# POTENCY OF ESREL AND RELDAN ON SOME BIOLOGICAL AND BIOCHEMICAL ASPECTS OF COTTON LEAF WORM *SPODOPTERA LITTORALIS* (BOISD.)

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## *Abstract*

The present study aims at comparing the effect among Esrel as plant growth regulator (PGR),Reldan (organophosphorus insecticide) on the lethal effects of tow compound in the cotton leaf worm *Spodoptera littoralis* larvea as well as the effects of sublethal on development and reproduction of surviving insects. and biochemical parameters . On basis of this knowledge it will be possible to design combinations and strategies for use of both ingredients in Integrated Pest Management Programmes.The response of, *S. littoralis* to lethal and sublethal concentrations of Esrel and the conventional insecticide reldan were determined using leaf dipping technique. Result showed that mortality of the newly molted 4th instar larvae increased with the concentration resulting $LC_{50}$ values of $2.89x10^5$ppm and 85.83 ppm after 5 and 2 days, for Esrel and reldan respectively. Sublethal effects were studied by treating the $4^{th}$ instar larvae with a concentration equivalent to $LC_{50}$. The larval development time from treatment until pupation was prolonged significantly in both treatments. The pupation period of the survivors was shorted in both sex but was significantly with female pupa and without significantly with male pupae with the two products treatments. The preoviposition egg period was shorted significantly with esrel and long without significant with reldan. The oviposition egg periods was shorted significantly in both treatments. The oviposition viable egg period was shorted in both treatment but significantly with reldan, and without significant with esrel. The fecundity was decreased significantly in both treatments but the egg viability not affected. The adult longevity for both products was shorted in both sex but in the case of female was significantly with esrel, and without significantly with reldan. The results of biochemical aspects showed that Reldan caused significant decrease of each of total Soluble Protein and Acetyl cholinesterase enzyme activity (Ach.E). Esrel, caused mild decreased in total proteins and Ach.E compared to the control treatment. **Conclusion:** The results indicated the toxic organophosphorus insecticide Reldan is more than Esrel on *Spodoptera littoralis* but (o.p)is not safty

so we suggest that usage of nature compound to control eg. Esrel although it less toxic to save environment.

**Key Words**:- *Spodoptera littoralis*, esrel, reldan, biochemical aspects .

## INTRODUCTION

The Egyptian cotton leaf worm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae), is a key pest of cotton and other many crops in the Mediterranean area and Middle Eastern countries (Domínguez 1993; Belda *et al.*, 1994). The fact that the insect infests more than 112 host plants belonging to 44 families (Moussa *et al.*, 1960; Brown and Dewhurst 1975) makes it a model of serious polyphagous pests. The lethal effects are the most frequently evaluated parameters, although sublethal effects on surviving insects should also be considered; for instance, insects not dying by product-insect interaction may have their physiological processes affected resulting in development and reproduction alterations.

Organophosphorus (OP) pesticides are the major chemical class of insecticides used in the world today. The primary mode of action for OP pesticides is initiated through irreversible inhibition of acetylcholinesterase (AChE), the enzyme responsible for degrading the neurotransmitter acetylcholine (Kousba *et al.*, 2003).

Ethephon [(2-chloroethyl) phosphonic acid] (ETF) is a major plant growth regulator (PGR) that promotes fruit ripening, abscission, flower induction, and other responses by releasing ethylene gas, a natural plant hormone (Tomlin, 1997; Haux *et al.*, 2000) which spontaneously decomposes at physiological pH (Zhang and Casida, 2002). ETF as an OP, even though it gives no cholinergic signs of poisoning, was tested the same way as an OP insecticide. It markedly inhibited plasma butyrylcholinesterase (BChE) with much less effect on brain or erythrocyte AChE in rats and mice both in vitro and in vivo.

## MATERIALS AND METHODS

### 1. Insects:-

Rearing technique: A laboratory colony of the *Spodoptera littoralis* continuously was reared away of insecticidial contamination in Plant Protection Research Institute.

Rearing of insects were conducted following the technique described by (El- Defrawi, *et al.*, 1964).

**2. Compounds used :-**

- **Pland growth regulator : Common Name:** Ethephon **48%SL wt/**vol SL. **Tread name**: Esrel

Sun date. Chemical industry Co., Singaphora

- **Organophosphorus insecticide** (OPs) Chloropyrifos-methyl 22.5% EC. **Tread name** (Reldan)

DOW AgroSciences - UK.

**3. Lethal effects:-**

Different concentrations of esrel ($350x10^3$, $300x10^3$, $250x10^3$, $200x10^3$ and $150x10^3$ ppm for *S. littoralis* and reldan (600, 400, 200, 100 and 80 ppm) were assayed against S. littoralis 4th larvae by using leaf dipping technique. Fresh castor bean leaves were dipped in each concentration for 10 second. Each concentration was replicated three times and each replicate contained 30 larvae. Control larvae were fed on caster bean leaves immersed in distilled water. Mortality was recorded every 24 hrs after treatment.

**4. Sublethal effects on reproductive:-**

Newly molted 4th instar of *S. littoralis* larvae was treated with LC50,s for esrel and reldan. Larvae fed on treated leaves during twenty four to forty eight hours. Control larvae were fed on caster bean leaves immersed in water. Males from the nighty after emergence were paired with 1 or 2-day-old virgin females in jars (6 cm in diameter and 12 cm high), which were supplied with parts of paper as an oviposition site, one pair per jar, and fed on 15% honey solution and maintained in the containers until they died. Egg production was recorded daily and eggs were allowed to hatch. All bioassays were conducted at 25±2 °C. Mortality was recorded every 24 hrs.

**5- Biochemical studies on *S. littoralis* :-**

Preparation of samples for biochemical analysis: Total body tissue samples were collected from late 6th larval instars treated as 4th instars fed on castor leave with LC50 values of two compounds. Samples were collected according to the method described by ( Ishaaya *et al.*, 1971).

a- Determination of Acetylcholinesterase (AchE) activity was measured according to the method described by (simpson *et al.*, 1964).

b- Determination of Total protein: Total proteins were determined by the method of **(Bradford, I976).**

**6. Analysis of data:-**

Median lethal concentrations ($LC_{50}$,s) were determined by linear regression analysis and a test was made for parallelism according to the relative potency estimation method (Finney, 1971), using the microcomputer program POLO-PC (Russell, *et al.* 1977). The larval and pupal development, preoviposition, oviposition period, total fecundity, egg viability and adult longevity data were analysed by ANOVA and comparison of means by the least significant difference test (LSD) were calculated.

## RESULTS AND DISCUSSION

**Lethal effects:-**

Results illustreted in the Tables (1 and 2) showed that the mortality percentage increased with increasing the concentration of both esrel and reldan. The larva mortality was started after 2 days of esrel and after one day when the larvae were treated witth reldan. The highest mortality percentage were 88.67% after 22 days and 90% caused by treating the larvae with esrel and reldan respectively.

Table 1. Mortality percentage in 4th instar of *S. littoralis* larvae treated with esrel.

| Concentration (ppm) | N | Mortality | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 2 days | | 5 days | | 7 days | | 15 days | | 22 days | |
| | | n | % | n | % | n | % | n | % | n | % |
| 0 | 30 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 350 x$10^3$ | 30 | 10 | 33.33 | 24 | 80 | 26 | 86.67 | 26 | 86.67 | 26 | 86.67 |
| 300 x$10^3$ | 30 | 7 | 23.33 | 12 | 40 | 12 | 40 | 23 | 76.67 | 23 | 76.67 |
| 250 x$10^3$ | 30 | 8 | 26.67 | 9 | 30 | 13 | 43.33 | 13 | 43.33 | 13 | 43.33 |
| 200 x$10^3$ | 30 | 5 | 16.67 | 7 | 23.67 | 7 | 23.67 | 11 | 36.67 | 11 | 36.67 |
| 150 x$10^3$ | 30 | 4 | 13.33 | 5 | 16.67 | 8 | 26.67 | 8 | 26.67 | 11 | 36.67 |

N = Treated larvae number. n = Died larvae number.

Table 2. Mortality percentage in 4th instar of *S. littoralis* larvae treated with reldan.

| Concentration (ppm) | N | Mortality | | | |
|---|---|---|---|---|---|
| | | 1 day | | 2 days | |
| | | n | % | n | % |
| 0 | 30 | 0 | 0 | 0 | 0 |
| 200 | 30 | 27 | 90 | 27 | 90 |
| 100 | 30 | 18 | 60 | 18 | 60 |
| 50 | 30 | 6 | 20 | 6 | 20 |

N = No. of treated larvae number. n = No of larvae mortality

Analysis by probit regression line revealed the following equations and $LC_{50}$s (with 95% confidence limits): $y = 0.46 x + 4.30$, $\chi^2 = 7.40$ (3df) and $2.89 \times 10^5$ ppm ($2.58 \times 10^5 \pm 3.45 \times 10^5$) for esrel (Table 3); $y = 4.05x + 3.53$, $\chi^2 = 0.004$ (1df) and 85.83 ppm (51.92±126.93) for reldan. (Table4). The adjustment was acceptable using the test $\chi^2$.

Table 3. Regression lines subjected to parallelism and $LC_{50}$ values against the fourth instar larvae of *Spodoptera littoralis* treated with esrel.

| days | $\chi^2$ | df | b | S.E. | $LC_{50}$ (ppm) | Confidence Limits 95% | |
|---|---|---|---|---|---|---|---|
| | | | | | | Lower | Upper |
| 2 | 0.58 | 3 | 1.76 | 0.91 | $6.57 \times 10^5$ | --- | --- |
| 5 | 7.40 | 3 | 4.30 | 0.92 | $2.89 \times 10^5$ | $2.58 \times 10^5$ | $3.45 \times 10^5$ |
| 7 | 11.93 | 3 | 3.80 | 1.73 | $2.65 \times 10^5$ | --- | --- |
| 15 | 4.55 | 3 | 4.70 | 0.88 | $2.26 \times 10^5$ | $1.99 \times 10^5$ | $2.52 \times 10^5$ |
| 22 | 7.11 | 3 | 3.94 | 0.85 | $2.17 \times 10^5$ | $1.84 \times 10^5$ | $2.45 \times 10^5$ |

Table 4. Regression lines subjected to parallelism and $LC_{50}$ values against the fourth instar larvae of *Spodoptera littoralis* treated with reldan.

| $\chi^2$ | df | b | S.E. | $LC_{50}$ (ppm) | Confidence Limits 95% | |
|---|---|---|---|---|---|---|
| | | | | | Lower | Upper |
| 0.0039 | 1 | 3.53 | 1.16 | 85.83 | 51.92 | 126.93 |

The laboratory colony of *S. littoralis* tested was susceptible to esrel and reldan are going in linewith those reported by (Barrania *et al.*, 2013) whom indicated high toxicity of reldanat two low concentrations of (1/2 & 1/4 field recommended rates) against the 4th instar of *S. littoralis* larvae at two cotton growing seasons 2011 and 2012. (El- Sheikh, 2012), found highly toxicity of organophosphoras and pyrethroids cpompunds against the 4th instar larvae of *S. littoralis* for both laboraotry strain and field strains. The present study results are accordance with those finding by El-Khayat *et al.*, 2003. They found that chloropyrifos caused high toxicity to *S. littoralis* 4th instar larvae.

**Sublethal effects**

Development period of the larvae treated with esrel and reldan were 7.81 and 7.92 days respectively and significantly prolonged than the control (6.83 days) (Table 5).

Table 5. Effects of esrel and reldan on larval development of *Spodoptera littoralis*.

| Insecticide | Concentration (ppm) | N | Time (days) | | |
|---|---|---|---|---|---|
| | | | Mean | Interval | S.E. |
| **Control** | 0 | 70 | 6.83 b | 5-9 | 0.28 |
| **Esrel** | $2.89 \times 10^5$ | 85 | 7.81 a | 5-8 | 0.30 |
| **Reldan** | 85.83 | 93 | 7.92 a | 5-9 | 0.25 |

Means followed by the same letter are not significantly different (LSD, p = 0.05).
S.E. = Standard error.

Resluts represented in the Table (6) cleaned that the development period of the pupae treated with each compound was shorter than the control pupae, while males tended to develop slower than females. Pupal development of females was significantly, decreased but the male pupae were not affected by the treatments.

Table 6. Effects esrel and reldan on pupal development of *Spodoptera littoralis*.

| Insecticide | Sex | Concentration (ppm) | N | Time (days) | |
|---|---|---|---|---|---|
| | | | | Mean | S.E. |
| **Esrel** | ♀ | Control | 34 | 9.50 a | 0.12 |
| | | $2.89 \times 10^5$ | 29 | 9.03 b | 0.13 |
| | ♂ | Control | 36 | 11.47 a | 0.14 |
| | | $2.89 \times 10^5$ | 34 | 11.26 a | 0.14 |
| **Reldan** | ♀ | Control | 34 | 9.50 a | 0.12 |
| | | 85.83 | 43 | 9.39 b | 0.10 |
| | ♂ | Control | 36 | 11.47 a | 0.14 |
| | | 85.83 | 42 | 11.14 a | 0.17 |

Means followed by the same letter are not significantly different (LSD, p = 0.05).

Pupation period of *S. littoralis* males was about two days longer than females, either resulted from treated or niether treated larvae, as have been previously reported by (Osman *et al.,* 2012 and Hatem, 2014). The esrel and reldan caused a significant reduction in the pupation period of females in both products, but it was no significantly reduction in male pupae in both products. (Osman *et al.,* 2012 and Hatem, 2014), reported that the pupation period of *S. littoralis* treated with Spinosad, *Bacillus thuringiensis* var. *aizawai*, chloropyrifos, camphor extract and their combination extended the developmental period of survivor larvae and pupal.

Resultes showen that the most of females were mated and most of them deposited viable eggs as, independently of treatment.

Table 7. Reproductive potential of *Spodoptera littoralis* larvae treated esrel and reldan according to different copulations.

| Combination | N | N° Eggs / ♀ | | N | % Viability | |
|---|---|---|---|---|---|---|
| | | Mean | Interval | | Mean | Interval |
| ♀ x ♂ | 10 | 1485.0 a | 1075-2050 | 9 | 74.89 a | 0-100 |
| $♀_{esrel}$ x $♂_{esrel}$ | 9 | 802.8 b | 450-1250 | 9 | 75.60 a | 44.44-100 |
| $♀_{reldan}$x $♂_{reldan}$ | 13 | 939.2 b | 550-1475 | 11 | 64.97 a | 0-92.86 |

Means followed by the same letter are not significantly different (LSD, p= 0.05). N= Mated female number.

As a summarized in the table (7), it was obvious that the reproduction of *S. littoralis* has been affected by the treatments of the two tested compounds different form according to the insecticide. The treatment with sublethal concentration of esrel and reldan caused a reduction of the fecundity in the resulted females. The coresponding number of eggs per treated female mated with treated male were 802.8 and 939.2 respectively compared with 1485.0 eggs per untreated female mated with untreated male. These were no significant differences in the eggs viability between the treatments and the control. El-Aw (2003) also found reduction in the *S. littoralis* fecundity as a result of insecticidal application.

Result presented in Table (8) showed that the mean of adults longevity of males did not differ significantly, among mating copulation when treated with esrel and reldan, respectively. The mean of adults longevity of females did not differ significantly among mating combinations when treated with reldan, but there differ significantly among mating combinations when treated with esrel as compared with thoseof untreated one.

**The data in table (9)** indicated that the used Reldan caused significant reduction each of total protien content and acetyl cholinesterase activity in the 4$^{th}$ larvae of *S.littoralis*. On the other hand, the Eserel caused mild reduction in soluble protein content and Ach.E as compared with control.

Table 8. Adults longevity of *Spodoptera littoralis* resulted from larvae treated with esrel and reldan according to different mating combinations.

| Combination | N | Males | | Females | |
|---|---|---|---|---|---|
| | | Mean | Interval | Mean | Interval |
| ♀ x ♂ | 10 | 11.20 a | 6-14 | 9.50 a | 6-11 |
| $♀_{esrel}$ x $♂_{esrel}$ | 9 | 10.22 a | 8-13 | 7.67 b | 5-10 |
| $♀_{reldan}$ x $♂_{reldan}$ | 13 | 10.15 a | 8-15 | 8.46 ab | 6-11 |

Means followed by the same letter are not significantly different (LSD, p= 0.05).

Table 9. Effect of Eserel and Reldan on the activitiy of Acetyl cholinesterase enzyme and total soluble protein in larvae of *S. littoralis*

| Treatment | Total soluble protein Mg/g.b.wt | | Acetyl cholinesterase activity *UgAchBr/min/g.b.wt* | |
|---|---|---|---|---|
| | Mean ±SE | % reduction | Mean ± SE | % reduction |
| control | 17.923±0.5562 | | 6.103±0.3939 | |
| Eserel | 15.066±0.6998 | 15.94 | 4.066±0.8386 | 33.38 |
| Reldan | 12.903±0.2514 | 28.01 | 3.343±0.4707 | 45.22 |

**II.Biochemical aspects in *S. littoralis* with the test compound :**

These results were in agreement with the results of (Osman 2006): she reported that the total protein was significantly decreased due to the treatment with protecto, chlorpyrifos, flufenoxuron and hexaflumuron as compared to control. The results are accordance with those obtained by (El- Sheikh 2012), who noticed that, the treatment of chlorpyrifos on the activity of Ach. E in different strain of *S. littoralis*. Reldan is an anticholinesterase this explain that it has significant decrease in acetyl cholinesterase enzyme activity, this results are accordance with the results of (Osman 2006) she reported that cholinesterase activity was significantly decreased in *S. littoralis* due to the treatment with protecto, Chlorpyrifos, Flufenoxuron and Hexaflumuron as compared to control .The results are supported with the results of (Osman and Osman 2014) who reported that *profenophos* treatment caused significantly reduction in the cholinesterase activity in *S. littoralis* .

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# فعاليه الايسرل و الريلدان على بعض الظواهر البيولوجيه والبيوكيميائيه لدوده ورق القطن

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استهدف هذا البحث مقارنه التاثيرات البيلوجيه والبيوكيميائيه بين مركبين احدهما منظم نمو نباتى يستخدم لاول مره فى مصر لمكافحه دودة ورق القطن وهو الايسريل او الايثفون والاخر من المركبات الفسفورية العضوية (OP) الريلدان(الكلوروبيروفوس ميثيل) وقد وجدإستجابة دودة ورق القطن للتركيزات القاتلة و تحت القاتلة لكل من الايسريل و الريلدان تم تحديدها عن طريق المعاملة بالغمر للعمر اليرقى الرابع. اظهرت النتائج زيادة فى معدل الموت لليرقات بزيادة تركيز كل من المركبين حيث كان التركيز نصف المميت لليرقات ٥٠ % من اليرقات هو ٢,٨٩ ×١٠° و ٨٥,٨٣ جزء فى المليون لكل من ايسريل و الريلدان على الترتيب. أوضحت النتائج أن فترة التطور اليرقى قد زادت فى المعاملتين بصورة معنوية بينما فترة التطور العذرى قد انخفضت فى كل من الجنسين و لكن انخفضت بصورة معنوية للاناث و بصورة غير معنوية للذكور فى كلتا المعاملتين. فترة ما قبل وضع البيض قد قصرت بصورة معنوية مع ايسريل و طالت بصورة غير معنوية مع الريلدان بينما فترة وضع البيض انخفضت بصورة معنوية لكلا المركبين. فترة وضع البيض المخصب قصرت فى كل من المعاملتين بصورة معنوية مع الريلدان و صورة غير معنوية مع ايسريل. الخصوبة إنخفضت بصورة معنوية لكلا المركبين. و لم تتأثر نسبة الحيوية فى كلتا المعاملتين. فترة حياة احشرة الكاملة انخفضت فى كل من الجنسين لكلا المركبين حيث قصر عمر الإناث المعاملة بصورة معنوية مع ايسريل و بصورة غير معنوية مع الريلدان مقارنه بالكنترول. وقد اظهرت النتائج من الدراسه البيوكيميائيه تاثرا واضحا حيث وجد نقص معنوى لليرقات المعاملة بالريلدان على كل من المحتوى الكلى للبروتين و نشاط انزيم الكولين استيريز بينما حدث نقص متوسط فى هذه الدلائل بالنسبة لليرقات المعاملة بالايسريل. ونستخلص من هذا البحث ان مركب الريلدان كان اكثر سميه على دودة ورق القطن من ايسريل ولكن وجد فى البحث الملحق انه غير امن على الثدييات لذلك نقترح الاهتمام باستخدام المكافحه بالمركبات الامنه على البيئه حتى ولو كان اقل سميه لتقليل الضرر

**الكلمات المفتاحية:** دودة ورق القطن – ايسريل – ريلدان – التغيرات البيوكيميائيه – التغيرات البيلوجيه