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TOXICOLOGICAL AND BIOLOGICAL EFFECTS OF LUFENERON AND DIFLUBENZURON ON PINK BOLLWORN *PECTINOPHORA Gossypiella* (SAUNDERS) (LEPIDOPTERA:GELECHIIDAE).

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ABSTRACT

Under the laboratory conditions, toxicological evaluation of two compounds lufenuoron & diflubenzuron against newly hatched larvae of *Pectinophora gossypiella* (Saund.) and biological effect of these compounds on larvae, pupae and adult emergence resulted from treated larvae were also studied. The results revealed that LC_{50} were 17.704 and 90.81ppm, for newly hatched larvae treated with lufenuoron & diflubenzuron, respectively. The obtained results show a prolongation in larval and pupal developments resulted from treated larvae by lufenuoron and diflubenzuron estimated by 21.43 and 23.16 days, respectively for larvae and 11.66 & 10.53 days for pupae. In contrast, in adult stage, the results indicated high reduction in total eggs laid, percentage of hatchability and longevity.

Keywords: lufenuoron and diflubenzuron, *Pectinophora gossypiella*, and bological study.

INTRODUCTION

Cotton is one of the major economics crops in Egypt. Throughout cotton growth season, it is attacking by many different pests. The pink bollworm (*Pectinophora gossypiella*) is considered the most destructive pest infesting cotton bolls causing severe damage resulting in high loss in both quality and quantity of cotton yield (Lohag and Nahyoon 1995).The first chitin synthesis inhibitor introduce into the market as a novel insecticide was benzoylphenylurea (BPU), or diflubenzuron (DFB) (Miyamoto *et al.* 1993). Some of the structural modifications (derivatives) of the compounds are more active than the parent compound. It was found to be effective on several insect species (Bayoumi, *et al.* 1998) and (El-Nemaky and Azab 2004). Since the introduction of DFB, a number of other BPU derivatives have been developed such as hexaflumuron, Flucycloxuron and Triflumuron (Bendjedou *et al.*, 1998) and (Rehimi and Soltani 1999). These compounds have been found to interfere with chitin biosynthesis (Soltani *et al.*, 1996). Lufenuoron and Diflubenzrona and its derivatives were effective against Coleoptera, Diptera and Lepidoptera (Edomwande, *et al.*, 2000) and (Butter, *et al*., 2003): they added that, it also effective against insect pests and mites infesting field crops and are relatively harmless to beneficial insect species.

The objective of the present study is amid to investigate the effect of lufenuoron & diflubenzuron on pink bollworm newly hatched larvae and the effect of these compounds on biological aspects of the first generation of treated *P. gossypiella*, including developmental duration, mortality, fecundity, fertility and adult emergence.

MATERIALS AND METHODS

Used Insecticides:-

a - Lufenuron

1- Common name: Lufenuron

2-Trade name: Match (5%)

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

b. **diflubenzuron**

1- Common name: diflubenzuron

2- Trade name: Dimilin (48%)

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

Insect used:

Pink bollworm, *P. gossypiella* 1st instar larvae laboratory strain was reared for several generations under the laboratory conditions (27±1^{0}C and 75±5 R.H. %), at Bollworms Research Department, Plant Protection Research Institute, Agriculture Research Center as a described by Rashad and Ammar (1985).

Used Larvae:

Four groups of freshly emerged moths of *P. gossypiella* each group 10 pairs(♂♂ X ♀♀) were confined in a glass chimney cage (17cm height and 7.12 cm in diameter), inside which a piece of cotton wool previously soaked in 20% sugar solution was suspended to be renewed 48 hr for moths' nutrition. The top and bottom of each cage were covered with screening mesh kept in position by rubber bands. Eggs were deposited thought the screening mesh, one piece of paper placed upper and lower the cages in open Petri-dish that served as an oviposition site. Eggs were collected daily and kept in glass jars (1/2 kg). Collected eggs were maintained at 27±1^{0}C and 75±5 R.H. % until hatching and newly hatching larvae were used.

Procedure:

Toxicity effect:

Pilot experiment was conducted to evaluate LC_{50} for each compound. Serial concentrations dilution, (50, 25, 12.5, 6.25 and 3.125 ppm) for Lufenuron and (240, 120, 60, 30 and 15 ppm) for diflubenzuron were freshly prepared from the stock solution of each compound (1ml/1 liter water)

To evaluate the effect of two insect growth regulators (IGRs) (Lufenuron and diflubenzuron**)** on the newly hatched larvae of PBW, the diet was poured into glass tubes (2X7.5 cm) each tube contained 3gm diet. Three replicates of

50 tubes/concentration of the tested (IGRs) were used in addition to 50 tubes containing untreated diet (control). One drop = 0.02 ml of the tested concentration was added to the surface of the diet in each tube. Distilled water was added to the untreated control. All the tubes were held uncapped for one hour to allow absorption and then newly hatched larvae of pink bollworm was transferred into each tube using fine hair brush and capped by cotton wool. All tubes were kept at 27±1°C and 75±5 R.H. % for 24 hrs. LC_{50} and slope values of both IGRs were calculated according to Finney (1971).

Studies of some biological aspects:

In order to some biological aspects of treated larvae, treating neonate larvae of PBW treated with LC_{50}. LC_{50} of the two tested IGRs were applied on the upper surface of the diet/ tube, as mentioned before. A (300 neonate were used/ treatment), 100 tubes were used for control.

The newly hatched larvae of PBW were placed individually into each tube treated and untreated using a fine hair brush and then capped by a piece of cotton wool and incubated at 27±1°C and75±5 R.H%. The tubes were examined to determine some biological aspects such as: percentage of larval mortality, larval malformation, larval duration, pupal duration, percentage of adult emergence, malformation and sex ratio.

Newly emerged moths resulted from newly hatched larvae treated by LC_{50} Lufenuron and diflubenzuron were sexed and transferred to chimney glass cage (five pairs /cage). Each treatment was replicated three times. The moths were fed on 20% sucrose solution. Cages were examined daily to record pre-ovipostion, ovipostion and post-ovipostion periods and the numbers of eggs laid in addition to percentage of hatchability and estimated the females and males longevity for each treatment.

The obtained data were statistically analyzed with one-way analysis of variance (ANOVA) ($P< 0.05$) (Snedecor, 1952) and Duncan's multiple range test means was used (Duncan's, 1955).

RESULTS AND DISCUSSION

1- Toxicological effects of tested IGRs:

Table (1) shows the susceptibility of newly hatched larvae of *P.gossypiella* towards the tested IGRs (lufenuoron & diflubenzuron). Based on LC_{50} values lufenuron was more effective against newly hatched larvae than diflubenzuron as the LC_{50} values were 17.704 and 90.81 ppm for lufenuoron and Diflubenzuron, respectively.

Also data in Table (1) revealed that the LC_{25} , LC_{50s} and LC_{90} values for newly hatched larvae have variations with two compounds.

Table (1): Toxicological evaluation of lufenuoron and diflubenzuron against first instar larvae of pink bollworm.

Stage used	Treatment	Toxicity		
		LC_{25}(ppm)	LC_{50} (ppm)	LC_{90}(ppm)

newly hatched larvae	Lufenuoron	4.95	17.704	199.02
	diflubenzuron	23.01	90.81	1232.64

2-Biological effects of the two tested compounds:

Data in Table (2) show the duration of larvae, pupae and adult emergence resulted from newly hatched larvae treated with LC_{50} of the two compounds (lufenuoron and diflubenzuron).

Larval stage:

It is clear that the two tested compounds significantly prolonged the duration of the larval stage resulted from treated newly hatched larvae than that of the untreated (check). Table (2) revealed that larval duration was 21.43 and 23.16 days after newly hatched larvae treated with lufenuoron and diflubenzuron, respectively, compared with 14.33days in the control. Kostandy, *et al.* (1999): recorded that the insect growth inhibitor prolonged the larval stage when newly hatched larvae of *P. gossypiella treated*.

Mortality and malformation:

As shown in Table (2) the high percentage of mortality and malformed larvae appeared in larvae resulted from newly hatched larvae treated with lufenuoron and diflubenzuron. The lowest percentage of mortality and malformation 56.5 and 6.3%, respectively, recorded by diflubenzuron, while it increased to 67.23 and 11.33 %, respectively, when larvae treated by lufenuoron compound

Pupal stage:

Data in Table (2) revealed significant increase in pupal duration of *P.gossypiella* resulted from the treated newly hatched with both lufenuoron and diflubenzuron, this durations were 11.76 and 10.53 days, respectively, compared with 8.97 days in control.

Pupal malformation:

Data presented in Table (2) indicate that Diflubenzuron, caused more malformation in pupal than Lufenuoron, malformation percentages were 8.6 and 5.4 %, respectively. While in case of untreated larvae percentage decreased to 1.5 %.

Total duration of immature stages:

Data in Table (2) showed that the two tested compounds significantly prolonged the duration of total immature stages than that of control. Total duration of immature stages was 31.4 and 33.9 days, when larvae treated with lufenuoron & diflubenzuron, respectively, compared with 23.3 days in control.

It is obvous that both Lufenuoron & diflubenzuron used in this study were significantly affected on different biological parameters as compared to control. The increase in mortality percentages, malformed, prolonged the duration in larval and pupal (total immature) stage and the decrease in the adult emergence. These data are similar to the data obtained by many authors using different IGRs against many Lepidopterous insects such as *P. gossypiella* , *Spodoptera littoralis* and the black cutworm, *Agrotis ipsilon* (Abdel-Aal, 2003), (Moawad and Khidr, 1982) and (Shaurub, *et al*., 1999).

Kandil, *et al*. (2005) recorded that chitin synthesis inhibitors increased the total immature stages of pink bollworm *P. gossypiella* (Sound.)

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Adult stage:

Adult emergence:

Data given in Table (2) showed significant reduction in the moth emergence percentage compared with control. The percentages of adult emergence were 82.3% and 77.66% adults resulted from treated larvae with lufenuoron & diflubenzuron, respectively compared with 97.0% in control. El-Barkey *et al*. (2009) recorded that the IGR hexaflumeroun reduced the percentages of adult emergence of *P. gossypiella.*

Ovepositional period

Pre-ovipostion, ovipostion and post-ovipostion periods, adult longevity, total number of deposited eggs (fecundity) and the total number of hatching larvae from the eggs (fertility) for the two tested compounds in comparison to the control were recorded in Table (3). It is clear that the pre-ovipostion period was highly significant increased by both tested compounds. This period were 5.0 and 4.8 days for females resulted from larvae treated with lufenuoron and diflubenzuron, respectively, while it was 2.9 days in control.

The two tested compounds caused high significant increase in ovipostion periods**;** 16.77 and 18.3 days resulted from treated larvae with lufenuoron & diflubenzuron, respectively compared to 14.2 days for control in table (3).

Adult longevity:

Female's longevity was highly significant affected by lufenuoron and diflubenzuron adult females longevity were 29.87 and 28.96 days/♀ resulted from larvae treated with the tow componds, respectively, compared to 19.63 days/ female in control. Also, the males' longevity resulted from PBW treated larvae were prolonged than the control. The recorded means were 21.1 & 24.56 days/♂ from larvae treated with lufenuoron & diflubenzuron, respectively, compared with 17.73 days/ ♂ in control (Table, 3). Rashad *et al.* (2006) indicated that treating adults of *P. gossypiella* with diflubenzuron, caused prolonged the longevity for female and male compared to the control.

Reproductive potential:

Data presented in (Table, 3) show high reduction in numbers of eggs laid by females resulted from treated larvae. The mean numbers of laid eggs value were 152.7 and 169.33 eggs/ female resulted from larvae treated with the two compounds, respectively, compared with 235.7 eggs/ female in control.

As shown in Table (3) the percentage of eggs hatchability were 53.66 and 49.1% in case of treatments with lufenuoron & diflubenzuron, respectively, compared with 95.67 % in control. These resulted agree with those of Abdel-Aal (2006) who reported that fecundity and egg- hatchability percent of treated cotton leafworm *S. littoralis* female with IGR_S compounds decreased as compared with control. Also, Rashad *et al.* (2006) indicated that treating adults of *P. gossypiella* with diflubenzuron, caused reduction in female fecundity and fertility**.**

Saenz-de-Cabezon *et al*., (2006) showed that lufenuron has activity on *L. botrana* in contact treatment. El-Barkey *et al*. (2009) stated that IGR_S cussed high reduction in fecundity and egg- hatchability of *P. gossypiella.* Lyra, *et al.* (1998): recorded that the chitin synthesis inhibitors caused highly reduceed on reproduction of *Spodoptera littoralis*. Also, Yasir *et al.,* (2012) recorded that the fecundity and egg hatchability were reduced at all concentrations of Lufenuron used against *T. castaneum* larvae.

Table (3): Effect of Oviposition period, fecundity and longevity treating newly hatched larvae of *P. gossypiella* with LC_{50} concentrations of lufenuron and diflubenzuron compounds

Treatment	Pre-oviposition period	Oviposition period	Post-ovipoxition period	Eggs/ Female ± SE	% hatchability ± SE	Adult longevity (days) ± SD	
						♀	♂
Lufenuron	5.00± 0.12	16.77± 0.84	7.80± 0.48	152.7±7.6	53.66 ±2.73	29.87 ± 0.18	21.1±1.18
diflubenzuron	4.80 ± 0.11	18.3± 1.27	7.56± 0.9	169.33 ±1.44	49.1± 1.18	28.96± 1.44	24.56±1.94
Control	2.9 0± 0.1	14.2± 0.9	2.40 ± 0.1	235.0± 5.58	95.67± 3.68	19.63± 0.41	17.73
LSD (5%)	0.335	1.371	0.135	5.988	3.573	1.160	2.731
P	**	*	*	***	**	**	**

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التأثيرات التوكسوكولوجية والبيولوجية لمركبات الليوفنيورون(الماتش)والداى فلوبنزيورون (الديملين) على دودة اللوز القرنفلية

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**** معهد بحوث وقاية النباتات – مركز البحوث الزراعية – الدقي – الجيزة**

تم دراسة التأثير السام للمركبين الماتش والديملين (أحدى منظمات النمو الحشرية) وقد أظهرت النتائج ان التركيزات المؤدية لموت ٥٠% من الفقس الحديث لدودة اللوز القرنفلية كان ١٧.٧٠٤ ، ٩٠.٨١ لمركب الماتش والديميلين على التوالي. وقد تم متابعة تأثير المركبين على النواحي البيولوجية. وقد أظهرت النتائج حدوث أطالة في عمر اليرقات وكذلك العذارى من حيث زيادة في نسبة الموت وكذلك التشوه في كلا الطورين لكلا المركبين. كذلك أظهرت النتائج حدوث تأخير في وضع البيض للإناث لكل من المركبين وأدى ذلك إلى حدوث نقص شديد في كمية البيض الموضوع لكل أنثى حيث كان متوسط البيض الموضوع لكل انثى١٥٢.٧ &١٦٩.٣٣ لكل من الاناث الناتجة من اليرقات المعاملة بالماتش والديميلين على الترتيب بينما كان متوسط البيض الموضوع لكل انثى فى الكنترول٢٣٥ .وكذلك حدوث نقص في نسبة الفقس مقارنة بالكنترول.

قام بتحكيم البحث

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Table (2): Survivors, mortality percentages and periods of immature stages of *P. gossypiella* exposed as newly hatched larvae to LC_{50} concentration of lufenuron and diflubenzuron under controlled conditions (26 ±1 ˚c and 75±5 % R.H.).

Treatment	LC^{50} Concentration (ppm)	Larval stage				Pupal stage				Total immature duration	zaAdult stage			
		% Total Mortality after 15-20 days	% Malformation	Duration (days) Means ± SE	Weight of larvae (g)	% Pupation	% Malformation	Duration (days) Means ± SE	Weight of pupae (g)		% Adult emergence	% Malformation	%Sex Ratio	
													♀	♂
Lufenuron	17.704	67.23 ±3.05	11.33± 0.68	21.43 ± 0.32	0.024 ± 0.001	85.3 ± 4.7	5.4 ±0.1	11.76 ± 0.14	0.019 ±0.001	31.4 ±3.34	82.3 ± 2.43	5.6 ±0.211	51.33 ±0.95	48.66 ±2.9
Diflubenzuron	90.81	56.0.5 ±5.9	6.3 ±.740	23.16 ± 1.40	0.0253 ±0.001	80.0 ±3.34	8.6 ±0.21	10.53 ± 0.62	0.0211 ±0.002	33.9 ±2.16	77.66 ± 0.9	3.3 ± 0.10	68.3 ± 6.9	43.0 ±6.1…4
Control	–	2.7 ±0.16	0	14.33 ± 0.38	0.0345 ±0.001	100.0 ±0.0	1.5 ±0	8.97 ± 0.6	0.03 ±0.001	23.3 ±1.1	97 ± 1.3	0	44.5 ±1.9	45.5 ±2.54
LSD	_	2.011	1.660	0.988	0.0001	3.517	0.761	0.397	0.0001	1.070	2.851	1.352	4.710	0.957
P	_	**	***	*	**	*	*	**	*	*	*	**	**	**