

## **TOXICOLOGICAL AND BIOLOGICAL STUDIES OF LUFENURON AND DIFLUBENZURON AGAINST *Pectinophora gossypiella* (SAUNDRS) EGGS**

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### **ABSTRACT**

Under laboratory condition, one day old eggs Of laboratory strain of *Pectinophora gossypiella* (saund) were treated with LC<sub>50</sub> values of lufenuron and diflubenzuron, 6.489 and 67.36ppm respectively to study their effects on percentage of hatchability and duration of subsequent larval, pupal, immature stages and longevity, fecundity and fertility of resulted adults. The obtained results clear that the percentage of hatchability of treated eggs were 51.9 and 54% for the two tested compounds, respectively oppose to 95% in untreated control. The incubation period estimated by 6.9 and 6.1 days / lufenuron and diflubenzuron; while in control it recorded 3.2 days. Also, the larval period prolonged to 20.7and 24.6 days in contrary it recorded 15.3 days in control; the pupal period estimated, respectively in treatments and control, by 11.3,13.2 and 7.9 days. On the other hand, the adult stage resulted from treated eggs was highly affected by the used two tested compounds. The respective fecundity reduced to 198.3 and 175.6 eggs/♀ compare to 238.6 eggs/♀ in control. The percentage of hatchability were 60.3 & 59.7 %, respectively compared with 89.3 % in control.

**Keywords:** *Pectinophora gossypiella*, (PBW), Toxicity , LC50 , Biological studies

### **INTRODUCTION**

The pink bollworm, *Pectinophora gossypiella* (saund) is one of the most serious insect pests infesting cotton plants in Egypt. It causes serious damage in cotton bolls resulting in high reduction in quantity and quality of cotton yield. Insect growth regulators are a unique class of insecticides with selective effects on various life stages of some order of insects. El Shennawy (2009) and Al-Shannaf *et al* (2012). Insect growth regulators (IGRs) have been developed due to their high activity and selectivity against insects with inherently low toxicity to non-target wildlife, Butter *et al* (2003).

Otherwise, some authors studied the biochemical changes in treated some insects by IGRs (Kandil *et al* 2005). They found that the chitin can be synthesized from glucose, glucosamine or (N- acetylglucosmine), protein and carbohydrate. This compound is the immediate precursor of chitin. If the (N-acetylglucosmine) moiety in transferred directly to the growing chitin inhibitor, (El-Barkey *et al* 2009; Mossan *et al* 1995; Moawad *et al* 1990 and Abd El-Megeed *et al* 1987).

The present study was carried out under laboratory condition to determine the toxicity effect of lufenuron and diflubenzuron on (one day old eggs and larvae) of *P. gossypiella*, and subsequently, some biological aspects for immature and adult stages under laboratory condition.

## MATERIALS AND METHODS

### Insect used:

In the present study a day old eggs of strain laboratory pink bollworm *P. gossypiella* were obtained from rearing laboratory of Bollworm Department, Plant Protection Research Institute; Agriculture Research Center (ARC). This insect reared for several generations away from any contamination with insecticides on artificial diet that previously described by Rashad and Ammar (1985).

### Pesticides used:

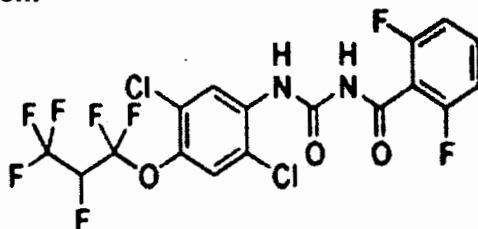
Two insect growth regulators (IGR<sub>s</sub>) were experimentally used in this study:

1- Common name: lufenuron

Trade name: Match (5% EC)

Chemical name: N-[2,5 d:chloro-4-(1,1, 2,3,3,3- hexafluoropropoxy)phenyl] amino]carbonyl]- 2,6-difluorobenzamide.

Stricter formulation:

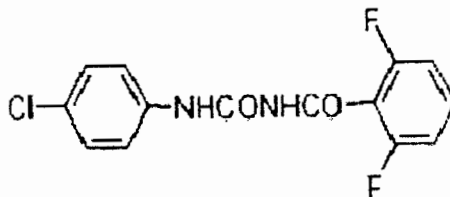


2- Common name: diflubenzuron

Trade name: Dimilin (48%)

Chemical name: - [(4-chlorophenyl) amino]-carbonyl] - 2, 6-diflorobenzamide]

Structural formula:



### Toxicity effect:

The ovicidal activity of the two experimental compounds, lufenuron and diflubenzuron tested against *P. gossypiella* eggs (one day old). Six Fresh serial concentrations of each compound were prepared as follows: 12.5, 6.25, 3.25, 1.61, 0.805 & 0.4025 ppm / lufenuron, and 200, 100, 50 & 25 ppm / diflubenzuron. Six treatments of the paper strip (150- 200 eggs / strip) / compound dipped for 15 seconds in each concentration and replay three times. The treatments left under room temperature to dry; the dried strips

kept separately in glass tubes (9.3 x1.5 cm) capped with cotton stopper and hold under incubation ( $26 \pm 1^\circ\text{C}$  and 75-80% R.H.) until hatching. Under the same conditions, three replicates of similar eggs in numbers and age were prepared and dipped in water as control. The percentage of hatchability estimated. Data of the two tested compounds were corrected and  $LC_{25}$ ,  $LC_{50}$  &  $LC_{90}$  of the two compounds were calculated by using Proban software.

**Biological studies:**

The estimated  $LC_{50}$  of the two-tested compound used to determine some biological effect. As the previous manner, three replicates of paper strip of one-day-old eggs of pink bollworm (150- 200 eggs / strip) dipped for 15 seconds in concentration of both the two tested compounds at  $LC_{50}$ . To following up effect on imature and mature stages newly hatched larvae resulted from treated eggs were transferred individually to glass tubes ( 2 x 7.5 cm ) using camel hair brush, each tube containing 3 gm of artificial diet described by Rashad & Ammar ( 1985 ). Three replicate of 20 diet tubes prepared for each tested compound. All the tubes kept at  $26 \pm 1^\circ\text{C}$  and 75-80% R.H. The same procedure was done with the newly hatched larvae resulted from untreated eggs used as a control. Larval, pupal duration, weights, adult emergence and sex ratio were determined. Newly emerged moths resulted of the two treatments as well as the control were sexed and transferred to chimney glass cage (5 pairs/ cage). Each treatment replicated three times. The moths fed on 20% sucrose solution. Cages were inspected daily to estimate the oviposition period, fecundity, % hatchability and longevity.

Obtained data was subjected to analysis of variance (ANOVA) with one-way by using SAS program (SAS, 1988).

## RESULTS AND DISCUSSION

**Toxicity of lufenuron and diflofenzeruon on *P. gossypiella* eggs (one-day- old):**

Data in Table (1) showd the  $LC_{50}$  and  $LC_{90}$  values resulted from (one-day-old eggs) of *P. gossypiella* treated with lufenuron, and diflofenzerun. The  $LC_{50}$  value of diflofenzeruon was higher than Match, where  $LC_{50}$  values were 6189 and 113.8 ppm, respectively. The same trend found at the level of  $LC_{25}$  and  $LC_{90}$ . Horowitz *et al.* (1992) recorded that, the  $LC_{50}$  values of hexaflumuron, flufenoxuron, teflubenzuron and diflofenzeruon were 0.420, 1.085, 5.966 and 18.493 ppm where dipping eggs 24- 48 hours age of *E. insulana*. Also, EL- Shennawy (2009) recorded that  $LC_{25}$ ,  $LC_{50}$  and  $LC_{90}$  for two chitin synthesis inhibitors flufenoxuron and Lufenuron were low active to treated 4-day old eggs of *P. gossypilla*.

**Table (1): LC<sub>50</sub> and LC<sub>90</sub> values of two tested compounds, lufenuron and diflubenzuron when applied on one-day- old eggs of *P. gossypiella***

Compounds	LC <sub>50</sub>	95% confidence limits	LC <sub>90</sub>	95% confidence limits
Lufenuron	6.189	3.895 – 13.648	788.463	13.648- 1492.13
Diflubenzuron	67.36	48.92-58.39	1308.26	921.9- 2431.6

**Effect of lufenuron and diflobenzeruon on hatchability and incubation period**

Data presented in Table (2) summarized the efficacy of lufenuron and diflobenzeruon on percentage of hatchability and incubation period of *P. gossypiella* eggs. It is obvious that the tow compounds at the level LC<sub>50</sub> caused high reduction in the percent of hatchability to reach 51.9 and 54.0 % when the eggs compared to 85.0% in control. The present results agree with Abdel- Megeed *et al.* (2009) who found that newly laid eggs proved more sensitive than older ones when they studied the activity of two nonsteroidal ecdysone agonists against the cotton leafworm, *Spodoptera littoralis* (Boisd).

In addition, data in Table (2) showed that the LC<sub>50</sub> treatment of lufenuron, and diflobenzeruon prolonged the incubation period of *P. gossypiella* eggs significantly than control. These periods were 6.4and 6.8 days, respectively, oppose to 3.2 days in control.

**Biological activity:**

**Larval duration and weight:**

Data in Table (2) illustrated the LC<sub>50</sub> latent effect of lufenuron, , and diflobenzeruon on *P. gossypiella* larval duration and weight resulted from treated and untreated eggs .The two tested compounds prolonged the duration of larval stage, significantly. These periods estimated by 20.7 and 24.6 days/larvae compared with 15.3 days in control. On the other hand, the weight in larvae reduced to 0.00198 and 0.00216 compared with 0.0365mg in control.

**Pupul stage:**

As shown in larval duration the two tested compound caused high significant increase in pupal duration of *P. gossypiella* resulted from one-days-old eggs with lufenuron and diflobenzeruon, the presented duration were 11.3 and 13.7 days / pupa, respectively compared to 7.9 days in control.

**Total immature stage:**

It is obvious that the total immature stage of *P. gossypiella* resulted from treated eggs highly elongated to 38.9 and 34.9 days for lufenuron, and diflobenzeruon, respectively, compared with 26.4 days in control (Table 2).

**Adult longevity:**

Data in table (3) showed that the ovipositional period significant affected by lufenuron and diflobenzeruon treatment. The mean pre-oviposition period were 3.97 and 3.77 days, respectively when the *P. gossypiella* adults resulted from eggs treated with LC<sub>50</sub> of lufenuron and diflobenzeruon respectively, compared with 3.3 days in control. Data indicated that females may taking the same period to lay the first eggs when reared when treated by both compounds.

**Table (2): Biological aspects of *P. gossypiella* immature stages resulted when applied LC90 of lufenuron and diflubenzuron on one-day- old eggs of *P. gossypiella***

compound	Conc. ppm	Egg hatchability %	Larvae died inside egg* %	Incubation period egg days (Mean ±SE)	Larval stage		Pupal stage		Total Immature Stage days (Mean ±SE)	Duration from eggs to pupae days (Mean ±SE)
					Duration days (Mean ±SE)	Weight (g)	Duration days (Mean ±SE)	Total immature stage		
Lufenuron 5%	6.189	51.9	23.7	6.9 <sup>a</sup> ±0.4 (4-8)	20.7 <sup>b</sup> ±2.07	0.00198	11.3 <sup>b</sup> ±0.7	38.9±3.07	29.6 <sup>a</sup> ±2.36	35.13±3.04a
Diflobenzuron5%	67.36	54	22.3	<sup>b</sup> 6.1±1.13(5-7)	24.6 <sup>a</sup> ±1.71	0.00216	13.2 <sup>a</sup> ±0.3	43.9±2.07	35.46 <sup>a</sup> ±1.8	39.7±1.59a
Control	-	95		3.2 <sup>c</sup> ±0.8(3-5)	15.3 <sup>c</sup> ±0.41	0.00365	7.9 <sup>c</sup> ±0.75	26.4±1.06	23.3±1.4	27.1 <sup>b</sup> ±2.19
LSD	-	-		0.269	1.538	0.0001	1.336	2.864	3.534	3.5375
P				*	**	*	**	***	0.003***	0.005***

Means followed by the same letters are not differ significantly

**Table (3): Effect of lufenuron and diflobenzeruon on fecundity and longevity induced to treated one-day- old eggs P. gossypiella**

Compound	Conc ppm	Oviposition period(days ±ES)			Fecundity		Longevity	
		Pre-oviposition	ovipositio n	Post-oviposition	Total eggs /♀	hatchabilit y %	♀	♂
Lufenuron 5%	6.189	3.97±1.01 <sup>b</sup>	10.67 <sup>b</sup> ± 3.2	5.3 <sup>a</sup> ±2.9	198.3± 3.5	60.3	25.97 <sup>a</sup> ±6.6	18.1 <sup>a</sup> ±4.58
Diflobenzeruon 5%	67.36	3.77±1.2	10.6b±1.5	4.6 <sup>b</sup> ±1.6	175.6± 2.7	59.7	29.37±2.04	20.5 <sup>a</sup> ±1.44
Control	-	3.2±0.16	13.3a±0.4	3.3 <sup>c</sup> ±0.34	238.6 <sup>a</sup> ±2.5	89.3	19.0 <sup>a</sup> ±0.58	15.2 <sup>b</sup> ±0.53
LSD		0.101	1.332	0.099	5.746	-		2.477
P		**	*	*	**	-		0.0064

Means followed by the same letters are not differ significantly

**Oviposition period:**

The oviposition period for mating female of *P. gossypiella* lasted 10.67 and 10.6 days when females resulted from eggs treated with lufenuron and diflobenzeruon, relatively shorter periods than 13.3 days in control.

In addition, data in Table (3) cleared that the tested compounds elongated the post-oviposition period of *P. gossypiella* significantly from 3.3 days in control to 4.6 days at diflobenzeruon treatments, respectively, and highly elongated to 5.3 days/ female resulted from lufenuron treatment. The results indicated that the two tested compounds caused elongation in post-oviposition period approximately, 1.5 times more than control.

Data in Table (3) showed that female's longevity was no significant affected by lufenuron, and diflobenzeruon, the adult females longevity were 19.87 and 18.97 days, respectively, compared to 19.80 days / control.

**Reproductive potential:**

Data presented in (Table 3) showed high reduction in numbers of eggs laid (fecundity) by females resulted from eggs treated by lufenuron and diflobenzeruon treatment. The main numbers of laid eggs value for lufenuron, and diflobenzeruon was 198.7 and 176.6 egg/female, respectively, compared with 238.6 eggs/ female in control

**Percentage of hatchability:**

As shown in Table (3) the percentage of eggs hatchability were 60.3 and 59.7 and on lufenuron and diflobenzeruon, respectively, compared with 89.3-% in control.

Generally, treating one-day-old eggs of *P. gossypiella* by the two tested compounds related to chitin synthesis inhibitor and /or moulting hormone agonists reflected high effects on immature and mature stages and produce reduction in fecundity and hatchability in comparison with control, this agree with Rashad *et al.* (2006) indicated that treating adults of *P. gossypiella* with diflubenzuron, caused reduction in female fecundity and fertility.

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## دراسات سمية وبيولوجية لمركبي لوفينرون و دفلوبينزرون ضد دودة اللوز القرنفلية (ساتدرز)

أيمن محمد محي الدين عدلى

مركز البحوث الزراعية - معهد بحوث وقاية النباتات - دقى - جيزة

تحت الظروف المعملية تمت دراسة تأثير LC50 لكل من مركب لوفينرون ومركب  
الدفلوبينزرون والتي قيمت بـ ٦,٤٨٩ / لوفينرون ، ٦٧,٣ / دفلوبينزرون وذلك على بيض  
السلالة المعملية لدودة اللوز القرنفلية فى عمر يوم واحد ومتابعة التأثير على فقس البيض المعامل  
فى الأطوار اليرقية والتعذير والطور اليافع . اوضحت النتائج ان نسبة الفقس للبيض المعامل بلغت  
٥١,٩ % / لمركب لوفينرون ، ٥٤ % لـ دفلوبينزرون مقارنة بنسبة ٩٥% للمقارنة وأن  
مدة حضانة البيض بلغت ٦,٩ يوم / لوفينرون ، ٦,١ يوم / دفلوبينزرون مقارنة بمدة ٣,٢ يوم /  
للغير معامل. متابعة التأثير على الفقس اوضحت النتائج أن ان مدة الطور اليرقى إمتدت إلى ٢٠,٧  
يوم / لوفينرون، ٢٤,٦ يوم/ دفلوبينزرون مقارنة ١٥,٣ يوم / الغير معامل. امتد التأثير على مدة  
التعذير حيث كان تحت تأثير مركب لوفينرون ١١,٣ يوم ، وتحت تأثير مركب دفلوبينزرون امتد  
التعذير ١٣,٢ يوم مقارنة ٧,٩ يوم للغير معامل. كما تأثرت إناث الطور اليافع بالمعاملات فكان  
متوسط عدد البيض الموضوع ١٩٨,٣ /معاملة لوفينرون ، ١٧٥,٦ / معاملة دفلوبينزرون مقارنة  
١٧٥,٦ / الغير معامل .