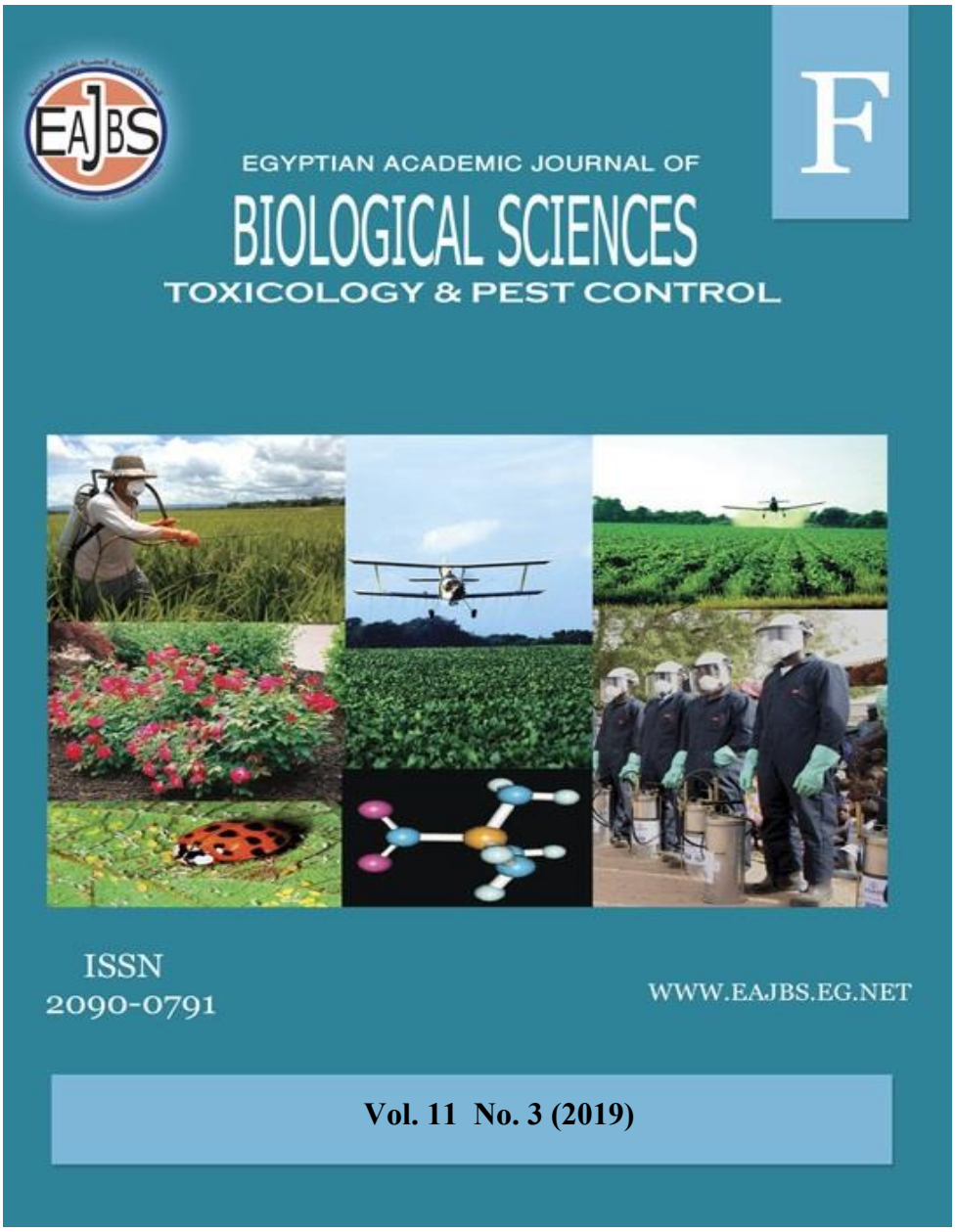

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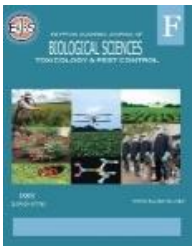
www.eajbs.eg.net

Egypt. Acad. J. Biolog. Sci., 11(3): 171-180 (2019)

Egyptian Academic Journal of Biological Sciences
F. Toxicology & Pest Control
ISSN: 2090 - 0791
http://eajbsf.journals.ekb.eg/



Latent Effect of Certain Ovicidal Compounds LC_{50} on Some Biological and Biochemical Parameters of Pink Bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae)

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ARTICLE INFO
Article History
Received:29/11/2019
Accepted:29/12/2019

Key words:
IGR, biological effects, biochemical effects, pink bollworm

ABSTRACT

Latent effect of LC_{50} of Growth Regulators (IGR's)(Teflubenzuron, Lufenuron, and Flufenoxuron) against pink bollworm *Pectinophora gossypiella* (Saund.) under laboratory conditions was studied. Tested compounds were evaluated against one-day-old eggs of *P. gossypiella*. LC_{50s} were 33.17, 18.65, and 14.36 mg/liter, for Teflubenzuron, Lufenuron, and Flufenoxuron, respectively. The biological effect of these compounds on larvae, pupae, and adult emergence resulted from treated eggs was studied. The obtained results showed prolongation in development periods of larval and pupal stages resulted from treated eggs compared with control. Results indicated prolongation in adult females and males' longevities, there was also a significant reduction in the number of eggs laid by each female and percentages of hatchability of these eggs compared with control. The latent effect of these compounds on some biochemical parameters in larvae resulted from treated eggs was analyzed for total protein, Free-amino acid, chitinase activity, N-acetyl- glucosamine and Phenoloxidase activity. The results showed significant reduction in all biochemical parameters but chitinase was significantly high compared with control. In general, the tested compounds had latent significant biological and biochemical effects on the pink boll worm.

INTRODUCTION

In Egypt, cotton is one of the most important economic crops; it is attacked by many insect species. Cotton bollworms are the most destructive pests infesting cotton plants such as**;** the Pink bollworm, *Pectinophora gossypiella* (Saunders), (Lepidoptera: Gelechiidae). *P. gossypiella* considered one of the important pest factors that influence cotton production and cause economic damage to the square and fruits and the damage-causing losses to the crop yield (El- Khayat *et al.,* 2015).In Egypt, cotton production depends on successful and efficient pest management programmers, which reduce the disaster of crop losses particularly caused by insect- pests. The effectiveness of different pesticides against bollworms was studied by several authors (El- Khayat *et al.,* 2015 and Abbas *et al.,* 2017). The group of pesticides known as insect growth regulators (IGRs) is

potent insecticides, owing to is potent insecticides, which causes inhibiting growth in insects (Ghoneim *et al.,* 2017). Insect Growth Regulators (IGR's) disrupt and impede the life cycle of insects in the egg and larvae stage of development. Insect growth and development is regulated by hormones produced by endocrine glands, hormones are chemical substances transported in the insect hemolymph that influence physiological processes. IGRs cause the abnormal formation of the endocuticle that accumulates during the molting process, specifically uridines' diphospho-N-acetylglucosamine thereby preventing chitin synthesis and breakdown of metamorphosis process (Ghoneim *et al.,* 2017), this produce a weaken cuticle and causes mortality. Insect growth regulators are most active on the most stages of insects that undergo complete metamorphosis (egg, larva, pupa, and adult) (Kandil 2013 and Said *et al.,* 2017).

Some authors recorded that IGRs, may be quite selective in their mode of action and potentially act only on the target species (Sabry and Abdou, 2016), and lead to various abnormalities that impair insect survival. The toxic or activity of IGRs depends on the insect's development and metamorphosis. Therefore, evidence of activity may be slower than with typical contact insecticides, especially with differenced the stages treatment (Kandil 2013).

The objective of the present study was to investigate the effect of ovicidal compounds on some biological and biochemical parameters of pink bollworm, *P. gossypiella*.

MATERIALS AND METHODS

Insect Used:

The laboratory strain (eggs) of pink bollworm, *P. gossypiella* used in this investigation was reared for several generations under the laboratory conditions at 26±2^{0}C and 65±5 R.H. at Bollworms Research Department, Plant Protection Research Institute, Agriculture Research Center as a described by Rashad and Ammer (1985). The experiments were carried out at Bollworms Research Department, Plant Protection Research Institute, Agriculture Research Center in December 2018.

Insect Growth Regulators (IGR) Used:

Classification: - Benzoylphenylurea

1- Common name: Teflubenzuron (benzoylurea). Trade name: Nomolt® 15% Suspension Concentrate (SC). Rate of application: the field 50 cm^3 / 100 L. Basic product: BASF Co.

2- Common Name: Lufenuron. Trade name: (Match 5% EC). Rate of application: The field rate is 160 ml/feddan (feddan = 4200m2)

3- Common name: Flufenoxuron. Trade name: (Caseced 10% DC). The field rate is 200 ml/feddan

Prepared the Tested Compounds:

The ovicidal activities of all compounds (IGRs) were tested against eggs (1-24hr after deposition) of *P. gossypeilla*. A serial of concentrations for each pesticide was prepared as follows:

1-150, 75, 37.5, 18.7, 9.3 and 4.65 mg/liter of Teflubenzuron.

3-120, 60, 30, 15, 7.5 and 3.7 mg/liter of Lufenuron.

4-50, 25, 12.5, 6.2, 3.1 and 1.5 mg/liter of Flufenoxuron.

Toxicity of the Tested Compounds Against the PBW Eggs:

All previous compounds were tested against eggs (1-24hr after deposition) of *P. gossypeilla*. The dipping technique was used against one-day-old eggs of *P. gossypiella*. Three replicates/ concentration (concentration/compound), as well as control, were used.

Each replicate contained 200 eggs (1 day old), deposited on a piece of paper. The eggs were dipped for 10 seconds in each concentration of each tested compound. Another group used as a control was done by dipping pieces of paper containing eggs in distilled water for the same time. After treatment, papers containing treated and untreated eggs were left to dry, then were placed in glass tubes (5 × 12.5cm) and held under constant conditions of 26±2°C and 65±5 % R.H. until hatching. Percentages of hatchability were estimated. Data were recorded and LC_{50} of the studied compounds was calculated by Proban program version 1.1.

Biological Studies:

In this experiment, three replicates of 50 glass vials, (each tube 2 X 7.5 cm) containing 3 gms of diet were used. First instars' larvae resulted from treated eggs with LC_{50} of Teflubenzuron, Lufenuron and Flufenoxuron were transferred individually to the diet glass vials by camel hairbrush. The same was done with the first instars' larvae resulted from untreated eggs. The glass vials were capped with cotton and kept under the previous conditions in an incubator and inspected daily until pupation. Pupae resulted from each treatment were removed from all tubes and placed in clean tubes until adult emergence. Some parameters were estimated such as % of larval mortality, malformation, larval duration, pupal duration, and percentage of adult emergence were determined.

Resulted moths were sexed and transferred to a chimney glass cage (five pairs /cage). Each treatment was replicated three times. The moths were fed on a 20% sucrose solution. Cages were examined daily to record pre-oviposition, oviposition, post-oviposition periods, fecundity, and percentage of hatchability. Also estimated females and males longevity for each treatment. Fecundity was calculated according to Crystal and Lachance (1963) as follows:

$$\% \text{ Fecundity} = \frac{\text{No. eggs/ treated female}}{\text{No. eggs/ untreated female}} \times 100$$

Biochemical Analyses:

Preparation of Samples for Biochemical Assay:

Samples of *P. gossypiella* larvae resulted from eggs treated by LC_{50} of Teflubenzuron, Lufenuron or Flufenoxuron tested were collected after 10 days from different treatments to study the latent effects of tested compounds in full-grown larvae, these samples were homogenized in distilled water. The homogenates were centrifuged at 5000 r. p. min. at $5C^0$ in a refrigerated centrifuge. The supernatants were kept in a deep freezer at $-20C^0$ till used for biochemical assays. Larvae were analyzed chemically for each compound with untreated check in Physiological Dept. of Plant Protection Researches Institute, (P.P.R.I.).

Analyses Technique:

The colorimetric determination of total soluble protein, in total homogenate *P. gossypiella* larvae was carried out, as described by Bradford (1976). Total free amino acids were colorimetrically assayed by ninhydrin reagent according to the method described by Lee and Takabashi (1966). The determination of chitinase activity was prepared according to Bade and Stinson (1981). Determination of N-acetyl- glucosamine by the sensitive method of Waterhouse *et al.* (1961). Phenoloxidase activity was determined according to the modification of Ishaaya (1971)

Statistical Analysis:

The obtained data were statistically analyzed by one- way ANOVA test at 0.05 of probability using Costat program (version 11) and range test of means used Duncan's multiple in the same program.

RESULTS AND DISCUSSION

Effect of Tested IGRs on Immature Stages:

The LC_{50} and LC_{90} values of various (IGRs) Teflubenzuron, Lufenuron, and Flufenoxuron on the one-day-old eggs, are given in Table (1). The LC_{50} values of tested (IGRs) on one-day-old eggs are given in Table (1), LC_{50} values for one-day-old eggs of pink bollworm were 15.63, 14.36 and 33.17 mg/liter for Lufenuronand, Flufenoxuron and Teflubenzuron, respectively.

Table 1: Toxicological evaluation of Teflubenzuron, Lufenuron, and Flufenoxuron against eggs of pink bollworm under laboratory conditions

Ages of eggs	Compounds used	Toxicity: 95%Confidence limits			
		LC_{25} (ppm)	LC_{50} (ppm)	LC_{90} (ppm)	Slope
One day old	Teflubenzuron	13-6	33.17	180.48	1.742±0.28.
	Lufenuron	7.42	15.65	140.23	1.858±0.15
	Flufenoxuron	4.33	14.36	47.63	1.295±0.11

The data in Table (2) recorded the high effect of Teflubenzuron, Lufenuron and Flufenoxuron on hatchability and incubation period of pink bollworm eggs. It is obvious that all compounds at the tested level LC_{50} reduced percentages of hatchability with 48.0 , 52 and 45 % for one day old eggs, respectively, compared with 98 % in control.

The LC_{50s} values for one-day-old eggs of pink bollworm were nearly similar for both Lufenuron and Flufenoxuron, with an LC_{50s} were 15.63 and 14.36 on contrast there was more variation with LC_{50} values of Teflubenzuron this variable values estimated by 33.17 ppm. This data revealed that one-day-old eggs less susceptible to Lufenuron and Flufenoxuron than Teflubenzuron treated. This data may be elucidated to the different or lower penetration of Teflubenzuron through the chorine of deposited eggs from 6 to24 hr.

The results of our study confirmed with Nehad *et al.* (2009) whose found the newly laid eggs may be slightly less susceptible to Hexaflumuron action than two days old eggs and pre hatched for *P. gossypiella*.

Table 2: Effect of Teflubenzuron, Lufenuron and Flufenoxuronn on Hatchability and Incubation period of pink bollworm eggs

Type of treatments	LC_{50}	% Hatchability	% Reduction in hatchability %	One day old eggs				
				Incubation period	N	F value	P value	LSD
Teflubenzuron	33.17	48.0	52.0	6.0±0.6 b	3	8.221	0.021 *	0.74
Lufenuron	18.65	52.0	48.0	7.0±0.5 a				
Flufenoxuron	14.36	54.0	46.0	6.1±0.57 b				
Control		98.0	2	3.8±0.5 c				

Means followed by the same letter in the same column have no significant differences.

The incubation period of pink bollworm eggs was high significant affected by LC_{50} treatment of Teflubenzuron, Lufenuronand Flufenoxuron (Table 1). These incubation periods estimated by 6.0, 7.0, and 6.1 days when one-day-old eggs treated with three compounds, respectively, compared with 3.8 days for control. Also, data indicated that control eggs completed hatching by 3.8 days, while the treated eggs required more 2 times (ranged from 5 to 8 days) for hatching.

This results can be concluded that treated one-day-old eggs with the tested compounds(IGRs), cussed high decreased in egg hatchability and increased in the incubation period of eggs (approximately up to 2 times), these results may be due to the penetration of these compounds into the eggs and prevent hatching by interfering with embryonic cuticle synthesis (Kandil et al. 2013).

The data recorded in Table (3) showed the difference in duration and weights of larvae & pupae, total immature stage, and malformed individuals resulted from eggs dipped in LC_{50} of Teflubenzuron, Lufenuron, and Flufenoxuron. It is clear that the tested compounds significantly prolonged the duration of the larval and pupal stages than that of the untreated check, which led to the longest total immature stages periods, which also caused a reduction in both larval and pupal weight, in addition to malformations in both larvae and pupae.

The larval duration was 21.3, 18.7, and 19.9 days/ larvae resulted from treated eggs (one-day-old eggs), respectively for Teflubenzuron, Lufenuron, and Flufenoxuron, respectively. It was observed that there was no significant difference between the tested compounds and this may be similar to chemical composition.

Table 3: larval and pupal stages of *Pectinophor agossypiella* when resulted from eggs treated with $LC_{50}$for each compound.

Tested copmpounds	Conc. (%)	Larval stage parameter		%Pupation	Pupal stage		Total immature	% Malformed L &P
		Duration times	Weigh		Duration times	Weigh		
Teflubenzuro n n	33.17	21.3±0.64 a	0.0213±0.001 b	84.3±1.7a b	8.66±0.33 b	0.0166±0.001 b	29.96±1.18 a	9.0±1.16 a
Lufenuron	28.65	18.7±0.45 b	0.0273±0.002 b	72.3±4.7 b	10.26±0.37 a	0.0255±0.002 ab	28.98±0.71 a	10.0±2.1 a
Flufenoxuron	14.36	19.9±0.66 ab	0.0225±0.005 b	73±6.42 b	10.8±1.2 a	0.0163±0.004 b	30.5±0.4 a	10.63±0.2 a
Control	-	14.95±0.54 c	0.0375±0.037 a	93.3±0.3a	8.3±0.66b	0.0346±0.001 3a	23.3±1.17 b	2.33±0.33 b
N	-	3	3	3	3	3	3	3
F value	-	23.879	6.6693	6.0416	7.126	11.3981	9.171	19.11871
P value	-	0.0002***	0.0144*	0.0188*	0.0120*	0.0029**	0.0057**	0.0005***
LSD	-	1.907	0.009	13.313	1.397	0.0093	2.595	3.739

Means followed by the same letter in the same column have no significant differences.

For the pupal duration of *P. gossypiella* resulted from the treated eggs with both compounds (IGRs) Lufenuron and Flufenoxuron, the results showed a significant increase in duration, it was 10.26 and 10.8 days, respectively, compared with control 8.3 days, but no significant difference in case of Teflubenzuron, the duration estimated by 8.66 days.

The tested compounds prolonged the durations of all immature stages compared with control, The total developmental periods required from larvae to adult emergence estimated by 29.96, 28.98, and 30.**5** days compared with 23.3 days in control.

The larval and pupal weights were 0.0213, 0.0273 and 0.0225mg/ larvae and 0.0166, 0.0255 and 0.0163mg/ pupae for Teflubenzuron, Lufenuron and Flufenoxuron compounds respectively, compared to 0.0375mg/ larva and 0.0346 mg/pupa for control (Table 3).

Data in Table (3) and Figure (1) Indicate that Teflubenzuron, Lufenuron, and Flufenoxuron caused a high increase in malformation of larval and pupal than control.

The percentage malformed recorded by 9.0, 10.0, and 10.63 %, respectively, compared with 2.33% in control. The most morphological deformation was pupal-adult intermediate resulted from IGRs treatments.

Previous results indicate that the tested (IGRs) compounds generally caused significant prolongation in both larval and pupal durations. It also caused a significant reduction in both larval and pupal weights, (Kandil et al., 2013; Ghoneim 2017 and Said et al. 2017). In addition to increased incidence of morphological malformations, alternations of the ovipositor, and inhibition of the ovarian growth (Tanani and Bakr 2018).

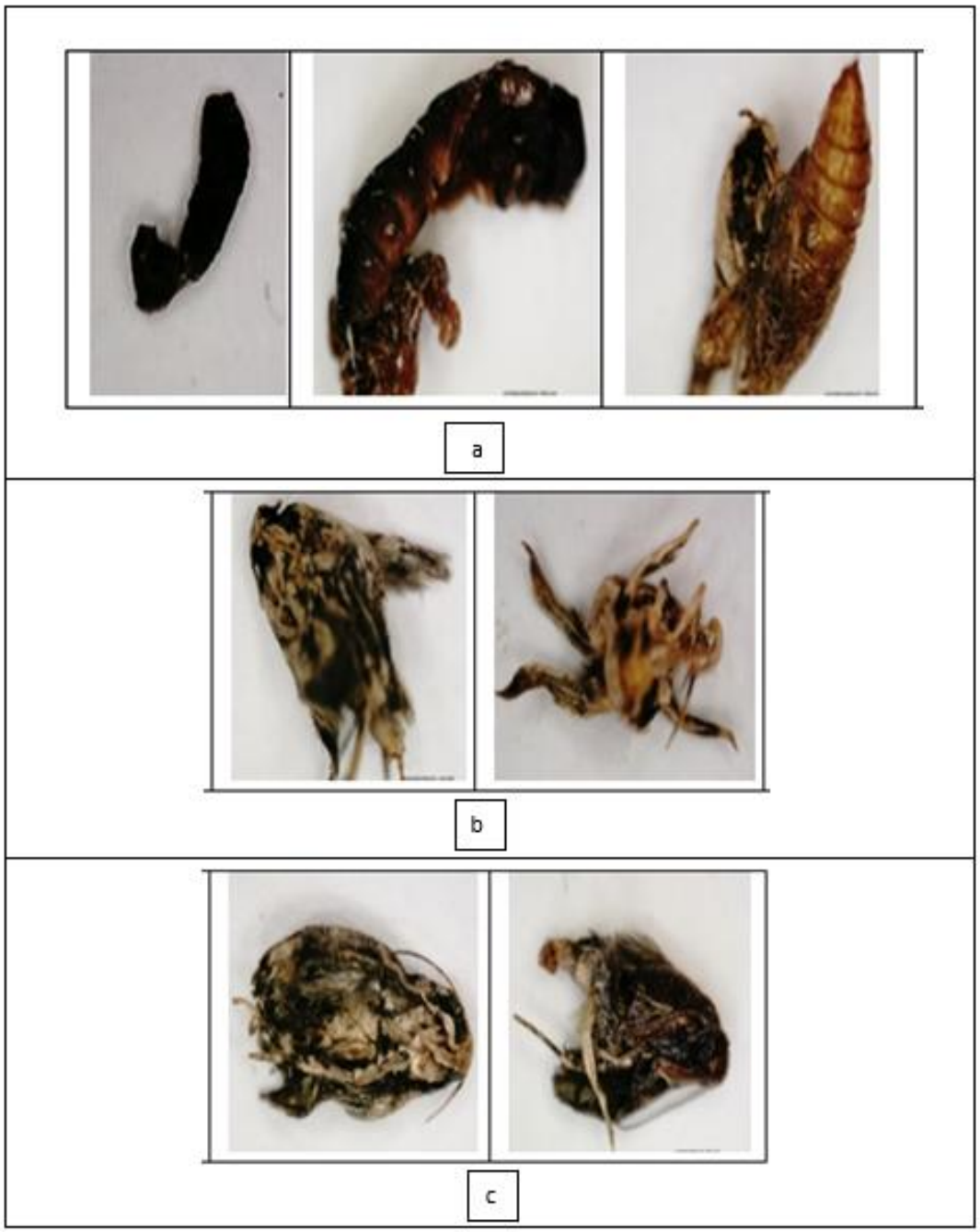

Fig.1: Malformations of different stages as results to treatment with tested compounds.(a) Teflubenzuron (Larvae and pupa) ; (b) Lufenuron (Adults) and (c) Flufenoxuron (Adults)

Effect of Tested IGRs on Adult Stage:

Pre-oviposition, oviposition, post-oviposition, and longevity periods for the tested compounds in comparison to the control were recorded in Table (4). In general, the tested compounds increased the different periods of adults and reduced the number of deposited eggs of females resulted from eggs treated with Teflubenzuron, Lufenuron, and Flufenoxuron (figure 2). Hatchability percentages also were highly affected by the treatments. The results show those pre-oviposition periods were significantly increased. These periods were 3.6, 4.3, and 3.3 days, respectively for Teflubenzuron, Lufenuron, and Flufenoxuron., respectively, compared with 2.66 days in control. Also, oviposition periods were 13.0, 15.6, and 14.3 days Teflubenzuron, Lufenuron, and Flufenoxuron, respectively compared with 10.2 days for control. Post-oviposition periods were 3.3, 2.6, and 4.6 days for Teflubenzuron, Lufenuron, and Flufenoxuron compared with control 2.7 days. As a result of the increase in previous periods, the longevity of adult females increased, longevities of females were 20, 22.7, 21.3, and 16 days for Teflubenzuron, Lufenuron, and Flufenoxuron and control, respectively. Longevity for males was 15.66, 18.3, 18, and 13.3 for Teflubenzuron, Lufenuron, and Flufenoxuron and control, respectively.

The mean numbers of deposited eggs were 103.3, 137.7 and 104.0 eggs/female resulted from treated eggs with LC_{50} of Teflubenzuron, Lufenuron, and Flufenoxuron, respectively. The hatchability percentages were 61.0, 65.3, and 74.0% Teflubenzuron, Lufenuron, and Flufenoxuron, respectively, compared with control (95.6%).

This data indicated that latent toxic effect in females and males resulted from eggs treated with previous compounds, these compounds caused increased in the adult stage (Khatter, 2014 and Gado et al., 2015).

Also, tested compounds have an effect on the adult performance and reproductive potential after treatment of 1-day old eggs with sublethal concentrations. Generally results indicated that all treatments decrease adult emergence, reduction in longevity and fecundity and eggs laying/female (Tanani and Ghoneim, 2018).

Table 4: Effect of tested compounds (IGRs) on egg production of *P. gossypiella* females resulted from eggs one day old treated with LC_{50} of tested IGRs.

Tested compounds	Conc. (%)	Female adult stage			Longevity		Fecundity		% Hatch ability %
		Pre-oviposition	Oviposition	Post - oviposition	Female	Male	Total no. of eggs/famale	Mean no. of eggs/female/day	
Teflubenzuron n	33.17	3.6±0.3ab	13.0±0.57b	3.3±0.88a	20.0±1.7ab	15.66±0.8b	103.3±14.1b	7.95±2.06	61.0
Lufenuron	18.65	4.3±0.3a	15.6±0.7a	2.6±00.2a	22.7±0.98a	18.3±0.3a	137.7±8.1b	8.8±1.5	65.3
Flufenoxuron	14.36	3.3±0.4ab	14.3±0.8ab	4.6±0.3a	21.3±2.18a	18.0±0.47ab	104.0±13.6b	7.4±0.9	74.0
Control	-	2.66±0.4b	10.2±0.3c	2.7±0.9a	16.0±0±1.15b	13.3±0.89c	244.0±11.26a	23.9±1.3	95.6
N	-	3	3	3	3	3	3	-	-
F value	-	4.333	13.6389	0.6111	3.4869	11.3958	30.2257	-	-
P value	-	0.0432*	0.0016**	0.6265ns	0.0701ns	0.0029**	0.0001***	-	-
LSD	-	1.0871	1.883	2.662	5.128	2.174	39.347	-	-

Means followed by the same letter in the same column have no significant differences'

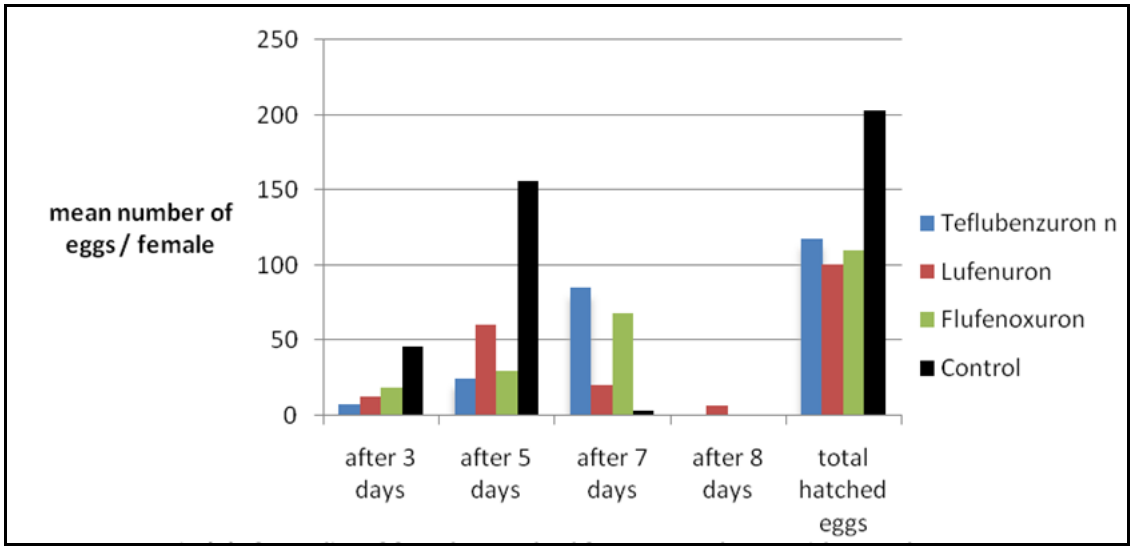

Fig.2: Fecundity of females resulted from treated eggd with tested IGRs

Effect of Tested IGRs on Some Biochemical Parameters of Larvae Resulted from Treaded Eggs:

The present data in the Table (5) indicated that tested IGRs caused high decreased in the level of total protein, Free-amino acid, Phenoloxidase, N- acytel-glucceamine in larvae resulted from eggs treated with Teflubenzuron, Lufenuron, and Flufenoxuron, respectively, compared with control, but high increased occurred in Chitinase activity.

Table 5: Changes in some biochemical parameters in larvae resulted from eggs treated with Teflubenzuron, Lufenuron, and Flufenoxuron.

Biochemical aspects	Teflubenzuron		Lufenuron		Flufenoxuron		Control	n	F value	P value	LSD
	Treatment	decrease (-)or increased (+)%	Treatment	Decrease (-)or increased (+)%	Treatment	Decrease (-)or increased (+)%					
Total protein (mg/g.b.wt)	15.0±1.10 b	-27.88	12.3±0.5 c	-40.87	16.7±0.21 b	-19.71	20.8±0.9 a	3	25.587	0.082 ***	2.296
Free-amino acid (µg D,L-alanine/g.b.wt)	265.0±4.1 c	-43.01	233.0±11.2 d	-50.54	302.0±16.25 b	-35.05	465.0±4.9 a	3	784.344	0.000 ***	11.995
Phenoloxidase (O.D. units/g.b.wt)	13. 0±0.28 c	-43.48	18.0±2.3 b	-21.74	16.9±0.5 b	-26.52	23.0±1.2 a	3	23.415	0.0003 ***	2.769
N- acytel-glucceamine (µg NAGA /g.b.wt)	105.3±6.4 c	-57.19	97.6±3.4 c	-60.33	124.3±5.7 b	-49.47	246.0±3.5 a	3	442.229 7	0.000 ***	10.767
Chitinase (µg NAGA x10^3/min/g.b.w)	467.0±7.44 ab	+68.59	369.0±10. 20 ab	+33.21	644.0±11.34 a	+132.49	277.0±4.9 b	3	2.403	0.143 ns	218

Means followed by the same letter in the same row have no significant differences.

The obtained results indicated a decrease in the level of total protein 15.0, 12.3, and 16.7(mg/g. b.wt) respectively, compared to 20.8 (mg/g. b.wt) in control. Also, in free amino acid high reduction occurred, the mean values were 265.0, 230.0, and 302.0 (mg/g. b.wt) in larvae resulted from eggs treated with Teflubenzuron, Lufenuron, and Flufenoxuron, respectively, compared with control 465.0 (mg/g. b.wt). The same effect occurred with Phynoloxidase and N- acytel- glucceamine which is necessary for chitin formation, for all treatments. The mean values For Phynoloxidase activity were 13.0 , 18.0 , 16.9 and 23.0 (O.D.units/g.b.wt) , for Teflubenzuron and Lufenuron, Flufenoxuron and control, respectively .For N- acytel- glucceamine the mean values were (µg 105.3 ,

97.6, 124.3, and 264.0 (µg NAGA /g.b.wt) for Teflubenzuron Lufenuron, Flufenoxuron, and control, respectively. In addition, the chitinase activity of *P. gossypiella* resulted from eggs treated with three IGRs Teflubenzuron, Lufenuron and Flufenoxuron,caused highly increased to 4567.0, 369.0 and 466.0 (µg NAGA x103/min/g.b.wt / larvae), respectively, compared to (277.0 µg NAGA x103/min/g.b.wt / larvae) control (El-Sheikh et al., 2013; Said et al., 2017; El-Naggar, 2013; Bakr et al., 2013and Tanani et al., 2015).

Conclusion

Based on the recoded results, Teflubenzuron, Lufenuron, and Flufenoxuron caused long-term inhibition effects on larvae, pupae and the adult longevity and reproductive capacity of the pink bollworm *P. gossypiella,* in addition to, tested IGRs caused a defect in molting process and also caused malformations at different stages of the tested insect .Tested compounds maybe can be involved in the integrated control program to reduce the population of this pest in the fields. Also, these compounds need future studies to show more physiological effects on the insect

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