Biological and biochemical changes in pink bollworm, *Pectinophora gossypiella* after treatment with Hexaflumuron and Chlorfluazuron.

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Abstract

The effects of two insect growth regulators (IGR_S), Hexaflumuron and Chlorfluazuron were evaluated on some biological aspects; pre-oviposition, oviposition and post oviposition periods, total number of deposited eggs (fecundity), percentage of hatchability (fertility) and longevity' in addition to some biochemical parameters such as total protein, total lipids and amylase as well as glutamic oxaloacetic transaminase (GOT = ALT) and glutamic pyruvic transaminase (GPT = AST) enzymes in one and three day old adults of pink bollworm, *Pectinophora gossypiella* (Saunders). This laboratory study was done under conditions of 26 ± 1 C° and 75 ± 5 R.H. Data revealed that one and three day old adults were more susceptible to Hexaflumuron than Chlorfluazuron at two concentrations of LC₅₀ and LC₂₅ values. Also, Hexaflumuron showed more than Chlorfluazuron' significant increase in pre-oviposition, post oviposition period, percentage of hatchability and longevity of both females and males. On the other hand, there was a significant decrease in oviposition period and total numbers of deposited eggs. Again, Hexaflumuron caused more reduction than Chlorfluazuron in total protein and glutamic pyruvic transaminase enzyme. In contrast, Chlorfluazuron caused more reduction than Hexaflumuron in total lipids and glutamic oxaloacetic transaminase.

Key words: Hexaflumuron, Chlorfluazuron, Pectinophora gossypiella and biological study

Introduction

The pink bollworm *Pectinophora gossypiella* (**Saunders**) is an important insect pest that attacks the cotton bolls in Egypt and other countries.

The first chitin synthesis inhibitor introduced into the market as a novel insecticide was benzoylphenylurea (BPU), or diflubenzuron (DFB) (**Miyamoto** *et al.* **1993**).

Insect growth regulators are a unique class of insecticides with selective effects on various life stages of some orders of insects. Chitin synthesis inhibitors are group of IGRs that interfere with the formation of new cuticle, (Hoffmann and Lorenz, 1998). Many authors recorded that IGRs play an important role in different developmental stage in insects. Abdel-Aal (2006) reported that fecundity and egg- hatchability percent of treated cotton leaf worm S. Littoralis female with IGR compounds decreased when compared with control. Also, Rashad et al. (2006) indicated that treating adults of P. gossypiella with diflubenzuron, caused reduction in female fecundity and fertility. Recently, Yasir et al., (2012) recorded that the fecundity and egg hatchability were reduced at all concentrations of Lufenuron used against *Tribolium castaneum* larvae.

The present study was carried out to determine the toxicity of Hexaflumuron and chlorfluazuron on one and three day old adults of *P. gossypiella*. Subsequently, some biological aspects, as well as biochemical studies for treated adults were studied.

Materials and methods

Pesticides used:

Two Insect growth regulators (IGR_S) were experimentally used in this study:

1. Hexaflumuron:

Trade name: - Consult

Classification: - Benzoylphenylurea.

Serial concentrations of the two compounds were prepared in water.

2. Chlorfluazuron:

Trade name: Atabron, Fertabron (Caprice 5% EC) **Chemical name:** N-[[3,5-dichloro-4-] [3-chloro-5-(trifluoromethyl)2pyridinyl]oxy]phenyl]amino]carbo nyl]-2,6-difluorobenzamide.

Insect used:

The susceptible laboratory strain of pink bollworm, *P. gossypiella* was reared for several generations in the laboratory under conditions of $26\pm1C^0$ and 75 ± 5 R.H at Bollworms Research Department, Plant Protection Research Institute, Agriculture Research Center as described by **Rashad** and Ammar (1985).

Toxicological studies:

The toxicity of the two experimental compounds, Hexaflumuron and chlorfluazuron were tested against *P. gossypiella* adults (one and three days old after emergence). Fresh serial concentrations from the stock solution of each compound (1ml/liter water) were prepared as follows: 12.5, 6.25, 3.125, 1.61, 0.805 & 0.4025 ppm of Hexaflumuron and chlorfluazuron.

The newly emerged moths were sexed and separated. Three replicates were used for each concentration, each contained five pairs (female and male) of newly emerged moths and also after 3 days of emergence. A piece of cotton wool soaked in 10 % sugar solution treated with one concentration of the tested compounds was hung inside the jar near its upper opening for the moth feeding. On the other hand, three replicates were used as control and adults were fed on untreated 10 % sucrose solution. After one day the jars were examined for dead and alive moths in each jar. Alive moths were transferred to clean jars contained another piece of cotton wool soaked in untreated 10 % sucrose solution. Adult mortalities were recorded three days after treatment. The mortality percentages were estimated. Data were corrected and LC25, LC50 & LC90 of the two compounds were calculated by using Proban software.

Biological studies:

For some biological studies, the newly emerged moths (one and 3 days after emergence) were sexed and separated to be fed on 10 % sucrose solution treated with LC_{50} of Hexaflumuron and chlorfluazuron for 24 hr. Three replicates were used for each concentration, each contained 10 pairs. The same procedure was done with untreated adults fed on 10 % sucrose solution to be used as control.

The moths treated previously with two compounds as well as control were transferred to chimney glass cages (5 pairs/cage). Each treatment was replicated three times. The moths were fed on 10% sucrose solution. Cages were inspected daily to estimate the oviposition period, numbers of eggs laid/female, % hatching and longevity of adult (female and male).

The recorded data were statistically analyzed with one-way analysis of variance (ANOVA) (P < 0.05) (**Snedecor, 1952**) and Duncan's multiple range test means was used (**Duncan, 1955**).

Biochemical analysis:

To study the effect of Hexaflumuron and Chlorfluazuron on some biochemical parameters, of *P. gossypiella* moth, the samples of PBW moth were collected after 7 days of each treatment and kept in clean tubes at 4 °C for chemical analysis.

Total protein, total lipids and amylase, glutamic oxaloacetic transaminase (GOT = ALT) and glutamic pyruvic transaminase (GPT = AST) enzyme activities were determined colorimetrically according to Koller (1984), Drevon and Schmitt (1964), Trinder (1969) and Murray (1984), respectively.

Results and discussion

Toxicological effect of Hexaflumuron and Chlorfluazuron:

The LC₂₅ and LC₅₀ values for one day and three day old adults varied between two compounds. The LC₅₀ and LC₂₅ values of Hexaflumuron were 2.11 and 0.48 ppm for one day old adult and 1.67 and 0.91 ppm for three day old adult. These values were 3.17 and 1.88 ppm for one day old and 3.06 and 2.031 ppm for three day old adults treated with LC₅₀ and LC₂₅ values of Chlorfluazuron compound (Table 1).

These data revealed that one and three day old adults were more susceptible to Hexaflumuron than Chlorfluazuron according to LC_{50} and LC_{25} values. This results agree with **Kandil (2005)**

Table 1. LC_{25} and LC_{50} values of Hexaflumuron and Chlorfluazuron compounds when treated adults of *P*. *gossypiella*.

Treatments	Ages of adult (days)	Toxicity		
		LC_{50}	LC ₂₅	
Hexaflumuron	One day old	2.11	0.48	
	three day old	1.67	0.91	
Chlorfluazuron	One day old	3.17	1.88	
	three day old	3.06	2.031	

Pre – oviposition period:

Data in Table 2 showed that the pre – oviposition period was significantly affected by using Hexaflumuron and Chlorfluazuron compounds.

The preoviposition period of one day treated adults with LC_{50} values of Hexaflumuron and Chlorfluazuron compounds were 3.76 and 4.36 days/ \bigcirc , respectively in comparison with 2.96 days/ \bigcirc in the control. These compounds had no significantly effect on the preoviposition period when adults were treated at three days old. This period was 2.93 and 2.6 days/ \bigcirc , respectively, compared with 2.96 in the control.

Oviposition period:

Hexaflumuron and Chlorfluazuron highly reduced the oviposition period in the treatment of one day old adults to 10.5 and 9.93 days (1.2 - 1.3 time shorter than that of the control), respectively, while, these periods prolonged to 13.46 and 14.4 days when females were treated after 3 days by Hexaflumuron and Chlorfluazuron, respectively, compared with 12.52 days/ \bigcirc in the control.

Post - oviposition period:

Data in Table 3 recorded high significant effects of assayed compounds on post - oviposition period. The averages of post – oviposition period were 9.87 and 7.3 days/ \bigcirc in female treated after one day and 8.63 and 5.33 days/ \bigcirc in female treated after three days with both compounds, respectively. These data indicated that treating the adult females with these

compounds caused significant prolongation in post oviposition period, being 2 to 4.5 times more than that of the control.

Table 2. Effect of LC₅₀ values of two tested compounds on longevity, fecundity and fertility of *P. gossypiella* at $26 \pm 1 \text{ C}^{\text{O}}$ and 75 - 80 % RH.

Time After	compounds	Oviposition period (day±SE)			Fecundity		longevity	
Treatments		Pre-	ovipositi	Post	Total	%	<u> </u>	33
Age Of		oviposi	on	ovipositi	Eggs/♀	Hatchabili		
Adult		tion		on		ty		
ONE DAY	Hexaflumuron	3.76±0.	10.5±0.8	9.87±1.1	147.3±	49.3±9.27	22.4±0.	19.93±0
		01	3	8	7.2		47	.41
	Chlarflugrung	4.36±0.	9.93±0.5	7.3±1.01	156.0±	57.0±1.88	21.6±1.	16.8±0.
	Chlorfluazuron	2^*			3.5		29	4
CONTROL		2.96±0.	12.52±0.	2.88±0.1	228±3.	94.67±0.6	17.33±	15.13±0
CONTROL		09	14	1	58	8	0.9	.7
LSD		0.56	1.53	2.75	14.01	9.13	3.65	1.62
Р		**	**	**	***	***	**	**
THREE DAYS	Hanaflumunan	2.93±0.	13.46±0.	8.63±0.3	190.6±	60.3±1.39	25.33±	21.76±0
	Hexaflumuron	11	45	4	8.1		0.4	.41
	Chlorfluazuron	2.6±0.1	14.4±0.2	5.33±0.1	179.0±	68.7±4.01	23.13±	17.76±0
	CIIIOIIIuazuioii	6	9	1	4.9		1.3	.34
CONTROL		2.96±0.	12.52±0.	2.88±0.1	228±3.	94.67±0.6	17.33±	15.13±0
		09	14	1	58	8	0.9	.7
LSD		N.S.	1.45	1.22	19.52	11.08	1.53	1.51
Р		-	**	***	**	***	***	***

Fecundity and Fertility:

The total numbers of eggs laid/ \bigcirc in all treatments were significantly less than the control. The total numbers of eggs were 147.3 and 156.0 eggs/ \bigcirc treated with Hexaflumuron and Chlorfluazuron after one day of emergence. While adult treated after three days with Hexaflumuron and Chlorfluazuron deposited 190.6 & 179.0 eggs/ \bigcirc , respectively, compared with 288 eggs/ \bigcirc in the control (Table, 2).

The total deposited eggs were 1.9 & 1.8 times fewer in the treatments of the two compounds to one day old adults and 1.5 & 1.6 times fewer for treated three day old adults, respectively.

Analysis of variance of the data given in Table 2 showed highly significant difference in fertility of eggs deposited by PBW moth fed on two compounds after one and three days compared to the control. The reduction was very highly significant and ranged between 49.3 & 57.0 % and 60.3 & 68.7 % for the females treated after one and three days with Hexaflumuron and Chlorfluazuron, respectively.

Longevity of adults:

Female and male longevities were highly significantly affected with Hexaflumuron and Chlorfluazuron after one & three days, respectively. The adult female longevities were 22.4 & 21.6 days/ \bigcirc compared with 17.33 days in the control and 19.93 & 16.8 days/ \bigcirc compared with 15.13 days in the control treated after one day of emergence, respectively. While, by treatment after three days

with Hexaflumuron and Chlorfluazuron, the longevity was significantly prolonged to 25.33 & 23.13 days/ \bigcirc compared with 17.33 days in the control and 21.76 & 17.76 days/ \bigcirc compared with 15.33 days in the control, respectively.

These data indicated that prolonged longevity of both females and males were increased by using the two tested compounds after one & three days.

These results were in agreement with **Abdel-Aal** (2006) who reported that fecundity and eggshatchability percent of treated *S. littoralis* female with IGRs compounds was decreased as compared with control. Also, **Rashad** *et al.* (2006) indicated that treating adults of *P. gossypiella* with diflubenzuron, caused reduction in female's fecundity and fertility.**Saenz-de-Cabezon** *et al.* (2006) showed that lufenuron has ovicidal activity on *L. botrana* in contact treatment. **Oouchi** (2005) and **El-Barkey** *et al.* (2009) stated that IGRs had ovicidal effects on *P. gossypiella.* **Yasir** *et al.* (2012) recorded that the fecundity and eggs hatchability were reduced at all concentrations of Lufenuron used against *T. castaneum* larvae.

Biochemical analysis:

Total soluble protein:

Data in Table 3 revealed that the two tested compounds, Hexaflumuron and Chlorfluazuron when used at LC_{50} values caused high reduction in soluble protein content in adult female than male compared

to control. The total soluble proteins were 5.7 and 6.8 mg/ml/ female and 6.1 and 7.88 mg/ml/ male in Hexaflumuron treatments at one & three days old of adults, respectively, compared with 10.93 and 8.566 mg/ml / female and male in control. In addition, Chlorfluazuron caused a decrease in soluble proteins. The content of protein were 5.05 & 6.5 mg/ml/ female and 7.6 & 7.1 mg/ml/ male after treatment of one & three day adults, respectively.

The high reduction recorded was 53.7 % in the one day adult female treated with Chlorfluazuron and the lowest one was 8.01 % in the three days old of male adult treated with Hexaflumuron. The present

result is in agreement with Kunkle and Nordin (1985).

Total lipid:

Data in Table 4 indicated that tested Hexaflumuron and Chlorfluazuron caused high reduction of lipids. Total lipid contents after one & three days of emergence were 9.87 and 13.6/female and 12.3 and 11.3 mg/ml male when adult female and male were fed on LC_{50} values of Hexaflumuron. While, in Chlorfluazuron treatment after one & three days of adult emergence, the lipid contents were 5.05 and 6.5/female and 7.6 and 7.1 mg/ml male.

Table 3. Effect of two tested compounds on total protein, lipid, ALT and AST of P. gossypiella adult.

Table 5. Effect of two tested compounds on total protein, npid, ALT and AST of F. gossyptetta adult.										
compounds	Old		Prote	%	Lipid	%	ALT	%	AST	%
	of	Sex	in	reductio	(mg/m	reductio	(mg/m	reductio	(mg/m	reductio
	adult		(mg/	n	1)	n	1)	n	1)	n
			ml)							
Hexaflumuron	1 day	44	5.7	-48	9.87	-49.4	23	-51.06	25	-47.9
	old	33	6.1	-28.7	12.3	-14.2	31	-13.8	39	-
	3 day	<u> </u>	6.8	-37.8	13.6	-30.2	27	-42.5	34	-29.16
	old	33	7.88	-8.01	11.3	-21.19	31	-13.88	21	-46.15
Chlorfluazuron	1 day	<u> </u>	5.05	-53.7	8.16	-58.1	19	-59.57	29	-39.6
	old	33	7.6	-11.28	10.33	-27.96	27	-25.0	36	-7.69
	3 day	<u> </u>	6.5	-40.5	11.93	-38.8	31	-34.04	29	-39.58
	old	33	7.1	-8.57	13.2	-7.69	23	-36.1	21	-46.15
control		<u> </u>	10.93	-	19.5	-	47	-	48	-
		33	8.56	-	14.34	-	36	-	39	-

The two compounds caused high reductions in total lipid compared with control 19.5 and 14.34mg/ml/ female and male, respectively.

The high reduction was 58.1 % recorded in one day old adult female treated with Chlorfluazuron and the lowest was 7.69 % recorded in three days old adult male treated with Chlorfluazuron.

On contrary to the present result, **Keeley (1985)** and **Kunkle and Nordin (1985)** recorded that the increase in total lipid caused increase on the mean number of eggs and hatchability percentage. In insect adult females, the major function of the fat body is the synthesis and release of vitellogenic proteins and lipids for yolk formation during oocyte maturation.

Transaminase enzymes (GOT and GPT or ALT and AST).

Data in Table (3) showed a reduction in the transaminase enzymes activity of PBW adults treated with LC_{50} of Hexaflumuron and Chlorfluazuron.The levels of ALT after treatment of one day old were 23.0 & 31.0 mg/ml and after three days old adult were 27.0 & 31.0 mg/ml for both female and male, respectively, treated with Hexaflumuron compared with 47.0 & 36.0 mg/ml in the control for both female and male, respectively. In the treatment of Chlorfluazuron, the levels of ALT after one day old adult were 19.0 & 27.0 and after three days old adult

were 31.0 & 23.0 mg/ml for both female and male, respectively.

In the treatments of both compounds on the one and 3 day old adults, AST was notably decreased when compared with the control. In Hexaflumuron treatment, AST level was 25& 39 mg/ml in one day old female and male, respectively and 34 & 21 mg/ml in 3 days old female and male, respectively. On the same manner, in the treatment of Chlorfluazuron, the level of AST was 29 & 36 mg/ml in one day old female and male adults, respectively and 29 & 21 mg/ml in 3 days old female and male adults, respectively when compared with 48 & 39 mg/ml female and male in control, respectively.

The high reduction in ALT enzyme was 59.57 % in one day old adult female treated with Chlorfluazuron and the lowest reduction was 13.8 % in one day old adult male treated with Hexaflumuron. In case of AST enzyme, the high reduction was 47.9 % in the one day old adult female treated with Hexaflumuron. On the other hand, Hexaflumuron treatment had no effect on the level of AST enzyme in the one day male adult.

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التغيرات البيولوجية والبيوكيميائية بعد معاملة حشرة دودة اللوز القرنفلية بمركبى Hexaflumuron and Chlorfluazuron

ميرفت عبد السميع قنديل محمد سالم محد سالم ، أيمن محمد محى الدين عدلى معهد بحوث وقاية النباتات-مركز البحوث الزراعية-الدقى-الجيزة

تم معاملة الحشرة الكاملة عمر يوم وثلاثة ايام لدودة اللوز القرنفلية بمركبى Hexaflumuron و Chlorfluazuron ودراسة تأثيرهما على بعض النواحى البيولوجية (فترة ما قبل وضع البيض – فترة وضع البيض – فترة ما بعد وضع البيض وعلى الكفاءة النتاسلية و نسبة فقس البيض بالاضافة الى عمر الحشرة الكاملة ذكورا وانائككما تم دراسة تأثيرهما على بعض النواحى البيوكيميائية مثل المحتوى البروتينى والدهون الكلى وعلى انزيمى AST و ALT.

اظهرت النتائج وجود تاثيرات معنوية عند استخدام المركبين على الحشرات الكاملة عمر يوم وثلاثة ايام فقد ادى الى زيادة فترة ما قبل وضع البيض وفترة ما بعد وضع البيض ونسبة الفقس وزيادة عمر ا لذكور والاناث لدودة اللوز القرنفلية بينما حدث نقص لكلا من عدد البيض الذى تضعة الانثى (الكفاءة التناسلية) ونسبة الفقس (الخصوبة).

اظهر المركب Hexaflumuron نسبة خفض اعلى من مركب Chlorfluazuron في المحتوى البروتيني الكلى وانزيم AST داخل الحشرة. بينما اظهر مركب Chlorfluazuron نسبة خفض اعلى من مركب Hexaflumuron في محتوى الدهون الكلى وانزيم ALT داخل الحشرة.