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Impact of Gamma Radiation on Male Proboscis of *Rhynchophorous ferrugineus* (Olivier, 1790) (Coleoptera: Curculionidae)

Eman Ahmed MAHMOUD[1] Ahlam GABARTY[2*]

[1]Biological Applications Dept., Nuclear Research Center, Atomic Energy Authority, Cairo, EGYPT
[2*]Natural Products Research Dept., National Center for Radiation Research and Technology, Atomic Energy Authority, Cairo, EGYPT, e-mail: ahlamalgabarty@yahoo.com

ABSTRACT

Red palm weevil, *Rhynchophorous ferrugineus* (Olivier, 1790), (Coleoptera: Curculionidae) is the most dangerous pest for date palms in the Middle East, especially the Arab Gulf countries. The proboscis sensilla in males are studied by using Scanning Electron Microscopy (SEM) to assess the effect of gamma irradiation with different doses. Eight types of sensilla have been observed; four types of coeloconic sensilla (I, II, III, IV) are distributed on the lateral and frontal cuticular surface of the proboscis with a chemoreceptors function. Trichodea sensilla (I, II) and coeloconic sensilla (V) are concentrated on the dorsal surface of proboscis and are arranged in two rows to serve an olfactory and mechanosensory function. A very few number of squamiform sensilla (I) are scattered on the anterior-lateral side of proboscis to perform a mechanoreception function. Such malformation that gave rise to morphological structures of sensilla after exposing the pupae to the doses 10, 15, and 20 gray of gamma radiation are discussed. Irradiation with dose 10 Gy had a slight effect on the different types of sensilla as compared to the control, whereas at the radiation doses (15, 20 Gy) percentage of malformation are increased. The information gained from this study shows that the doses 15, 20 Gy have affected most types of sensilla and that the coeloconic sensilla (IV) are the only type that is not affected by any dose of irradiation.

Key words: Gamma radiation, *R. ferrugineus*, proboscis, sensilla.

INTRODUCTION

Infestations of red palm weevil (RPW) have been reported in over 50% of the date palm growing countries, sparing none in the Middle East (Faleiro, 2006). Injured trees release highly volatile compounds that attract male red palm weevils (Gunawardena *et al.,* 1998). After their arrival, males secrete aggregation pheromones that attract all males and females. Transfer of date palm offshoots as planting material has played a major role in rapid proliferation of the pest in the Middle East (Abraham *et al.,* 1998). RPW is considered as a wound parasite because infestation usually starts in the wounds. One of the main causes of wounds is the detachment of offshoots from the mother tree. Infestations, therefore, mostly occur in the lower part of the trunk, less than one-meter above the soil surface (Lukmah and Alquat, 2002).

Chemical communication between insects and host plants is one of the aspects of insect chemoecology. By clarifying the mechanism of chemical communication between an insect and its host plant, chemoecological research provides the theoretical basis for integrated management. Receptors are the main tools of insect chemical communications and are mainly located in the antenna (Liu, 2006). Adult antennae of many insect species possess various types of sensilla with different functions that play important roles in various behaviors during adult life (Gao *et al.*, 2007). Antennal sensilla, detecting various stimuli, are implicated in the recognition of food, hosts, or partners (Onagbola *et al.*, 2008). Receptors adjust feeding preferences (De Bore, 2006), recognize host plant odors (Skiri *et al.*, 2005), and play important roles in insect survival. The types of sensilla on head and rostrum of RPW were studied by Sharaby and Al-Dosary (2006). They identified three types of sensilla; trichoid sensilla I, II and coeloconic sensilla. These sensilla may be recognized as pheromone-receptors or mechanoreceptors.

The main purpose of this paper is to describe the divergence in morphological characteristics of proboscis sensilla and their socket in males RPW by gamma irradiation, as a more careful step to understand the dynamic effect of rays on the chemical communication between insect-insect and insect-plant.

MATERIALS AND METHODS

Insects

Red palm weevil adults were obtained from cocoons collected from infested date palm trees in the Menasheet El Keram and Tel Bani Tamim-Qalyubia-Egypt. The maintained pupae are used in laboratory of Atomic Energy Authority-Inshas-Central Labs-Kebab Building, according to the method described by (Mahmoud and Shoman, 2009). The pupae were transferred in cylindrical plastic dishes (15cm in depth x 37cm in diameter) with an amount of moist tissue fibers of sugarcane output from adult and larvae feeding. They were also kept at controlled laboratory conditions (27°C and 85% RH) and daily inspection was carried out until the adult emergency. The adult weevils were transferred into cylindrical glass jars (16cm in depth x 8 cm in diameter) with pieces of sugarcane as food, which was renewed almost every two or three days. Jars were covered with a network of metal wires.

Irradiated Treatments

From 25 to 30 RPW pupae (about 25 days) were kept in plastic containers (11cm in depth, 16 cm in diameter) and irradiated with 10, 15, 20 Gy of gamma radiation, using gamma cell-40 (cesium-137 irradiation unit), at the National Center for Radiation Research and Technology (NCRRT, Cairo).The one-day-old emerged males were separated and they fed on reeds. Three males were taken from each group. The dose rate was 0.758 rad/sec at the time of the present investigation.

Scanning Electron Microscopy Studies

As for the examination of the proboscis of freshly killed normal and irradiated adult males, RPW of 5 old days were removed from the head cleaned in distilled water, immersed in 2.5% glutaraldehyde for 24 hours and then washed three times with 0.1 M phosphate buffered saline (PBS) (pH 7.4). The samples were fixed in osmic acid for 2 hrs and then underwent a series of ethanol concentrations (50, 70, 80, 90, 95, and 100%) for 15 mins for each (Kang *et al.*, 2012). The proboscis was attached vertically to aluminum stubs and air-dried before getting coated with gold in a vacuum evaporator (Hazaa, 2010). The examination was conducted by using the Scanning Electron Microscope (SEM) (Joel JSM 5400) with an accelerating voltage of 10 kV.

Statistical Analysis

All data obtained were statistically analyzed and the variance ratios were calculated. The method of ANOVA is involved by using (SPSS) computer program, ver.15.0, and the significance among the samples was calculated at $P \leq 0.05$.

RESULTS

The head of red palm weevil is a prognathus type, bearing two large compound eyes as photoreception; there is one compound eye at each lateral side at the base of the proboscis of the head (Fig. 1). Optical dierentiation between male and female adults from the morphological feature of the proboscis and the hairbrush-like structures at the terminal 1/2 of the dorsal side for males, are absent in females. These brush hairs may be recognized as pheromone or mechanoreceptors (Fig. 1). There are different kinds of sensilla on this proboscis as follows:

Coeloconic Sensilla (CI)

Large number of CI is distributed on the lateral and frontal surface of the proboscis recorded as 176.7±25 (Fig. 2B and Table 1). Under high magnification, the sensilla appear to be formed of multiple straight tubules directed upward and giving a blunt shape, opening at the apex with many ridges on the surface wall of the sensilla whether they are broad or not. Each one arises from a narrow socket. These sensilla range from about 30.7 to 39.8 μm in length, and from about 53 to 63.6 μm in diameter at its base (Fig. 2C).

Coeloconic Sensilla (CII)

CII located in few numbers on the lateral surface of the proboscis recorded about one. The fingers are elongated and taper apically, with some tilt around the other, and appear to be directed downwards towards the basal margin of proboscis (Table 1). They emerge from a well-defined wide socket (finger-like). These sensilla range from about 18.9 to 21 μm in length, and are about 15.4 μm in diameter at its base (Fig. 2D).

Coeloconic Sensilla (CIII)

These sensilla found on the anterior part of the lateral and frontal surface of the proboscis recorded about 40.3±0.9 (Table 1). The finger-like processes of the sensilla are long and fused together and their tip is blunt. They extend with the same diameter and decrease apically in size. Most of the sensilla grow out from wide sockets with an amount of cuticular glands attached to some sensilla at bases and are more perpendicular on the antenna than other coeloconic sensilla. They outnumber the sensilla type CII and extend vertically on the proboscis surface at a different length. These sensilla range from about 15 to 26.9 μm in length, and from about 3.7 to 5 μm in diameter (Fig. 3A).

Coeloconic Sensilla (IV)

The sensilla IV are represented as 5 rows found on the frontal surface and as one or two rows around the trichiod sensilla on the lateral surface of the proboscis as about 62.3 (Figs. 2A, 3B, Table 1). These immovable hairs resemble sensilla CI but have various sizes. In addition, some of their tips are pointed and some are broad and long. They grow out from very narrow sockets where their bases of fingers occupy all cavities, attached to the margin of socket and are more perpendicular on the antenna than other sensilla. They appear pyramidal in shape, and their height gradually increases, ranging from about 31.4 to 93 μm, and about 71 μm in diameter as illustrated in Fig. 3B.

Squamiform Sensilla (SI)

A very few number of SI was found on the anterior lateral surface of proboscis, equal to about one sensilla (Fig. 2D, Table 1). The sensilla are smooth-walled hairs, arising from cuticular raised glands at its base with wide articulatory socket. Their height gradually and slightly increases, with a diameter at the base of about 1.9 μm. to be about 6.5 μm and grow less in size to about 4.2 μm at the tip. These sensilla are more curved from the base over the whole surface of the proboscis. They arise nearly from the center of the fine, broad-tipped, finger-like process at its end base, measuring about 6.5 μm in length and about 1.5 μm in diameter.

Trichoid Sensilla (TrI, TrII)

The sensilla TrI, TrII with different lengths are illustrated on the dorsal surface of proboscis. They are arranged clearly in two rows, with narrow spaces between them of approximately 192 μm, and combined the rows from the front, whereas the two sides remained as separated posterior (Figs. 2B, 4A). Each row is a set of rows ranging in number from three to four. The surface of sensilla appeared striated; they consist of fine long tubules that unite together (Fig. 3C). The sensilla seem vertical on the proboscis surface, near the base, forming an angle of about 45° and back again in a vertical-ascending in curvature; they are adherent to each other and dense. They stand aside from an articulatory and prominent socket with an amount of glands (Figs. 2B, 3C). They are long or short with blunt tipped sensilla. TrI were recorded at about 21.7±0.8 and ranged from 166 to 216 μm in length, 23 to 26 μm in diameter, whereas TrII were recorded about 160.3±1.5 and ranged from 63 to 83 μm in length, 15 to 21 μm in diameter (Table 1). The shortest sensilla are found in the frontal and lateral parts while the longest sensilla are behind them. They are short only in the distal row (Fig. 2B).

From the same socket of TrI, TrII arise CV attached to their base (Fig. 5A).Some of these sensilla resemble the sensilla (CII) with some differences in size, about 76.1 μm in length and nearly 30.4 μm in diameter at their base (Fig. 3C). The other parts of CV are pyramidal in shape and are 26.6 μm in length and about 29.7 μm in diameter at their base (Fig. 5A).

Apodemes

The surface of cuticle in RPW contains a large number of internal processes or bands (gab through the cuticle), which appear to be light or dark. Arcuate patterns seem to be superimposed in these bands. These bands contain a matrix of tinny protrusions (or filaments) on their internal margin that is internally oriented (Figs. 3B, D) and meet with the other filaments on the opposite side as funnel in shape. The protrusions appeared to be of an open type in the normal state (Fig. 3D). These bands are with different size and length along the cuticular surface between the different sensilla. Their orientation is in all direction at the anterior and lateral surface while most of them are in the dorsal surface between the two rows of Tr that are horizontal in direction (Figs. 2B, C).

Glands

A number of glands could be illustrated on the anterior and lateral side of proboscis between CI, CII (Fig. 2D). However, they were found among CIII in few numbers. Most of them are circular in shape while few are rectangular and vary in their diameters (Figs. 2D, 3D).

The Effects of Gamma Radiation

Proboscis Sensilla

It is obvious that irradiation with dose 10 Gy had a slight effect on the features of the different types of sensilla as to with the normal state. Some CI, CIII changed in appearance and recorded 27.7±1.9, 5±0.6 (Table 1). The base of sensilla became less dense especially in the central part, but it become flattened at the apex, diffused and grew less dense and blunt. A number of tubules-like processes of CI and CIV became aggregated in two groups and their apical parts were ruptured (Fig. 4B). Also some CIII, cuticular glands attached to its bases are disappeared. Whereas the tubules of TrI, TrII seemed to be slightly separated on the terminal part and recorded about 8±0.6, 55±2.9 respectively (Fig. 4A).

On the other hand, with the increase of radiation doses (15, 20 Gy), the changes in CI are as in dose 10 Gy but with an increase in number of malformations from about

42.3±1.5 to 65.3±4.2 (Figs. 5B, 6B). As the sensilla CIII appeared to grow out from dipped sockets, small numbers of cuticular glands were attached to the base of sensilla and a percentage of malformations increased from about 9±0.6 to 13.3±0.8 (Figs. 5C, 6C). Additionally, in dose 15 Gy some of TrI, TrII were clearly and drastically affected where their tubules were completely separated and some of them were ruptured and recorded 15±0.5 and 93±4, respectively, (Fig. 5A). Most of TrI, TrII lost their normal curvature and adherence at the dose level 20 Gy and also recorded about 19±0.3, 137.7±4.6, respectively, in significant to control at ($p \leq 0.05$) (Fig. 6D, Table 1).

Apodemes

The appearance of podemes differed due to the use of different doses of gamma radiation. The variances in size and depth were illustrated and increased with the increase of the doses (Figs. 4C, 5B, 6C). They appeared to be of an open type in the dose 10 Gy (Fig. 4C) and seemed to be few in the dose 15 Gy (Fig. 5C), whereas the majority of closed apodemes appeared in the use of doses 15 and 20 Gy (Figs. 5B, 6B, C).

Table 1. The deformity induced by gamma irradiation on the different types of male proboscis sensilla of *R. ferrugineus*.

Dose (Gy)	Types of Sensilla Mean±SE						
	CI	CII	CIII	CIV	TrI	TrII	SI
Control	176.7±14.5	1	40.3±0.9	62.3	21.7±0.8	160.3±1.5	1
10	27.7±1.9*	-	5±0.6*	-	8±0.6*	55±2.9*	-
15	42.3±1.5*	-	9±0.6*	-	15±0.5*	93±4*	-
20	65.3±4.2*	-	13.3±0.8*	-	19±0.3*	137.7±4.6*	-

The mean equals 3 replicate for each group-SE is the stander error-the mean difference is significant at the 0.05 level.

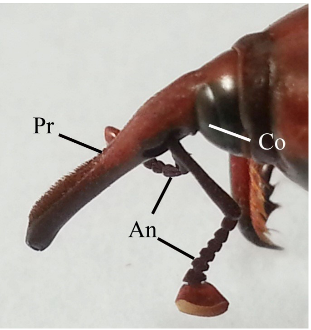

Fig. 1. Photograph of male head *R. ferrugineus* showing the sensilla at the dorsal side of proboscis. (antennae=An, proboscis=Pr, compound eyes=Co).

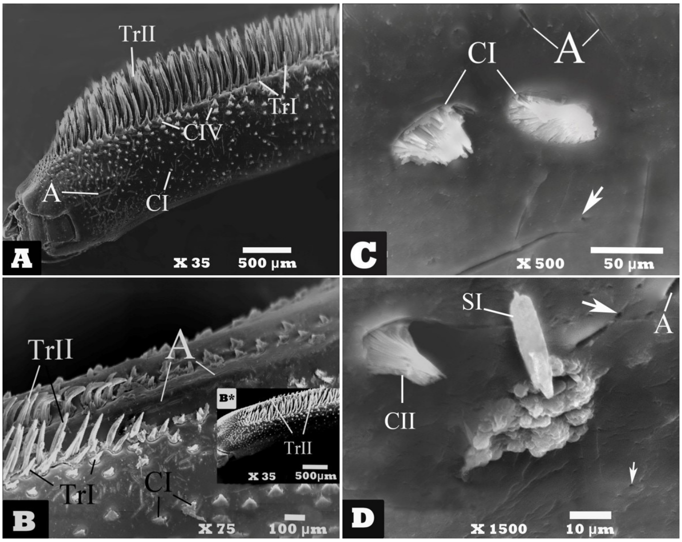

Fig. 2. (A) Types of sensilla located on the lateral view to normal proboscis. (B) Type I, II of trichoid sensilla (Tr) located on dorsal view. (C) Type I of coeloconic sensilla (C) were distributed on the lateral and frontal surface. (D) Type II of coeloconic sensilla and squamiform sensilla (SI) were located in very few numbers on the anterior lateral surface (Apodemes=A and the arrows represented different size for glands).

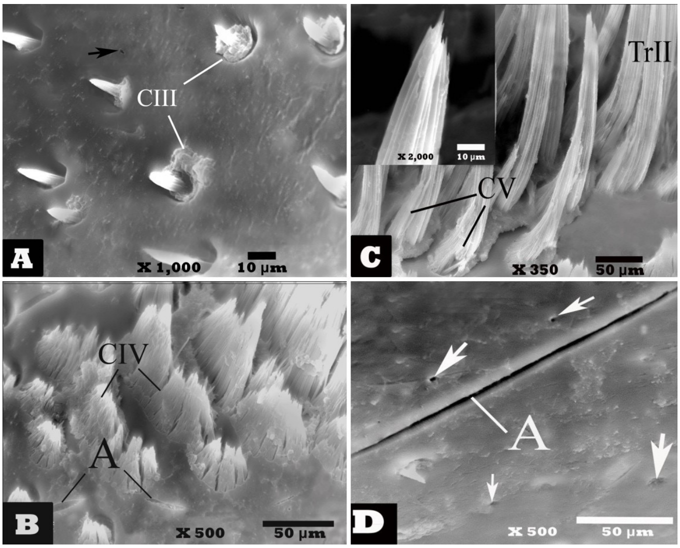

Fig. 3. (A) Type III of coeloconic sensilla arising from wide sockets with amount of cuticular glands attached to its bases. (B) Type IV of coeloconic sensilla represented as 5 rows found on the frontal surface. (C) From the same socket of trichoid sensilla arise type CV of coeloconic sensilla attached to their base. (D) The opened apodemes contain matrix of tinny protrusions on its internal margin oriented perpendicular internally and the arrows represented different size for glands.

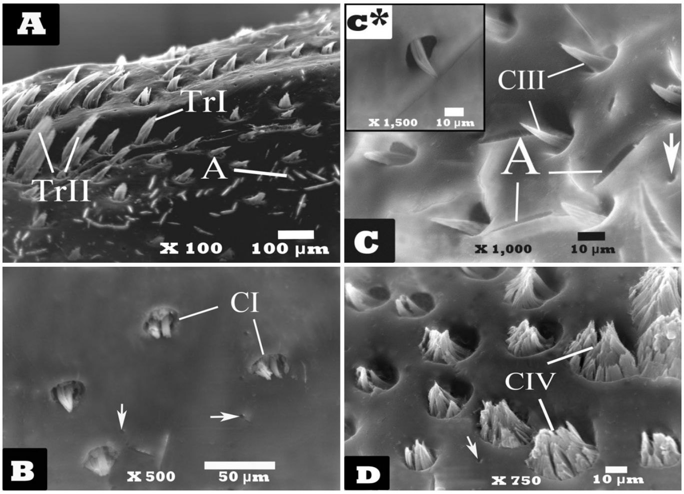

Fig. 4. Irradiated *R. ferrugineus* with 10 Gy of gamma rays. (A) A number of tubules TrI, TrII seemed to be slightly separated on the terminal part. (B) The fingers of CI became aggregated in two groups and their apical parts were ruptured. (C) In most CIII, cuticular glands attached to its bases were disappeared. (D) Ruptured to some apical parts of tubules-like processes in CIV.

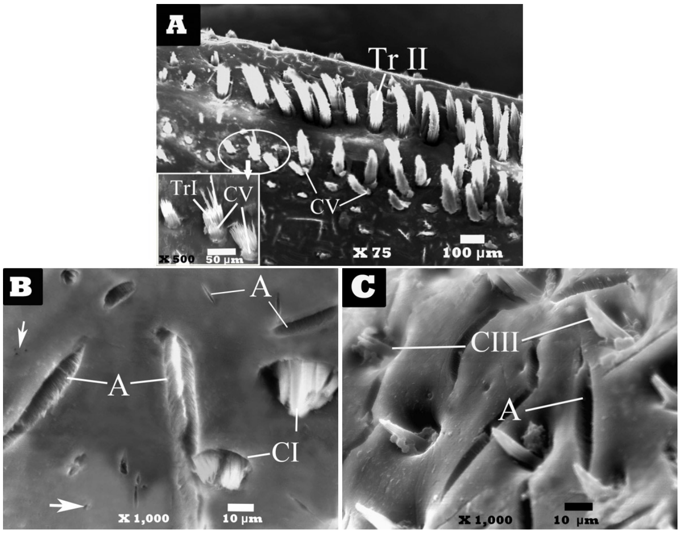

Fig. 5. Irradiated *R. ferrugineus* with 15 Gy of gamma rays. (A) Malformation on TrI, TrII. (B) The varying in sizes and deep of apodemes. (C) Irregular tubules of CI, CIII.

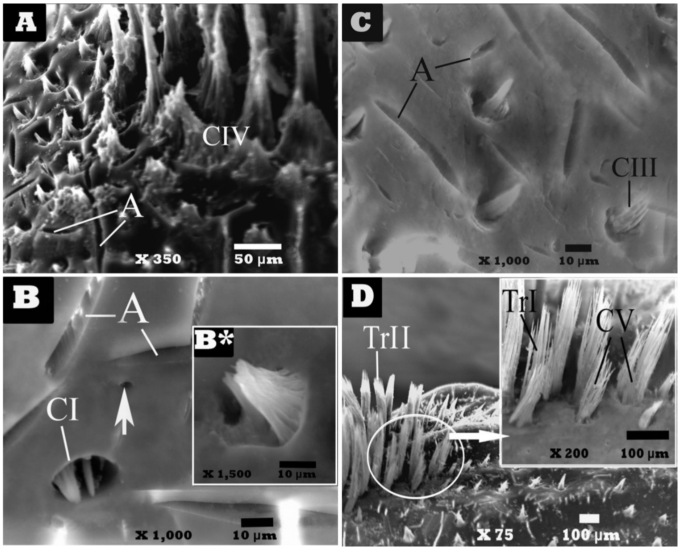

Fig. 6. Irradiated *R. ferrugineus* with 20 Gy of gamma rays. (A) shrinkage and atrophy of CIV. (B) Highly irregular sizes of closed apodemes. (C) increased malformations in CIII. (D) Most of TrI, TrII tubules were completely separated and some of them were ruptured and the arrow represented the gland.

DISCUSSION

The obtained results indicated that there were eight different sensilla identified along the proboscis of male's *R. ferrugineus*. All sensilla observed, in this study could be displayed external morphologies similar to those displayed by the previous studies performed on antenna (Mahmoud *et al.*, 2011), and ovipositor of (Sharaby and Al-Dosary 2006, 2007) of *R. ferrugineus*. According to Baker and Ramaswamy (1990) the striations on the sensilla surface are regarded as one of the characteristic features of trichod sensilla. It has been suggested by Merivee *et al.* (1997) that asymmetries in the distribution pattern of olfactory sensilla on antennae might be due to the peculiarities of their behavior (waiting, walking, flying, antennal movement), which cause certain areas of the antennal surface to catch the wind-borne odour molecules more effectively than the others. These results are largely in conformity with those results reported in our study; the most numerous trichoid sensilla are arranged into two rows that exist on the dorsal surface of proboscis. Each row is a set of rows ranging in number from three to four. These sensilla are considered as pheromone receptors in the beetles *Hylobius abietis* (Mustaparta, 1973), and *Psacothea hilaris* (Castrejon-Gomez *et al.*, 1999).

The coeloconic-sensilla distributed along the proboscis of RPW resemble the sensilla recorded on the ants and wasps, whose behavior is guided by variations in temperature. Coeloconic sensilla are chemoreceptors that respond to air temperature changes (Rychty *et al.*, 2009). Interestingly, the coeloconic sensilla are reported to have receptor neurons that respond to host plants volatile compounds as described by Pophof (1997).

In addition, very few mechanoreceptor squamiform sensilla were distributed on the anterior lateral surface of proboscis. The same sensilla elucidated on adults of rice water weevil, *Lissorhoptrus oryzophilus* with fluted surfaces, occurring on the escape, pedicel and funicular flagellum (Kang *et al.*, 2012). Likewise, squamiform sensilla were found on elytra surface of *Harmonia axyridis Pallas* and was known as the micro-hair with a length of 8 µm, which was developed in the depression structure and located in the center (Li *et al.*, 2012). The squamiform sensilla I, II were also found on the rostrum, antenna, elytra and tibia of *Oryzophagus oryzae* (Martins *et al*., 2012). In Hix *et al.* (2003) it was confirmed that its function might have to do with in mechanoreceptor and proprioreception.

The microscopic observations of the males *R. ferrugineus* proboscis revealed a number of the glands most of which are circular in shape while few are rectangular. These glands defined by Chen *et al*. (2012) as glandular pores or glands on *Dendroctonus valens* antennal sensilla. Similar glands were also observed in *Chilopod subtaxa* as epidermal glands of differences size, named 'flexo-canal epidermal glands (Müller *et al.*, 2009). These glands have been thought to represent either a kind of lubricant for the antennae and their sensilla or appeasement glands or pheromone glands (Weis *et al.*, 1999). In this work no significant changes could be observed by irradiation in the features of these glands in the treated males. Unfortunately we did not find any research performed on the effect of gamma ray on the insect's proboscis but a lot of research papers have examined its impact on the antennae.

In all insects, the rigidity of the exoskeleton is increased by four deep culicular invaginations, known as apodemes, which meet internally to form a brace for the exoskeleton and for the attachment of muscles (Chapman, 2003). The apodemes reported in RPW have different size and length along the cuticular surface between the different sensilla. The trend of the internal protrusions appeared inward (in funnel-shaped) with the doses of 15 and 20 Gy and this explains its function which may play an important role in regulating the evaporation of water from the body to overcome the rise in temperature. It also helps water balance to preserve its life where the sensitivity of the RPW to dry conditions was studied by Nirula (1956).These apodemes resemble to that illustrated in *Drosophila* where the muscle has detached from one of its apodemes. These effects show that the behavioral studies suggesting a dominant suppression are incomplete (Naimi *et al.*, 2001).

Some malformations were observed on proboscis sensilla of RPW male by gamma irradiation which increased with the increasing dose rays from 10, 15 to 20 Gy. However, only coeloconic sensilla IV were not affected by any dose. Irradiation with gamma rays on first generation of *Spodoptera littoralis* antennal sensilla showed that the trichoid sensilla became low in number and also showed the loss of the central pegs from some coeloconic sensilla. With the increase of doses some spines of the coeloconic were knobbed, plus point, the central pegs were lost (El-Shall *et al.*, 2004).

Furthermore, Hazaa (2010) found that there are no malformations on squamiform or coeloconic sensilla by low doses of gamma rayes but the trichoid sensilla showed swelling of their base on *S. littoralis* or slight warping or that they twisted together with loss in number. With increase of radiation dose, the malformation of sensilla increased as the trichoid sensilla disoriented and collected forming bundles of sensilla and the terminal parts nodulations and the coeloconic shrank in many areas. At the high doses (150 Gy) the density of trichod sensilla became lower than in control at first generation to *Galleria mellonella* male (El-Kholy and Mikhaiel, 2008). Gharieb and El-Degwi (2002) recorded that the length and diameter of trichodea sensilla are affected by irradiation in *Trogoderma granarium* antenna.

Hussien *et al.* (2001) stated that substerilizing doses of gamma radiation (5-12 Krad) reduced the density of trichoid sensilla and caused malformation to some of them in the antenna of the male moth of black cutworm, *Agrotis ipsilon*. The authors observed that the response of these irradiated males to sex pheromones was lower than in control males and its perception decreased with the increasing of the dose applied. These radiation effects were more pronounced in the parental generation than in F1 generation.

CONCLUSION

This result identified eight different sensilla along the proboscis of *R. ferrugineus* male. Most of the trichoid sensilla are either arranged into two rows or distributed with coeloconic and squamiform sensilla along the proboscis. Sensilla can play a role as mechanoreceptor, chemoreceptors and pheromone receptors. A large number of apodemes with different sizes and lengths was also observed on the integument. These apodemes may play an important role in regulating the evaporation of water from the body to overcome the rise in temperature and making water balance to preserve its life. The effect of the doses 10, 15 and 20 Gy of *gamma radiation* on the proboscis were tested. The percentage of malformations increased with the increase of dose, and this may affect feeding behavior and the insect's plant interaction. Moreover, feeding behavior studies are recommended to detect the effect of these deformations on the host seeking behavior of irradiated males.

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