

**FIELD AND LABORATORY EVALUATION OF
A NEW INSECT GROWTH INHIBITOR LUFENURON
AGAINST COTTON LEAF WORM
SPODOPTERA LITTORALIS (BOISD.) IN RELATION
TO ITS ANTIPHENOL OXIDASE ACTIVITY**

By

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ABSTRACT

Field and laboratory experiments were carried out at Etay El-Baroud region, Behera Governorate during the cotton season 1997 and Agriculture Research Station Farm during season 1998 to evaluate the efficiency of Lufenuron (Match); a new insect growth inhibitor. Field and laboratory toxicity data showed that Lufenuron had a high initial deposit with low initial reduction in insect counts. Population increased after 5 or 7 days post treatments up to 98%. Also toxicity data revealed that dipping method using 1/8 field concentration resulted in more initial effect than that of spray with field concentration. Data of enzymatic studies indicted that phenol oxidase activity was high in 4th instar larvae and decreased with larval development. Data also indicated that phenol oxidase activity was highly affected especially at the moulting time even though the absolute activity was low. Data suggested that phenol oxidase is not the sole site of action. However, Lufenuron overall target mechanism resulted in death of the treated larvae.

INTRODUCTION

The cuticle affords support and protection through its rigidity and hardness. After ecdysis the cuticle is soft and flexible, but the outer part

subsequently becomes hard by a process known as tanning or sclerotization. The phenol oxidase system seems to play an important role in this process. the scheme which is generally accepted is that ortho diphenols become oxidized to quinones by means of the phenol oxidase present in the cuticle, the quinones react with a valuable free amino groups in the cuticle proteins linking adjacent poly peptide chains in a tight network, thereby converting a soft and soluble protein into a hard insoluble material called sclerotein. Quinones derived from various ortho diphenols are such as protocatechuic acid (Brunet and Kent, 1955).

The diversity of processes may result from the use of different groups of insects and/or the existence of different systems involved in the sclerotizing process. Studying the enzymatic systems involved in the cuticle build up would probably throw some light on the insect cuticle formation and could be used for finding ways of weakening the protection afforded by the cuticle through inhibition of a key enzyme involved in the process (Ishaaya, 1972).

The aim of the present work is to evaluate and to determine the phenol oxidase activity in larvae of the Egyptian cotton leafworm treated with a new insect growth inhibitor (IGI) to throw some light on its residual activity and also to show its acute and accumulative effect on both toxicity and enzyme inhibition.

MATERIALS AND METHODS

I. Test insect:

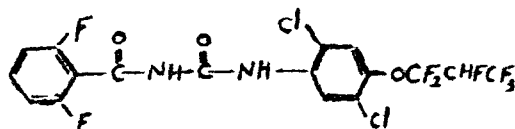
A field strain of the Egyptian cotton leafworm *Spodoptera littoralis* (Boisd.) was reared in the laboratory on cotton leaves as described by El-Defrawi *et al.* (1964).

II. Insecticide used:

Trade name	:	Match 50% EC
Common name	:	Lufenuron
Chemical name	:	<i>IUPAC</i> (RS)-1-[2,5-dicloro-4-(1,1,2,3,3,3-hexafluoro-propoxy)-phenyl]-3-(2,6-difluorobenzoyl)-urea

Empirical formula : $C_{17}H_8Cl_2F_8N_2O_3$

Structural formula :



Molecular weight : 511.2

III. Field efficacy evaluation of Lufenuron against cotton leafworm.

Successive field trials were carried out at Miniet Bany Mansour, Etay El-Baroud, Behera Governorate during the cotton season (1997). An area of about 3 feddans of cotton was sprayed with the tested compound. Larval counts of cotton leafworm/100 plants were done before and after different periods post spray. Reduction percent of cotton leafworm infestation were calculated according to Henderson and Telton equation (1955).

IV. Assessment of phenol oxidase activity:

a. Preparation of larval homogenate:

The whole body of about 300 mg of the tested instar of *S. littoralis* were homogenized with 10 ml of 0.03 M phosphate buffer pH 6.7 using glass tissue grinder with Teflon pestle and homogenized in a light glass homogenizer with auto cooling pestle. The homogenate was centrifuged for 15 min. at 3000 rpm using cooling centrifuge. The upper fatty layer was removed and the supernatant was centrifuged again for 30 min. at 8000 rpm. The final supernatant was used for assay of phenol oxidase activity (El-Sebae *et al.*, 1981).

b. Determination of phenol oxidase activity:

The method used was according to Thomson and Sin (1970) with some modifications. The total volume of the reaction mixture was 3.5 ml.

The reaction mixture contained 2.5 ml of 0.03 M phosphate buffer pH, 6.7, 0.5 ml of enzyme solution and 0.5 ml of adrenaline solution (2.0 mg/ml). A blank contained 3 ml of 0.03 M phosphate buffer pH 6.7, 0.5 ml of the enzyme solution. The mixture was then incubated for 15 min. After the incubation period, the absorbance was measured spectrophotometrically at 475 nm using Spectronic 201. The enzyme activity (specific activity) was expressed as extension unit/mg protein/min (El-Gendy, 1977).

The protein level in the enzyme preparations was estimated, after TCA precipitation and overnight dialysis, by the Lowry method (Lowry *et al.*, 1951).

V. Bioassay of tested insecticide:

Four experimental plots, one kerate each, were selected in a field of cotton at Etay El-Baroud Research Station Farm. All plots were sprayed with the insecticide on June 1998 using Knapsack sprayer with one nozzle at the rate of 200 Liters of the diluted spray per feddan (400 ml 50% EC/fed.). Treatment I a group of 4th instar larvae were fed daily on treated cotton leaves. Treatment II 4th instar larvae were fed once on treated cotton leaves which were replaced with untreated leaves after 24 hrs. Treatment III a group of 4th instar were fed daily on new cotton leaves which were previously dipped in 1/8 field concentration to estimate the accumulative effect of Lufenuron. The mortality counts were recorded at different time intervals *i.e.* one day, three, five and seven days. Percentage of kill was corrected according to the percentage of kill in the control (Abbotts, 1925).

RESULTS AND DISCUSSION

The results of field evaluation Table (1) showed that Lufenuron compound had a low initial effect at the beginning of treatment. On the other hand, high reduction in insect counts at 5, 7, 9, 12 and 15 days post spray were obtained. They were 98.6, 97.1, 99.5 and 99.6 reduction percent respectively. Also, acute and accumulative toxicities Table (2) showed the same trend as in treatments I and III. Treatment II also

indicated that Lufenuron has initial deposit with low initial effect. The accumulative toxicities after 5 and 7 days showed that continuous and increase in feeding periods on the treated cotton leaves had highly kill percent 91.74 and 96.33 respectively and support data in Table (1) treatment III showed that dipping method had high initial effect than spray with high concentration.

All previous results obtained showed that the lower initial effect of Lufenuron (IGI) may be related to feeding periods on the treated cotton leaves or the delay of mortality to the time of moulting. The same effect on *S. littoralis* was obtained by Ramesh Arora *et al.* (1996) who concluded that the increase in feeding period on the treated cotton resulted in a large increase in the toxicity of diflubenzuron resulted in accumulative mean of mortality. The high residual mortality may be due to delayed mortality at the time of larval moult. The same conclusion were noted by Ramesh Arora *et al.* (1996) who reported that 4th and 5th instar larvae of *S. littoralis*, in addition to the mortality recorded at the next moult, there was delayed mortality at the time of larval-larval and larval-pupae moult.

Data in Table (3) and Fig. (1) showed that the specific activity of phenol oxidase was inhibited in the three groups A**, B** and C** after one day of spraying. It was 57.5 E. unit/mg/min. while it was 149.9 E. unit/mg/min. in control. The percent of inhibition was 61%. After 3 days, although the specific activity in control was lower than after 1 day. Post spray data clearly showed the accumulative effect of daily feeding in treatment A**, the percentage of inhibition of phenol oxidase activity was 91.13, while in treatment B** (fed. one time at zero time for 24 hrs) caused 76.98% inhibition. These results indicate the high initial deposit of Lufenuron. After 5 days the activity of phenol oxidase increased in A** and B**. The inhibition percent was somewhat lower than it was after 3 or 7 days. These results may be interpreted either by the foregoing finding of Deloach *et al.* (1981) and Ahmed and Mostafa (1989) who showed that IGI's act as inhibitors for a number of enzyme systems and also reduced the larval enzymatic protein due to the inhibition of DNA synthesis as suggested by Meala and Mayer (1980). This mode of action of this group of insect growth inhibitors IGI's results in retardation of

protein biosynthesis leading to slower metamorphosis accompanied by malformation (Ahmed *et al.*, 1990). It can be suggested that the enzyme activity within the first 3 days represents the same level of activity, as progressive inhibition was shown, after moulting the sub sequence cycle started with low specific activities 41.8 for control and 29.8, 30.3 and 27.0 in treatments A**, B** and C** which were clearly less affected. These data are in agreement with Ramesh Arora *et al.* (1996) who concluded that high mortality was correlated with moulting time. Low increase in mortality after 5 days in Table (2) treatments 1 and 2 and the high residual enzyme activity after 5 days in Fig. (1) support these above mentioned discussion. On the other hand; treatment C** showed that Lufenuron had the same effect against phenol oxidase activities 1 and 3 days after spraying. It caused inhibition of (61%). After both 5 days and 7 days the phenol oxidase activity were gradually increased as shown in Fig. (1).

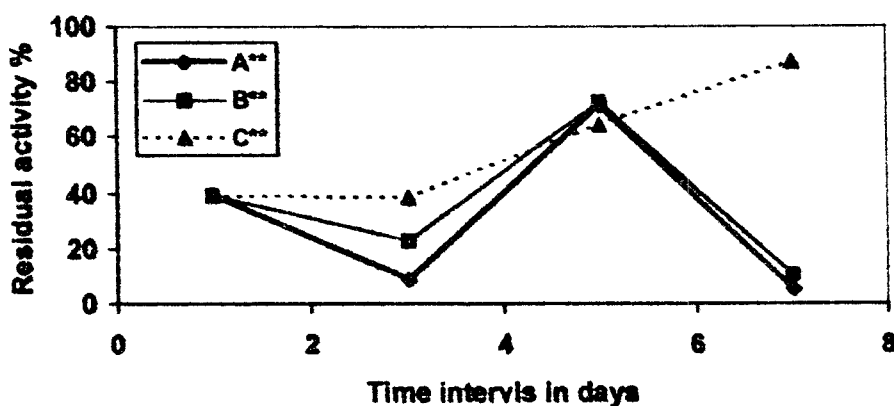


Fig. 1: The residual activity of phenol oxidase in fourth instar larvae of *S. littoralis* after different time intervals from feeding on field concentration of Lufenuron.

Table (1): Effect of Lufenuron field treatment against cotton leafworm *Spodoptera littoralis* (Boisd.) infesting cotton plants (1997 season).

Treatment	Rate /200 L water	Prespray count/ 100 Plants	Number and percentage of reduction postspray by												General mean of reduction
			3 days		5 days		7 days		9 days		12 days		15 days		
			26/6/1997		28/6		30/6		2/7		5/8		7/8/97		
			A	B	A	B	A	B	A	B	A	B	A	B	
Lufenuron	400	2600	2600	96.7	1400	76.6	54	98.6	84	97.1	10	99.5	3	99.6	90.2%
Control	cm	2900	4500		3152		2040		1500		1050		400		

A : No. of larvae/100 cotton plants B: Percentage reduction

Table (2): Acute and accumulative toxicities against 4th instar larvae of cotton leafworm *S. littoralis* (Boisd.) treated with IGI Lufenuron.

Period Treatment	% mortality			
	One day	3 days	5 days	7 days
I*	4.89	28.61	68.70	91.74
II**	7.70	48.88	62.61	87.16
III***	27.97	69.18	83.47	96.33

- I* : Larvae were fed daily on cotton leaves sprayed with field concentration.
 II** : Larvae were fed once for 24 hrs on cotton leaves sprayed with field concentration and replaced with untreated leaves until counts.
 III*** : Larvae were fed daily on cotton leaves dipped in 1/8 field concentration.

Table (3): The effect of field concentration of Lufenuron on the activity of phenol oxidase from the 4th instar larvae of *S. littoralis*.

Sampling Date in Day	Enzyme activity *(S.A.) x 10 ³									
	Un-treated	A**			B**			C**		
	Chick Control	Treated Larvae	Residual activity %	Inhibition %	Treated Larvae	Residual activity %	Inhibition %	Treated larvae	Residual Activity %	Inhibition %
1 day	149.9	57.4	39.3	61.7	57.5	38.36	61.64	58.4	38.96	61.04
3 days	41.7	3.7	8.87	91.13	9.6	23.02	76.98	16.0	38.37	61.63
5 days	41.8	29.8	71.29	28.71	30.3	72.5	27.5	27.0	64.59	35.41
7 days	47.6	1.2	2.52	97.48	4.99	10.48	89.52	41.61	87.37	12.63

* Specific activity (S.A.) = Extension unit/mg/min. A **group of larvae were fed daily on cotton leaves sprayed with field concentration.

B** group of larvae were fed one time on cotton leaves sprayed with field concentration at zero time and replaced with untreated leaves after 24 hrs.

C** group of larvae were fed one time on cotton leaves sprayed with field concentration after 3, 5 and 7 days for 24 hrs.

Table (4): Accumulative effect of Lufenuron on phenol oxidase activity of 4th instar of *S. littoralis* in groups of larvae using dipping method.

Treatment	Enzyme activity (S.A.) x 10 ³								
	One day			3 days			5 days		
	Treated larvae	Residual Activity %	Inhibition %	Treated Larvae	Residual activity %	Inhibition %	Treated larvae	Residual Activity %	Inhibition %
1/8 field dose*	83.9	59.46	40.44	23.1	49.6	50.4	39.9	120.91	20.91
Control **	140.7	-	-	46.6	-	-	33.0	-	-

* Larvae were fed daily on a new cotton leaves dipped in 1/8 field concentration.

** Larvae were fed daily on untreated cotton leaves.

The results in Table (4) showed that activity of phenol oxidase decreased one day after feeding on cotton leaves treated with 1/8 field concentration, while the activity decreased more sharply after the next 3 days. Also the specific activity (S.A.) increased more than the untreated check larvae after 5 days of continuous daily feeding on the same concentration. This may be due to the induction effect of the sublethal doses of Lufenuron in the surviving individuals (the more tolerated larvae).

It may be concluded that Lufenuron has a high initial deposit that caused gradual increasing mortality in which phenol oxidase act not as the sole site of action but as the overall target mechanism leading to death.

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الملخص العربي

التقييم الحقل والمعمل لمنظم النمو الحشري الليفينيرون ضد دودة ورق القطن
Spodoptera littoralis (Boisd) وعلاقة ذلك بتنشيط نشاط
إنزيم الفينول أوكسيداز

د. عبد السلام إسماعيل محمد الصردى

محطة البحوث للزراعة بإيتاي البارود
معهد بحوث وقاية النباتات - مركز البحوث الزراعية

أجريت التجارب الحقلية والمعملية في منطقة إيتاي البارود محافظة البحيرة أثناء موسم القطن ١٩٩٧ وفي مزرعة محطة البحوث الزراعية بإيتاي البارود في الموسم ١٩٩٨ لتقييم كفاءة مبيد الليفينيرون (الماتش) كمبيد جديد من مثبطات النمو.

أظهرت بيانات الحقل والمعمل أن هذا المبيد له متبقى أولى عالى مع تأثير فوري ضعيف حيث أن معدل الانخفاض في تعداد الحشرات ازداد بعد خمس أو سبع أيام بعد المعاملة حتى ٩٨%. كذلك أظهرت دراسات السمية معمليا بوضوح أن طريقة الغمر باستخدام ١/٨ تركيز الحقل كان لها تأثير فوري عالى عن الرش بتركيز الحقل.

أظهرت بيانات الدراسات الإنزيمية أن نشاط إنزيم الفينول أوكسيداز كان عالى في يرقات العمر الرابع وتناقصت مع التطور اليرقى. أظهرت البيانات أيضا أن نشاط إنزيم الفينول أوكسيداز تأثر كثيرا خاصة قبل واثاء فترات الانسلاخ حتى إذا كان النشاط المطلق منخفضا. النتائج تقترح أن الفينول أوكسيداز ليس هو مكان التأثير الوحيد للمبيد ولكنه يعتبر الميكانيكية العامة التي تجب كل الاحتمالات الأخرى.