Comparative study of antennal sensilla in irradiated male and female fall armyworm, Spodoptera frugiperda with gamma radiation

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Article

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Abstract

Morphology of the Fall Armyworm (FAW) *Spodoptera frugiperda* adult antenna was photographed by Scanning Electron Microscope (SEM). The type of the antenna is filiform, and consists of three main segments: the scape, pedicel and flagellum. Scanning of antennae revealed the presence of eight types of sensilla in male and seven in female which are Sensillum Squamiformia, Scales, Sensillum Styloconica, Sensillum Trichodea, Sensillum Chaetica lateral and central, Sensillum Coeloconica, Sensillum Auricillica and Sensillum Basiconica which are distributed among the antennal segments. These types of sensilla found in both sexes except Sensillum Styloconica. The effect of four doses of gamma radiation (50, 100, 150 and 200 Gy) on the antennal sensilla were studied. These studies revealed that these higher doses in both sexes have certain malformation features in the sensilla. The magnitude of these malformations was dose dependent as they increased by increment of the doses applied. These malformations in the sensilla and its role in courtship and mating inhibition were discussed.

Introduction

The Fall Armyworm (FAW) *Spodoptera frugiperda* (Biosduval), is native to tropical and subtropical areas of the Americas. More than 80 plant species can be consumed by the larvae (Polyphagous insect) causing major damage as maize, rice, sorghum, sugarcane, vegetable crops and cotton. FAW has several generations per year and has the ability to fly up to 100 km per night causing significant crop losses if not managed well. FAW was first discovered in early 2016 in Benin, Nigeria, Sao Tome and Principe, and Togo in Central and Western Africa. It was then reported and confirmed throughout all of mainland Southern Africa (excluding Lesotho), in Madagascar, and in the Seychelles (Island State). By 30 January 2018 FAW had been detected and reported in almost all Sub Saharan African countries, except Djibouti, Eritrea, and Lesotho [1]. In May 2019, the Agricultural Pesticide Committee (APC) of the Ministry of Agriculture in Egypt reported the first case of FAW presence in a maize field in Aswan Governorate, Upper Egypt, after which it spread too many areas and caused significant losses for this crop. The larvae consume the vegetative and reproductive parts of the plants, the young larvae of which prefer the epidermal tissue of the leaves and make holes in them. Also, dead heart is a symptom caused by feeding of young larvae. As for the mature larvae in the flowers of old plants, they can feed on the corn cob and its kernels, thus reducing yield and quality **[2, 3.]** This insect has marching behavior similar to that of the army causing havoc loss to the crops that come in its path [4]. The FAW is extremely destructive, and **CABI** [5] has predicted that if control measures are not implemented, the pest might result in a loss of 6.1 billion US dollars in solely African countries.

Antennae in insect are organs of taste, smell and stimulation [6]. Other authors [7, 8, and 9] have suggested a possible role of sensory stimuli (olfactory, gustatory or mechanical) in prey, or mate detection. The antennae also play kinetic roles and normally keep the nervous system in a state of tone in which it responds to stimuli of all kinds. Antennae of insects vary greatly in length, overall size, size of the individual segments, segmentation, setation and other aspects with the structures being closely related to their function [10]. The antennal sensory organs of the Fall Armyworm, *S. frugiperda* are responsible for locating the host for laying eggs or feeding, as they are filled with numerous receptors (sensilla) responsible for the vital communication between the insect's nervous system and the outside environment. **Byers** [11] **and Shields & Hildebrand** [12] showed that the design of specialized receptor cells present in these sense organs is convenient for detecting and transmitting information about the nature of the surrounding environment to the central nervous system, which leads to different insect behaviors.

In the field of insect taxonomy, sensilla can be used for classification. However, knowledge about insect sensilla is also necessary for insect control, including biological and chemical control. **Faucheux** [13] who investigated morphology and distribution of sensilia on the male and female antennae of the European sunflower moth, *Homoeosoma nebulella* (Lepidoptera: Pyralidae) by using SEM. Many other researcher studied the external antennal morphology such as **Mayer et al.** [14] by study the antenna of The cabbage looper, *Tricoplusia ni*. Also **Salama et al.** [15] studied the Ultrastructure of chemoreceptors of the American bollworm moth, *Heliothis armigera* antenna.

Many authors had studied the effect of gamma radiation on the sensilla of different insects. **Haiba** [16] on the legs of potato tuber moth, *Phthorimae operculellaea*; **El-Akhdar** [17] on wings and mouth parts of the Mediterranean fruit fly, *Ceratitis capitata*; **Hazaa** [18] on antennal sensilla in the male moth of the cotton leaf worm, *Spodoptera littoralis* and **Zahran** [19] on ovipositor sensilla of the peach fruit fly, *Bactrocera zonata*. These authors found many of malformations and changes in the shape of the sense organs as a result of the effect of gamma radiation.

The aim of this work is to investigate the effect of gamma radiation (50, 100, 150 and 200 Gy) on the external structure and the types of the antennal sensilla on *S. frugiperda* adults (males and females) by using Scan Electron Microscopy (SEM). This information may be useful for controlling this pest by know whether gamma radiation affects the main role of antennae of the released moths for location of the host plant for feeding and other biological behaviors.

Materials And Methods

Insects Culture.

Insects were obtained from Plant Protection Research Institute, Agriculture Research Center, Giza, Egypt. Colonies of *Spodoptera frugiperda* larvae were collected from infected maize plants and reared in plastic boxes (30 × 15 × 10 cm) covered with muslin cloth and contain maize leaves as food which replaced every 3 days. Larvae were reared individual in small glass vials from 3rd instar to overcome a phenomenon of cannibalism and incubated at 26 ± 1°C, 65 ± 5% RH, and 14L: 10D photoperiod until pupation. Pupae were kept in the same incubator until moths emerged. After emergence of moths, each pair of one male and one female were put into glass cage for mating and oviposition and incubated under the previous condition for many generations according to those described by **Kruger** et al. [20].

Radiation technique.

The males and females of *S. frugiperda* pupae (3-day-old) were irradiated with (50, 100, 150 and 200 Gy). The irradiation technique was carried out by a Gamma Cell (Co^{60} source) irradiation unit Model 220 located at National Center for Radiation Research and Technology (NCRRT). At the time of the experimental research, the dose rate was 0.950 KGy/hour.

Scanning electron microscopy.

For examination with Scanning Electron Microscope (SEM), *S. frugiperda* males and females pupae (3-day-old) were irradiated with (50, 100, 150 and 200 Gy). Whole the head of unirradiated and irradiated emerged adults at all doses and control were carefully cut by means of a sharp razor blade; the specimens were quickly dried then mounted on specimen stubs with gold conducting paints. Samples were gold coated in a layer of approximately 300 A°, using a fine gold coating apparatus, ion sputtering device (ZEISS-EVO-15).

Examinations of *S. frugiperda* antenna were carried out by a (ZEISS-EVO-15) scanning electron microscope (SEM) with an accelerating voltage of 30 kV. The antennal sensilla were viewed and photographed directly from the SEM video monitor.

Results

Morphology and function of the normal male and female antenna.

The male and female antennae of fall armyworm, *Spodoptera frugiperda* is filiform type and consists of a basal scape, a pedical and a flagellum. The scape is inserted into membranous region of the head wall and pivoted on a single marginal point, the antennifer, so that it is free to move in all directions. The flagellum is divided into a number of similar sub segments jointed to each other by membranes so that the flagellum as a whole is flexible. These sub segments or flagellomers are small cylindrical and they diminish in length and diameter from the base to the apex of the antennae. The results of the SEM experiments on *S. frugiperda* antennae clearly showed that the female antenna was longer than the male antenna. The flagellum of female has 65–79 segments and 42–53 for male densely packed with sensilla distributed on the ventral surface and lateral edges of the flagellum.

Morphology And Distribution Of Antennal Sensilla In Both Sexes

Examination of the structure of flagellum segments revealed the presence of eight different types of sensilla in male, while in female are seven. They are Sensillum Squamiformia (S. Sq.), Scales (Sc.), Sensillum Basiconica (S.Ba.), Sensillum Trichodea (S. Tr.), Sensillum Chaetica lateral (S.ChI), Sensillum Chaetica 2 central (S.ChII), Sensillum Coeloconica (S.Co.), Sensillum Auricillica (S.Au.) and Sensillum Styloconica (S.St.) which found only in male antenna (Figs. 1&2). On all segments, sensilla trichodea and chaetica were randomly distributed and were the most frequent types.

1. Sensillum Squamiformia (S. Sq.): They typically exist among the flagellum's scales and take the shape of thin scales. These sensilla might be thought of as having a mechanical function because they are able to detect stress in the cuticle brought on by mechanical deformation **Mayer et al.** [14] figure (1A&2A,C).
2. Scales (Sc.): Scales exist with the sensilla on the surface of the noctuid antennae. However, **Van Der Pers et al.** [21] argued that scales do not shield the sensilla from mechanical damage and instead that their position aids an insect's capacity to determine the stimulus's direction. Also, **Wall** [22] contended that moth scales may serve as a mechanism to capture and concentrate odorous chemicals, figure (1A&2A).
3. Sensillum Basiconica (S.Ba): The smallest and least numerous sensilla are these ones. They are rounded at the apex and wider in the base figures (1E, G&2C, D).
4. Sensilla trichoidea (T): It’s a hair-like protrusion of the cuticle that is free to move because it is articulated with the body wall by a membranous socket. It is the sensilla variety that is most prevalent figures (1&2 A to G).
5. Sensillum Chaetica lateral (S.ChI): Its blunt type and the most conspicuous of the antennal sensory organs. They are mainly located near the segment's center and are distributed rather equally all around it. These sensilla are wide at the basal portion, straight, and curved apically, with the tip truncated. The base is put into a cuticular structure-based socket, figures (1A, F, G &2 A, F).
6. Sensillum Chaetica 2 central (S.ChII): They scattered on the scape and pedicel and they shorter than S.ChI, figures (1A, B, C &2 A to G).
7. Sensillum Coeloconica (S.Co): This sensillum are small, sometimes known as pit pegs. They are mainly located in the intermediate and distal parts of the segment. The pegs might or might not stick out through the cuticle's surface circular holes. Sensillum coeloconica's surface has deep grooves running longitudinally, figures (1D, E &2 A to F).
8. Sensillum Auricillica (S.Au): Usually, these sensilla can be seen between the scales. They typically resemble a rabbit's ear or are simply dorsoventrally flattened, but they occasionally ometimes are biforked at the tip, figures (1E &2 B, C, G).
9. Sensillum Styloconica (S.St.): It’s always present at the upper-middle region of each subsegment of the flagella. This type of sensillum neither socket nor pores but has a smooth petiole and a conic extremity with one to three apical features, figure (1A, B, E, G).

Effect of gamma radiation on male antenna.

It is obvious that irradiation dose 50 Gy had a slight effect on the different types of the located sensilla as compared with the control. In (Fig. 2A&B) the sensilla squamiformia, coeloconica, basiconica and auricillicadid not show any malformations, while sensilla trichoidea and chaetica showed some nodulation on their edges. Also disorientation and slight warping in trichoidea sensilla was happened. The styloconica shrank in many areas and its end became impaired. Also, some fallen scales were observed at certain parts of the flagellum segments. On the other hand, with increase of radiation dose, the sensilla malformations were increased as observed at 100 Gy where the nodulation of trichoid sensilla was increased but not noticed in chaetica. Some of trichoid sensilla were twisting together. The coeloconica sensilla lacked their central peg. The cuticle of sub segments was swollen in some parts and some pores were observed. Also, the scales have been observed to have teethed edges, (Fig. 2C&D).

At 150 Gy, the swollen of cuticle and pores have increased significantly than the lower doses. There is some twisted trichoid found at the tip of the flagellum, (Fig. 2E&F). Male antenna resulted from pupae irradiated with 200 Gy showed highly malformed sensilla as many nodulation and swollen bases of chaetica. The cuticle of flagellum segment was liquefied in many areas leaving undistinguished shape (Fig. 2G&H).

Effect of gamma radiation on female antenna.

Irradiated female with 50 Gy of gamma radiation showed that either antennae or sensilla were not affected by irradiation as their structure was comparable to the unirradiated (control) except a few nodulation in trichoid sensilla at the apical sub segment and some pores (Fig. 4A&B). The deformation of the antenna and sensilla structures start with the exposure to 100, 150, and 200 Gy of gamma radiation. Where deformations in 100 Gy were induced in sensilla chaetica as swollen the base and nodulation, moreover dwarfed and twisted trichoid however the other sensilla were not affected (Fig. 4C&D). At dose of 150 Gy, the antennal cuticle were looks like melt, disrupted, impaired of trichoid, swollen the base and nodulation of chaetica,, empty pores and liquefaction cuticle (Fig. 4E&F). Treatment of 200 Gy had more malformation features than the previous treatments. Trichoid sensilla had adequate or abundant number with formal distribution, and these trichoid bended at the basal end or at the distal end or twisted at their mid and may swell at their base, and they, also, may be collected together forming bundles. Sensilla chaetica also showed many forms of malformation like trichoid, which are swollen and loosely attached base. Moreover, central peg was absent from coeloconica sensilla. The scales may also fall leaving empty sockets or pores and the cuticle was swollen in most parts of the flagellomeres and may be liquefied. Also, this dose caused deformation for the apical crown (Fig. 4G&H).

Discussion

Since, the irradiation affected mechanosensitive, chemosensitive, olfactory, auditory and gustatory sensilla, this may be make the fall armyworm, *Spodoptera frugiperda* failed in their communication to seek their favorite hosts for feeding, courtship and mating behavior with non-irradiated males and females in the field during the application of SIT program.

In this investigation the flagellum of *S. frugiperda* female has 65–79 segments with seven different types of sensilla; while male have 42–53 segments with eight different types of sensilla. **Seada** [23] found that the flagellum of female cotton leaf worm, *S. littoralis* has 65–80 segments with eight types of sensilla.

Male and female of the same insect species have different antennal topologies, sensillum kinds, sizes, widths, and numbers. The dimorphism between insect antennal sensilla number are due to different feeding habits between the sexes or to the male's attraction to the female's pheromone **Chapman** [24]. **Malo et al.** [25] studied the olfactory system of *S. frugiperda* male and female by investigated the morphology and distribution of antennal sensilla and evaluating the responses of both sexes to conspecific female sex pheromone components and plant volatiles by using electroantennography (EAG).

The different sensilla on *S. frugiperda's* antennae are comparable to those on the antennae of other *Spodoptera* species in both type and distribution **Jefferson et al.** [26]; **Ljungberg et al.** [27]; **Monti et al.** [28]; **Aruna et al.** [29]. However, *S. frugiperda* antennae only present one type of sensilla trichodea, whereas, on the antennae of both sexes of *Spodoptera littoralis*, *Spodoptera latifascia* and *Spodoptera descoinsi*, the sensilla fall into two different size classes **Ljungberg et al.** [27]; **Monti et al.** [28]. Three types of trichodea were identified on the antennae of both sexes of *Spodoptera exigua* and *Spodoptera ornithogalli* **Jefferson et al.** [26].

Sensilla trichodea contain receptor neurons responding to plant odors and sex pheromones. For example, in *S. littoralis*, large sensilla contain receptor neurons to sex pheromone components **Ljungberg et al.** [27], whereas detecting neurons to the plant odor are located in short sensilla trichodea **Anderson et al.** [30]. The sensilla trichoidea may have an important role in courtship behavior. The surface texture of these sensilla is distinctly multiporous for possible olfactory perception of an air-born chemical stimulus **Ronald and Michael** [31]. Electrophysiological studies show that each of the nerve fibers reaching the tip of a trichoid sensillum reacts to a particular class of compounds. The perception of chemicals is important in many aspects of the life of the insects. For instance, smell may assist the insect in finding food or a mate. It has been demonstrated that in several lepidopeteran moths the long trichoids sensilla on the antenna of males are receptors of the sex pheromone of the females **Seada** [23]. However, **Jefferson et al.** [26] stated that in moths all types of trichoids are probably olfactory in function. They are stimulated by contact with chemical substance. Contact chemoreception may be important in final recognition of the food, an oviposition site or a mate **Chapman** [32].

In this study S.ChI mainly located near the segment's center and are distributed rather equally all around it. According to **Chapman** [24] **and Zacharuk** [33] the sensilla on insect antennae are not dispersed randomly. Their pattern may reflect the impact of many interacting selection pressures in which size of the individual, developmental stages, sex, feeding habits and habitats are of considerable significance. Sensilla chaetica as trichoid may play an important role in finding the other sex and the host, since they have a primary olfactory function **Kline and Axtell** [34]. Also, in other noctuid moths these sensilla were suggested to be a contact chemoreceptor as in *Trichoplusia ni* and *Prodenia ornithogalli* **Jefferson et al.** [26]. The results obtained revealed that sensilla chaetica of *S. frugiperda* are similar to those in other noctuid in structure **Liu and Liu** [35].

Several authors observed pores in basiconica sensilla of moths **Fauchex** [13] and **Cuperus** [36]. The presence of these pores suggests an olfactory function perhaps the reception of volatile odors of plant **Van Der Pers** [21]; **Inouchi, et al.** [37] and **Mohamed** [38] or it may be receptors concerned with the perception of irritant substances such as ammonia chlorine and essential oils.

Styloconica sensilla of *S. frugiperda* are short with pores and it has a ramified stick. These pored sensilla were thought to be chemoreceptors in the tortricid moth, *Choristoneura fumiferna* **(Van Der Pers 1981)**, or contact chemoreceptors in the noctuid moth, *Heliothis zea* **(Callahan, 1969)**. However, in yponomeutid moths they may have some other sensory functions because they are located under scales where contact chemoreceptors are not possible **Van Der Pers et al., (1980)**.

Also, sensilla coeloconica are olfactory receptors, possibly sensitive to volatile plant odour **Van Der Pers** [2]. However, in other lepidopteran moths these sensilla have been considered to be temperature, carbon dioxide, acids, hydroxides and natural organic salts receptors as indicated by **Salama et al.** [15] in the American bollworm, *Heliothis armigera.* The coelonic sensilla are receptors of humidity as basiconica. They may respond simply to water molecules impinging on the surface in the same way that chemoreceptors are presumed to act **Chapman** [32]. Generally the structure, location and function of these sensilla in *S. frugiperda* are similar to other noctuid as *S. unipuncata* [35].

Sensilla squamiformia of S. frugiperda are similar to those in other noctuid moth as *Copitarsia consueta* [39] and to those in Pyralid moth **Fauchex** [13]. These squamiformia sensilla may be considered as mechanical in function where it may perceive stress in the cuticle resulting from mechanical deformation (**Fauchex** [13].

Sensilla auricillica may represent chemoreceptors in function. **Boeckh et al.** [40] and **Kaissling** [41] suggested that auricillica are considered to be the main receptors for plant volatiles but physiological types may respond only to secondary plant metabolites **Bogner and Boppre** [42]. The olfactory function in the antennal segments has been suggested by **Salama et al.** [15] in the form of styloconic, chaetic, trichoid, basiconica and coeloconic sensilla.

Investigating the impact of gamma radiation doses, which cause hereditary sterility or F1 sterility in insects but did not affect their health or ability to reproduce, is urgently or absolutely important. It was observed that the effect of radiation appears clearly on the sense organs, depending on the dose used. Considering the results obtained, falling of scales, and malformation of other sensilla was noticed in some areas as affected by gamma radiation. Since these sensilla have a mechanical, chemical or olfactory function, when the radiation affects them, it causes insects to fail to find their appropriate place or to fail in courtship behavior and thus mating. These undesirable effects of radiation were noticed by many authors as **Hussien et al.** [43] who confirmed that substerlizing 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*, and according to this, the response of these irradiated males to sex pheromones were lowered and its perception decreased with increasing the dose applied. Also, **El-Shall et al.** [44] and **Hazaa et al.** [45] suggested the same effect of gamma radiation on *Spodoptera littoralis* male moth antennal sensilla and tarsal sensilla. Also, **Hazaa** [18] revealed that the sub-sterilizing doses exposed to full grown male pupae of *S. littoralis* have certain malformation features in the sensilla and the magnitude of these malformations was dose dependent as they increased by increment of the doses applied. Finally, it is clear from the examination the undesirable effects of gamma radiation on the shape, function and distribution of the sensilla. Therefore, it is preferable to use lower radiation doses to achieve a sufficient level of sterility with less distortion.

Conclusion

In conclusion, the study showed that gamma radiation can be used in SIT programs to reduce or eradicate the number of *Spodoptera frugiperda* as an ecofriendly method by affecting their antennal sensory organs which is responsible for olfactory or taste function, and thus reaching the appropriate host for feeding or reaching to the opposite sex to complete the mating process.

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Figures

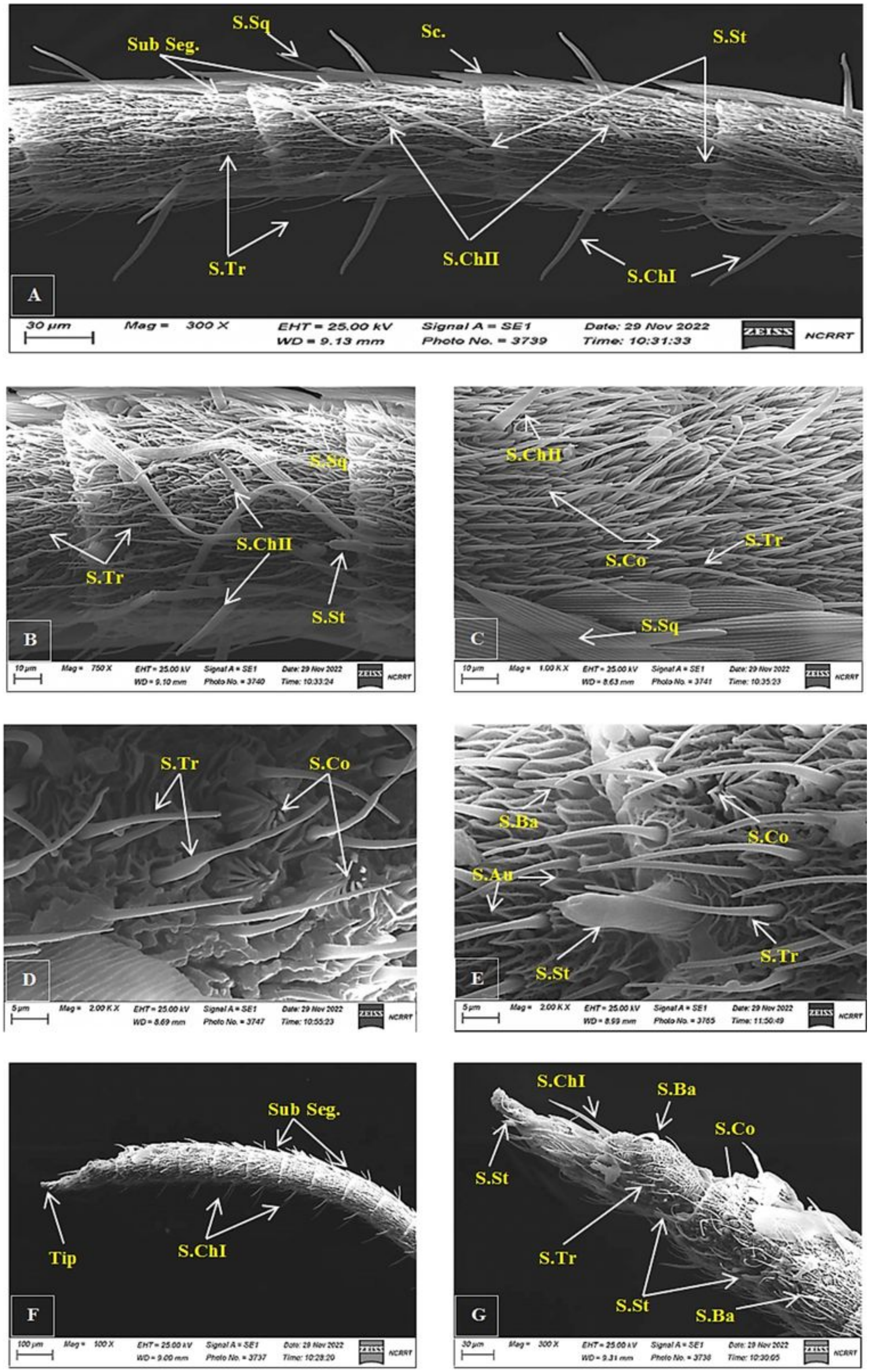

Figure 1

(A to G) Scanning electron microscope of normal FAW male flagellum segments showing Sub Segments (Sub Seg.), Sensillum Squamiformia (S. Sq.), Scales (Sc.), Sensillum Styloconica (S.St.), Sensillum Trichodea (S. Tr.), Sensillum Chaetica lateral (S.ChI), Sensillum Chaetica central (S.ChII), Sensillum Coeloconica (S.Co.), Sensillum Auricillica (S.Au.) and Sensillum Basiconica (S.Ba.).

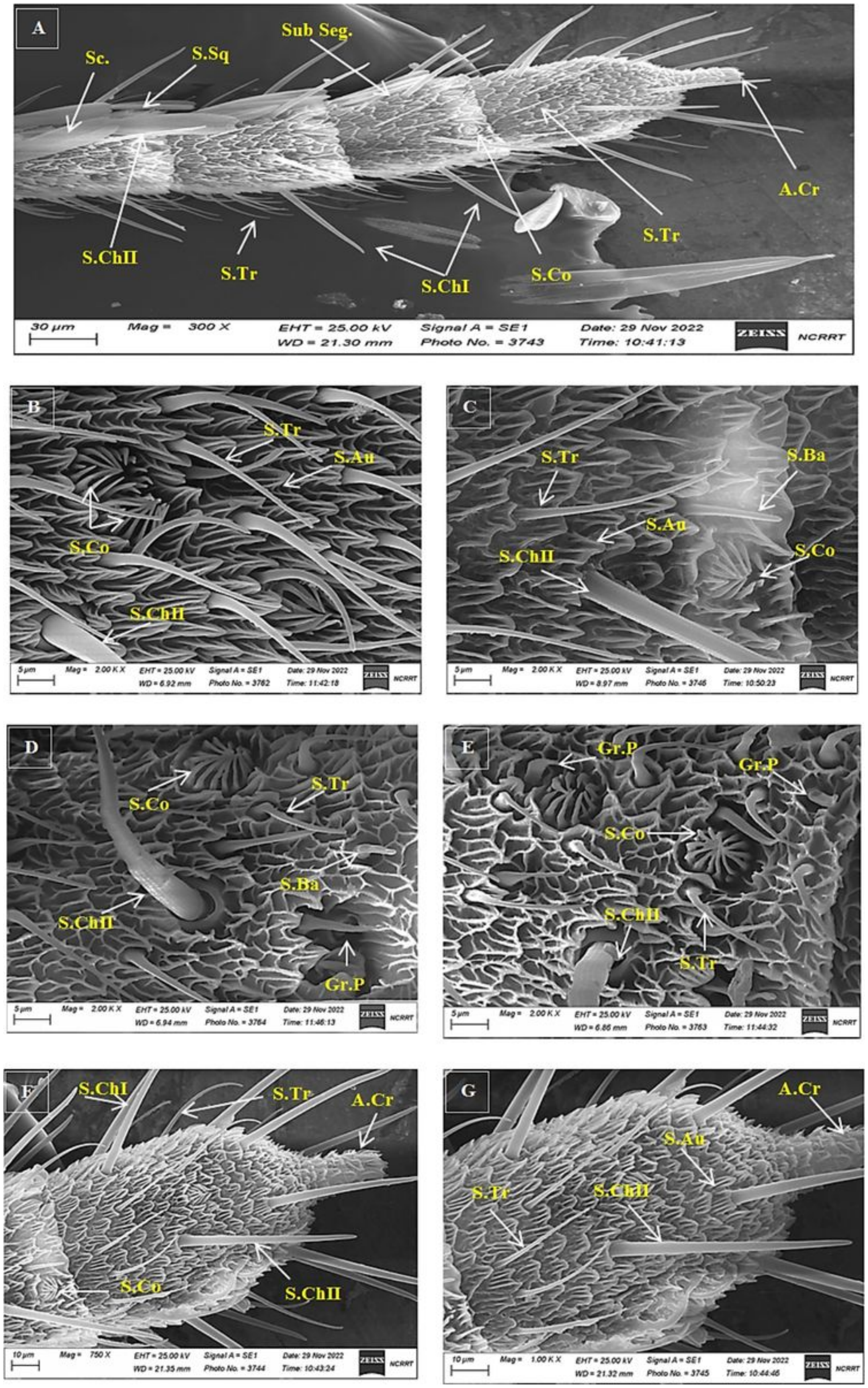

Figure 2

(A to G) Scanning electron microscope of normal FAW female flagellum segments showing Apical crown (A.Cr) Sub Segments (Sub Seg.), Sensillum Squamiformia (S. Sq.), Scales (Sc.), Sensillum Trichodea (S. Tr.), Sensillum Chaetica lateral (S.ChI), Sensillum Chaetica central (S.ChII), Sensillum Coeloconica (S.Co.), Sensillum Auricillica (S.Au.) Grooved peg (Gr.P.) and Sensillum Basiconica (S.Ba.).

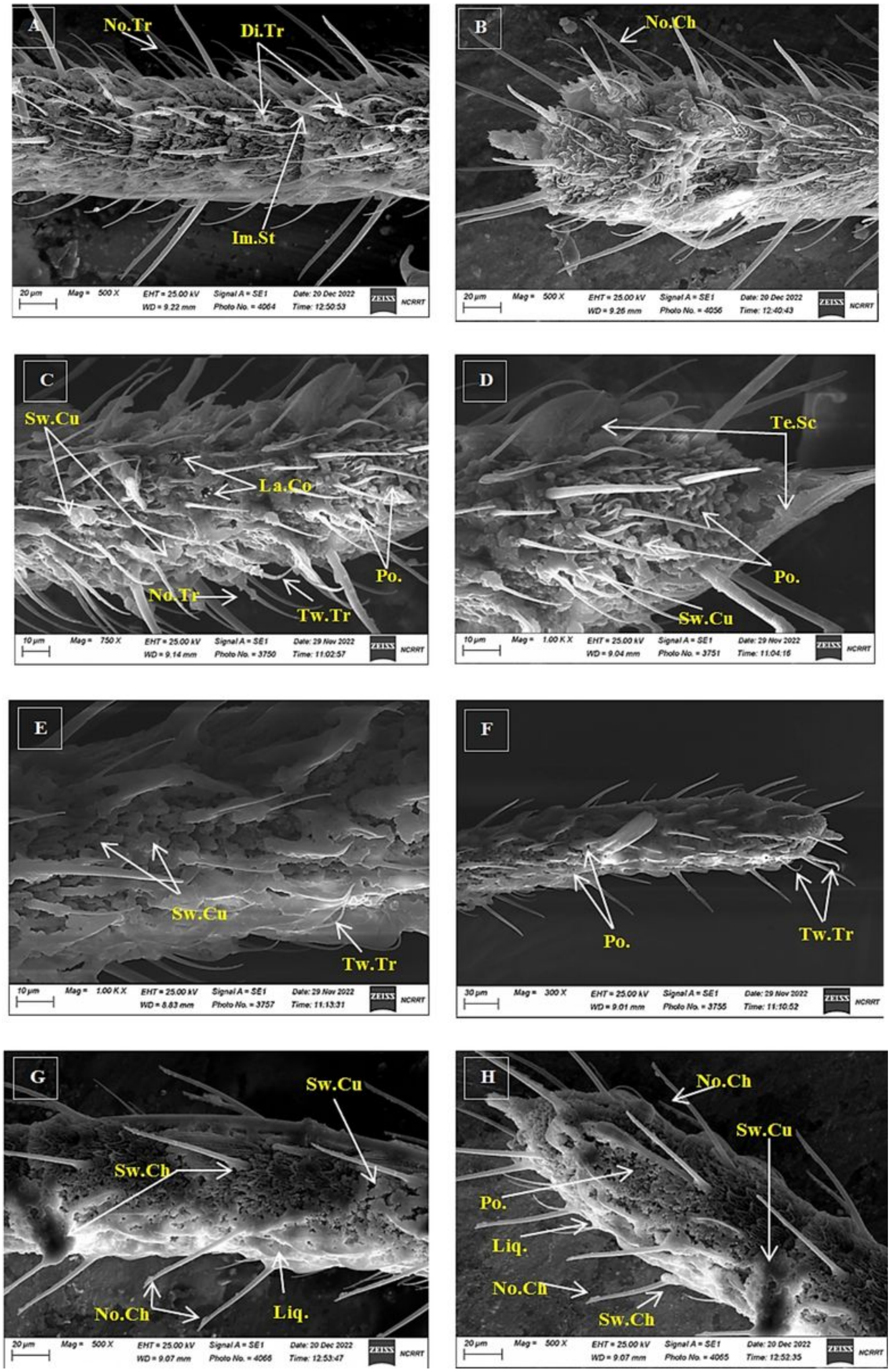

Figure 3

(A&B) Scanning electron microscope of the flagellum segments of the antenna in FAW male moths treated as full grown pupae with 50 Gy showed some nodulation in sensilla trichoidea and chaetica on their edges (No. Tr& No. Ch), disoriented of trichoidea (Di. Tr) and impaired of styloconica (Im.St). **(C&D)** 100 Gy showed swollen of the cuticle (Sw.Cu), lack of ceoloconic central peg (La.Co), pores (Po.), twisted trichoidea (Tw.Tr) and teethed edges of scales (Te.Sc). **(E&F)** 150 Gy showed (Sw.Cu), (Tw.Tr) and pores

(Po.). **(G&H)** 200 Gy showed swollen base chaetica (Sw.Ch), (Sw.Cu), (No. Ch), liquefaction of cuticle (Liq.) and pores (Po.).

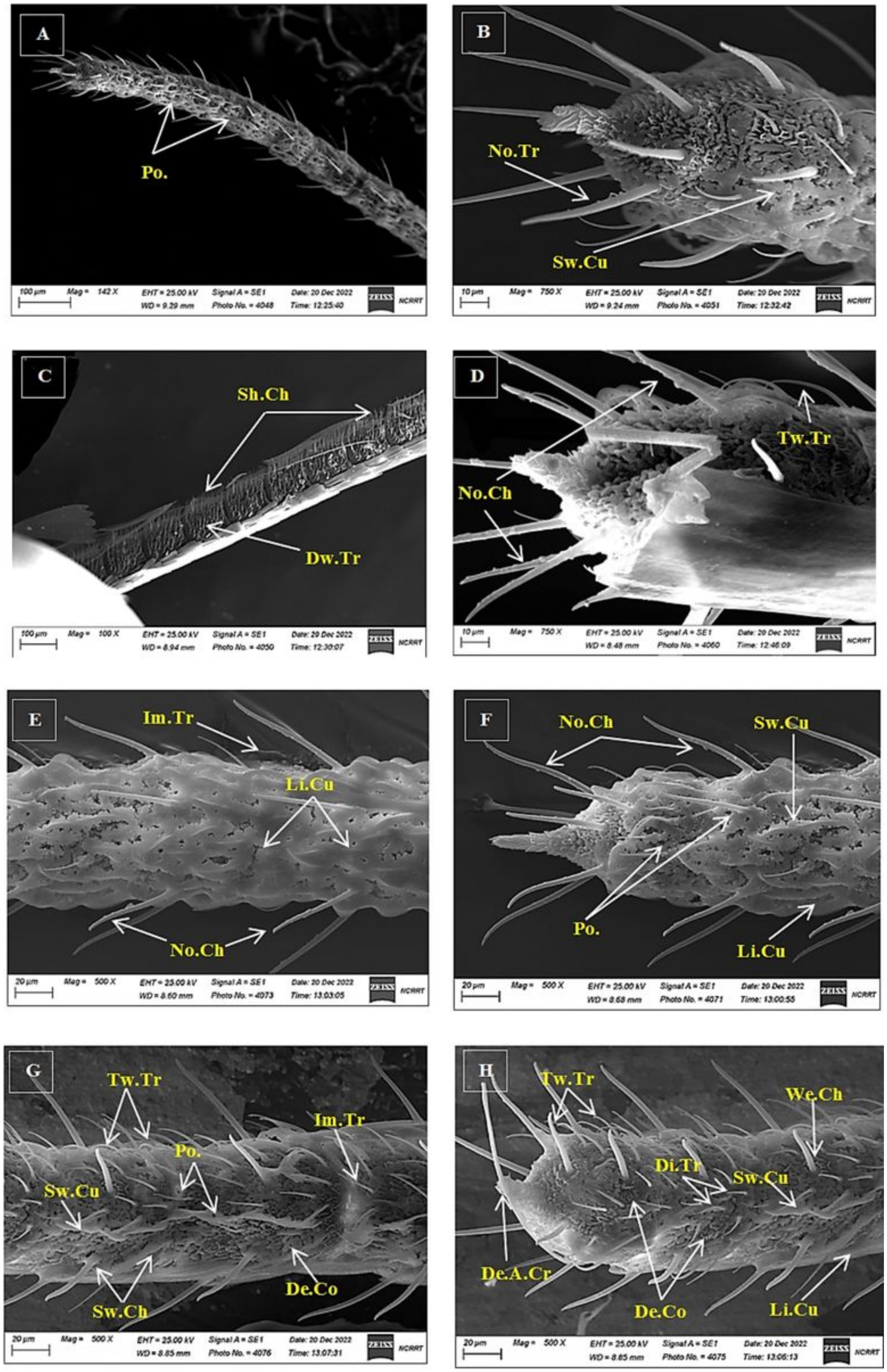

Figure 4

(A&B) Scanning electron microscope of the flagellum segments of the antenna in FAW female moths treated as full grown pupae with 50 Gy showed pores (Po.), some nodulation in sensilla trichoidea on their edges (No. Tr) and swollen base chaetica (Sw.Ch). **(C&D)** 100 Gy showed shrinkage of chaetica (Sh.Ch), dwarfed trichoid (Dw. Tr), nodulation in sensilla trichoidea on their edges (No. Ch) and twisted trichoidea (Tw.Tr). **(E&F)** 150 Gy showed impaired of trichoidea (Im.Tr), liquefaction of cuticle (Liq.), (Po.) and swollen of the cuticle (Sw.Cu). **(G&H)** 200 Gy showed (Tw.Tr), (Sw.Cu), (Im.Tr), (Po.), (Sw.Ch), deformed of ceoloconic (De.Co), weakly attached base of chaetica (we.Ch), disoriented of trichoidea (Di.Tr), (Liq.) and deformed of apical crown (De.A.Cr).