

Biological and behavioral effects of Pyriproxyfen on pheromone production and perception. of *Tribolium castaneum* (Coleoptera: Tenebrionidae).

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ABSTRACT

Pyriproxyfen (Admiral) is an insect growth regulator (IGR) acts as anti-juvenile hormone. The present work aims to investigate the toxicological effect of Pyriproxyfen on both sexes of rust red flour beetle, *Tribolium castaneum* resulted from treated 4th larval instar with LC₅₀ value (2.4ppm) and its effect on female production and male's perception to pheromone. The results indicated that both treated and untreated sexes could secrete a pheromone that was able to stimulate the opposite sex as well as its own sex. But production and responsiveness of pheromone in untreated groups were significantly higher than treated one.

Females secreted a pheromone is a sex pheromone, While the pheromone secreted by males is an aggregation pheromone.

Keywords: *Tribolium castaneum*, pyriproxyfen, pheromone production and perception, daytime, age, hunger, temperature and antennal sensillae.

INTRODUCTION

The rust red flour beetle, *Tribolium castaneum* (Herbst), is one of the serious pests of flour and other cereal products in Egypt and other countries. Mixing chemical protectants with grains is currently one of the main methods for controlling insect pests in such stored products. One promising way to fulfill this need is through the use of insect growth regulators (IGRs). IGR was introduced to describe a new class of bio-rational compounds. IGRs have very low toxicity to mammals and other non-target organisms and, usually, are rapidly degraded in the environment (Kostyukovsky *et al.*, 2000). These characteristics make IGRs as potential alternatives to conventional insecticides.

Pyriproxyfen (Admiral) is a new juvenile hormone analogue (JHAs); acts as anti-JHs which artificially enhances JH levels preventing insect development to the adult stage (Leighton *et al.*, 1981). It is an effective pesticide against

Hymenoptera, Dictyoptera and Heteroptera (Mojaver & Bandani, 2010).

Pheromones must be considered a major mode of intraspecific communication in insects that acts to elicit a specific behavioral or developmental response from other organisms of the same species (Nordlund, 1981).

The aim of this study was clarify the possibilities of using IGR (Pyriproxyfen) and sex pheromone in pest control.

MATERIALS AND METHODS

Insect colony:

A laboratory colony of the red flourbeetle, *T. castaneum* was maintained 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).

Juvenile hormone analogue "JHA":

Juvenile hormone analogue (10%EC), Pyriproxyfen (Admiral) was tested in the present study. Its chemical formula is (2-[1-methyl-2-(4-phenoxyphenoxy) ethoxy] pyridine).

Bioassay test:

Fourth larval instar were obtained from the synchronized population reared on flour media, and then transferred to a treated freshly diet for feeding. The feeding technique was used according to Oberlander, (1997). An appropriate stock concentration was prepared in distilled water and mixed with diet. Four replicates were performed for each concentration. A preliminary experiment was carried out to determine the effect of LC₅₀ of Pyriproxyfen as a Juvenile hormone analogue against 4th larval instar of *T. castaneum*. A wide range of concentrations ranging from 0.1 to 10 ppm (0.1, 0.5, 1, 5 and 10 ppm) were used. In addition, a corresponding untreated control was used. Mortality was scored 48 hrs after feeding. A total of 100 beetles were tested per concentration.

Evidence of pheromone production on *T. castaneum* adult treated as 4th larval instar by LC₅₀ of Admiral and untreated one:

The olfactometer used in the present study was a vial type similar to that used by Burkholder (1970). It consisted of a glass vial (15x1.5cm), 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 that tested for response was placed on the bottom of the vial. The distance between the two insects was 4 cm.

Ten replicates each one contains 10 vials and in each vial two individuals (male and female) were placed

separately. The tested males and females were 8-10 days old.

Hexane was the solvent used for extracting pheromone in the following experiment at (0.3) female equivalents (FE) per 10 μ of solvent according to Hussien (1982).

Statistical analysis

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

RESULTS AND DISCUSSION**Effect of Pyriproxyfen on 4th larval mortality**

Table (1) showed the percentages of larval mortalities as 1.00, 3.00, 7.00, 13.00 and 15.00 at the Admiral concentrations of 0.1, 0.5, 1, 5 and 10 ppm, respectively. This result is similar to that obtained on nymphal stage by (Hitoshi *et al.*, 1989) on cockroach, (Hatakoshi *et al.*, 1991) on *Myzus persicae*, (Ishaaya & Horowitz, 1992) on sweet potato white fly, (Wood & Godfrey, 1998) on *Aphis gossypii*, (Elbert & Nauen, 2000) on *Bemisia tabaci*, and on larval stage, (Boina *et al.*, 2010) on 5th larval instar of *Diaphorina citri*. Larval mortality may be as a result of competing of JHA with JH in binding to the JH receptors or to the JH carrier proteins, injuring the corpora allata cells, or interfering with JH biosynthesis (Leighton *et al.*, 1981). Also, percentages mortality of both pupal and adult stage were significantly ($P < 0.01$) increased to record 9, 11, 28, 35 and 39 % for pupae and 0.0, 1, 11, 13 and 19 % for adults.

The percentage of adult emergence decreased with the increase of concentrations showing 90, 86, 65, 52 and 46 decrease. The present results indicated that there was gradual inhibition for adult emergence percentages of 10, 14, 35, 48 and 54 with the tested concentrations of 0.1, 0.5, 1, 5

and 10 ppm. Similar effects were obtained by Hitoshi *et al.* (1989) on cockroach, Miyamoto *et al.* (1993) on Mosquito larvae, Dhadialla *et al.* (1998), Mojaver and Bandani (2010) on *Eurygaster integriceps* and Boina *et al.* (2010) on 5th larval instar of *Diaphorina citri*.

The LC50 value of the tested IGR Pyriproxyfen (Admiral) against the 4th larval instar of *T. castaneum* was 2.4ppm. Latent effect of Admiral on larval, pupal and adult malformation of *T. castaneum* treated as 4th larval instar.

Results obtained in the same table and represented in Fig. (1) declared that larval, pupal and adult malformation percentages had a positive relationship with the different concentrations of Admiral except with percentage of adult malformation at 5 ppm. These data are similar with those obtained by Kostyukovsky *et al.* (2000), Mojaver and Bandani (2010) on *Eurygaster integriceps* and Boina *et al.* (2010) on 5th larval instar of *Diaphorina citri*.

Table 1: Effect of Admiral against *Tribolium castaneum*, treated as 4th larval instar.

Conc. (ppm)	% larval mortality ±SE	% larval malformation ±SE	% pupal mortality ± SE	% pupal malformation ±SE	% adult mortality ±SE	% adult malformation ±SE	% emerged adult ±SE	% inhibition of adult emergence
0.1	1.00±0.00	00.00	9.00±0.63	8.00±0.71	00.00	00.00	90.00±0.75	10.00
0.5	3.00±0.49	00.00	11.00±0.25	11.00±0.25	1.00±0.25	1.00±0.25	86.00±0.48	14.00
1	7.00±0.47	3.00±0.48	28.00±0.29	20.00±0.71	11.00±0.43	8.00±0.00	65.00±0.57	35.00
5	13.00±0.50	8.00±0.82	35.00±0.85	27.00±1.11	13.00±0.72	7.00±0.85	52.00±0.48	48.00
10	15.00±0.49	15.00±0.49	39.00±0.48	39.00±0.48	19.00±0.48	19.00±0.48	46.00±1.31	54.00
Control	00.00	00.00	00.00	00.00	00.00	00.00	100	00.00
p-value	**	**	**	**	**	**	**	-

Latent effect of Admiral on certain biological aspects (larval and pupal duration, fecundity, fertility, sterility and oviposition deterrent index) of *T. castaneum* treated as 4th larval instar:

Larval and pupal durations were increased significantly ($P < 0.01$) as shown in table (2). This result is in conformity with that obtained by Reid *et al.* (1994) on German cockroach, Liu and Chen (2001) on *Lipaphis erysimi* and Liu (2003) on *Thrips tabaci*.

Prolongation in larval duration may be as a result of an increase in hemolymph total proteins and carbohydrates and decrease of total lipids and cholesterol Bakr *et al.* (2004). Generally, such prolongation is dependent on special hormone condition (Slama *et al.*, 1974) on last nymphal instar of *Schistocerca gregaria*.

On the other hand results tabulated in Table (2) showed that admiral induced reduction in both fecundity and fertility, on contrast, (O.D.I) and sterility showed a positive relationship with the increasing of concentrations. The present work are coincide with that obtained by Kawada *et al.* (1989) on last-instar of *Blatella germanica*, Liu and Chen (2001) on *Lipaphis erysimi*, Boina *et al.* (2010) on 5th larval instar of *Diaphorina citri* and Liu (2003) on *Thrips tabaci* who thought that reduction of hatchability could be caused by sterilizing eggs, reducing survival of viable eggs, or reducing fecundity of the adults. This reduction may be also due either to an effect on some later steps in the differentiation and function of follicular cells (Gelbic and Sehna, 1973) or to derangement of humoral control of oviposition (Matalin and Gelbic, 1975).

Table 2: Biological activity of Admiral against *Tribolium castaneum*, treated as 4th larval instar.

Concentrations (ppm)	larval duration (days) $\pm$ SE	pupal duration (days) $\pm$ SE	No. of eggs/female (fecundity) $\pm$ SE	% fertility $\pm$ SE	% sterility	% oviposition deterrent index (O.D.I)
0.1	14.00 $\pm$ 0.26	8.44 $\pm$ 0.10	255.00 $\pm$ 1.76	84.31 $\pm$ 0.62	17.94	1.35
0.5	16.15 $\pm$ 0.37	9.92 $\pm$ 0.08	244.00 $\pm$ 1.71	81.56 $\pm$ 1.66	24.04	3.56
1	17.11 $\pm$ 0.32	10.81 $\pm$ 0.12	195.00 $\pm$ 0.60	75.90 $\pm$ 1.27	43.51	14.66
5	17.89 $\pm$ 0.41	10.91 $\pm$ 0.14	176.00 $\pm$ 1.26	69.89 $\pm$ 1.18	53.05	19.63
10	8.90 $\pm$ 0.44	6.54 $\pm$ 0.08	138.00 $\pm$ 1.41	65.94 $\pm$ 0.66	65.27	31.00
Control	14.17 $\pm$ 0.18	9.44 $\pm$ 0.05	262.00 $\pm$ 1.29	100 $\pm$ 0.0	00.00	00.00
p-value	**	**	**	**	-	-

P-value of ANOVA:

= significantly different at $P < 0.01$ **Morphological abnormalities**Larvae affected by Admiral**

Failures of larval development events as a result of treatment with Admiral are shown in Plate (1) as larva was dark with wing buds on both sides and also larva was dark and swollen in whole body, these abnormalities due to Admiral interfere with JH biosynthesis and consequently prevent larval development to adult stage.

Pupae affected by Admiral after treatment 4th larval instar

Gradation in morphogenic changes in pupae is shown in Plate (2) such as abnormal pupal appearance with stretched, transparent wings, also, Larval-pupal monstrosity with larval cuticle patches and shrinkage in pupal body.

Adults affected by Admiral after the 4th larval instar treatment

Symptoms of adult emergence failure induced by feeding of larvae on Admiral are illustrated in Plate (3) such as adult with transparent elytra and with longed, stretched hind wing.

Effect of LC₅₀ (2.4ppm) of Admiral on responsiveness and production of pheromones in male and female adult beetles which resulted from treated 4th larval instar:**Evidence of pheromone production:-**

Results on the response of treated virgin females and males of *T. castaneum*, to pheromone produced by either treated sexes (8-10 days old), under constant conditions of 30°C and 70 %R .H. are given in Table (3).

Table 3: Response of virgin *Tribolium castaneum* males and females (8-10 days old) to adults of both sexes produced by treated 4th larval instar by LC₅₀ of Admiral.

Types of Experiment	Percentage of response					P- Value
	Treated	Corrected experiment	Untreated	Corrected experiment	With only solvent	
Male tested against female	40	34.78 $\pm$ 0.32	80	78.26 $\pm$ 0.93	8	**
Male tested against male	28	21.74 $\pm$ 0.20 b	66	63.04 $\pm$ 0.76	8	**
Female tested against female	18	12.77 $\pm$ 0.20	54	51.06 $\pm$ 0.56	6	**
Female tested against male	14	08.51 $\pm$ 0.40 a	42	38.30 $\pm$ 0.45	6	**
P- Value	**	-	**	-	N.S	-

ANOVA P-Value:

**= Significantly different at $P < 0.01$.
non Significantly different.

Student's (t) test:

a- significant difference between male tested against males N.S= treated group (b) at $P < 0.01$.
b- significant difference between female tested against males treated group (b) at $P < 0.01$.

Male response behavior to female:-

The level of response 34.78 % was reached when treated males were tested against treated females. While in untreated one and used solvent only the response reach 78.26 and 8.00 %, respectively.

The response behavior of treated male beetles to treated female consisted of a sequence of increasing levels of excitation similar to that resulted from adults produced by treated 4th larval instar by Atabron (Bakr *et al.* 2010).

Male response behavior to male:-

Treated males also responded at a level of 21.74 % to treated male beetles. but response of untreated one and used solvent only were 63.04 and 08.00 %, respectively. The response of treated male beetles to their own sex also consisted of a sequence of events which is similar to that resulted from adults produced by treated 4th larval instar by Atabron (Bakr *et al.* 2010).

Female response to female:-

Treated females tested against their own sex showed a level of 12.77 % of response. While response of untreated one and used solvent was only 51.06 and 6.00 %, respectively.

The treated females exhibited a sequence of events similar to that resulted from adults produced by treated 4th larval instar by Atabron (Bakr *et al.* 2010).

Female response behavior to male:-

The level of response 08.51 % was reached when female beetles were tested against males but response of untreated one and used solvent only were 38.30 and 6.00 %, respectively. In this case, females also exhibited a sequence of events similar to those mentioned in male response behavior to male.

The present study indicates that virgin female adults of *T. castaneum* produce the sex pheromone. Sex pheromone- producing females have

been reported for another untreated related species (Oceallachain and Ryan, 1977 on *T. confusum*).

The results indicated that both treated and untreated sexes of the rust red flour beetle could secrete a pheromone that was able to stimulate the other sex as well as its own sex. But responsiveness and production of pheromone in untreated groups were significantly higher than treated one. The degree of response varied according to the source of pheromone. Thus, females secreted a pheromone that stimulated and highly excited males more than females. Thus the female pheromone appeared to be a sex pheromone. On the other hand, the pheromone secreted by males seemed to be an aggregation pheromone and both sexes were affected by this pheromone for aggregation. Results obtained in the present study are in agreement with those results obtained by (Narayanan and Nadarajan, 2005 in *Antigastra catalaunalis* and Ruther *et al.*, 2007 in jewel wasps, *Nasonia vitripennis*) and Bakr *et al.* 2010.

Pheromone extraction by different solvent:-

The obtained results on the response of males of *T. castaneum* to extracts by different solvents (hexane, diethylether, acetone and chloroform) of virgin females are given in Table (4).

According to the percentage of treated male response to extracts of treated virgin females, the tested solvents could be arranged descendingly in the following manner: hexane 32.61 %, diethylether 30.85 %, acetone 25.00 % and chloroform 18.37 %. While in untreated one, the response reach 76.09, 72.34, 62.50 and 61.22 %, respectively.

Statistical analysis of the data indicated that the difference in response between extracts by either hexane and diethylether, or between acetone and chloroform was not significant and the

difference between the two groups of solvents was significantly different at both treated and untreated case.

The present study proved that hexane is the most effective solvent. This was found true by Abdel Kader *et al.* (1986b) on untreated *T. castaneum*.

Table 4: Efficiency of different solvents in sex pheromone extraction of (8-10 days old) virgin *Tribolium castaneum* females produced by treated 4th larval instar by Admiral.

Types of Solvent	Percentage of male response					P- Value
	Treated	Corrected experiment	Untreated	Corrected experiment	With only solvent	
Hexane	38	32.61±0.20	78	76.09±0.87	8	**
Diethylether	32	30.85±0.37	74	72.34±0.43	6	**
Acetone	28	25.00±0.20	64	62.50±0.98	4	**
Chloroform	20	18.37±0.32	62	61.22±0.24	2	**
P- Value	**	-	**	-	N.S	-

Bioassays were conducted at 30°C and 70% R.H.

ANOVA P-Value:

**= Significantly different at P<0.01.

N.S= non Significantly different.

Effect of pheromone concentration on male response:-

The response of males of *T. castaneum* to different pheromone concentrations or titers of virgin females is given in Table (5). According to the percentage of male response to different concentrations of virgin females extracts, the obtained results showed that the male response increased progressively and significantly with the increase of pheromone titers at both treated and untreated case.

The treated male response started with low level 10.87 % at 0.1 female equivalent and increased with the increase of female equivalents to reach the maximum level of response of 51.11 % at 0.9 female equivalent. While in untreated one the lowest response was 47.83 % and the highest response was 88.89 %.

These observations was similar to those obtained by Fatzinger and Asher (1971) on *Dioryctria obietella* (Lepidoptera: Pyralidae).

Table 5: Response of male *Tribolium castaneum* to pheromone concentrations (female equivalents) of virgin females, (Both sexes were 8-10 days old) produced by treated 4th larval instar by Admiral.

Different pheromone concentrations (titers)	Percentage of male response				
	Treated	Corrected experiment	Untreated	Corrected experiment	With only solvent
0.1	18	10.87±0.20	52	47.83±0.65	8
0.2	26	21.28±0.24	66	63.83±0.87	6
0.3	38	32.61±0.37	78	76.09±0.34	8
0.5	48	44.68±0.37	84	82.98±0.86	6
0.8	52	48.94±0.37	88	87.23±0.35	6
0.9	56	51.11±0.24	90	88.89±0.76	10
P- Value	**	-	**	-	N.S

Bioassays were conducted at 30°C and 70% R.H.

ANOVA P-Value:

**= Significantly different at P<0.01.

N.S= non Significantly different.

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Plate 1: showed the Effect of Admiral on 4th larval instar.



(A)



(B)



(C)



(D)



(E)



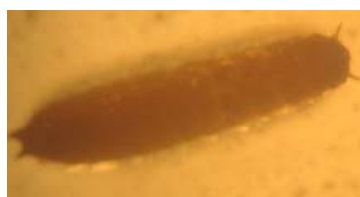
(F)



(G)



(H)



(I)

- A) Normal larva.
- B) 5th larval instar, with 4th larval exuvium adhering to terminal abdomen (unsclerotized) (arrow).
- C) 4th larval instar became dark brown, depressed and failed to develop to the next instar.
- D) 5th larval instar with 4th larval instar exocuticle and inhibited to complete moulting.
- E) Larval exuvium was adhering to head capsule, abdominal end and larva become thinner and twisted.
- F) Larval exuvium was adhering to head capsule.
- G) Larva with small, dark head and large, light, unsegmented abdomen.
- H) Larva was dark with wing buds on both sides.
- I) Larva was dark, shrink and swollen in whole body.

Plate 2: showed the Effect of Admiral on pupal stage after treatment 4th larval instar



(A)



(B)



(C)



(E)



(D)



(F)



(G)



(H)



(I)

- (A)- Normal pupa.
 (B)- Pupa with transparent wing at right side of body.
 (C)- Pupa failed to emerge from old exuvium to convert into adult.
 (D)- Pupal- adult intermediate with adult head capsule, thorax and unable to free from the pupal exuvium during moulting.
 (E)- The last larval instar was unable to free the head capsule from exuvium during transformation into pupa.
 (F)- Abnormal pupal appearance with stretched, transparent wings.
 (G)- Pupal- adult intermediate with adult head , thorax and wings, the internal organs also adhering to the abdominal tip.
 (H)- Pupal- adult intermediate with adult head capsule and thorax.
 (I)- Larval- pupal monstrosity with larval cuticle patches and shrinkage in pupal body.

Plate 3: Effect of Admiral on adult stage after treatment 4th larval instar



(A)



(B)



(C)



(D)



(E)



(F)

- (A)- Normal adult.
 (B)- Abnormal adult took horse- shape.
 (C)- Normal adult showing incomplete cuticular left elytron.
 (D)- Normal adult with incomplete elytra and with very short left hind wing.
 (E)- Adult with short, transparent elytra and the pupal exuvium remains adhering to the abdominal end.
 (F)- Adult with transparent elytra and with longed, stretched hind wing.

ARABIC SUMMARY

التأثيرات البيولوجية والسلوكية للبيروبروكسفين على إنتاج وإدراك الفيرمون
لحشرة خنفساء الدقيق

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- 1- قسم علم الحشرات - آلية العلوم - جامعة بها
- 2- قسم علم الحشرات - كلية العلوم - جامعة عين شمس .
- 3- قسم الاحياء - كلية العلوم - جامعة الملك خالد- ابها - المملكة العربية السعودية -

البيروبروكسفين هو منظم لنمو الحشرات بمثابة المضاد لهرمون الحداثة . ويهدف هذا العمل دراسة تأثير السمية للبيروبروكسفين على كلا الجنسين لخنفساء الدقيق . وحشرة خنفساء الدقيق الناتجة من معاملة الطور اليرقى الرابع بالتركيز القاتل للنصف (2.4 جزء من المليون) وتأثيرها على إنتاج الاناث للفيرمون وإدراك الذكور لها . أشارت النتائج الى ان كلا الجنسين المعالجة وغير المعالجة يمكن ان تفرز فيرمون قادر على تحفيز الجنس الآخر فضلا عن ممارسة الجنس الخاص بها . ولكن كان الانتاج والقدرة على الاستجابة للفيرمون فى المجموعات غير المعالجة أعلى بكثير من المعالجة . وتفرز الانث فيرمون يدعى فيرمون الجنس فى حين ان الفيرمون الذى يفرزه الذكور هو فيرمون التجميع .