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Effect of chlorfluazuron and pyriproxyfen on the antennal morphology, pheromone production and response of surviving adults of *Tribolium castaneum* treated at the LC_{50} level during the pupal stage

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ABSTRACT

Initial experiments were carried out to determine the dosage mortality response of the rust-red flour beetle *Tribolium castaneum* to two insect growth regulators, the chitin synthesis inhibitor chlorfluazuron and the juvenile hormone analogue pyriproxyfen. The response was measured as the proportion of adults developing from individuals treated during the pupal stage. For males, at the LC_{50} level, chlorfluazuron was more toxic than pyriproxyfen with LC_{50} values of 10.6 and 12.6 ppm respectively. For females, pyriproxyfen was more potent than chlorfluazuron with LC_{50} values of 7.1 and 8.3 ppm.

Subsequent experiments were carried out using adults that had survived after treatment at the LC_{50} level during the pupal stage. Both responses to pheromone and the production of pheromone, by adults of both sexes that had been treated during the pupal stages with pyriproxyfen, were significantly more affected than those treated with chlorfluazuron.

Both treatments caused abnormalities in the antennae of adults of both sexes.

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1. Introduction

Insect pests of stored grains cause high economic losses in many countries of subtropical and tropical regions. The rust-red flour beetle, *Tribolium castaneum* (Herbst), is one of the most serious pests of flour and other cereal products in Egypt and other countries. Treatments to control insect pests in such stored products must be active against the pests, and safe to humans and environmentally-friendly organisms. One promising way to fulfill this need is through the use of insect growth regulators (IGRs).

The termIGR was introduced to describe a new class of biorational compounds. IGRs are divided into three main groups; juvenoids, which mainly affect larval metamorphosis by mimicking juvenile hormone; ecdysteroids, which affect molting, and chitin synthesis inhibitors (CSIs), which interfere with cuticle formation (Post et al., 1974).

Chlorfluazuron is a novel chitin synthesis inhibitor that belongs to benzoylphenylureas (BPUs) group and acts as an anti-molting agent and inhibits biosynthesis of chitin of an important constituent in insect cuticle. The cuticle loses elasticity and there is abnormal endocuticular deposition and abortive molting (Dhadialla et al., 2005). It is also known as an active larvicide with a broad spectrum of activity on various insects, including Lepidoptera, Coleoptera, Homoptera, Hymenoptera, and Orthoptera (Bakr et al., 2005).

Pyriproxyfen is a new juvenile hormone analogue (JHA). It acts as an anti-JH which artificially enhances JH levels preventing insect development to the adult stage. It competes with JH in binding to the JH receptors or to the JH-carrier of proteins, and injuring the corpora allata cells or interfering with JH biosynthesis (Leighton et al., 1981). It is an effective pesticide against Hymenoptera, Dictyoptera, and Heteroptera (Mojaver and Bandani, 2010).

Pheromones provide a major mode of intraspecific communication in insects and act to elicit a specific behavioral or developmental response from other individuals of the same species (Nordlund, 1981).

The objective of this study was to clarify the possibilities of using IGRs and sex pheromone in pest control.

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Table 1
Toxicity of the tested IGRs against pupal stage of *T. castaneum*.

IGRs	Chlorfluazuron		Pyriproxyfen	
Pupal stage	Male	Female	Male	Female
LC_{50} ppm	10.6	8.3	12.6	7.1
Slope	$(0.73 \pm 9.2) \times 10^{-3}$	$(0.72 \pm 8.73) \times 10^{-3}$	$(1.02 \pm 1.45) \times 10^{-3}$	$(0.9 \pm 1.07) \times 10^{-2}$
Chi square	3.8	4.7	7.2	8.1
Degree of freedom	3	3	3	3
Probabilities	0.23	0.17	0.05	-1.78×10^{-2}
Toxicity index at LC_{50}	100	85.54	84.13	100

2. Materials and methods

2.1. Insect colony

A laboratory colony of the red flour beetle, *T. castaneum*, was maintained for many generations under constant conditions of temperature at 30 °C and 70% R.H. in the Department of Entomology, Benha University. The rearing medium was wheat flour mixed by weight with brewer's yeast (95: 5 w:w).

2.2. Treatments

Chlorfluazuron was prepared by aqueous dilution of an emulsifiable concentrate containing 50 g/L active constituent (Atabron) and pyriproxyfen from an emulsifiable concentrate containing 100 g/L active constituent (Admiral). Aqueous dilutions of both compounds were used to produce concentrations of 0.1, 0.5, 1, 5 and 10 ppm.

Pupae of uniform age were obtained using the sieve of 495 μm which separates pupae from adults. Pupae were segregated into males and females and were dipped for 10 s then were transferred into suitable media. Four replicates (25 pupae for each) were run for each concentration. In addition; a corresponding untreated control group was used. The pupae were examined after eight days. Percentage mortality was calculated on the base of the number of adult emerged in relation to the number of pupae per petri dish. Uncompleted emerging adults were counted as dead. To determine the effect of LC_{50} of chlorfluazuron and pyriproxyfen against *T. castaneum*, subsequent experiments were carried out on adults treated at these dose levels during the pupal stage. All tests were performed at constant conditions of temperature at 30 °C and 70% r.h.

2.3. Pheromone production and response

Studies were carried out using a vial type of olfactometer similar to that used by Burkholder (1970). It consisted of a glass vial (15 × 1.5 cm), which had a rubber plug with a movable glass rod. The latter had a broad inner end at which a small piece of masking tape was fixed. The insect tested for pheromone production was held by the masking tape, while the one tested for response was placed on the bottom of the vial. The distance between the two insects was 4 cm. There were ten replicate vials set up in this way, each vial containing two individuals (a male and a female). The tested males and females were at the age range of 8–10 days old. Pheromone extraction was prepared by placing 30 treated beetles of the same sex and of known age (8–10 days) in one mL of hexane. The latter was confined in a screw-top glass vial of 5 mL in capacity. The tops of the vial caps were foil-lined to avoid solvent loss or contamination. The beetles were held in solvents for 24 h in a refrigerator, after which they were removed. Extracts were stored in a deep freezer at 20 °C until used. A vial containing 30 untreated beetles was held under the same conditions and served as a control. The concentration of the female extracts was (0.3 μ) female equivalents (FE) per 10 μ solvent, according to Hussien (1982).

2.4. Scanning electron microscope studies on adult antennae

The fine structure and distribution of various types of antennal sensilla at the age range of 8–10 day old females and males resulting from treated pupae were compared with those of untreated individuals by using scanning electron microscopy (SEM). This was done using high vacuum mode at the Regional Center of Mycology and Biotechnology, Cairo, Egypt.

2.5. Statistical analysis

The results obtained were evaluated using one-way analysis of variance "ANOVA" (Snedecor, 1971) using Pro. Lab. Version 7.5. The statistical program was set at the 1% level of significance ($P < 0.01$). The data were subjected to Probit Analysis (Finney, 1971) to calculate LC_{50} values.

3. Results

3.1. Toxicity of the tested insect growth regulators against the pupal stage

Chlorfluazuron exhibited higher toxic action than pyriproxyfen against male pupae of *T. castaneum*, giving LC_{50} values of 10.6 and 12.6 ppm, respectively (Table 1). On the other hand, pyriproxyfen was more potent than chlorfluazuron against female pupae of *T. castaneum*, recording the lowest LC_{50} value of 7.1 ppm.

Forming a toxicity index, when the male pupal stage treated with chlorfluazuron was considered as the standard rather than pyriproxyfen, the potency of pyriproxyfen was 84.31% lower than the standard. On the other hand, when the female pupal stage treated with pyriproxyfen was considered as the standard rather than chlorfluazuron, the potency of chlorfluazuron was 85.54% of the standard.

3.2. Effect of LC_{50} of the tested IGRs on responsiveness and production of pheromones in male and female adults

Figures 1 and 2 illustrate that the responsiveness and production of pheromone in treated groups with pyriproxyfen were significantly higher than those treated with chlorfluazuron.

3.2.1. Male response behavior to female

For chlorfluazuron, when treated males were tested against treated females, the level of response was 60% compared to the control response of 82%. For pyriproxyfen, the responses were 52% and 84% respectively. The response behavior in the first group consisted of a sequence of increasing levels of excitation. The first

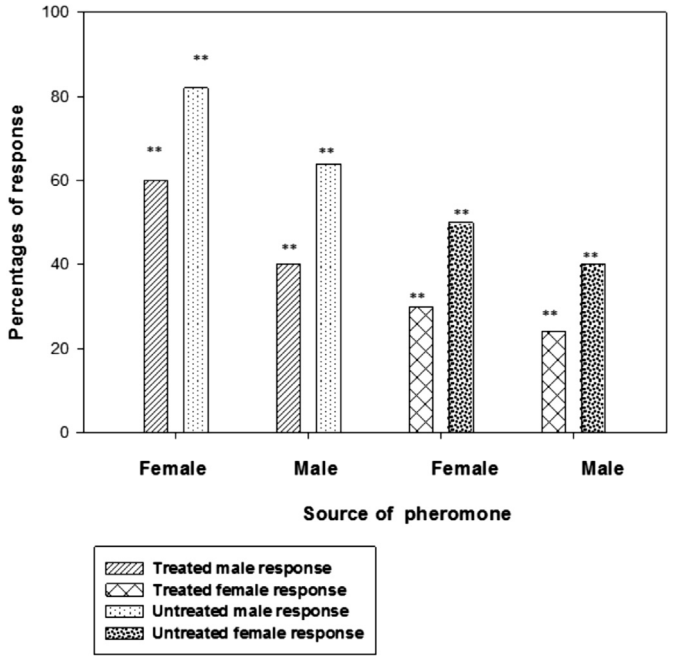

Fig. 1. Response of virgin *Tribolium castaneum* males and females (8–10 days old) to adults of both sexes produced by pupal stage treated by chlofluazuron.

level of response included the raising of antennae, head, and thorax. The second level of response included moderate activity of circular running in contrast to the high activity in untreated beetles. In third level of excitation, the treated male was bobbing up and down on the surface of the olfactometer vial. During the excitation, antennal and leg vibrations occurred. The duration of any level of excitation was variable.

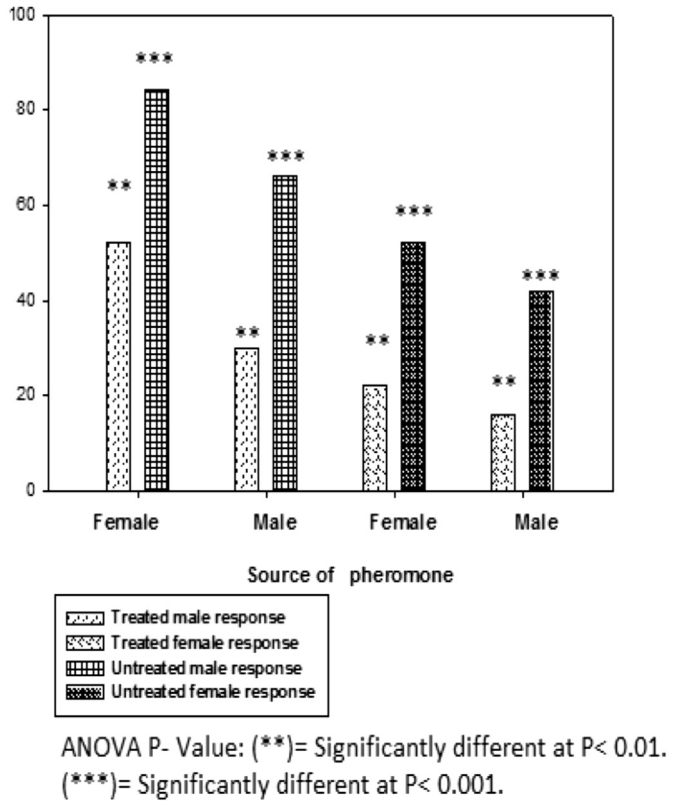

Fig. 2. Response of virgin *Tribolium castaneum* males and females (8–10 days old) to adults of both sexes produced by pupal stage treated by pyriproxyfen.

3.2.2. Male response behavior to male

For chlorfluazuron, when treated males were tested against treated males the level of response was 40% compared to the control response of 64%. For pyriproxyfen, the responses were 30% and 66% respectively. In the second group, the response of treated male beetles to their own sex also consisted of a sequence of events. The first level of response included the raising of antennae, head, and thorax. The second level of response included low activity of circular running while the activity moderated in untreated beetles. No vibration or bobbing appeared.

3.2.3. Female response behavior to female

For chlorfluazuron, when treated females with their own sex the level of response was 30% compared to the control response of 50%. For pyriproxyfen, the responses were 22% and 52% respectively. The treated females exhibited a sequence of events which also included the raising of antennae, head, and thorax. Movements were generally slow. The duration of any level of excitation was rather short compared to untreated beetles.

3.2.4. Female response behavior to male

For chlorfluazuron, when treated females were tested against treated males, the level of response was 24% compared to the control response of 40%. For Pyriproxyfen, the responses were 16% and 42% respectively.

In this group, females also exhibited a sequence of events similar to those mentioned in the second group.

3.3. Effect of LC_{50} of chlorfluazuron and pyriproxyfen on the antennal sensilla of adult T. castaneum

The antennae of these beetles consist of the scape, pedicel, and eleven flagellomeres. Scan electron microscopy showed that seven types of sensillae were found in male antennae (Fig. 3), while three types only of sensillae were present in female antennae (Fig. 4).

Scanning electron microscopy of the antennae of adult male and female beetles emerging from pupae treated with chlorfluazuron showed that the antennae kept their normal capitate shape. Male antennal segments from 7 to 11 appeared to have incompletely shed the old cover during molting (Fig. 3). In females, the enlarged last three antennal segments lost a number of sensillae (Fig. 4).

On the other hand, pupae treated with pyriproxyfen showed that adult male antennal segments were normal except for some segments with wrinkled tissue surface and with a reduced number of sensillae (Fig. 3), while female antennal segments had only a very few fragile sensillae and retained much of the old cover from the molt (Fig. 4).

4. Discussion

4.1. Toxicity and effect of LC50 of the IGRs on responsiveness and production of pheromones in male and female beetles emerging from treated pupae

At the LC_{50} level chlorfluazuron exhibited higher toxic action than pyriproxyfen against male pupae of *T. castaneum*, but pyriproxyfen was more potent than chlorfluazuron against female pupae. Generally, the two IGRs used at the same concentration both had latent effects leading to reduction of population. These results are in conformity with those obtained by Bakr et al. (2005) against *Monomorium pharaonsis*.

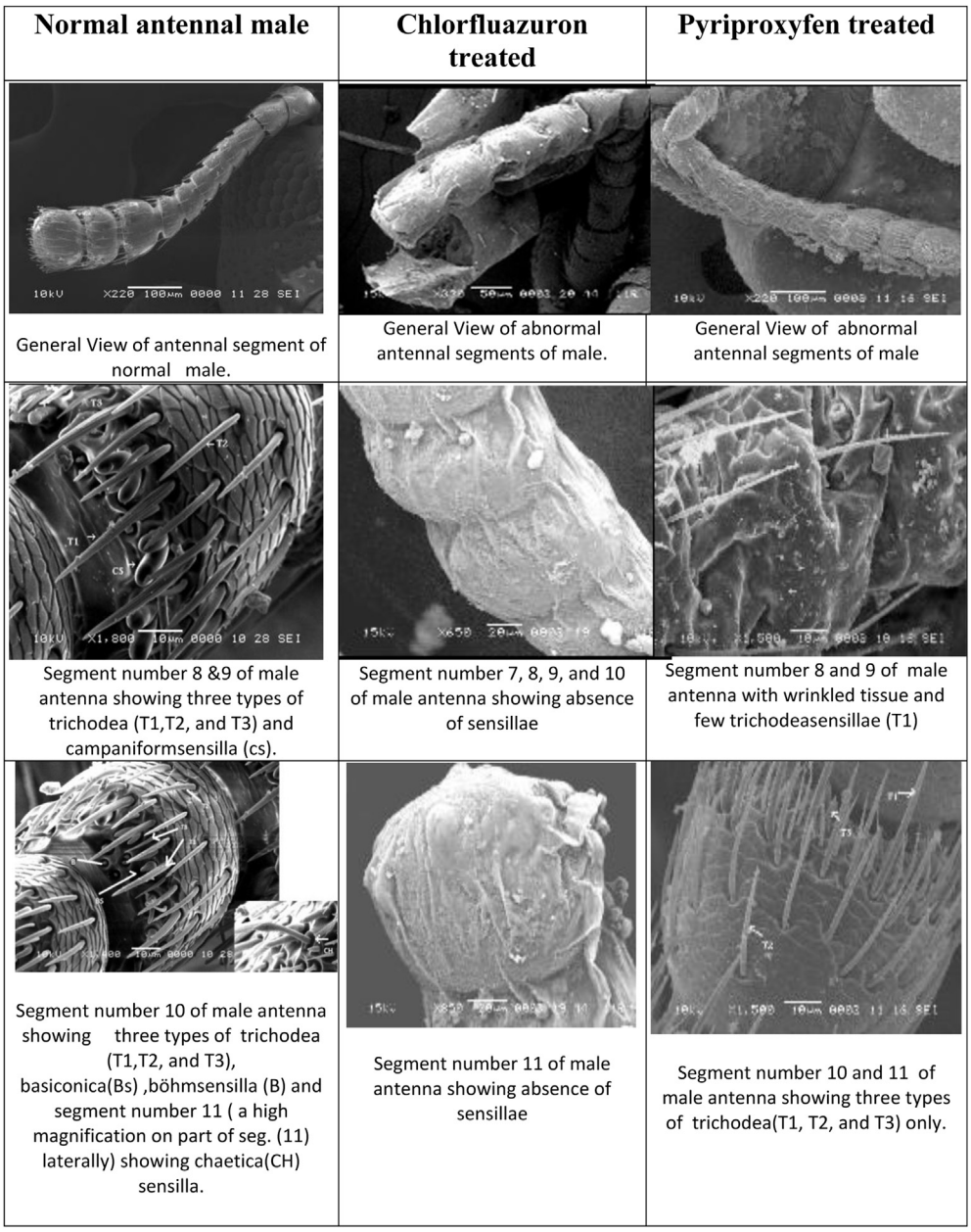

Fig. 3. Scanning electron microscopy of antennal sensillae of male *T. castaneum* which resulted from treated pupal stage.

Results obtained in the present paper indicate that both treated and untreated sexes of the rust-red flour beetle could secrete a pheromone which is able to stimulate the other sex as well as its own sex. Responsiveness and production of pheromone in untreated groups were significantly higher than treated ones. Females secrete a sex pheromone which is much more attractive to males than to females. Males, on the other hand, secrete an aggregation pheromone which is attractive to both sexes.

In treated sexes, the male response to sex pheromone was much more affected by chlorfluazuron and pyriproxyfen than the level of pheromone produced by females. This is attributed to the fact that male sense organs responsible for pheromone perception may be more affected by treatment of the pupae at the LC_{50} of both IGRs than are the pheromone producing glands of females. Similar results were obtained by Menon (1978) on the cockroach, *Nauphoeta cinerea* and Hussien (1982) on *T. castaneum*.

4.2. Effect of LC_{50} of chlorfluazuron and pyriproxyfen on the antennal sensilla of adult T. castaneum

The previous results showed that normal male antennae have seven types of sensillae; Trichodea (T1, T2, and T3), Campaniform sensilla, Bohm sensilla, Basiconic sensilla, and Chaetica sensilla; while normal female antennae have only three types of Trichodea (T1, T2, and T3). Similar results were obtained for antennal sensillae of both sexes of the ground beetles *Bembidion lampros* and *Platynus dorsalis* (Merivee et al., 2000, 2001). Generally, male antennae contain more types of sensillae than female antennae, and most likely contain the receptor sites for the female sex pheromone.

In treated beetles, fusion of some sensillae was observed among treated sensillae trichodeae and some pores appeared in sensillae basiconicae. These abnormalities may be attributed to the effect of

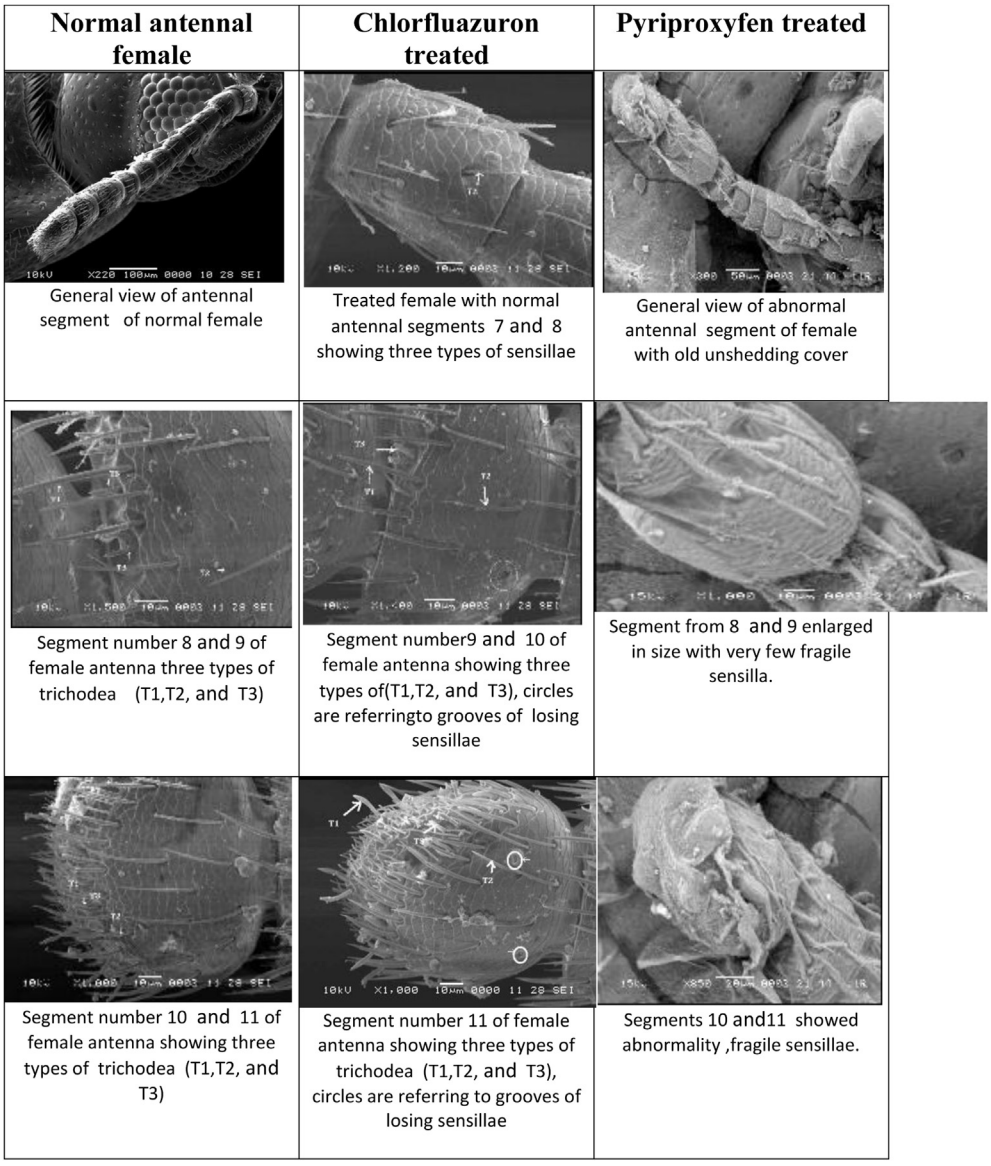

Fig. 4. Scanning electron microscopy of antennal sensillae of female *T. castaneum* which resulted from treated pupal stage.

insect growth regulator indirectly on the release of ecdysteroids, through interfering with the neuroendocrine sites responsible for the release of this hormone. These results were similar with those obtained by Said et al. (2003) who showed that the treatment of adult beetles palm weevil, *Rhynchophorus palmarum* (Coleoptera, Curculionidae) with 9 ppm of lufenuron caused abnormalities in the shape of antennae and except for the Böhm bristle sensillae, the sensillae were longer than for untreated specimens. Similarly, results obtained by Hussien et al. (2001) showed malformed antennae of males of *Agrotis ipsilon* after treatment of pupae by gamma radiation. These results also are in conformity with those obtained by Hazarika and Baishya (1997) on antennae of *Melano gryllus* and *Dicladispa armigera* as affected by the juvenile hormone analogue methoprene.

In summary, abnormalities shown by scanning confirmed that responsiveness and production of pheromones in groups treated with pyriproxyfen were significantly higher than those treated with chlorfluazuron. Also, these results confirm that the effects will hinder the two sexes getting together for mating and reproduction, disrupting the intraspecific communication between males and females and will therefore be of help in pest control by reducing population increase.

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