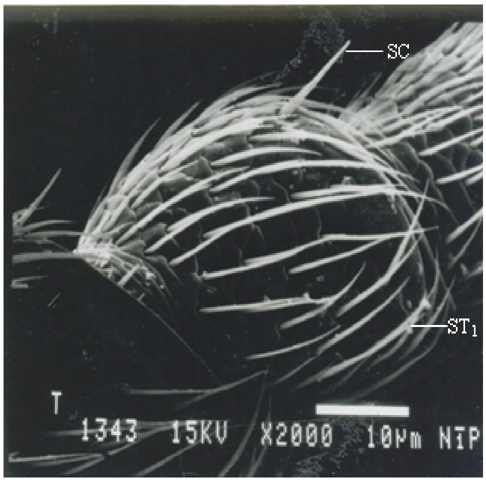

Fig. (4): SEM photomicrograph of treated adult of *Callosobruchus maculatus* with Lufenuron in scape region showing sensilla trichodea (ST_1) and sensilla chaetica (SC). Bar 100 µm.

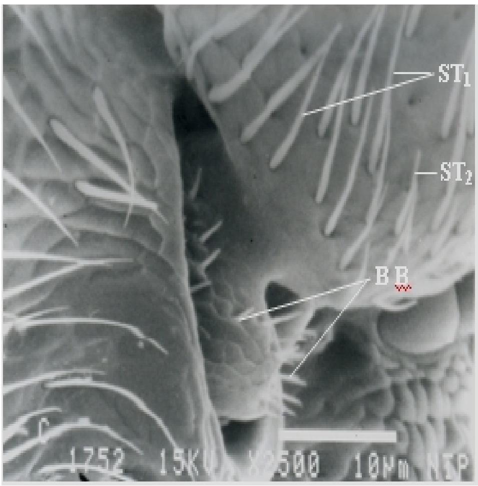

Fig. (5): SEM photomicrograph of untreated adult of *Callosobruchus maculatus* in pedicel region showing (ST_1, ST_2) sensilla triochodea, Böhm bristles sensilla (BB) Bar 100 µm.

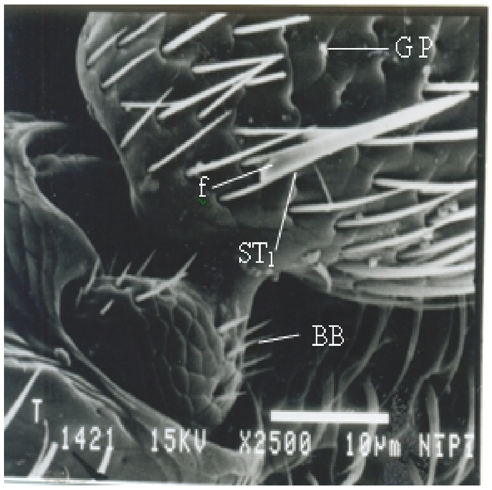

Fig. (6): SEM photomicrograph of treated adult of *Callosobruchus maculatus* with Lufenuron in pedicel region showing fusion (f) of sensilla trichodea (ST_1), Böhm bristle (BB) and grooved peg (GP). Bar 100 µm.

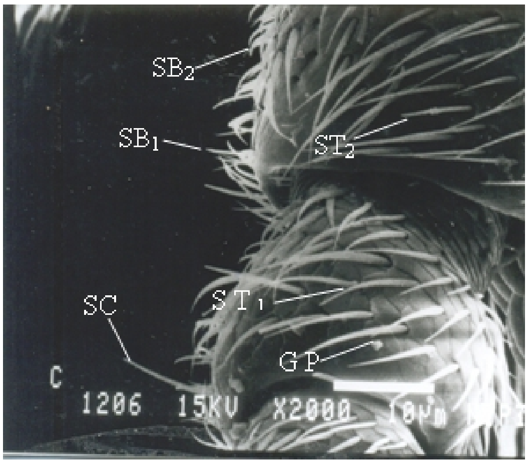

Fig. (7): SEM photomicrograph of untreated adult of *Callosobruchus maculatus* of flagellar segment showing sensilla trichodea (ST_1, ST_2), grooved peg (GP) sensilla chaetica (SC) sensilla basicanica (SB_1, SB_2). Bar 100 µm.

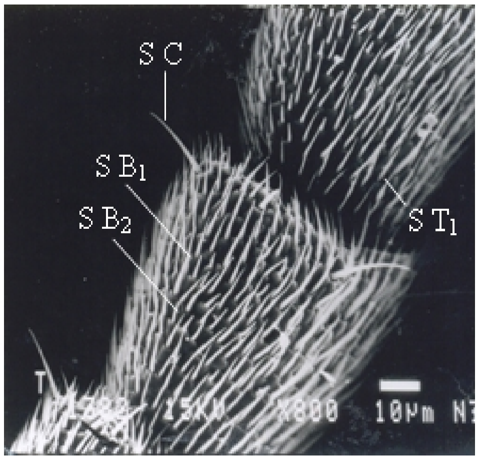

Fig. (8): SEM photomicrograph of treated adult of *Callosobruchus maculates* with Lufenuron of flagellar segment showing sensilla trichodea (ST_1), sensilla chaetica (SC) and sensilla Basiconica (SB_1, SB_2). Bar 100 µm.

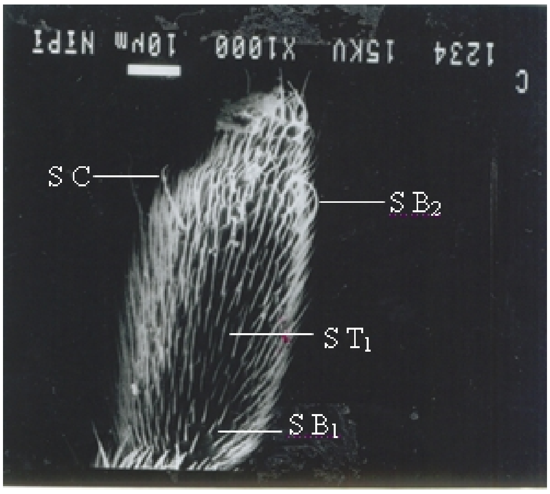

Fig. (9): SEM photomicrograph of untreated adult of *Callosobruchus maculatus* of last flagellar segment (9) showing sensilla trichodea (ST_1), sensilla chaetica (SC) and sensilla Basiconica (SB_1, SB_2). Bar 100 µm.

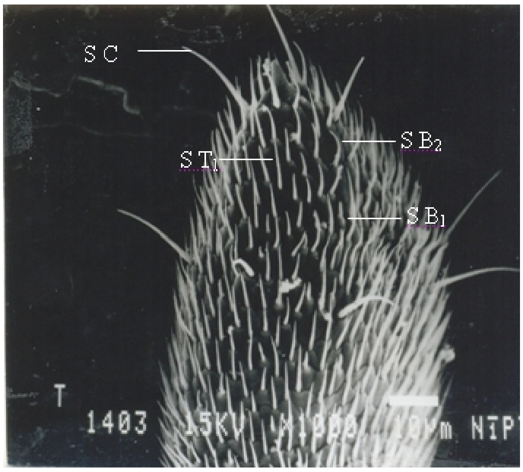

Fig. (10a): SEM photomicrograph of treated adult of *Callosobruchus maculatus* with Lufenuron in flagellar segments showing sensilla basiconica (SB_1, SB_2). sensilla trichodea (ST_1) and sensilla chaetica (SC). Bar 100 µm.

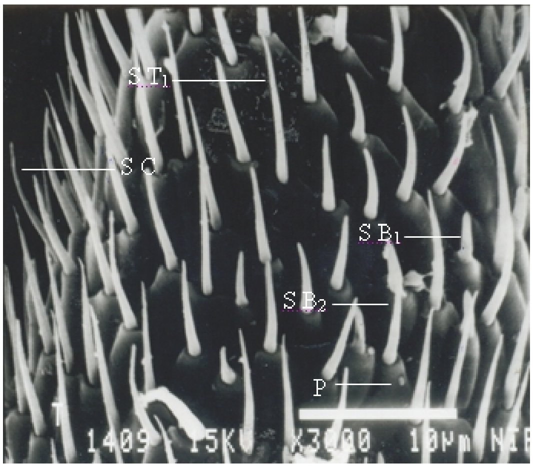

Fig.(10b): Higher magnification of photomicrograph of SEM of treated adult of *Callosobruchus maculatus* with Lufenuron in flagellar segments showing pores (P) in sensilla basiconica (SB_2). sensilla trichodea (ST_1), sensilla chaetica (SC) and sensilla Basiconica (SB_1, SB_2) Bar 100 µm.

ARABIC SUMMARY

التغيرات المورفولوجيه المستحدثه في قرون استشعار خنفساء اللوبيا كالوزوبروكس ماكيولاتس(فابريشيس) (كوليوبترا: بروكيدى) بعد المعامله باللوفينرون.

سريا الطنطاوي حافظ – رجاء قطب عبد الجابر حامد - ليلى سيد حموده
قسم علم الحشرات - كليه العلوم – جامعه عين شمس

استهدف البحث دراسه تأثير مثبط النمو الكيتينى (لوفينرون) على حلقات قرون الاستشعار و شعيراتها الحسية لخنفساء اللوبيا كالوزوبروكس ماكيولاتس (فابريشيس) باستخدام الميكروسكوب الالكترونى الماسح حيث تم تميز سبعة أنواع من الشعيرات الحسيه لقرون الاستشعار فى كل من الخنافس غير المعاملة والمعاملة بتركيز 9 جزء من المليون من اللوفينرون و هذه الشعيرات(Böhm bristles (BB و نوعين من الشعيرات Tricodea (ST_1,ST_2)، **ونوع** sensilla chaetica (SC) ونوعين من شعيرات (SB_1,SB_2) Basiconica , **ونوع** groove peg (GP).

ولقد تم مناقشه وظائف هذة الشعيرات تبعا لموقعها على قرون الاستشعار. **ولوحظ اختلاف معنوى فى** اطوال أغلب حلقات قرون الاستشعار بين الخنافس المعامله و غير المعامله. وسجل اختلاف معنوى فى طول وعرض كل الانواع تقريبا. وكانت أكثر زيادة فى طول الشعيرات للشعيرة من النوع (chaetica) ثم الشعيرة (Tricodea).

وهذه الانواع تستخدم بواسطة الحشرات كمستقبلات حسية ميكانيكية، مستقبلات للفرمونات الخاصة بالجنس و كمستقبلات تذوقيه التى تساعدها فى اتخاذ القرار لاختيار أماكن تغذيتها و وضعها للبيض، مما يوضح امكانية استخدام هذا المركب فى مكافحة هذه الآفة.