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**Some factors affect the perception and production of pheromone and ultrastructure of antennae after treatment of *Tribolium castaneum* with Chlorfluazuron**

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### ABSTRACT

Chlorfluazuron (Atabron) is an insect growth regulator (IGR) used for controlling the major stored product pests. This study investigate the effect of Chlorfluazuron on both sexes of *Tribolium castaneum* which resulted from treated 4<sup>th</sup> larval instar with LC<sub>50</sub> values (1.2ppm for 4<sup>th</sup> larval instar).

The maximum production and perception of pheromone on both sexes resulted from treated 4<sup>th</sup> larval instar were at 8-10 days old while at 4-6 days old in untreated sexes.

The maximum and minimum pheromone production and perception by sexes resulted from treated 4<sup>th</sup> larval instar were at 30°C, while it decline to its lowest titer at 15°C.

The study revealed that both sexes resulting from treated 4<sup>th</sup> larval instar with LC<sub>50</sub> of Chlorfluazuron caused the formation of abnormal antennae and effect on number and distribution of the sensillae.

**Keywords:** *Tribolium castaneum*, Chlorfluazuron, pheromone production and perception, Age, Temperature, scan antennae.

### INTRODUCTION

The red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), is a common insect pest associated with food- processing facilities worldwide Mills and Pedersen (1990), so we must control this pest by safe means.

Nowadays, alternative methods are being appreciated. One of these methods may be the inclusion of insect growth regulators (IGRs) such as Chlorfluazuron . These compounds are highly effective against various insects attacking stored products and other pests that have become resistant to organic insecticides. Meanwhile, all these compounds are less toxic to mammals and non target organisms (Horowitz, 1998).

Chlorfluazuron is effective as chitin synthesis inhibitors (CSI) ingredient that used to eliminate *Coptotermes acinaciformis* in Australia because it disrupts chitin deposition during ecdysis (Peters and Fitzgerald (2003), also, Abd El-Mageed and Elgohary (2007) used Chlorfluazuron in pest management strategies as safety environmental compound for controlling the two corn borers *Sesamia cretica* Led. and *Ostrinia nubilalis* Hun.

Pheromones are a class of semiochemicals that insects and other animals release to communicate with other individuals of the same species Mueller (1995).

This study investigate the possibilities of using Chlorfluazuron and sex pheromone in control this destructive pest.

## MATERIALS AND METHODS

### **Rearing insect and bioassay test:**

Red flour beetle, *T. castaneum* was reared for many generations under constant conditions 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). Chlorfluazuron (Atabron) "5% EC" was diluted in water at the concentrations of 0.1, 0.5, 1, 5 and 10ppm, then transferred into suitable media. Four replicates (25 4<sup>th</sup> larval instar) were run for each concentration and a control with water was used for comparison in the diet. A preliminary experiment was carried out to determine the effect of LC<sub>50</sub> of Chlorfluazuron as a chitin synthesis inhibitor against 4<sup>th</sup> larval instar of *T. castaneum*.

### **Factors influencing sex pheromone production and perception:**

#### **a- Effect of age on *T. castaneum* adult treated as 4<sup>th</sup> larval instar on pheromone production and perception**

To investigate the effect of age on pheromone production and perception, hexane extracts were prepared from treated virgin females at 0.3 equivalents (FE) per 10 $\mu$  of solvent of various ages (0-2, 2-4, 4-6, 6-8, 8-10, 10-12 and 12-14 days). Each treated female age group extract was tested in ten replicates each one contains 10 treated males (10-12 days old) placed individually in 10 vials. One hundred treated males of various ages (0-2, 2-4, 4-6, 6-8, 8-10 and 10-12 days old) were tested for single treated female extract (10-12 days old) as previous manner. Untreated males and females were used according to the previous manner for control.

#### **b- Effect of temperature on *T. castaneum* adult treated as 4<sup>th</sup> larval instar on pheromone production and perception**

To find out the effect of temperature on extractable pheromone content. Treated virgin female pupae reared at 30°C, until reach adult were incubated at different temperatures (15, 25, 30 and 40 °C). Then treated adult females were removed and were extracted for pheromone production after 8-10 days from adult emergence at each temperature. The extracts were tested against treated males 8-10 days old reared at 30 °C. For each extract, ten replicates each one contains 10 treated males placed individually in 10 vials were used. Untreated males and females were used according to the previous manner for control.

#### **c- Scanning examination of antennal sensillae of adult *T. castaneum*.**

The fine structure and distribution of various types of antennal sensilla at 8-10 days old female and male resulting from 4<sup>th</sup> larval instar treated with LC<sub>50</sub> of Chlorfluazuron was compared with those of untreated individuals by using scanning electron microscopy (SEM) with high vacuum mode at the Regional Center of Mycology and Biotechnology, Cairo, Egypt.

### **Statistical analysis**

The results obtained were evaluated using one way analysis of variance "ANOVA" (Snedecor, 1971) on origin Pro. Lab (version 7.5) statistical program at one level of significance (P< 0.01).

## RESULTS AND DISCUSSION

The treated 4<sup>th</sup> larval instar showed a high sensitivity to Chlorfluazuron where LC<sub>50</sub> value was 1.2 ppm. These results similar to those obtained by Bakr *et al.* (2005) on *Monomorium pharaonsis*. These results may be due to Chlorfluazuron is a chitin

synthesis inhibitor that belongs to benzoylphenyl- -ureas (BPUs) and acts as an anti-molting agent, inhibit biosynthesis of chitin of an important constituent in insect cuticle larvae (Dhadialla *et al.*, 2005).

We applied the LC<sub>50</sub> of Chlorfluazuron under some factors to illustrate these sensitivity on both sexes.

#### I) Effect of age on pheromone production by females at different ages:-

Normal female produced maximum sex pheromone titer at 4- 6 days old. While female produced from treated 4<sup>th</sup> larval instar with LC<sub>50</sub> of Chlorfluazuron appeared to produce lesser amounts of a sex pheromone which significantly ( $P < 0.01$ ) increases gradually to reach a peak at 8-10 days old. On the other hand, production of pheromone in untreated virgin females extract were significantly higher than treated one at different ages from 0-14 days old. These data are represented graphically in Fig (1).

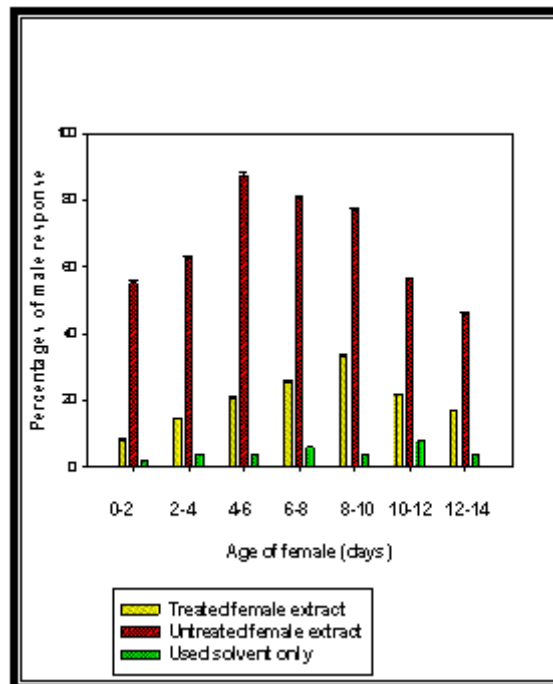


Fig. 1: Average male response of *Tribolium castaneum* to pheromone extracts of females at different ages produced by treated 4<sup>th</sup> larval instar by Atabron.

These results agreed with Barratt (1974) who indicated that the maximum pheromone production by the normal female drug store beetle, *Stegobium paniceum* (L.), 3 to 4 days old. In contrast results obtained by Xiang *et al.* (2010) which reported that the percentage of sex pheromone production by female black cutworm moth, *Agrotis ipsilon* increased in 1 to 3 days-old females and decreased in 3 to 7 days-old females. Females advanced their pheromone production time with increasing age.

#### II) Effect of age on pheromone perception by males at different ages:-

The data obtained on the response of males resulted from treated 4<sup>th</sup> larval instars with LC<sub>50</sub> of Chlorfluazuron at different ages to treated female extracts are represented graphically in Fig. (2).

The response of treated males started at a low level 06.12 % for young males 0-2 days old. It increased as the males became older 2-8 days old. The maximum response 31.25 % was reached when treated males were 8-10 days old.

Much older treated males showed variable levels of response, that were somewhat lower than that of 8-10 days old males. The level of male response dropped to 14.29 % at the age of 12-14 days old.

No significant difference between the maximum response of treated males 8-10 days old and the response of treated male 6-8 days old. A significant difference, was found among the maximum response of males 8-10 days old and the response of males (0-2, 2-4, 4-6, 10-12 and 12-14 days old).

These results indicated that the optimum age of treated male and treated female for perception and production sex pheromone was likely 8-10 days old.

Although responsiveness to pheromone in untreated virgin males was significantly higher than treated one at different ages from 0-14 days old. The maximum responsiveness to pheromone in untreated virgin males was at 4-6 days old. Similar result obtained by Spurgeon (2003) on boll weevil.

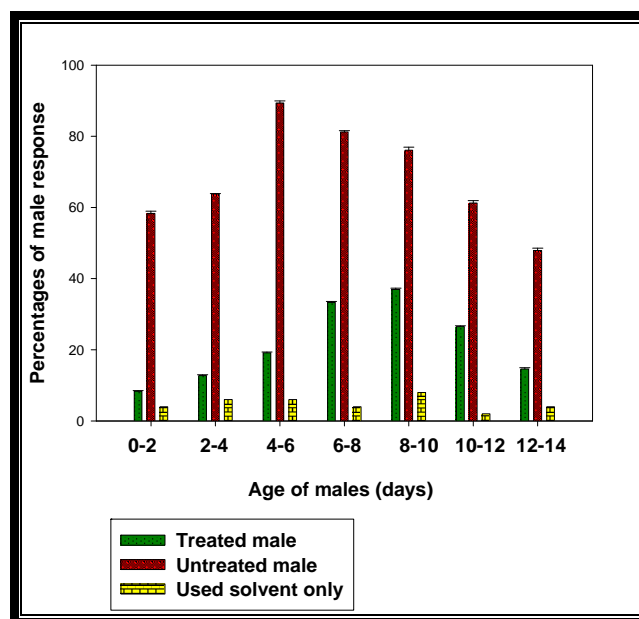


Fig. 2: Average male response of *T. castaneum* at different ages to female extracts produced by treated 4th larval instar by Atabron.

### III) Effect of Temperature:-

Males resulting from treated 4<sup>th</sup> larval instar were exposed to pheromone extracts of treated females reach lowest level at 15°C and the male response increased gradually to reach its maximum at 30°C, but, the male response started to decrease at 40°C. The difference, however, was not significant from the response level at 30°C. These results represented graphically in Fig. (3). Although responsiveness and production of pheromone in untreated groups were significantly ( $P < 0.01$ ) higher than treated one at different rearing temperatures.

Results of the present study indicated that beetles of *T. castaneum* are active throughout a wide range of temperature and none of the tested temperatures could inhibit pheromone production. This indicates that temperatures favourable for its life are also favourable for sex pheromone production; and that higher temperatures are more favourable than lower one. These results similar to those obtained by Webster and Carde (1982) who showed that the decrease of the temperature of untreated females of the omnivorous leafroller moth, led to reduction in the pheromone titer inside the sex pheromone gland.

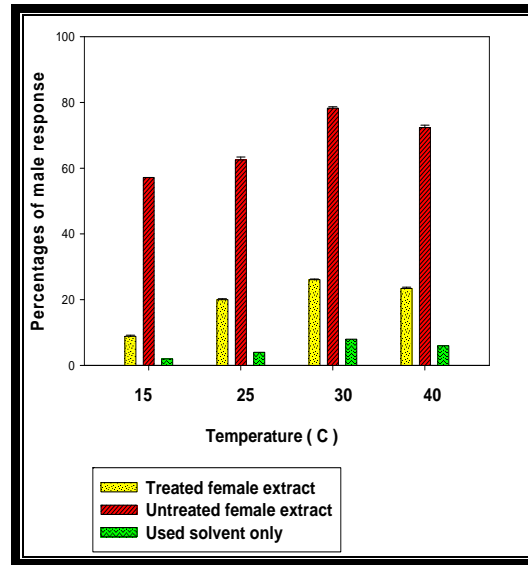


Fig. 3: Response of male *Tribolium castaneum* produced after treated 4<sup>th</sup> larval instar with Atabron to sex pheromone extracts of females kept at different temperatures

#### Effect of Chlorfluazuron on the Antennal Sensilla of adult Male *T. castaneum* :

The long capitata antennae of these beetles consist of the scape, pedicel and eleven flagellomeres, even the last three segments at the tip of antennae are abruptly larger than the preceding one. Types of antennal sensillae in both males and females are different.

By using scan electron microscopy, seven types of normal male antennal sensillae were found in antennae: male antennae has (Trichodea type I “T<sub>1</sub>”, Trichodea type II “T<sub>2</sub>”, Trichodea type III “T<sub>3</sub>”, Chaetica sensillae “CH”, Böhm sensillae “BS”, Campaniform sensillae “CS”, and Basiconica sensillae “B”) as shown in plate (1-a) and the last segment contains all the previous types of sensillae except Campaniform sensillae as shown in plate (1-b). Similar results obtained by Merivee *et al.*, 2000 on antennal sensillae of the male ground beetle *Bembidion lampros*, while male adult antennae resulted from treated 4<sup>th</sup> larval instar with LC<sub>50</sub> of Atabron revealed that, the antennae of treated group lost their normal shape. The number of male antennal segment decreased with weak and fragile sensillae at dorsal side as shown in Plate (1-c) and the last three segments confused and without sensillae as shown in Plate (1-d).

#### Effect of Chlorfluazuron on the Antennal Sensilla of adult Female *T. castaneum*:

Normal female antenna with three types only of sensillae (Trichodea type I “T<sub>1</sub>”, Trichodea type II “T<sub>2</sub>” and Trichodea type III “T<sub>3</sub>”), as shown in plate (2-a) and the last segment has the three previous types of sensillae, as shown in plate (2-b), Similar results obtained by Merivee *et al.* (2001) on antennal sensillae of the ground beetle *Platynus dorsalis*.

The previous results illustrated that antennae of male have more types of sensillae than female, so, it may contain the receptor sites for the female sex pheromone, that agreed with Timothy (2005) who reported that an increased number of sensillae, through lengthening, branching or thickening of antennal segments would increased sensitivity.

On the other hand female adult antennae resulted from treated 4<sup>th</sup> larval instar with LC<sub>50</sub> of Atabron showed antennae with highly confused segments without sensillae as shown in Plate (2-b) and the last three segments confused but without

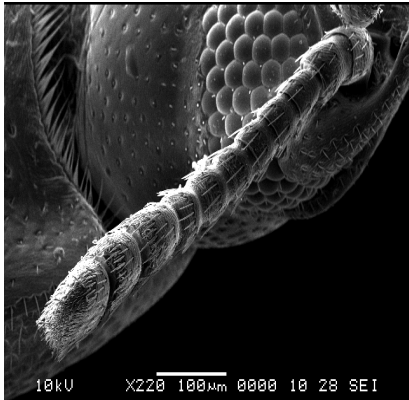
sensillae as shown in Plate (2-d). Similarly, obtained by Zohry (2008) who revealed that LC<sub>50</sub> of flufenoxuron on 3<sup>rd</sup> larval instar of *Spodoptera littoralis* caused the formation of abnormal antennae and affect the length and distribution of sensillae.

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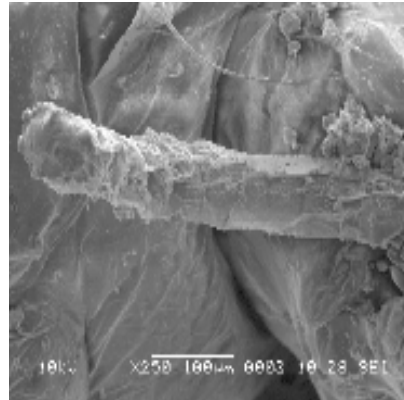
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**Plate1: Scanning electron microscopy of antennal sensillae of male *T. castaneum*.**

Normal Male

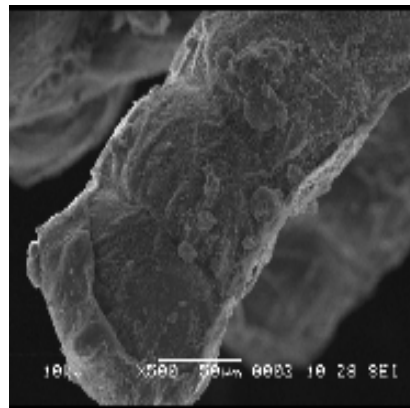
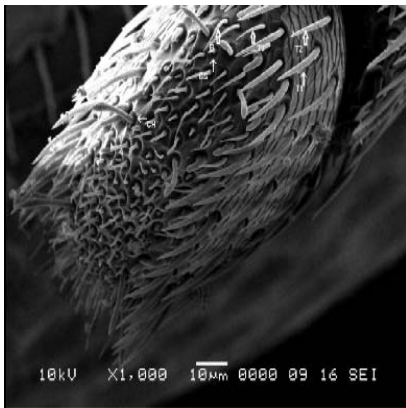


Treated Male



a) General View of antennal segment of normal male

b) General View of antennal segment of treated male

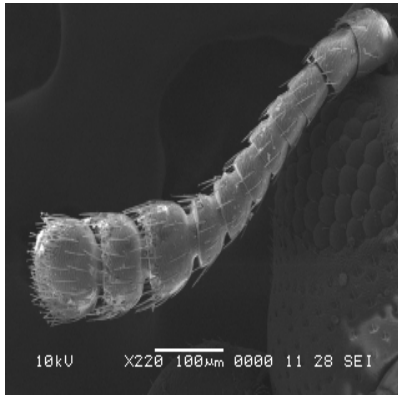


c) Last segment (No. 11)

d) Last 3 segments (No. 9, 10 & 11)**Plate 2:**

**Plate2: Scanning electron microscopy of antennal sensillae of female *T. castaneum*.**

Normal Female

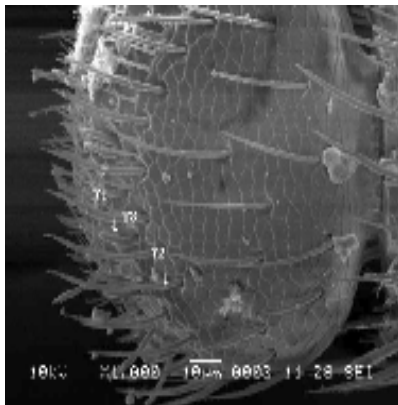


Treated Female

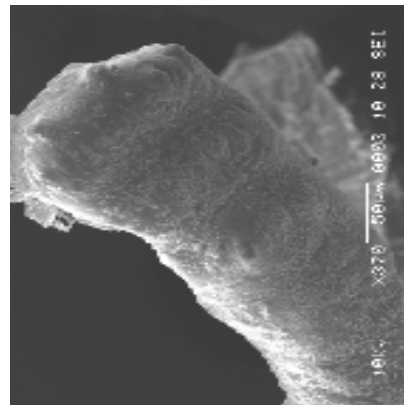


a) General view of antennal segment of normal female.

b) General View of abnormal antennal segments.



c) Segment number 11 of female antenna showing three types of trichodea (T1,T2 &amp; T3)



d) Last segments of female antennae showing absence of sensillae with destroyed tissue.

## ARABIC SUMMARY

بعض العوامل المؤثرة في إنتاج وإدراك الفيرومون والتركييب الدقيق لقرون الاستشعار لحشرة خنفساء الدقيق الناتجة من المعاملة بالكورفليوزورون

- الفت محمد المنيرى<sup>1</sup> - نهاد محمد البرقى<sup>1</sup> - رضا فضيل على بكر<sup>2&3</sup> - نانسى مجدى بيومى الشوربجى<sup>2</sup>
- 1- قسم علم الحشرات - آية العلوم - جامعة بنها  
2- قسم علم الحشرات - كلية العلوم - جامعة عين شمس-  
3- قسم الاحياء - كلية العلوم - جامعة الملك خالد- ابها - المملكة العربية السعودية

الكورفليوزورون هو منظم نمو حشري والذي ينتمى لمجموعة بينزويل فينيل يوريا و يستخدم لمكافحة العديد من افات الحبوب المخزونة. هذه الدراسة تبحث فى تأثير هذا المركب على انتاج و ادراك الفيرومون على الطور البالغ لحشرة خنفساء الدقيق الصدئية والناتج من معاملة العمر اليرقى الرابع بالتركيز الخاص بالجرعة القاتلة للنصف لهذا المركب و هى (1.2 جزء من المليون للعمر اليرقى الرابع تحت تأثير بعض العوامل. و قد وجد ان اعلى انتاج و استجابة للفيرومون فى الافراد الناتجة من معاملة العمر اليرقى الرابع بالتركيز الخاص بالجرعة القاتلة للنصف لهذا المركب من 8- 10 ايام . و قد لوحظ ايضا ان اعلى انتاج و استجابة للفيرومون فى الافراد الناتجة من معاملة العمر اليرقى الرابع بالتركيز الخاص بالجرعة القاتلة للنصف كان عند درجة حرارة 30 م° بينما اقل انتاجية و ادراك للفيرومون عند انخفاض درجة الحرارة عند 15 م°. و بفحص قرون الاستشعار للحشرة وجد ان الذكور لديهم 7 انواع من الشعيرات الحسية على قرون استشعارهم بينما الاناث لديهم 3 انواع فقط من الشعيرات الحسية. و عند المعاملة فى العمر اليرقى الرابع بالتركيز الخاص بالجرعة القاتلة للنصف وجد ان الكورفليوزورون يسبب تشوه للقرون لكلا الجنسين كما يؤثر على عدد و توزيع الشعيرات الحسية الموجودة على قرون الاستشعار.