Effect of Three Insect Growth Regulators on Some Biological Aspects of the Cotton Leafworm, *Spodoptera littoralis (Boisd)*

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ABSTRACT

*Effects of three insect growth regulators (IGRs) chlorfluazuron, flufenoxuron and pyriproxyfen on certain biological in **Spodoptera Littoral's** parameters were evaluated. The two chitin synthesis inhibitors, chlorfluazuron and flufenoxuron were the first and second most toxic IGRs, respectively for the larvae, whereas the juvenile hormone mimic, pyriproxyfen was the least toxic compound. Chlorfluazuron and flufenoxuron decreased the mean larval duration in the parental generation (the generation during which the treatment was carried out) and in both the first and second generations (the generations following the parental generation) as compared to non-treated larvae. Such decrease in larval duration was only significant in chlorfluazuron – treated larval in the parental generation. However, pyriproxyfen resulted slightly increased in the parental and first generation the slightly decrease in the second-generation .In the same context, all treatments resulted in a slight increase in the pupal duration as compared to parallel control. However, these treatments slightly decreased in pupal weight and such decrease was generation-dependent.*

Both sexes of Spodoptera littoralis moths survived after treatment of the 4th instars treated with the LC_{50} values of chlorfluazuron, flufenoxuron and pyriproxyfen lived shorter than non-treated moths in the three generations. Such decrease was only significant for chlorfluazuron-insects in all generations, and for the male and female moths treated with flufenoxuron in both the parental and second generations, respectively. The percent of egg-hatch also was decreased as a result of treatment with the three tested IGRs in the three generations. However, chlorfluazuron exhibited the highest effect in suppressing the reproductive potentials of the female moths.

Key words: Growth Regulators, Cotton Leafworm, *Spodoptera littoralis*.

INTRODUCTION

The cotton leafworm, ***Spodoptera littoralis*** (Boisd.) is considered one of the most serious insect pests in Egypt, causing a great damage to many agricultural economic crops especially cotton.

The main way for controlling this pest is still by using Insecticide. There are many problems appeared with the repeated use of Insecticide include hazards to man and his animals and the appearance of resistant strains to Insecticide. The foregoing problems and hazards that have arisen as a result of the use of conventional insecticides are incentives for researching alternative control agents with new modes of action. Among these agents are the insect growth regulators (IGRs). Moreover, IGRs elicit their primary action on insect metabolism, ultimately affecting development and growth of the target insect, particularly when such compounds are applied during the sensitive period of insect development Essawy, *et al* (1985). They induce morphological abnormalities as well as death of treated insects Ahmed, *et al* (1990). These characteristics made IGRs one of the most promising new control agents Mauchamp, (1974); El-Guindy and Bishara (1976); Abid, *et al* (1978); Barnby, and Klocke (1987); Abdel-Azeem, (1989); Ignoffo , and couch (1989); Gadalla, *et al* (1990); Corbitt, *et al* (1989); Moawad, *et al* (1990); Abdalla, and Sammour, (1992); Fisk, and Wright, (1992b); Fisk, and Wright, (1992a); Abo El-Ghar, (1993); Guyer, and Neumann (1998) and Guerra, *et al* (1999) .

The present study aims to investigate the following points: Evaluation of three insect growth regulators (chlorfluazuron, flufenoxuron and pyriproxyfen) for controlling *Spodoptera littoral's* larvae. This can be attained by determining their possible larvicidal effects as well as determining their possible latent effects on certain biological aspects.

MATERIALS AND METHODS

1. Rearing technique:

Larvae of ***Spodoptera littoral's*** surface contamination of the artificial diet (Shory and Hall 1965) were obtained from the Research Division of the Cotton Leaf worm, Plant Protection Research Institute. Newly hatched larvae were transferred to clean glass jars covered with muslin held in position with rubber bands. They were fed on artificial diet, at 27±2°C and 65 ± 5% RH and examined daily.

2. Insect growth regulators:

Commercial formulations of three insect growth regulators (IGRs) obtained from Sumitomo Chemical Co., Ltd. were used during the present work. Two of which are chitin synthesis inhibitors and the third is a juvenile hormone analogue.

Chitin synthesis inhibitors:

Chlorfluazuron (IKI-7899, 10% EC).

Juvenile hormone analogues: Flufenoxuron (10% EC) 2.2.

Pyriproxyfen (Admiral, 10% EC)

Experimental technique:

Toxicity test:

Susceptibility of ***Spodoptera littoral's*** larvae to the IGRs was carried out with use of the leaf-dipping technique (Abo El-Ghar *et al.*, 1994b). Different aqueous concentrations of the IGRs (chlorfluazuron, flufenoxuron and pyriproxyfen) were prepared. Castor oil leaves, *R. communis*, were dipped in each concentration level, left to dry at room temperature and then were offered to the newly moulted 4th instars. Four replicates each of 50 larvae were used for each concentration. Larvae that fed on castor oil leaves dipped in distilled water only were used as control. Treated larvae were allowed to feed for 24 hr. Then, they were provided with fresh, clean and untreated castor oil leaves until pupation. The mortality percentages of treated larvae were corrected against those of the control by Abbott's formula (Abbott, 1925).

The data were then subjected to probit analysis (Finney, 1971) to give values of LC_{50}. The toxicity index of each of the tested IGRs was determined according to Sun (1950).

Biological studies:

Newly moulted 4th instar larvae treated with the LC_{50} of the three tested compounds as described above were examined daily to determine the larval and pupal durations, pupal weight and adult emergence. The longevity, fecundity and fertility of ***Spodoptera Littoral's*** moths that survived each treatment were determined and compared with those of untreated insects.

The percentage of egg-hatch or fertility also was determined. Sterility was calculated according to Toppozada *et al.* (1966). All the foregoing biological parameters, which were determined for the parental generation (P), were extended and determined for the first (F_1) and second (F_2) generations. The "P" generation refers to the generation to which the IGRs were applied and (F_1) and (F_2) refer to the first two generations surviving treatment of "P" generation. Four replicates each of 50 larvae each were carried out.

RESULTS AND DISCUSSION

Biological studies:

Toxic effect:

Table (1) shows the susceptibility of 4th instar of ***Spodoptera Littoral's*** towards the tested IGRs. Based on LC_{50} values the toxicity of the IGRs can be arranged in a descending order as follows: chlorfluazuron > flufenoxuron > pyriproxyfen. The toxicity index indicates that chlorfluazuron (standard IGR) was about 2.12 and 3846 times as toxic as flufenoxuron and pyriproxyfen, respectively. The differences between the LC_{50} value of chlorfluazuron and that of pyriproxyfen were significant. There was no significant difference between the LC_{50} value of chlorfluazuron and that of flufenoxuron.

Latent effect:

Larval duration:

The obtained data (Table 2) shows that treatment with chlorfluazuron significantly decreased the larval duration of the parental generation of ***Spodoptera littoralis***, as compared to control. Treatment with chlorfluazuron and flufenoxuron slightly decreased the larval duration of both the first and second generations, as compared to control. Pyriproxyfen also followed the same pattern, as compared to control in the second generation only. The reverse was true for larval duration of the first generation treated with this JHA where such duration was slightly increased. The data showed that the larval duration of ***Spodoptera littoralis*** treated insects with chlorfluazuron, flufenoxuron and pyriproxyfen in the second generation was about 87.74, 91.51 and 97.17% of the control, respectively.

Pupal duration:

The chitin synthesis inhibitors, chlorfluazuron and flufenoxuron slightly increased the pupal duration of the parental generation of ***Spodoptera littoralis***, as compared to control. Also, pupal duration was slightly increased in pyriproxyfen-treated larvae as compared to control (Table 3). The same pattern was also obtained for the pupal duration of both the first and second generations as compared to parallel values control due to treatment with the three tested IGRs.

Pupal weight:

Treatment of 4th instar larvae of *Spodoptera littoralis* with the chitin synthesis inhibitors, chlorfluazuron and flufenoxuron resulted in a decrease of the weight of resulted pupae in the parental, first and second generations, as compared to control. However, the juvenile hormone analogue, pyriproxyfen did not, approximately, affect the pupal weight of *Spodoptera littoralis*, as compared to control in all the three generations (Table 4). The pupal weight of *Spodoptera littoralis* was decreased progressively from one generation to another in IGRs-treated insects.

Adult longevity:

Table (5) shows that the adult longevity of both sexes of ***Spodoptera littoralis*** which survived after treatment of the 4th instar larvae with the three tested IGRs was decreased as compared to control during the three generations except of mal larvae-treated with pyriproxyfen with no change .It must be noted that Chlorfluazuron exerted the highest magnitude in decreasing the longevity. The effect of flufenoxuron on the moths' longevity was sex and generation-dependent. In this context, the significant decrease in longevity of moths was obtained for the males of the parental generation and for the females of the second generation.

Fecundity:

Table (6) shows the fecundity of ***Spodoptera littoralis*** female moths survived after treatment of the 4th instar larvae with chlorfluazuron, flufenoxuron and pyriproxyfen. The results obtained showed that the lowest number of eggs laid per female and consequently the highest oviposition deterrent index were obtained for treated females mated with treated males, followed by treated females mated with normal males, as compared to control. On the other hand, the highest number of eggs laid per female and consequently the lowest oviposition deterrent index were occurred when the males were the treated sex only. This may indicate that the females were more sensitive to IGRs than the males. In all the mating combinations, chlorfluazuron was the most effective IGR; whereas pyriproxyfen was the least effective one. The fecundity of the treated females, relative to the generation, was linear. Thus, the fecundity obtained for the females of the second generation was about twice more than that obtained for the females of the parental generation in case of treatment with chlorfluazuron. However, in case of treatment with either flufenoxuron or pyriproxyfen, the fecundity of the second generation females was about 1.5 times more than that of the parental generation females. In the second generation, the fecundity of treated female moths approached that of non-treated female moths.

Fertility:

Table (7) shows that in all the mating combinations the egg-hatch percent was decreased, as compared to control. This reduction was much obvious in case of chlorfluazuron treatment, followed by flufenoxuron and pyriproxyfen treatments. This is also true for the percentage of sterility. The fertility followed the same pattern as that of fecundity, i.e., the lowest egg-hatch was obtained in the mating combination containing treated females. The females were more sensitive to IGRs than the males. The egg-hatch was steadily increased up to the second generation in treated insects and became very close to that of non-treated insects.

Table (1): Susceptibility *Spodoptera littoralis (Boisd.)* 4th instar larvae to chlorfluazuron, flufenoxuron and pyriproxyfen.

IGR's	LC_{50} (ppm)	95% Fiducial limits		Slope ± S.E.	Toxicity index
		Lower	Upper		
Chlorfluazuron	0.1890	0.1710	0.3150	1.82 ± 0.258	100
Flufenoxuron	0.4014	0.1980	0.6890	1.99 ± 0.294	0.4708
Pyriproxyfen	725.30	528.74	1042.58	1.65 ± 0.301	2.6×10^{-2}

Table (2): Effect of LC_{50} of chlorfluazuron, flufenoxuron and pyriproxyfen on larval duration of *Spodoptera littoralis (Boisd).*

IGRs	# Mean larval duration (days) ± S.E.		
	P	F_1	F_2
Chlorfluazuron	9.10 * ±0.42	10.41^{ns} ± 0.74	10.47^{ns} ± 0.75
Flufenoxuron	10.03^{ns} ± 0.41	10.21^{ns} ± 0.39	10.35^{ns} ± 0.56
Pyriproxyfen	12.20^{ns} ± 0.38	12.21^{ns} ± 0.85	10.42^{ns} ± 0.34
Control	11.84 ± 0.40	11.06 ± 0.22	11.99 ± 0.75

Ns: not significant. P: Parental generation.; F_1: First generation.; F_2: Second generation.

Table (3). Effect of LC_{50} of chlorfluazuron, flufenoxuron and pyriproxyfen on pupal duration of *Spodoptera littoralis (Boisd.).*

IGRs	Mean pupal duration (days) ± S.E.		
	P	F_1	F_2
Chlorfluazuron	15.52 ^{ns} ± 3.59	16.58 ^{ns} ± 0.67	15.87 ^{ns} ± 0.24
Flufenoxuron	16.57 ^{ns} ± 0.29	17.87 ^{ns} ± 0.82	16.94 ^{ns} ± 0.64
Pyriproxyfen	18.64 ^{ns} ± 0.80	19.58 ^{ns} ± 0.64	17.37 ^{ns} ± 0.39
Control	14.47 ± 0.60	14.88 ± 0.28	14.89 ± 0.72

ns: not significant.
P: Parental generation.; F_1 : First generation.; F_2 : Second generation.

Table (4). Effect of LC_{50} of chlorfluazuron, flufenoxuron and pyriproxyfen on pupal weight of *Spodoptera littoralis (Boisd.)*

IGRs	Mean pupal weight (mg) ±S.E..		
	P	F_1	F_2
Chlorfluazuron	381.19 ^{ns} ± 5.65	375.08 ^{ns} ± 4.97	368.70 ^{ns} ± 12.64
Flufenoxuron	395.25 ^{ns} ± 4.54	359.85 ^{ns} ± 4.51	358.86 ^{ns} ± 13.52
Pyriproxyfen	402.80 ^{ns} ± 4.80	399.82 ^{ns} ± 4.62	349.52 ^{ns} ±10.41
Control	412.62 ± 3.99	417.70 ± 3.60	399.79 ± 3.79

ns: not significant.
P: Parental generation.; F_1 : First generation.; F_2 : Second generation.

Table (5). Effect of LC_{50} of chlorfluazuron, flufenoxuron and pyriproxyfen on adult longevity of *Spodoptera littoralis (Boisd.).*

IGR's	Mean moth longevity (days) ± S.E.					
	P		F_1		F_2	
	Males	Females	Males	Females	Males	Females
Chlorfluazuron	11.0** ± 0.42	13.87* ± 0.74	10.30** ± 0.34	13.60* ± 0.85	14.75* ± 0.49	10.72** ± 0.84
Flufenoxuron	12.8** ± 0.94	15.08 ^{ns} ± 0.95	15.85 ^{ns} ± 0.6	15.50 ^{ns} ± 0.12	15.51 ^{ns} ± 0.56	13.46 * ± 0.55
Pyriproxyfen	15.89 ^{ns} ± 0.95	15.40 ^{ns} ± 0.78	15.72 ^{ns} ± 0.34	15.60 ^{ns} ± 0.54	16.30 ^{ns} ± 1.54	15.54 ^{ns} ± 1.54
Control	16.13 ± 0.18	16.62 ± 0.58	16.52 ± 0.34	16.35± 0.41	16.28 ± 0.54	16.33 ± 0.14

ns: not significant; * Significant at P≤ 0.05.; **: Highly significant at P≤ 0.01.
P: Parental generation; F_1: First generation.; F_2: Second generation.

Table (6). Effect of LC_{50} of chlorfluazuron, flufenoxuron and pyriproxyfen on fecundity of *Spodoptera littoralis (Boisd.).*

IGR's	Mating combination								
	$T_{Females}$ x T_{Males}			T_{Female} x N_{Males}			N_{Female} x T_{Males}		
	Oviposition deterrent index %								
	P	F_1	F_2	P	F_1	F_2	P	F_1	F_2
Chlorfluazuron	62.3	44.0	22.7	66.4	49.7	31.3	54.2	31.7	28.2
Flufenoxuron	45.2	32.4	28.5	41.2	31.7	22.2	34.5	20.5	16.8
Pyriproxyfen	38.4	25.7	19.2	25.4	15.8	10.7	19.6	9.4	5.9
Control	-	-	-	-	-	-	-	-	-

ns: not significant; * Significant at P≤ 0.05.; **: Highly significant at P≤ 0.001; **: very Highly significant at P≤ 0.001.
P: Parental generation; F_1: First generation.; F_2: Second generation.
N:Normal; T:Treated

Table (7). Effect of LC_{50} of chlorfluazuron, flufenoxuron and pyriproxyfen on fertility of *Spodoptera littoralis (Boisd.)*.

IGR's	Mating combination								
	$T_{Females} \times T_{Males}$			$T_{Female} \times T_{Males}$			$T_{Female} \times T_{Males}$		
	Sterility%								
	P	F_1	F_2	P	F_1	F_2	P	F_1	F_2
Chlorfluazuron	62.3	50.4	22.5	61.0	40.1	21.3	45.0	34.3	18.1
Flufenoxuron	52.8	28.3	15.9	48.2	25.4	18.5	39.8	15.2	9.20
Pyriproxyfen	35.2	19.7	09.5	29.3	09.7	05.9	17.8	04.5	01.2
Control	-	-	-	-	-	-	-	-	-

P: Parental generation; F_1: First Generation; F_2: Second generation.
N: Normal; T: Treated

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دراسة تأثير ثلاثة من منظمات النمو الحشرية علي بعض الجوانب البيولوجية لدودة ورق القطن

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هدفت الدراسة إلى توضيح تأثير ثلاثة من منظمات النمو الحشرية هم الكلورفلوازيورون والفلوفينوكسيورون والبايريبروكسيفين علي بعض الجوانب البيولوجية لدودة ورق القطن. وجد أن المركبين الكلورفلوازيورون والفلوفينوكسيورون هما أكثر المركبات سمية لليرقات بينما كان المركب المشابه لهرمون الحداثة وهو البايريبروكسيفين هو أضعفهم من حيث السمية لليرقات. تبين كذلك أن المعاملة بكل من الكلورفلوازيورون والفلوفينوكسيورون قد قللا من متوسط عمر اليرقات لأفراد جيل الآباء (الجيل الذي تم عنده المعاملة بالمركبات) وكذلك يرقات أفراد الجيلين الأول والثاني (الأجيال التي تلت جيل الآباء) وذلك بالمقارنة باليرقات غير المعاملة وكان النقص في عمر اليرقات غير معنويًا باستثناء النقص الذي تم الحصول عليه في يرقات جيل الآباء عند المعاملة الكلورفلوازيورون حيث كان نقصا معنويا. تم الحصول علي نتائج مشابهه لذلك بالنسبة للعمر اليرقي لأفراد الجيل الثاني المعامل بالبايريبروكسيفين حيث كان النقص في عمر اليرقات أيضا غير معنويا.

وعلي النقيض، فقد أحدثت المعاملة بالبايريبروكسيفين زيادة في متوسط عمر يرقات أفراد جيل الآباء والجيل الأول غير أن تلك الزيادة لم تكن معنوية بالمقارنة باليرقات غير المعاملة. أيضا قللت المعاملة بكل من الكلورفلوازيورون والفلوفينوكسيورون من متوسط عمر العذارى لأفراد جيل الآباء والجيلين الأول والثاني، غير أن هذا النقص لم يكن معنويا وذلك بالمقارنة بالعذارى غير المعاملة، ومن ناحية أخري فلم يكن تقريبا للمعاملة بمركب البايريبروكسيفين أي تأثير يذكر علي متوسط عمر العذارى لأفراد الأجيال الثلاثة. وقد انطبق هذا تقريبا علي متوسط وزن العذارى الناتجة بعد المعامله بالثلاث مركبات من منظمات النمو الحشرية.

كانت الفراشات من كلا الجنسين لأفراد الأجيال الثلاثة الناتجة من معاملة الطور اليرقي الرابع بالكلورفلوازيورون والفلوفينوكسيورون والبايريبروكسيفين أقصر عمرا من مثيلاتها غير المعاملة وكان النقص في عمر فراشات الأجيال الثلاثة معنويًا فقط لكلا الجنسين عند المعاملة بالكلورفلوازيورون وكذلك كان هذا النقص معنويا أيضا فقط لذكور فراشات جيل الآباء وإناث فراشات الجيل الثاني عند المعاملة بالفلوفينوكسيرون. تبين كذلك أن عدد البيض الذي تضعه الأنثى الواحدة من الفراشات ونسبة الفقس لهذا البيض قد نقص نتيجة للمعاملة بالثلاثة مركبات لمنظمات النمو الحشرية لكل أفراد فراشات الثلاث أجيال.