

Effect of three insect growth regulators (Chlorfluazuron, Tebofenozoid and pyriproxyfen) on fecundity of *S. littoralis*, histopathological and some biochemical aspects of moth ovary.

Aziza E. Abdel-Aal

Plant Protection Research Institute, A.R.C., Dokki-Giza, Egypt

ABSTRACT

The insecticidal and biological effects of three insect growth regulator (chlorfluazuron, Tebofenozoid and pyriproxyfen) were evaluated on 4th instar larvae of *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). Based on the LC₅₀ values Chlorfluazuron is the more toxic to *S. littoralis* than that of the two other compounds. Female longevity, fecundity and fertility were significantly reduced at all treatments compared to control. Furthermore, different levels of significant changes in the total protein, carbohydrate contents of the female ovaries pre-treated as 4th instar larvae by LC₅₀ of used IGRs were recorded. Moreover, different abnormal histological structures of ovary were noticed.

Keywords: Insect growth regulators (IGR's), Toxicity, LC₅₀, *Spodoptera littoralis*, biological, biochemical and histological aspects.

INTRODUCTION

The extensive use of insecticides to control *Spodoptera littoralis* (Boisd.) larvae has led to its resistance to various classes of insecticides (Tabashink *et al.*, 1987) residual toxicity and environmental pollution (Frank *et al.*, 1990) and negative effects on non-target organisms (Franz, 1974). A new approach to insect pest control is the use of substances that adversely affect insect growth and development. These substances are classified as insect hormone mimics or insect growth regulators (IGRs) owing to their effects on certain physiological regulatory processes essential to the normal development of insects or their progeny. They are quite selective in their mode of action and potentially act only on target species. The action of IGRs, however, should not be confused with other synthetic insecticides, such as organophosphates and carbamates, since these chemicals interfere with other physiological processes but do not regulate the development of normal insects. Also these compounds causes

histological aberration of the mid gut of insects Thabit *et al.* (2010). There are three types of IGRs, each of which has a different mode of action:

- 1- Chitin synthesis inhibitors: These prevent the formation of chitin, a carbohydrate that is an important structural component of the insect's exoskeleton. When treated with one of these compounds, the insect grows normally until the time to molt. When the insect molts, the exoskeleton is not properly formed and it dies. Death may be quick, but in some insects it may take several days. As well as disrupting molting, chitin synthesis inhibitors can kill eggs by disrupting the normal development of the embryo e.g. Chlorfluazuron.
- 2- Anti juvenoid: This group of compounds induces a premature and lethal larval moult by direct stimulation of ecdysteroid receptors, especially in larval Lepidoptera e.g. (tebofenozoid).
- 3- Juvenile hormone analogue: When applied to an insect, these abnormal

sources of juvenilizing agent can have striking consequences e.g. pyriproxyfen.

The aim of the work to evaluate three insect growth regulators (chlorfluazuron, Tebofenozoid and pyriproxyfen) for controlling *S. littoralis* larvae. This can be attained by determining their possible larvicidal effects as well as determining their possible latent effects on certain biological aspects (adult longevity, fecundity and fertility) As well as latent effect on histopathological and biochemical studies of the ovaries.

MATERIALS AND METHODS

Insect Rearing Technique:

Egg masses of the cotton leafworm, *Spodoptera littoralis* were obtained from Plant Protection Research Institute without any insecticidal pressure. Newly hatched larvae were transferred to clean glass jars covered with muslin held in position with rubber bands. They were fed on castor bean leaves, *Ricinus communis*, L. at 27 ± 2 °C and $65 \pm 5\%$ RH El-Defrawi *et al.* (1964).

Insect growth regulators:

Two insect growth regulators (IGRs) obtained from Sumitomo Chemical Co., Ltd. were used during the present work. One of which are chitin synthesis inhibitors Chlorfluazuron (IKI-7899, 10% EC) and the second is a juvenile hormone analogue Pyriproxyfen (Admiral, 10% EC). The third is Tebufenozide (Mimic®, Confirm®, 24% E.C.) obtained from Rohm & Haas Co.

Relative susceptibility of 4th instars of *S. littoralis* to tested IGRs:

From the maintained insect culture, the 4th instar larvae were obtained. Then, they feed on castor oil leaves treated with different concentrations of the tested compounds. The percentages of mortality in untreated and treated larvae were recorded and calculated per each concentration,

corrected using Abbott's formula (1925) if necessary. The corrected percentage mortality of the compound was statistically computed according to Finney (1971) to determine the LC₅₀, LC₉₀ and slope values of the tested compounds.

The latent effect on adult:-

The pupae resulted from fourth instars treated with LC₅₀ of all toxicants were sexed and then placed in pairs in the glass globes in one of the following combinations : treated male x treated female, treated male x untreated female and treated female x untreated male, in addition to untreated male x untreated female as control ,in these cases the adults were confined in a glass jar containing a cotton pad soaked with 20% sugar solution as a food and stripes of paper as an ovipositional substrate. The eggs deposited per each mated were collected daily and counted to give an estimate of fecundity and the deterrent index was calculated according to Lundgren (1975) as:

$$\text{Deterrent index} = \frac{(A - B)}{(A + B)} \times 100$$

Where A: the total number of eggs per female in control.

B: the total number of eggs per female in treatment.

The percentage of egg hatch or fertility was determined and subsequently, the percentage of sterility was calculated according to Crystal (1968) as sterility = $(1 - fh) \times 100$, where "f" is the corrected decimal fractions of the percentage of fecundity. "h" is the corrected decimal fractions of the percentage of fertility. The corrected percentage of either fecundity or fertility was then calculated as $(A / B) \times 100$, where A and B are the treatment and control effect of either fecundity or fertility, respectively.

Histopathological studies of the ovaries:-

The surviving virgin treated and untreated females were dissected in

ringer's solution on the first day of emergence. The ovaries were fixed in carnoy's solution, embedded in paraffin wax, and stained with heamatoxylin and aeosin.

Biochemical effects on the females ovaries:-

The effect on the ovaries of virgin females obtained from larval treatment with LC₅₀ values of the three tested IGRs was studied. The total protein, carbohydrate and lipid of the ovaries of both treated and normal females were determined according to Lowery *et al.*, (1951), Singh, Sinha (1977), Baronos and Blackstck (1973), respectively.

RESULTS AND DISCUSSION

Susceptibility of 4th instars of *S. littoralis* to tested IGRs:

Table (1) shows the susceptibility of the 4th instars of *S.littoralis* towards

the tested IGRs. Based on LC₅₀ values the toxicity of the IGRs can be arranged in a descending order as follows: chlorfluazuron > tebofenozoid > pyriproxyfen. The toxicity index indicates that chlorfluazuron (standard IGR) was about 1.8 and 4194 times as toxic as tebofenozoid and pyriproxyfen, respectively. The obtained results are confirmed with those obtained by Haga *et al.*, (1984) who reported that Chlorfluazuron is very toxic to insects because it metabolizes slowly inside the insect body. The toxicity of Chlorfluazuron against *S. littoralis* larvae was somewhat similar to that obtained by Farag (2001), Abdel Aal (2006) and on *A. ipsilon* (Shurab *et al.*, 1999). Also the toxicity of five insect growth regulators on 4th instar of *S. littoralis* was recorded by Abdel-aal *et al.*, (2009).

Table 1: Susceptibility of *S. littoralis* 4th instar larvae to chlorfluazuron,tebofenozoid and pyriproxyfen.

IGR's	LC ₅₀ ppm	95% Fiducial limits		Slope± S.E.	χ ² (d.f.)	Toxicity index
		Lower	Upper			
Chlorfluazuron	0.166	0.155	0.244	1.65± 0.3	1.290	1.00
Tebofenozoid	0.353	0.176	0.534	1.22 ± 0.3	0.162	0.55
Pyriproxyfen	756.19	464.60	1173.900	1.14+ 0.26	1.220	2.38 x 10 ⁻⁴

The latent effect on adult:-

Effect on adult emergence: The result in table (2) indicated that the adult emergence % of moth pretreated as 4th instar by tested IGRs reduced by 16, 6.5 and 2% as treated with chlorofluazuron, tebufenozoid and pyriproxyfen respectively. The decrease

in percentage of adult emergence may be due to the fact that toxin block the maturation of imaginal discs which are primordial for adult integumentary structures in endopterygote insects (Schneidermann 1972 and Suh *et al.*, 2000).

Table 2: Effect of Chlorfluazuron, Tebfenozoid and Pyriproxyfen on total oviposition periods and mean adult longevity of *S littoralis*.

Compounds	Total oviposition periods (days)			Adult emergence %	Mean of adult life span (days ± S.E.)
	pre	Ovi	Post		
Chlorfluazuron	2.2±0.10	3.9±0.95	3.3±0.31	84(16)	9.6±0.28 ***
Tebofenozoid	2.1±0.32	4±0.62	4.9±0.51	93.5(6.5)	11.00*±0.28
Pyriproxyfen	3.2±0.4	5.3±1.2	6±1.42	98(2)	14.5**±0.19
Control	2.3±0.21	4.6±0.14	5.4±1.21	100	12.3±0.1

Number between brackets is reduction in adult emergence

The latent effect on adult fecundity and fertility:

Table (3) shows latent effect on the fecundity of *S. littoralis* female moths

surviving treatment of as 4th instars with tested IGRs. The results obtained show that the lowest number of eggs laid per female and consequently the highest 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 lay per female and consequently the

lowest deterrent indexes were occurred when the males were the treated sex only. This may indicate that the females were more sensitive to toxicants than the males. The result indicated that chlorfluazuron was the most effective compound on fecundity of *S. littoralis*, while pyriproxyfen recorded the lowest one.

Table 3: Reproductive potential of *S. littoralis* (Boisd.) moths treated as 4th instar larvae with LC₅₀ of Chlorfluazuron, Tebfenozoid and Pyriproxyfen.

IGRs	Mating combination					
	T♀ x T♂		T♀ x N♂		N♀ x T♂	
	No. of egg / ♀ ± S.E.	Oviposition Deterrent index %	No. of egg / ♀ ± S.E.	Oviposition Deterrent index %	No. of egg / ♀ ± S. E.	Oviposition deterrent index %
Chlorfluazuron	151***± 4.4	46.0	170***± 5.8	41.0	180***±5.8	38.7
Tebfenozoid	221**±6.6	29.7	226***±7.2	28.0	251***±7.3	23.7
Pyriproxyfen	305** ±6.6	20.5	380** ± 2.8	18.6	390.7* ±5.8	14.3
Control	408 ±13.0	-	-	-	-	-

* Significant at P< 0.05. ** Highly significant at P< 0.001. ***Very highly significant at P< 0.001

Table (4) 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 tebfenozoid 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.

Table 4: Effect of LC₅₀ of Chlorfluazuron Tebfenozoid and Pyriproxyfen on fertility of *S. littoralis*.

IGRs	Mating combination					
	T♀ x T♂		T♀ x N♂		N♀ x T♂	
	Egg hatchability %	Sterility %	Egg Hatchability %	Sterility %	Egg Hatchability %	Sterility %
Chlorfluazuron	65.0	74.0	66.0	70.0	71.0	66.0
Tebfenozoid	65.0	64.0	68.0	59.0	73.0	55.1
Pyriproxyfen	78.0	47.4	82.0	38.7	94.0	23.0
Control	91.8	-	-	-	-	-

The suppression of egg production per treated female obtained in this study may be due, in part, to interference of the tested IGRs with oogenesis. Reduction in the percentage of egg-hatch obtained in the present study may be attributed to sterilization of either eggs and or/sperms; or may be due to inability of the sperms to be transferred to females during copulation (Ismail, 1980). Moursy and Bartlett (1991-1992)

reported that the JHA, pyriproxyfen decreased the number of spermatophores transferred to *P. gossypiella* females. Riddiford (1970) demonstrated that in endopterygote insects the juvenoids may cause female sterility when applied at a certain stage of oocyte development. Taher and Cutkomp (1983) suggested that the sterility of females seems to be attributed chiefly to a delay or reduction of ova giving same opportunities not for

retention but for possible re sorption of eggs in ovaries. They also added that although the cause for the delay is not known, it could be due, in part, to a lower metabolic rate.

Histological Effects:

In normal female moths Fig (1), the four convoluted ovarioles in each ovary are "8" polytrophic ovarioles and each ovariole consists of a chain of developing egg follicles. Histologically each follicle Fig. (2) consists of a growing oocyte accompanied interiorly by a few number of nurse cells. The oocyte is surrounded by somewhat columnar or cuboidal follicular epithelium, while the nurse cells are surrounded by squamous follicular epithelium.

Fig. (3) showed the effect of Chlorofluazuron treatment on the female

ovary. The histological abnormalities, Fig. (4) were in the form of oocytes shrinkage which leaving space around it and other were semi absorbed. The follicular epithelium became thin and slightly vacuolated.

Figs. (5& 6) showed that Tebofenozoid treatment because slight clumping of chromatin materials leaving space near the epithelial cells, absence of some nurse cells and also the oocytes were semi absorbed.

Biochemical aspects on the ovaries:-

From data in table (5) it is clear that chlorofluazuron , tebufenozoid and pyriproxyfen decreased the biothynthesis of total protein, carbohydrate and lipid contents of the ovarioles of *S. littoralis* females as compared wih normal females.

Table5: Effect of Chlorfluazuron, Tebfenozoid and Pyriproxyfen on total protein, Carbohydrat and lipid contents in the ovarioles of *S. littoralis* post 48h feeding 4th instar larvae

Compound	LC ₅₀	Totalprotein (mg/g) fresh tissues ±S.E.	Total carbohydrat (mg/g) fresh Tissues ±S.E.	Total lipid (mg/g) fresh tissues±S.E.
Chlorfluazuron	0.166	23.6*±.8	46.2***+0.9	88.3***+0.7
Tebofenozoid	0.353	21.6**±0.5	36.1***+0.6	55.2***+1.3
Pyriproxyfen	756.19	30.2 ^{ns} +o.12	82.3 ^{ns} +o.4	92.5 ^{ns} +0.5
Control	0.0	33.7±o.2	89.9±1.2	98.3±0.9

Ns means non significant

*Significant at P< 0.05.

** Highly significant at P< 0.001.

***Very highly significant at P< 0.001

Decreased total ovarian protien of *S. littoralis* following treatment is similar to the the results of soltani and Mazouni(1992) that diflubenzuron reduced the total protein per ovary in *Cydia Pomonella* Also the same result recorded by shurab *et al.*,(1999) that chlorofluazuron and flufenoxuron reduced the total (protein, carbohydrate and lipid) in ovary in *A. ipsilon* female. The decreaseed ovarian protein obtained in this study may be due to decreased larval haemolymph, protein as a result of treatment .This suggestion is confirmed by the data of many workers that larval haemlymph protein contributed in deveoping ova in lepidoptera (kong and

kim, (1988). Gglucose, obtained from the haemolymph is utilized by the oocyte for the synthesis of glycogen at the periplasma (Ramamurty, 1968). According to these findings decreased in total carbohydrate in the ovariole of *S. littoralis* treated with Chlorofluazuron and Tebofenozoid may be accounted for the histological damages of both the oocyte and follicular epithelium caused by these two compounds as shown in this study. Decreased in total lipid in the ovarioles of *S. littoralis* treated with Chlorofluazuron and Tebofenozoid may be interpereted by the damage of both the nurse cells and follicular epithelium as shown in this study, where these tissues

were found to contributed in lipid deposition to the devolving oocyte (Tiripthi and kumar, 1982).

Moreover, it is probably that these compounds affected the fat bodies of *S. littoralis* during the period following larval treatment with them which ultimately led to decreased lipid deposition in the devolving oocyte. In general, the development of oocytes in most insects is under hormonal control and involves the neurosecretory cells and corpora allata. The histological aberration in this study are agree with Abdel Aal and Abdel Wahab (2007) when he studied the Ovicidal effects of lufenuron on the cotton leafworm, *Spodoptera littoralis*.

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Fig. 1: Normal ovarioles of healthy female moth of *S.littoralis* Showing the shape of Oocyte. OV Ovariole.

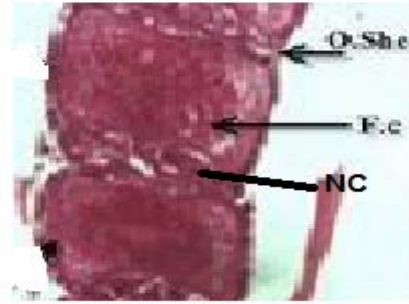


Fig. 2: Longitudinal sections in ovariole of normal *S. littoralis* adult (10X): nurse cell, F C: follicle cell, O.she: oocyte sheath.

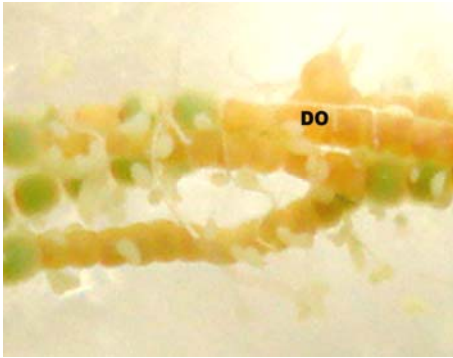


Fig. 3: Ovaries of female moths resulted from treated 4th instar larvae of *S. littoralis* with *Chlorfluazuron* showing the shape of degenerated Oocyte.

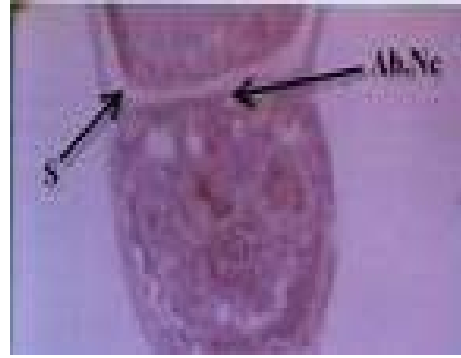


Fig. 4: L.S through female ovarioles of *S. littoralis* moth pretreated as 4th instar with LC₅₀ of Tebofenozoid. Ab.NC: abnormal nurs cell.



Fig. 5: Ovaries of female moths resulted from treated 4th instar larvae of *S. littoralis* with Tebofenozoid showing the shape of degenerated Oocyte.

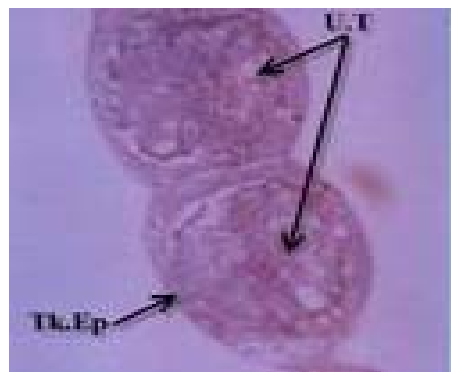


Fig. 6: L.S through female ovarioles of *S. littoralis* following feeding of 4th instars larvae for 48h on castor oil leaves treated with LC₅₀ Chlorfluazuron.

ARABIC SUMMARY

تأثير ثلاثة من منظمات النمو الحشرية كلوروفلوازورون وتبيوفينوزويد وبيريبروكسيفين على خصوبة وبعض التأثيرات الهستولوجية و البيوكيميائية لمبيض فراشة دودة ورق القطن دودة ورق القطن

عزيزة السيد عبد العال

معهد بحوث وقاية النباتات- مركز البحوث الزراعية – الدقى- جيزة.

هدفت الدراسة الحالية الى تقييم التأثير السمي، البيولوجي لبعض منظمات النمو الحشرية على العمر اليرقي الرابع لدودة ورق القطن سبودوترا ليتوراليس. اعتمادا على التركيز النصف مميت اثبتت النتائج ان مركب الكلوروفلوازورون اكثر سمية للعمر اليرقي الرابع لدودة ورق القطن من كلا المركبين الاخرين. أدت المعاملة بكل المركبات الى وجود نقص معنوي في كل من فترة حياة الفراشات ونسبة وضع البيض ونسبة الخصوبة مقارنة بالمقارنة. أيضا أوضحت النتائج تغيير معنوي في المحتوى الكلي للبيبيدات و للبروتين والكربوهيدرات مبيض انثى دودة ورق القطن. كما ادت المعاملات الثلاث الى وجود تشوهات في مبيض الفراشات التي عوملت مسبقا كعمر يرقي رابع .