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Evaluation of Two Insect Growth Regulator Activities on Egg and Larvae of *Earias insulana* (Boisd.) (Lepidoptera: Noctuidae)

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ABSTRACT

Under the laboratory conditions, toxicological evaluation of the ovicidal and larvicidal activities of two IGRs compounds; Triflumuron and Hexaflumuron against eggs or 1st, 2nd, 3rd and 4th instars larvae of *Earias insulana* (Boisd.). Also, some biological effects of these compounds on larvae, pupae treated as eggs were studied. In addition, some biochemical assays for protein, lipid, N- acytel-glucceamine and chitinase activity of *E. insulana* larvae were estimated

The results showed that Hexaflumuron was the most toxic IGR than triflumuron against eggs and larval stages of *E. insulana.* Eggs (1-3 days old) treated with Triflumuron had LC_{25} and LC_{50s} values (9.42 and 41.6 ppm). In contrast, It's had appeared a high susceptibility with Hexaflumuron, the LC_{25} value decreased to 7.3 ppm, while LC_{50} values had a highly decreased to 19.7 ppm. While treated the first and second instars larvae of *E. insulana* had highly susceptible to Triflumuron and Hexaflumuron compounds than 3rd and 4th instar larvae. All stages of *E. insulana* had less susceptible to Triflumuron than Hexaflumuron treatments.

The obtained results cleared that increase in times required for larval and pupal developmental stages treated as eggs (1-3 days old) of *E. insulana* treatment with Triflumuron and Hexaflumuron.

E. insulana larvae treatment with Triflumuron and Hexaflumuron had a highly reduction in the total soluble protein contents to 21.99 and 16.9 (mg/g.b.wt), respectively, compared with the control (32.5 mg/g.b.wt) with high decrease in the total lipid content that estimated by 20.6 and 14.6 (mg./ g.b.wt), respectively compared to 27.3 (mg./g. b.wt) in control. On the other hand, both of compounds occurred reduction in N- acytel - glucoseamine to 109 and 99 (µg NAGA /g. b.wt) compared to 171.5 (µg NAGA /g. b.wt) in control that led to increase the chitinase activity to 623.43 and 857.3 (µg NAGA x103/min/g.b.wt /larvae) compared to 560.2 (µg NAGA x103/min/g.b.wt / larvae) in control.

INTRODUCTION

Cotton considerable one of the major economic crops in Egypt, throughout the cotton growing season, attacking by different dangerous insect pests. The spiny bollworm (SBW), *Earias insulana* (Lepidoptera: Noctuidae) one of the most dangerous pests attacking

many crops especially cotton in various parts of Egypt. Different instar larvae of *E. insulana* feed on fruiting parts of the cotton plant especially the terminal buds (Khan, *et al.,* 2007). The eggs and different instar larvae of *E. insulana* were exposed to many insecticides during controlling some pests throughout the cotton growing season. The use of different pesticides against all bollworms was studied by many authors from these compounds, the chitin synthesis inhibitor (CSI) or Insect grows inhibitor (IGR) groups. It was studied against all bollworms by many authors (especially IGR) in the field or laboratory (El- Khayat, *et al.*, 2015 and Abbas, *et al.*, 2017 and Said, *et al.*, 2017). In addition, most IGR is considerably widely used in the cotton crop until now because it was a potent compound against the larvae develop until molting or fail to ecdyse different stages the arthropod (especially Lepidoptera). (El-Barkey *et al.,* 2009); for instance, when experimenting with many IGRs to some lepidopterous (*Pectinophora gossepeilla E. insulana,* and *Spodoptera littoralis* (Boisduval) eggs or larvae. it recorded a high increased the mortality and caused an increase in the time duration of larval and pupal stages for *P. gossepeilla,* or *E. insulana* (Kandil, *et al*., 2013; Tanani and Bakr 2018).

The objective of the present study was to investigate the effect of two compounds (Triflumuron and Hexaflumuron) on spiny bollworm eggs or larval stages. Also, the effect of these compounds on biological characteristics of *E. insulana* treated as eggs was observed. In addition, biochemical analysis for treated larvae with the two compounds was investigated.

MATERIALS AND METHODS

Insects:

Spiny Bollworm (SBW), *Earias insulana* (Boisd.):

The culture of *E. insulana*, eggs, and larvae used in the current experiment was obtained at Bollworms Research Department, Plant Protection Research Institute, reared on an artificial diet that was described by Amer (2015) for several generations away from any contamination with insecticides.

Compounds:

Triflumuron (Alsystin 48% SC), Benzoylphenylurea The field rate is 100 ml/feddan

Hexaflumuron (Camuoron 10% EC.), Benzoylphenylurea The field rate is 200 ml/ feddan

Procedure:

For evaluation of the ovicidal and larvicidal activity of Triflumuron and Hexaflumuron against eggs and 1st, 2nd, 3rd and 4th instar larvae of *E. insulana*, Serial concentrations of the two compounds in water were prepared. Six concentrations (50, 25, 12.5, 6.2, 3.1 and 1.5 ppm) for Flufenoxuron and six concentrations (25, 12.5, 6.25, 3.12, 1.56 and 0.78 ppm) for Hexaflumuron were freshly prepared for the stock solution of each compound (1ml/1 liter water).

Toxicity of the Two Compounds on *E. insulana* Eggs:

The dipping technique was used against eggs of *E. insulana* for 10 seconds. Three replicates from eggs age 1-3 days /concentration⁄compound. Each replicate contained 60 eggs (1-3 days old), deposited on a piece of paper. 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 25±2°C and 65±5 % R.H. until hatching. Percentages of hatchability and the time required to hatch (incubation eggs) were recorded for LC_{25} and LC_{50} estimation of the two compounds.

Toxicity of the Two Compounds on *E. insulana* Larvae:

For evaluation the toxicity of two tested compounds against different instars (1^{st}, 2^{nd} and 3^{rd} and 4^{th}) larvae of *E. insulana*, the serial number concentrations for each tested compound were sprayed on the surface of an artificial diet in Petri dishes. Three replicates were used, each replicates 20 larvae of the SPW for different instars' (1^{st} or 2^{nd} or 3^{rd} or 4^{th}) were allowed to feed on the treated diet for each compound and kept under constant conditions of 25 ± 1°C and 60±5 %RH. After 3-5 days from treatment with Triflumuron and Hexaflumuron, the dead larvae were counted to represent acute toxicity of the two tested compounds.

Toxicity was conducted to estimate LC_{25} and LC_{50} values by Probit analysis (proban software) according to Finney (1971).

Biological Aspects:

Estimated the LC_{25} value for each compound (sub-lethal dose) was prepared and dipping pieces of paper containing *E. insulana* eggs in the LC_{25} for each compound and kept under the constant conditions of 25±1°C and 60±5 %RH until hatching. The alive larvae hatched from each treatment eggs were transferred individually to the diet tubes (2x7.5 cm) each containing about 3 gm of artificial diet by camel hairbrush and another group used as a control. The tubes were capped with cotton and kept in an incubator under 25±1°C and 60±5 %RH and inspected daily until pupation.

The eggs hatchability and non-hatchability, incubation period, duration of larvae, pupal and total immature stages treated as eggs were recorded for each stage.

Biochemical Analyses:

Samples Preparation for Biochemical Assay:

Samples of *E. insulana* larvae resulted from eggs treated by LC_{25} of Triflumuron and Hexaflumuron tested were collected after 14 days from different treatments to study the latent effects of tested compounds in full-grown larvae were centrifuged at 5000 r.p. min. at $5C^{o}$ in a refrigerated centrifuge. The supernatants' were kept in a deep freezer at $-20C^{o}$ until use for biochemical assays. Samples of *E. insulana* larvae were analyzes chemically for treated and untreated in the Physiological Research Department of plant Protection Researches Institute, (P.P.R.I.).

Analyses Technique:

The colorimetric determination of total soluble protein and total soluble lipid, in total homogenate full-grown *E. insulana* larvae as described by Bradford (1976) and of chitinase activity was prepared according to Bade and Stinson (1981).

Statistical Analysis:

Biological and biochemistry data were statistically analyzed by ANOVA test at 0.05 of probability using Costat program (version 11) and range test of means used Duncan's multiple (Duncan, 1955) in the same program.

RESULTS AND DISCUSSION

Egg Treatments:

Toxicological Effect:

The susceptibility of Triflumuron and Hexaflumuron against *E. insulana* (1-3 days old) eggs under laboratory conditions was showed in Table (1). The LC_{25}'s values for 1-3 days old eggs were different for both Triflumuron and Hexaflumuron. The LC_{25} and LC_{50}s values were 9.42 and 41.6 for Triflumuron. It had appeared the highly variable values, the LC_{25} value decreased to 7.3; while, LC_{50} values had the highly decreased to 19.7 ppm for Hexaflumuron. This data revealed that *E. insulana* eggs had a highly susceptible to

Hexaflumuron than Triflumuron compound. The LC_{25} and LC_{50} values for Triflumuron were much less effective on eggs than Hexaflumuron.

Table 1: Toxicological evaluation of Triflumuron and Hexaflumuron against *E. insulana* (1-3 days old) eggs under laboratory conditions

Compounds used	Toxicity at 95% Confidence limits			
	LC_{25} (ppm)	LC_{50} (ppm)	LC_{90} (ppm)	Slope ±SD
Triflumuron	9.24	41.6	198.8	1.982 ±.0.43
Hexaflumuron	7.3	19.7	173.4	1.635±0.16

Hatchability and Incubation Eggs:

Date summarized in Table (2) that the effect of Triflumuron and Hexaflumuron on percent hatchability and incubation period of (1-3 days old) *E. insulana* eggs. It was obvious that the two compounds at the level of LC_{25} reduced the percentages of hatchability with 51and 49% for 1-3days old eggs, respectively compared with 96% in control. It can be concluded that the effect of Triflumuron and Hexaflumuron due to the delayed development or mortality of the embryonic inside the eggs. In addition, Table (2) showed that the incubation period of *E. insulana* eggs was high significantly affected by both compounds tested. The times required for egg hatchability were estimated at 4.6 and 5.1 days when eggs were treated with Triflumuron and Hexaflumuron compounds, respectively compared with 3.4 days for control.

Table 2: Effect of Triflumuron and Hexaflumuron (LC_{25}'s) on hatchability and incubation times of *E. insulana* (1-3 days old) eggs under laboratory conditions

Compounds used	1-3 days old eggs				
	LC_{25}	% Hatchability	% Reduction in hatchability	Incubation period ±SE	Rang in days
Triflumuron	9.24	51.0	49.0b	4.6±0.3 b	4-6
Hexaflumuron	7.3	49.0	51.0c	5.1±0.6 c	4-7
Control	-	96.0	4.0a	3.4±0.4a	3-4
L.S.D$_{0.05}$	---	6.713	1.387	0.254	--
P value		***	**	**	--

Larval Stage:

Data in Table (3) illustrated that the two tested compounds, Triflumuron and Hexaflumuron caused a high significant prolongation in *E. insulana* larval duration treated as eggs compared to untreated. The larval duration was 17.9 and 20.42 days treated as eggs with Triflumuron and Hexaflumuron, respectively.

Pupal Stage:

The obtained data in Table (3) indicated that the pupal stage of *E. insulana* treated as eggs with (IGR) Triflumuron caused less effect on the pupal duration than that Hexaflumuron treatment. The pupal durations were 9.76 and 11.3 days treated as Triflumuron and Hexaflumuron, respectively, compared with 7.6 days in control.

Total Immature Stage:

Present data in Table (3) indicated that two tested IGRs caused a high increase in the total immature stage of *E. insulana* treated as eggs with LC_{25} of Triflumuron and

Hexaflumuron compounds. It caused a high significant prolongation in duration to 27.66 and 31.7 days, respectively compared with 22.9 days in control.

Table 3: Effect of Triflumuron and Hexaflumuron (LC_{25}'s) on *E. insulana* larval and pupal stages treated as eggs (1-3 days old) under laboratory conditions

Treatments used	1-3 days old eggs				
	LC_{25}	Duration in days ±SE		Total immature stage	Life cycle
		Larvae	Pupae		
Triflumuron	9.24	17.90±1.6b	9.76±0.5 b	27.66±2.3b	32.26±2.4b
Hexaflumuron	7.3	20.4±1.3c	11.3±1.1c	31.70±1.9c	36.8±2.3c
Control	-	15.3±0.7a	7.6±0.3a	22.90±1.2a	26.3±1.3a
L.S.D$_{0.05}$	---	1.451	0.068	1.0558	1.571
P value	***	**	**	--	

Larval Treatments:

Toxicological Evaluation of Triflumuron and Hexaflumuron against *E. insulana* Larvae*:*

Present results in Table (4) showed that LC_{25} and LC_{50} values for *E. insulana* different instars larvae (1st, 2nd, 3rd & 4th) when treated with Triflumuron and Hexaflumuron compounds were 8.6, 26.8, 57.62 and 83.65 ppm, respectively when treated with Triflumuron and 5.9, 23.7, 54.38 and 79.7 ppm, respectively when treated with Hexaflumuron compound. In addition, Table (4) cleared that LC_{50}'s values for 1st, 2nd were nearly similar for both compounds with LC_{50}'s 13.0 and 39.0 ppm for Triflumuron and 11.64 and 36.4 ppm for Hexaflumuron. In contrast, there was more variation with LC_{50} values for 3rd and 4th instars larvae when treated with Triflumuron than Hexaflumuron. It was estimated at 103 and 170 ppm, respectively, with Triflumuron and 88.0 and 154 ppm, respectively with Hexaflumuron compound (Table 4). Data revealed that first and second instar larvae had highly susceptible to Triflumuron and Hexaflumuron compounds than 3rd and 4th instar larvae. All stages of *E. insulana* had less susceptible to Triflumuron than Hexaflumuron treatments.

Table (4). Toxicological of Triflumuron and Hexaflumuron against *E. insulana* larval stage under laboratory conditions

Compounds used	95%Confidence limits	Different instar			
		1st	2nd	3rd	4th
Triflumuron	**LC_{25} (ppm)**	8.60	26.8	67.62	83..65
	LC_{50} (ppm)	13.00	39.0	103.80	172.4
Slope ±SE		1.022 ±.0.13	1.124 ±.0.25	1.961 ±.0.73	2.982 ±.0.67
Hexaflumuron	**LC_{25} (ppm)**	5.90	23.7	54.38	79.7
	LC_{50} (ppm)	11.64	36.40	88.0	154
	Slope ±SE	1.37 ±.0.43	1.572 ±.0.62	1.924 ±.0.33	1.488 ±20.43

Biochemical Assays:

Data presented in Table (5) cleared that *E. insulana* larvae treated with Triflumuron and Hexaflumuron caused a highly significant decrease in the total soluble protein to 21.99 and 16.9 (mg/g.b.wt), respectively compared with 32.5 (mg/g.b.wt) in control. In addition, the same table showed a high decrease in the total lipid content to 20.6 and 14.6 (mg./g.b.wt) in treated larvae with Triflumuron and Hexaflumuron, respectively compared to 27.3 (mg./g.b.wt in control. However, this reduction in the total protein, lipid content that necessary for completed the development of larval and pupal stages caused a very small size with the death of larvae. Also, Data summarized in Table (5) recorded a higher reduction in N- acytel - glucceamine (µg NAGA /g. b.wt. It was estimated by 109.0 µg NAGA /g. b. wt) for Triflumuron treatment. While the high reduction recorded with Hexaflumuron treatment was 99.0 (µg NAGA /g.b. wt) compared to 171.5(µg NAGA /g.b.wt) in control. In addition, Data in Table (5) showed the percent of chitinase activity of *Erias insulana* larvae that treated as eggs with Triflumuron and Hexaflumuron increased to 623.43 and 857.3 (µg NAGA x103/min/g.b.wt/ larvae), chitinase activity percent compared with 560.2 (µg NAGA x103/min/g.b.wt / larvae) in control.

Generally, It can be concluded that eggs or different instar larvae of *E. insulana* had a highly significant affected on both compounds used. But, all the pest stages had highly susceptible when treated with Hexaflumuron than Triflumuron. The two treatments caused an increase in the incubation period and prolonged in larval and pupal stages. In addition, the biochemical analysis recorded the highest decrease in total protein and lipids with increased in chitinase activity may cause inhibition of the synthesis of the new cuticle specifically, chitin biosynthesis and so resulted in failure ecdysed or molt that may lead to death the different stages larvae during molting or non-completed the development. Effects on all parameters considerable are key factors in developing successful pest.

Table 5: Biochemical assays of *E. insulana* treated larvae with Triflumuron and Hexaflumuron**.**

Compounds used	Total protein	Total lipid	N- acytel-glucceamine	Chitinase
	(mg/g.b.wt)	(mg/g.b.wt)	(µg NAGA /g.b.wt)	
Triflumuron	21.99±1.51b	20..6±0.9b	109.37±10.8b	623.43±13.10b
Hexaflumuron	16.9±1.3a	14.6±0.7a	99.0±5.54a	857.3±12.9c
Control	32.5±1.5c	27.3±1.4c	171.5±14.6c	560.2±18.20a
L.S.D$_{0.05}$	3.611	2.447	4.802	9.573

The current study confirmed those previously obtained by Cabezon *et al.* (2006) found that lufenuron significantly reduced hatchability on all eggs age classes of *Lobesia botrana.* Kellouche and Soltani (2006) recorded that Hexaflumuron highly affected the growth and development of oocytes and egg viability for *Callosobruchus maculates*. Sammour, *et al.* (2008) showed the effect of Chlorfluazuron and Leufenuron on *S. littoralis*, the results indicated that all treatments increased the duration and decreased the hatchability. Kandil *et al*. (2013) found that increased in time required for both larval and pupal stages of *Pectinophora gossypiella* resulted from eggs treatment with LC_{50} concentration of Hexaflumuron and Chlorfluazuron. Said, *et al.* (2017) showed that Teflubenzuron with LC_{50} was prolonged the developmental period larval and pupal stages of *Pectinophora gossypiella* compared with the control. Also, a high reduction in total protein, lipid and chitin enzyme in full-grown larvae of *P. gossypiella* treated as 1st instar larvae with Teflubenzuron (IGR's) than control was found.

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ARABIC SUMMARY

تقييم نشاط إثنين من منظمات النمو الحشرية على بيض ويرقات دودة اللوز الشوكية

أيمن محمد محيى الدين عدلى
معهد بحوث وقاية النباتات – مركز البحوث الزراعية- الدقى – جيزة – مصر

تحت الظروف المعملية تم تقييم التأثير السمي لإثنين من منظمات النمو الحشرية (الترايفلوميرون - الهيكسافلوميرون) على البيض (عمر 1-3 يوم) واليرقات (من العمر الأول حتى الرابع) لدودة اللوز الشوكية *Earias insulana* (Boisd.). كما تم دراسة بعض التأثيرات البيولوجية لكلا المركبين على اليرقات والعذارى الناتجة من معاملة البيض إضافة إلى بعض القياسات الكيميائية الحيوية (البروتين- الليبيدات – الأسيتيل جلوكوزأمين- نشاط إنزيم الكيتينيز) فى يرقات دودة اللوز الشوكية.

أشارت النتائج أن مركب الهيكسافلوميرون أكثر سمية عن مركب الترايفلوميرون على بيض ويرقات دودة اللوز الشوكية. معاملة البيض (عمر 1-3 يوم) بمركب الترايفلوميرون سجلت قيم 9.42 و 41.6 جزء فى المليون لكلا من التركيز الربع والنصف مميت على التوالى. بينما ظهرت حساسية البيض لمركب الهيكسافلوميرون حيث إنخفضت قيم التركيز الربع والنصف مميت إلى 7.3 و 19.7 جزء فى المليون. بينما أظهرت معاملة العمر الأول والثانى ليرقات دودة اللوز الشوكية حساسية لمركبى الترايفلوميرون والهيكسافلوميرون عن العمر الثالث والرابع لليرقات. وعموما الأعمار اليرقية المعاملة أظهرت حساسية أقل لمعاملات الترايفلوميرون مقارنة بالهيكسافلوميرون.

أوضحت النتائج زيادة فى عمر اليرقات والعذارى المعاملة فى طور البيض لكلا المركبين. فى القياسات الكيميائية الحيوية حدث إنخفاض بشكل ملحوظ لمحتوى بروتين يرقات دودة اللوز الشوكية إلى 21.99 و 16.9 ملجم ومحتوى الليبيد إلى 20.6 و 14.6 ملجم للمعاملة بمركبى الترايفلوميرون والهيكسافلوميرون على التوالى مقارنة بالكونترول (32.5 ملجم للبروتين و 27.3 لليبيد). تسببت أيضا المعاملة بالمركبين (ترايفلوميرون – هيكسافلوميرون) خفض فى الأسيتيل جلوكوزأمين إلى 109 و 99 ملجم على التوالى مقارنة بالكونترول (171.5 ملجم) والذى بدوره أدى إلى زيادة نشاط إنزيم الكيتينيز إلى 623.43 و 857.3 ملجم/ يرقة المعاملة بالمركبين على التوالى مقارنة بالكونترول (560.2 ملجم/اليرقة).