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Toxicological and biochemical studies of lufenuron, chlorfluazuron and chromafenozide against *Pectinophora gossypiella* (Saunders)

Mervat A. kandil; A.F. Ahmed and Hemat Z. Moustafa
Plant protection research inistitute, A.R.C., Dokki-Giza, Egypt

ABSTRACT

In the present study, one day old eggs laboratory colony of *Pectinophora gossypiella*, were treated with estimated LC_{50} values of lufenuron 5%, chlorfluazuron 5% and chromafenozide 80% (3.471, 4.189 and 122.703 ppm 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 49.6, 51.0 and 53.0 for the three tested compounds, respectively oppose to 97% in untreated control. The incubation period of the hatched eggs was 6.5, 4.7, 6.2 and 3.2 days at three (IGR_S) and control, respectively. Also, the treatments affected the subsequent stages it caused significant effects on larval and pupal duration, weight and malformations. On the other hand, the adult stage resulted from treated eggs was highly affected by the three (IGR_S). All the compounds especially chlorfluazuron 5% caused increasing in adult longevity and reduced the respective fecundity and fertility compared to control. The biochemical effects of the tested insect growth regulators as chitin synthesis inhibitor (CSI) against larvae resulted from (treated one-day old eggs) was studied. The obtained results indicated that the tested IGR_S reduced the glucose, protein and carbohydrate contents. Also, the tested IGR_S elicited inhibitory effect on alanine amino transferase (ALT) and aspirate amino transferase (AST).

Keywords: *Pectinophora gossypiella*, PBW, IGR_S, Toxicity, LC_{50}, Biological studies, Biochemical analysis.

INTRODUCTION

The pink bollworm, *Pectinophora gossypiella*, 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 stags of some order of insects. Chitin synthesis inhibitors are group of IGR_S that interfere with the formation of new cuticle, (Hoffmann and Lorenz 1998). Many authors recorded that the IGR_S play an important role in different developmental period in insect; it caused prolongation in larval& pupal period. In addition, latent effect appear on the longevity, fecundity & fertility of adult stage of Lepidopteron insects as *Heliothis armegera*, *Earias insulana*, *Pectinophora gossypiella* and *Spodoptera litturales*, Mossan *et al.* (1995), Kandil *et al.* 2005 and El Shennawy (2009). Otherwise, some authors studied the chitin synthesis of treated insects with IGR_S. They found that the chitin synthesized from glucose, glucosamine or (N- acetylglucosmine), protein and carbohydrate. This compound is the immediate precursor of chitin. They added that biochemical changes could be happen if the (N-acetylglucosmine) moiety is transferred directly to the growing chitin inhibitor (Candy& Kilby 1962; Mills *et al*., 1967 and Marks & Sowa 1976).

The present study was carried out to determine the toxicity of lufenuron, chlorfluazuron and chromafenozide on

one day old eggs of *P. gossypiella*. Subsequently, some biological aspects for immature and adult stages as well as biochemical studies for larvae resulted from treated eggs were directed.

MATERIALS AND METHODS

Insect used:

One day old eggs of pink bollworm *P. gossypiella*, used in this study was obtained from laboratory colony of Bollworm Department, Plant Protection Research Institute; Agriculture Research Center (ARC), reared for several generations away from any contamination with insecticides on an artificial diet that previously described by Rashad and Ammar (1985).

Pesticides used:

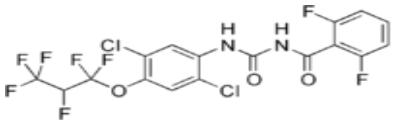
Three Insect growth regulators (IGR_S) were experimentally used in this study:

1-Common name: **lufenuron 5%:** a benzoylurea pesticide inhibits the production of chitin in larvae of lepidopterans.

Trade name: Match (5% E.C.)

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

Stucture formoula:

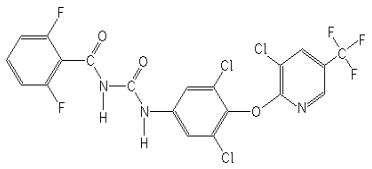


2-Common name: Chlorfluazuron 5%: a benzoyl phenyl urea insect growth regulator inhibiting the synthesis of chitin.

Trade name: Caprice, Atabron, Fertabron (Caprice 5% EC)

Chemical name: N-[[[3,5-dichloro-4-[[3-chloro-5-(trifluoromethyl) 2pyridinyl] oxy]phenyl] amino]carbonyl]-2,6-difluorobenzamide.

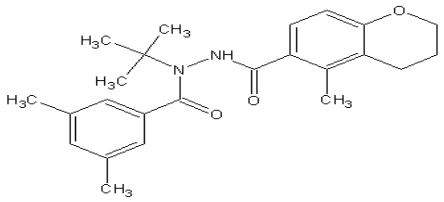
Structure formula:



3- Common name: Chromafenozide 80%: is a novel dibenzoylhydrazine and is categorized to be an insect hormone ecdysone (moulting hormone agonists)

Trade name: Virtu (80% WP)

Chemical name: 3, 4 dihidro-5-methyl-2H-1-benzopyran-6-Carboxylic acid 2-(3, 5-dimethylbenzoyl)-2-(1, 1-dimethyl) hydrozide



Toxicological studies:

The ovicidal activity of the three experimental compounds, lufenuron, chlorfluazuron & chromafenozide were tested against *P. gossypiella* eggs (one day old after deposition). Fresh serial concentrations from the stock solution of each compound (1ml/liter water) were prepared as follows: 6.25, 3.25, 1.61, 0.805 & 0.4025 ppm of lufenuron, chlorfluazuron and (0.5 g/liter water) were prepared as follows: (400, 200, 100, 50 & 25 ppm) of chromafenozide. Strips of muslin on which one day old eggs of pink bollworm had been deposited (100-150 egg/strip) were dipped for 10 seconds in each concentration of the three tested compounds.

Strips were left under room condition to allow evaporation of the excess water then dried strips of eggs were kept separately in glass tubes (9.3x1.5 cm) capped with cotton stopper and hold under constant condition of (26 ±1ºC and 75-80% R.H.) until hatching. Other three replicates of similar eggs numbers and age were dipped in water and left as control under the same conditions.

The percentage of hatchability was estimated. Data were corrected and LC_{25}, LC_{50} & LC_{90} of the three compounds were calculated by using Proban software.

Biological studies:

For some biological studies, newly hatched larvae of PBW resulted from treated one day old eggs with LC_{50} of lufenuron, chlorfluazuron and chromafenozide were transferred individually to glass tubes (2 x 7.5 cm) using camel hair brush, each tube containing 2 gm of artificial diet described by Rashad & Ammar (1985).

The same procedure was done with the newly hatched larvae resulted from untreated eggs used as control. The tubes were capped with cotton stopper and kept under the previous controlled conditions and inspected daily until pupation.

Larval & pupal durations & weights, adult emergence and sex ratio were determined. Newly emerged moths resulted of three compounds as well as the control were sexed and transferred to chimney glass cage (5 pairs/cage). Each treatment was replicated three times. The moths were fed on 20% sucrose solution. Cages were inspected daily to estimate the oviposition period, numbers of eggs laid, % hatchability and the longevity of adult females and males for each.

The recorded 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).

Biochemical analysis:

For the biochemical studies, 50 newly hatched larvae resulted from treated one day old eggs/compound and untreated ones were transferred individually to glass tubes (2 X 7.5 cm) containing 2 gm of untreated diet and kept under the previous controlled conditions. Twelve days later larvae/each treatment and untreated control were collected and kept in clean tubes in a refrigerator (7±1˚C) for chemical analysis.

Determination of enzyme activity:

Total protein, total lipids, glucose and amylase, glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) and chitin enzyme activities were determined colorimetrically according to Koller (1984), Drevon and Schmitt (1964), Henry and Chiamori (1960), Trinder (1969) and Murray (1984).

RESULTS AND DISCUSSION

Toxicity of three IGR compounds on *P. gossypiella* eggs (one day old):

Data in Table (1) show the LC_{25}, LC_{50} and LC_{90} values resulted from (one day old eggs) of *P. gossypiella* treated with the lufenuron, chlorfluazuron and chromafenozide. The LC_{50} values were 3.471, 4.179 and 122.703 ppm, respectively. According to LC_{25}, LC_{50} and LC_{90} values Caprice is considered the highest potent than Match and Virtu. Horowitz *et al.* (1992) recorded that the LC_{50} of hexaflumuron (0.420 ppm) was the highest in efficacy against eggs 24-48 hours age of *E. insulana* than other tested IGR_S.

Table 1: Toxicity of three IGRs compounds on *P. gossypiella* eggs (1day old)

Compound	Conc.	Conc. (ppm)	95% confidence limits		Slope
			Lower	Upper	
lufenuron (Match)	LC_{25}	1.864	0.558	2.631	2.891
	LC_{50}	4.179	1.873	11.257	
	LC_{90}	756.18	19.3398	1403.1601	
chlorfluozuron (Capris)	LC_{25}	0.392	0.101	0.745	3.144
	LC_{50}	3.417	2.275	5.65	
	LC_{90}	671.737	134.322	2656.829	
chromafenozide (Virto)	LC_{25}	30.603	19.145	43.113	1.118
	LC_{50}	122.703	93.131	162.823	
	LC_{90}	3627.56	1877.015	9776.817	

Also, Saenz de Cabezon *et al*. (2006) found that the chitin synthesis inhibitor lufenuron was highly active against *Lobesia botrana* eggs with greater effect on 1-day old eggs than on the other ages. In contrast, El- Shennawy (2009) recorded that LC_{25}, LC_{50} and LC_{90} for lufenuron were (0.6086, 2.276 and 16.07ppm) when treated 4-day old eggs of *P. gossypilla*.

Eggs hatchability and incubation period of *P.gossypiella* eggs treated with insect growth regultors (IGR_S):

Data presented in Table (2) summarize the efficacy of lufenuron, chlorfluazuron and chromafenazide on percentage of hatchability and incubation period of *P. gossypiella* eggs. It is clearly obvious that the LC_{50} of the tested compounds caused high reduction in the percent of hatchability to reach 49.0, 51.0 and 53.0 %, respectively compared with 97.0 % in control. The present results agree with Abdel- Megeed *et al.* (2009) who found that the newly laid eggs proved to be more sensitive than older ones when they studied the activity of two nonsteriodal ecdysone agonists against the cotton leafworm, *S. littoralis* (Boisd).

On the other hand, LC_{50} treatment of lufenuron, chlorfluazuron and chromafenozide prolonged the incubation period of PBW eggs significantly than control (Table 2). These incubation periods were 6.5, 4.7, and 6.2 days, respectively compared with 3.2 days in control. The present results indicated that the incubation period of *P. gossypiella* eggs when treated by lufenuron and chromafenozide nearly require 2 times long with low hatchability % than control.

Biological activity:

Table (2) shows the duration, weight of larvae & pupae, and total immature stage, for newly hatched larvae resulted from one day old eggs treated with LC_{50} value of lufenuron, chlorfluazuron and chromafenozide. Normal stages of PBW are shown in Figs. (1.1: 1.3), while Figs. (1.4: 1.17) show malformed larvae, pupae & adults resulted from eggs treated with lufenuron, chlorfluazuron and chromafenozide, respectively.

Table 2: Ovicidal activity of tested compounds on egg masses of *Spodoptera littoralis.*

compounds	Conc. ppm	Egg hatch-ability	% 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)	Pupal period (days) (Mean ±SE)	Weight (g)		
Lufenuron	4.179	49.6	19.7	6.5^{b} ± 0.4	19.1^{b}± 2.07	0.030^{b}± 0.003	10.5^{b}± 0.62	0.0238^{a}± 0.005	29.6^{a}± 2.36	35.13^{a}± 3.04
Chlofluazron	3.41	51.0	22.3	4.7^{a}± 1.14	23.4^{c}± 1.71	0.025^{b}± 0.005	12.06^{a}± 0.4	0.0020^{a}± 0.001	35.46^{a}± 1.877	39.7^{a}± 1.59
Chroma-fonazide	122.7	53.0	29.0	6.2^{b}± 0.55	21.14^{ab}± 1.69	0.025^{b}± 0.005	10.3^{b}± 0.61	0.0138^{a}± 0.003	31.73^{b}± 2.27	37.3^{a}
Control		97.0		3.2^{a}± 0.8	14.6^{a}± 0.41	0.039^{a}± 0.002	8.07^{c}± 0.75	0.0314^{b}± 0.002	23.3± 1.4	27.1^{b}± 2.19
LSD				1.4235	2.66	0.009	1.4016	0.0115	3.534	3.5375
P				0.0012**	0.003***	0.017*	0.0013	ns	0.003***	0.005***

Means followed by the same letters are not differ significantly

* Percentage of died larvae that developed inside the eggs and failed in emergence.

Larval and Pupul stages:

Data in Table (2) illustrates the LC_{50} latent effect of lufenuron, chlorfluazuron and chromafenozide on PBW larval & pupal period and weigh resulted from treated eggs compared with untreated eggs. The three tested compounds prolonged the duration of

larval stage, significantly. These periods estimated by 19.1, 23.4 and 21.14 days/larvae oppose to 14.6 days in control. Also, the used IGRs caused high significant increase in pupal period, the presented duration were 10.5, 12.06 and 10.3 days/ pupa, respectively, compared to 8.07 days in control .The total immature stage of PBW resulted from treated eggs highly elongated to 29.6, 35.46 and 31.73 days for lufenuron, chlorfluazuron and chromafonuzide, respectively, compared with 23.3 days in control (Table 2).

In addition, the average larval weight decreased significantly to reach 0.030, 0.025 and 0.025 g/larva for three IGRs, respectively, while it was 0.039 g/larva in control. Also, the pupal weight resulted from treated eggs decreased significantly in all treatments than control. Chlorfluazuron caused significant effect on pupal period and weight than the other two compounds (Table 2). Yasir *et al*., (2012) found that Lufenuron caused significant effects on larval mortality, larval weight and larval duration of *Tribolium castaneum.*

Adult stage:

Data in Table (3) show that the three tested compounds elongate significantly the pre-oviposit period of emerged females from one day old eggs treated by lufenuron, chlorfluazuron and chromafenozide to reach 3.97, 3.77 and 4.17 days, respectively, compared with 2.9 in control. In contrast, the oviposition period of emerged females from lufenuron and chromafenozide treatments was shortened to be 10.67 and 13.33 days, while no significant increase was recorded in treatment of chlorfluazuron (19.6 days) compared to 14.0 day in control.

Table 3: Effect of lufenuron chlorfluazuron and chromfenozide on longevity and fecundity of PBW females resulted from treated eggs.

Compounds	Conc ppm	Oviposition period(days ±ES)			Fecundity		Longevity	
		Pre-oviposition	oviposition	Post-oviposition	Total eggs/♀	% hatch-ability	♀	♂
Lufenuron	4.179	3.97^{b}±1.01	10.67^{b}± 3.2	10.27^{a}±2.38	106^{b}±24.1	67.3^{b}	25.97^{a}±6.6	18.1^{a}±4.58
chlorfluazuron	3.417	3.77^{a}±0.56	19.6^{a}±0.8	6.03^{b}±1.01	123.3^{b}±9.02	66^{b}	29.37±2.04	20.5^{a}±1.44
Chrom-fenozide	122.703	4.17^{c}± 0.38	13.33^{b}±1.5	6.13^{b}±1.7	142.7^{b}±4.3	70.7^{ab}	23.63^{b}±0.77	18.96±0.85
Control	-	2.9^{a}±0.18	14.0^{b}±0.59	2.67^{c}±0.34	214.1^{a}±5.52	90.67^{a}	19.0^{a}±0.58	15.2^{b}±0.53
LSD		1.040	5.526	2.554	49.458	21.957	7.567	2.477
P		0.011***	0.08***	0.008**	0.0048***	0.1004	0.264	0.0064

Also, data in Table (3) clear that the tested compounds elongated the post-oviposition period of *P. gossypiella* significantly from 2.67 days in control to 6.03, 6.13 &10.27 days/female at chlorfluazuron chromafenozide and lufenuron treatments, respectively. The results indicated that the three IGRs caused elongation in post-oviposition period from 2 to 5 times more than control.

Female's longevity was highly significant affected by lufenuron, chlorfluazuron and chromafenozide, the adult females longevity were 25.97, 29.37 and 23.63 days/♀, respectively, compared to 19.0 days/ female in control. Female longevity increased in treatments mostly due to the increase in post oviposition period. Also, the males longevity resulted from PBW treated eggs were longer than the control. The recorded means were 18.1, 20.5 and 18.96 days/♂ resulted from lufenuron, chlorfluazuron and chromafenozide,

respectively compared with 15.2 days/ ♂ (Table 3).

Reproductive potential:

Data presented in (Table 3) show high reduction in numbers of eggs laid by females from 33.64% in chromafenozide to 50.47% in lufenuron treatment. The main numbers of laid eggs value for lufenuron, chlorfluazuron and chromafenozide was 106, 123.3 and 142.7 egg/female, respectively, compared with 214.1 eggs/ female in control. As shown in table (3) the percentage of eggs hatchability were 67.3, 66.0 and 70.7 % on lufenuron, chlorfluazuron and chromafenozide, respectively, compared with 90.67 % in control.

Generally, treating one day old eggs of PBW by three compounds related to chitin synthesis inhibitor and /or moulting hormone agonists reflected high effects on immature stage and the adult stage and reduced fecundity and hatchability in comparison with control. Abdel-Aal (2006) reported that fecundity and egg- hatchability percent of treated cotton leaf worm *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 (2006) showed that lufenuron has ovicidal activity on *L. botrana* in contact treatment. Oouchi (2005) & El-Barkey *et al.* (2009) stated that IGR_S had ovicidal effects on *P. gossypiella.* Yasir *et al.,* (2012) recorded that the fecundity and egg hatchability were reduced at all concentrations of Lufenuron used against *T. castaneum* larvae.

Biochemical analysis:

Larvae resulted from one day eggs treated with lufenuron, chlorfluazuron and chromafenozide, as well as in control were chemically analyzed and results were as follows:

Total protein:

Data in Table (4) reveal that the three tested compounds caused high reduction in soluble protein in larvae resulted from eggs treated.The total soluble protein were 5.13, 4.18 and 4.89 mg/ml on Match, caprice and virtu, respectively, compared with 11.9 mg/ml in control.

The present result is in agreement with Assar *et al.* (2010) who found that the total protein content and total concentration of amino acids decreased in the house fly treated with match and consult. Also, Ghoneim *et al*. (2012) found that proteins in treated *Schistocerca gregaria* by insect growth regulators (IGR_S) were generally exhibited.

Table 4: Chemical composition of *P. gossypiella* larvae resulted from (one day old eggs) treated with LC_{50} of three IGR_S under controlled conditions.

Treatments	LC_{50} (ppm)	Total Protein mg/ml	Total lipid mg/ml	Glucose mg/ml	Enzymes			
					Chitinase activity (Mg.NAGA/ min/wg bwt) means±SE	Amylase mg/ml	Transaminase enzyme IU/L	
							GOT	GPT
Lufenuron (Match)	4.179	5.13±0.115	10.57±0.043	17.83	6.4±0.1	13.55	47	72
Chlorfluozuron (Capris)	3.417	4.18±0.18	11.34±0.0035	16.55	5.9±0.36	12.72	7	8
Chromafonozide (Virto)	122.703	4.89±0.32	12.37±0.02	12.11	7.8±0.13	28.33	16	17
Control	----	11.9±1.6	15.6±023	27.96	8.6±0.16	25.28	18	34

Total lipid:

Data in table (4) indicated that tested IGRs caused high lipid reduction from 20.68 to 32.24% in larvae resulted from treated one day old eggs of PBW by lufenuron, chlorfluazuron and chromafenozide. Total lipid content were 10.57, 11.34 and 12.37 mg/ml, respectively compared to 15.6 mg/ml in control. Hamadah *et al.* (2012) found a predominant inhibitory in lipid content of *S. gregaria* nymphs that treated with pyriproxyfen, tebufenozide or lufenuron.

Glucose:

Data in Table (4) show that glucose activity greatly reduced on larvae of *P. gossypiella* resulted from treating one day old eggs with lufenuron, chlorfluozuron and chromafenozide. The levels of activity were 17.83 and 16.55 and 12.11 mg/ml. comparing to 27.96 mg/ml in control. The present results agree with those of Assar *et al.* (2012) who found that treating the 4th instar larvae of *Culex pipiens* with (cyromazine) chitin synthesis inhibitor (CSI) caused high decrease in glucose quantity.

Chitin activity:

Data in table (4) recorded that chitinase activity of the PBW larvae resulted from treated one- day eggs were decreased relatively to control. It recorded 6.4, 5.9 and 7.8 units (µg N-acetylglucoseamine liberated x 103/min/g.b.wt.) for lufenuron, chlorfluezaron and chromafonozide, respectively, compared with 8.6 units in control. In contrast, Al-Shannaf *et al*. (2012) found that insect growth regulators (chlorfluazuron and pyriproxyfen) caused highly significant increases in the activity of chitinase enzyme (130 % times in larvae of American bollworm, *H. armigera*).

Carbohydrate hydrolyzing enzymes:

Amylase:

The results presented in Table (4) show that the amylase greatly reduced in PBW larvae resulted from one day old eggs treated by lufenuron and chlorfluazuron (53.60 and 50.32 %, respectively) while, the amylase activity increased to 112.06 % in chromafenozide compared to the untreated control. Al-Shannaf *et al.* (2012) recorded that chlorfluazuron give the lowest significant decrease in the activity of amylase enzyme (59.86 %) in treated larvae of American bollworm, *H. armigera* relative to control.

Transaminase enzymes (GOT and GPT or ALT and AST):

Data in Table (4) shows the transaminase enzymes activity on larvae of *P. gossypiella* resulted from one day old eggs treated with lufenuron, chlorfluozuron and chromafenozide. The levels of GOT and GPT were highly increased to 47 and 72 IU/L for one day old eggs treated by lufenuron compared with the control which were 18 and 34 IU/L. Greatly reduction in GOT and GPT activity of *P. gossypiella* treated with chlorfluozuron (7 and 8 IU/L) and (16 and 17 IU/L) in chromafenozide treatment compared with the control.

(Katumuma *et al*. (1968) recorded that the GPT enzyme acts as a catalytic factor in the metabolism of carbohydrate. Assar *et al*. (2010 & 2012) found that match induced inhibitory effect on the house fly, *Musca domestica* at 1000 ppm. Consult had no effect on the total activity of AST. With respect to the total ALT activity, match and consult elicited inhibitory effect on the total ALT activity.

In conclusion, the chemical changes explain the relationship between reduction in all larval enzymes and prolonged duration with less weight and production of deformed stages. The reduction in protein, lipid, carbohydrate especially glucose, amylase, GOT and GPT caused inhibition and/or reduced chitin contents in larvae as well as the reduction in reproductive potentiality of

PBW resulted from treating one day old eggs with IGRs.

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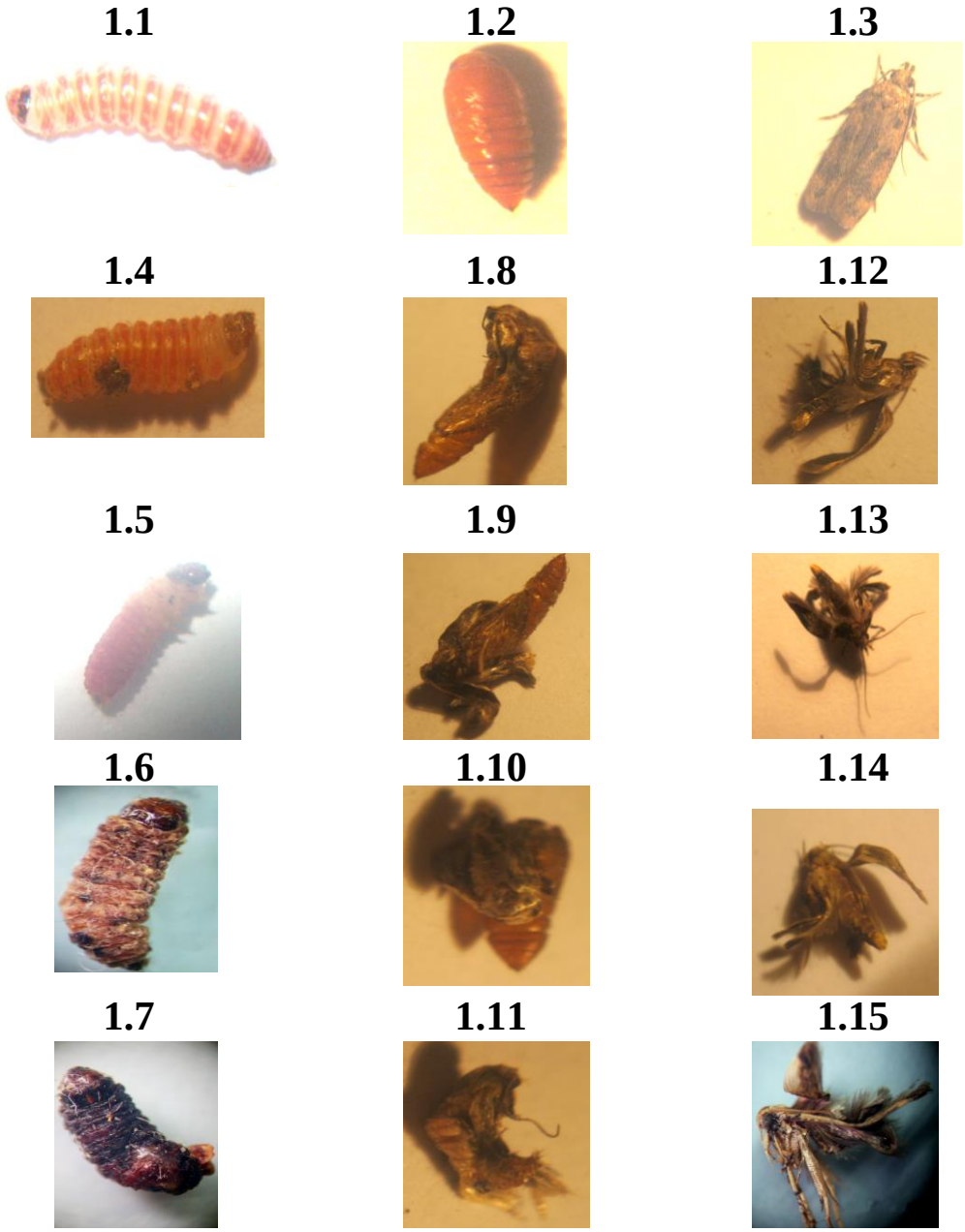

1.1, 1.2 and 1.3: Normal larvae, pupae and adult.
1.4: Larvae resulted from eggs treated with lufenuron.
1. 5: Larvae resulted from eggs treated with chlorfluazuron.
1.6 and 1.7: Larvae resulted from eggs treated with chromafenozide.
1.8, 1.9 and 1.10: Shape intermediate between pupae and adult resulted from eggs treated with lufenuron, chlorfluazuron and chromafenozide.
11: Adult resulted from eggs treated with lufenuron.
1.12, 1.13 and 1.14: Adult resulted from eggs treated with chlorfluazuron.
1. 15: Adult resulted from eggs treated with chromafenozide

Fig. 1: Morphological deformations of larvae, pupal – adult intermediate and adults resulted from one day old eggs of *P. gossypiella* treated with lufenuron, chlorfluazuron and chromafenozide in comparison to control.

ARABIC SUMMARY

دراسات تكسوكولوجية وبيوكيميائية لمركبات ليوفينورون، كلورفلوروزان وكرومافينوزيد لدودة اللوز القرنفلية

ميرفت عبد السميع قنديل ، أشرف فايز أحمد ، همت زكريا محمد مصطفي
معهد بحوث وقاية النباتات- مركز البحوث الزراعية – الدقى- جيزة.

اجريت هذه التجارب لدراسة تأثير ثلاث مركبات تنتمي لمجموعة منظمات النمو الحشرية ليوفينورون 5%، كلورفلوروزان 5% و كرومافينوزيد 80% ، وقد تم حساب الجرعة المميتة لنصف التعداد للبيض (عمر يوم) فكانت 3.471، 4.189 و 122.703 جزء في المليون. وتم متابعة الأطوار الناتجة من هذه المعاملات. وأوضحت النتائج أن نسبة الفقس المعامل كانت 49.6، 51.0 و 53.0 % للثلاث مركبات علي التوالي، مقارنة بقيمة 97 % للبيض غير معامل. وإستغرقت فترة حضانة البيض 6.5، 4.7 و 6.2 يوم للثلاث مركبات علي التوالي، في حين سجلت 3.2 يوم للمقارنة. وسببت جميع المعاملات تأثير معنوي علي إطالة العمر اليرقي والعذاري كما أدت إلي خفض الأوزان مع وجود تشوهات، ومن جهة أخري أثرت المعاملات خاصة الكلورفلوروزان 5% إلي زيادة عمر الحشرات الكاملة مع إنخفاض في الخصوبة ونسبة الفقس الناتج وأبرزت نتائج التقييم البيوكيميائي لليرقات الناتجة من معاملة بيض عمر يوم واحد للمركبات التي تم إختبارها كمثبطات تكوين الكيتين خفضت محتوي الجلوكوز، البروتين، إنزيمات الأميليز، الشيتين، ALT وAST.