RESEARCH | Egyptian Journal of Agricultural Research | Plant Protection

Effects of certain pesticides on the embryonic development of Pink Bollworm eggs and subsequent stages

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Received: 23-10-2023**; Accepted:** 24-01-2024 **; Published:** 09-03-2024 **DOI:** 10.21608/EJAR.2024.244278.1455

ABSTRACT

The pink bollworm, *Pectinophora gossypiella* (Saund.) is an important pest that causes severe economic loss for cotton crop. The potent bioactive properties of three tested compounds; the two IGRs compounds; Dimilin (Diflubenzuron) and Nomolt (Teflubenzuron), and the bio-pesticide compound Emacte (*Emamectin benzoate)*; were tested against two different ages of *P. gossypiella* eggs under laboratory conditions of 26±1°C and 70±5% RH. Results revealed that older eggs of *P. gossypiella* are more susceptible to pesticides than the younger ones where LC_{50} values were 155.4, 185.85, and 1.95 ppm. for 0–1-day-old eggs, while decreased to 81.42, 103.42, and 1.30 ppm, for 2-3 days-old eggs in Dimilin, Nomolt, and Emacte treatments, respectively. The incubation periods for eggs were prolonged as a result of the treatments, in comparison to the untreated check. In addition, the estimated developmental periods for the immature stages increased to 19.6, 17.9 and15.85 days for larva and 12.5, 11.8, and 9.76 days for pupa, in treatment of 0-1 day old eggs and to 20.2, 18.8, and 16.9 days for larva and 13.6, 11.98 and 10.36 days for pupa in treatment of 2-3 days old eggs in Dimilin, Nomolt and Emacte treatments respectively, compared to 14.55 days for larval stage and 8.03 days for pupal stage in the untreated. The present study noted also, light changes in the levels of the total protein, carbohydrate and chitin composition during embryogenesis for *P. gossypiella* eggs as a result of treatments in comparison to the untreated.

Keywords: *Pectinophora gossypiella*; eggs; Diflubenzuron; Teflubenzuron; Emamectin benzoate.

INTRODUCTION

The bollworm, *Pectinophora gossypiella* (PBW) (Lepidoptera: Gelechiidae), is a significant and devastative insect pest to cotton cultivations that lays its eggs on squares, flowers, or green bolls, (Radwan *et al*, 2019). The egg is oval-shaped with a rough surface about 0.55 mm long and 0.25mm wide, its color is whitish yellow when first laid and finally pink before hatching, (Sarwar, 2017). The egg stage is considered the most exposed to insecticidal applications in the cotton fields, as it requires 3 to 4 days before hatching, (Said and Abdelaal, 2020). Insect eggs contain large amounts of yolk proteins synthesized in extra-ovarian tissues for oocyte development (Tomino, 1985; Yamashita and Indrasith, 1988). During the incubation period, egg contents of protein, lipid, carbohydrate, and chitin are essentially required for embryogenesis, development, and hatching of larvae. Thus, it was necessary to know the effect of some chemicals used in the field during the development of larval structures (embryogenesis) inside the eggs.

Using conventional insecticides has developed a high level of resistance to insect pests (Ishaaya *et al*., 1995). Intensive studies have been carried out during the last decades to evaluate insecticides with a novel mode of action against insect pests with low environmental hazards (Ishaaya, 1990). However, Insect growth regulators (IGR_s) during the last three decades as novel insecticides include benzoyl phenyl urea (BPU) and diflubenzuron (DFB) (Miyamoto *et al.* 1993). Conversely, such compounds are considered selective and effective insecticides on different lepidopterous species such as *Spodoptera littralis, Earias insulana,* and *P. gossypiella* with relatively harmless effects on beneficial insect species. (Soltani and Soltani-Mazouni, 1992 and Khebeb *et al.,* 1997). The susceptibility of cotton bollworms to such IGRs was demonstrated by many authors (Said *et al*., 2019 and Adly, 2020).

The bio-pesticide *Emamectin benzoate* is a derivative of the natural Avermectin family produced by the fermentation of soil microorganism *Streptomyces avermitilis*, (Schulman, 1987). It demonstrated high efficacy against the developmental stages *P. gossypiella* insect (Moustafa, *et al* (2019)

The direct effects of tested Diflubenzuron (Dimilin), Teflubenzuron (Nomolt), and *Emamectin benzoate* (Emacte) on PBW eggs as well as the latent effects on the resulting stages were considered in this study.

MATERIALS AND METHODS

Laboratory experiments were conducted at Bollworms Research Department, Plant Protection Research Institute, Agriculture Research Centre, Ministry of Agriculture, Giza, Egypt.

Used Insect:

The eggs of *P. gossypiella* used in this experiment were obtained from a susceptible laboratory strain of pink bollworm, reared for several generations away from any contagion of insecticides under controlled conditions of (26±1°C and 75±5 % RH) on an artificial diet described by Rashad and Ammar (1985).

Two groups of *P. gossypiella* eggs were used for each treatment:

Group (1) (0-1) day-old eggs.

Group (2): (2-3) days-old eggs.

Used Pesticides:

I- IGR compounds:

1- Common name: Diflubenzuron.
Trade name: Dimilin (48 % SC).
Rate of application: 125 cm^3/feedan.

2- Common name: Teflubenzuron.
Trade name: Nomolt (15% SC).
Rate: 50 cm^3 / 100 L.

II- Bio-pesticide compound:

Common name: *Emamectin benzoate*.
Trade name: Emacte 2.15 % EC.
Rate of application: 150 cm3 / 200 L.

Experimental techniques:

Pesticides' Preparation:

Serial concentrations were prepared using sterilized water as follows: 240, 120, 60, 30, and 15 ppm for Dimilin; 150, 75, 37.5, 18.75, 9.38 and 4.69 ppm for Nomolt and 1.9, 0.95, 0.47, 0.23 and 0.12 ppm for Emacte to estimate LC values.

Toxicity tests on eggs:

Eggs of PBW, 0-1 day and 2-3 days old, were treated by dipping technique for 10 sec. in different tested concentrations of the three tested compounds. After dryness at room temperature, three replicates (150 -200 eggs/ replicate) were used for each concentration. Besides, similar three replicates for the two egg groups were dipped in water for the same time and used as untreated. Treated and untreated egg groups were kept in an incubator under constant conditions of 26 ± 1°C and 75±5 % RH. The number of hatched and unhatched eggs was counted. The hatchability percentage for all the treated and untreated eggs was estimated and corrected according to Abbott, (1925). The LC_{90}, $LC_{50,}$ and LC_{25} values were calculated by the LDP line program according to Finny, (1971). In addition, the toxicity index of different compounds was measured according to Sun's equation (Sun, 1950) as follows:

$$\text{Toxicity index (Ti)} = \frac{LC_{50} \text{ of A}}{LC_{50} \text{ of B}} \times 100$$

Potency levels (PL) = LC_{50} of C / LC_{50} B

ıst effective compound.
B: The other tested compound.
C: The least effective compound.

Biological Studies:

At the level of $LC_{50,}$ the indirect effects of the tested compounds in relation to certain biological parameters of hatched larvae were studied. Three replicates (150 -200 eggs/ replicate) for each egg group were treated by dipping technique for 10 sec. Besides, similar three replicates for each egg group were dipped in water for the same time and used as control checks. Treated and untreated egg groups were kept in an incubator under constant conditions of 26 ± 1°C and 75±5 % RH until hatching. The resulting larvae were reared on an artificial diet (described by Rashad and Ammar, 1985) and inspected daily until pupation. The total accumulative mortalities, malformations, durations, and weights for the resulting larvae and pupae were recorded. Pupation, as well as adult emergence percentages, were recorded. Five pairs of newly emerged moths of each treatment as well as untreated were placed in a glass chimney cage for mating. Three replicates were used for each treated and untreated. Fecundity (egg number per female) and hatchability (fertility) percentages were estimated. In addition, female and male longevities were determined.

Biochemical Analyses:

For each treatment, Samples of unhatched LC_{50}-treated eggs were used for biochemical analyses. Similar samples of untreated eggs were used as untreated. Treated and untreated egg samples (2000- 3000

eggs/treatment) were homogenized in distilled water. The homogenates were centrifuged at 8000 r. p. min. at $5C^0$ in a refrigerated centrifuge. The resulting supernatants were used to estimate some biochemical parameters:
-The total protein content of the whole homogenate was measured according to the method described by Bradford (1976).
- The Total carbohydrates were estimated according to Singh and Sinha (1977).
-Determination of chitinase activity was prepared according to Bade and Stinson (1981).
Statistical analysis:

Values of the obtained data were statistically analyzed with a one-way analysis of variance (ANOVA) and mean separated using Duncan's Multiple Range Test (Duncan, 1955), at $P < 0.05$ %.

RESULTS

1- Toxicity of tested Pesticides:

The toxic effects of Dimilin, Nomolt, and Emacte were evaluated against (0-1) day-old eggs and (2-3) day-old eggs of *P. gossypiella*. Data given in Table (1) indicated that the older eggs showed higher susceptibility to the three tested compounds than the younger ones. In addition, at the level of LC values, Emacte treatment exhibited remarkably high ovicidal efficiency against the two egg groups. The estimated values of LC_{25}, LC_{50}, and LC_{90} for Emacte treatment were 0.185, 0.529, and 3.906ppm for 0-1 day-old eggs, where recorded 0.046, 0.191, and 2.867ppm for 2-3 day-old eggs, respectively.

Table 1. Toxicity of Dimilin, Nomolt, and Emacte against *P. gossypiella* eggs.

Used eggs	Used Compound	LC_{25} (ppm)	LC_{50} (ppm)	LC_{90} (ppm)	Slope	*TI (LC_{50})	**PL (LC_{50})
(0-1) day-old eggs	Dimilin,	53.63	155.40	375.41	2.92	0.34	1
	Nomolt	15.51	36.63	196.63	1.7 4	1.44	4.24
	Emacte	0.185	0.529	3.906	1.48	100	293.76
(2-3) days old eggs	Dimilin,	47.57	81.42	222.89	2.6	0.24	1
	Nomolt	9.73	23.36	132.85	1.64	0.82	3.49
	Emacte	0.046	0.191	2.867	1.09	100	426.28

* Toxicity index (TI). ** Potency levels (PL).

On the other hand, remarkably recorded low curative ovicidal effects of Dimilin and Nomolt expressed respectively as LC_{50} of 155.40 and 36.63 ppm for (0-1) day-old eggs and 81.42 &23.36 ppm for (2-3) day-old eggs. This is confirmed by the values of TI (0.34& 1.44 for (0-1) day-old eggs and 0.24 &0.82 for (2-3) day-old eggs) while PL values recorded (1.0 &4.24 for (0-1) day-old eggs and 1.0 & 3.49 for (2-3) day-old eggs), for the same compounds, respectively. These results revealed low activity of the two IGR compounds, in comparison to many multiple values of TI (100) and PL (293.76 & 426.28) for Emacte in both egg ages, respectively (Table 1).

2- Biological Studies:

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2.1. Egg incubation period:

The egg incubation periods were significantly increased for both egg ages as a result of all treatments in comparison to the untreated checks, with more efficiency for the two IGR compounds (Dimilin and Nomolt) than the Emacte treatment. Table (2) showed that the incubation periods recorded 6.9, 6.5, and 5.36 days for (0-1) day-old eggs in Dimilin, Nomolt, and Emacte treatments, respectively with an increase of 2.1, 1.7, and 1.5 times than the untreated. In addition, the incubation periods of older aged eggs (2-3 days old eggs) were 5.8, 5.3, and 4.4 days for the three compounds, respectively, compared to 3.3 days for the untreated check with an increase of 1.5, 1.4, and 1.2 times.

2.2. Hatchability percent:

Present results show the inhibition effect of Dimilin, Nomolt (IGRs), and Emacte compounds regarding the hatchability percent of pink bollworm-treated eggs. Eggs' hatchability percentage was reduced to 50.2 &51.66 % for Emacte treatment followed by Dimilin (52.2 & 54.67 %), and lastly Nomolt (54.3 & 58.37%) for the one- and three-days eggs, respectively, compared to 96.7% for control Table (2).

Table 2. Effect of the three tested compounds on the incubation periods and hatchability percent of *P. gossypiella* eggs.

Conc. (LC_{50}) ppm		Egg Incubation periods (days)	Increase in time compared to control	%Total Hatchability
(0-1) day-old eggs	**Dimilin**	6.9±0.9[a]	2.1	52.2[a]
	Nomolt	6.5±0.8[a]	1.7	54.3[a]
	Emacte	5.36±0.3[ab]	1.5	50.2[a]
	Control	3.3±0.3[b]	-	96.7[b]
	P	.0243 *	-	*** 0000.
	LSD	2.228c	-	6.49
(2-3) days-old eggs	**Dimilin**	5.8±0.4[a]	1.5	54.67[c]
	Nomolt	5.3±0.33[a]	1.4	58.37[b]
	Emacte	4.4±0.3[b]	1.2	51.66[d]
	Control	3.3±0.3[c]	-	97.09[a]
	P	.0005 ***	-	.0000 ***
	LSD (0.05)	0.812	-	2.867

Numbers followed by the same letters were statistically not significant

In addition, most of the hatchability percent takes place in the first four days of the incubation period at all treatments as well as control checks. The effect of tested compounds was illustrated in Figs. (1&2). The exposure of one-day eggs to the tested compound led to the inability to develop embryos inside Fig1 (A, B, and c). However, despite the formation of the embryos at older ages of eggs, treatments resulted in the death of fully or partially developed larvae and their failure to break out of the eggshell (Fig. 2(A, B, and c), compared to the untreated check (Fig. 3).

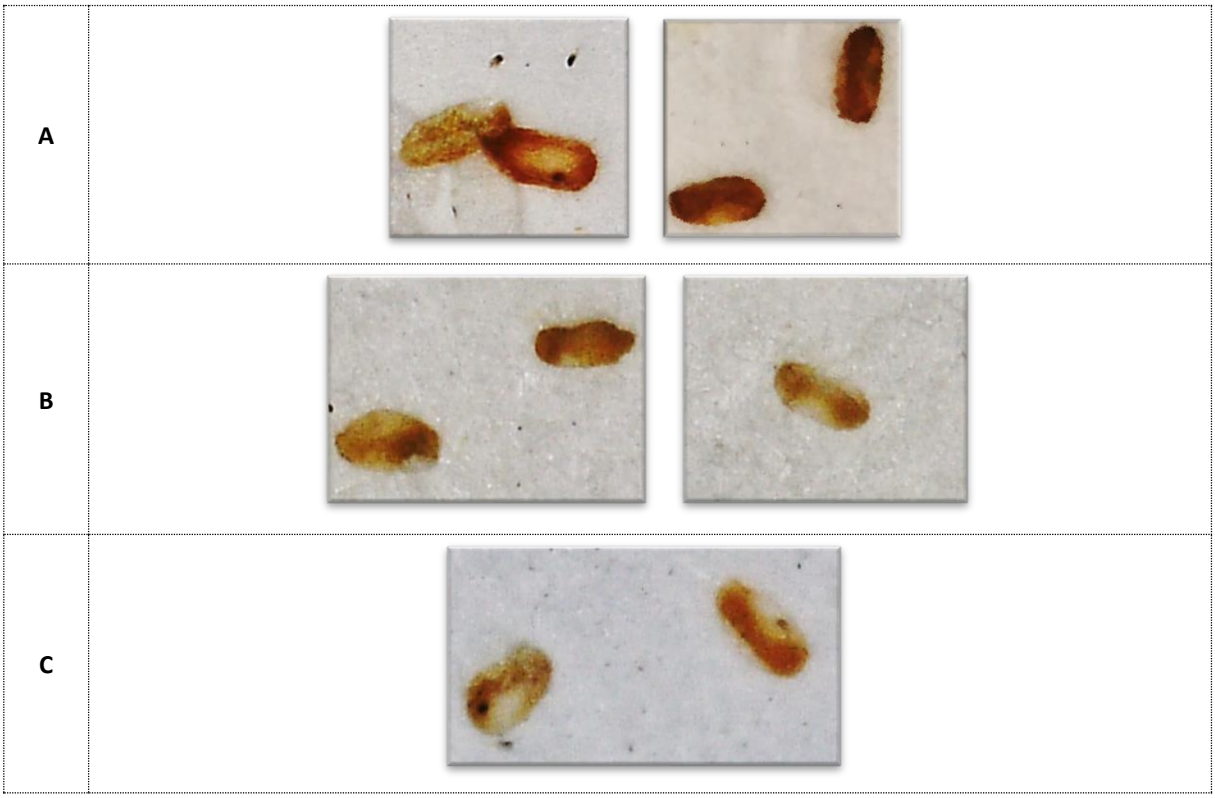

Fig. 1. Effect of tested compounds on 0–1-day *P. gossypiella* - treated eggs indicating clumping and coagulation of the inner yolk of the eggs on the extremities with the inability to develop embryos inside, (A): Dimilin treatment, (B): Nomolt treatment, and(C): Emacte treatment

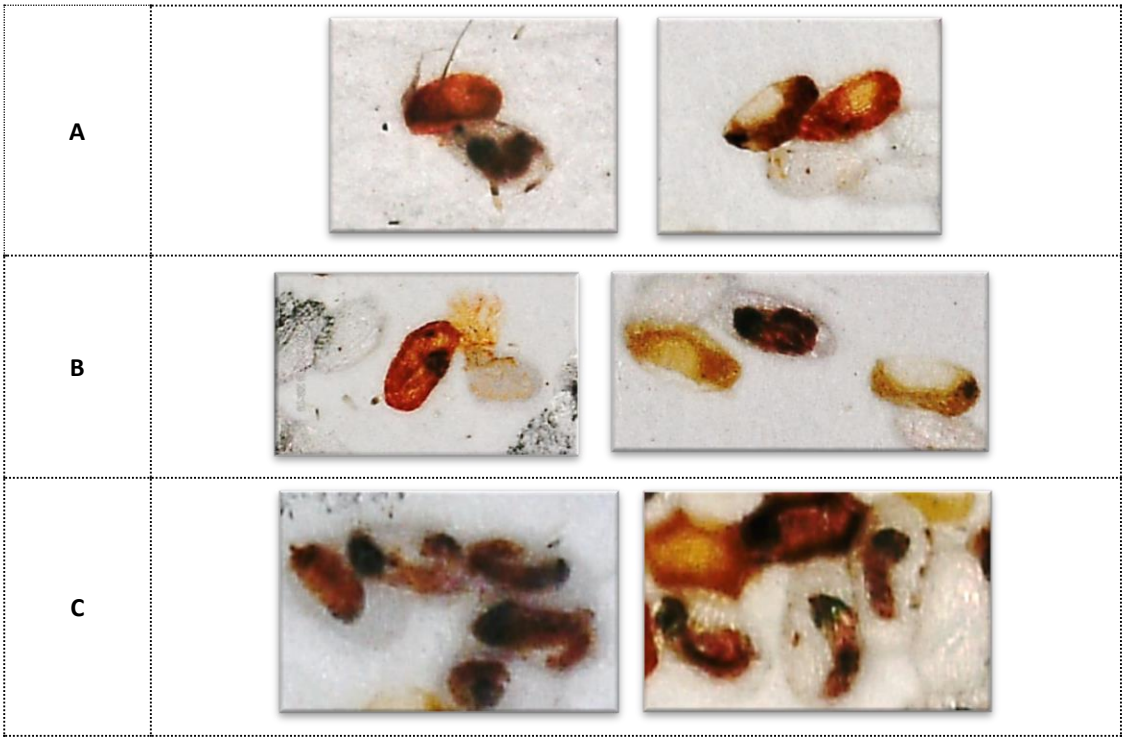

Fig. 2. Effect of tested compounds on 2-3 day *P.2–3-dayiella*-treated eggs indicating the death of fully or partially developed larvae and their failure to break out of the eggshell,
(A): Dimilin treatment, (B): Nomolt treatment, and(C): Emacte treatment.

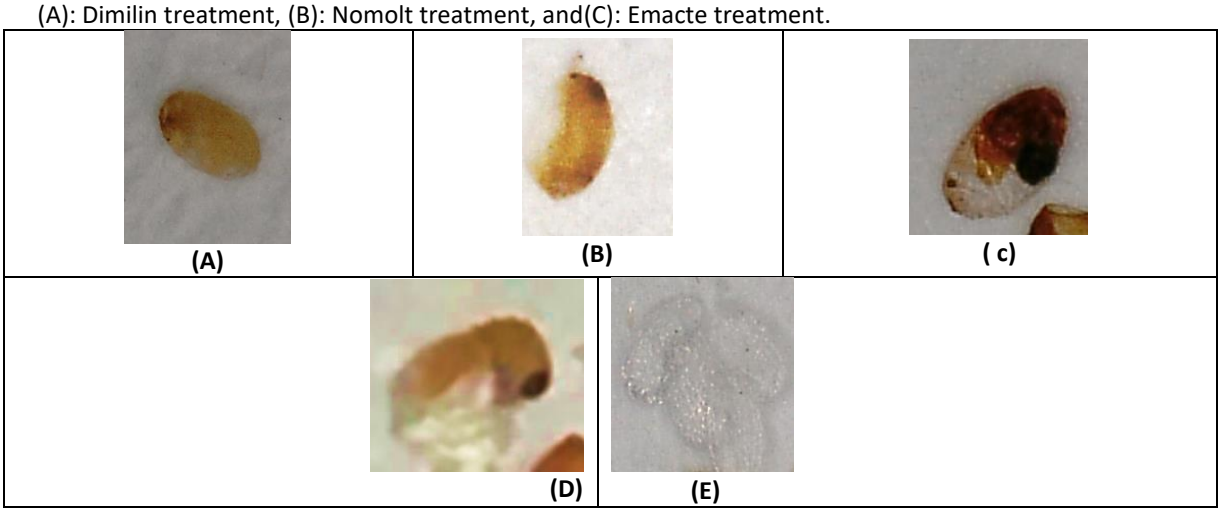

Fig. 3. Hatching process of *P. gossypiella*-untreated eggs indicated,
(A): Normal 0-1 day old untreated egg, (B): Egg with partially developed embryo (larva) inside egg, (c): Fully developed larva inside, (D): The fully developed larva break out the eggshell (Hatching process), (E): Egg sell after hatchability.

The larval and pupal developmental periods of *P. gossypiella* resulting stages recorded 19.6, 17.9 and15.85 days for larvae and 12.5, 11.8, and 9.76 days for pupae when one-day old eggs treated by Dimilin, Nomolt, and Emacte, respectively, compared to 14.95 days for larvae and 8. 3 days/pupae in control. In the case of 2-3 days eggs treatments registered longer times 20.2, 18.8 & 16.9 days/larvae and 13.6, 11.98 & 10.36 days for pupae, respectively in comparison to 14.55 days/larvae and 8.03 days/pupae in control, (Table 3).

Table 3. Latent effects of Dimilin, Nomolt, and Emacte on different immature stages resulted from pink bollworm-treated eggs.

Treatments		larval mortality %	Larval duration	Pupation%	Pupal duration	% Adult emerges.
(0-1) Day-old eggs	Dimilin	23.51^{a}	19.6±0.4^{a}	73.66^{c}	12.5±0.25^{a}	58.98^{c}
	Nomolt	19.42^{b}	17.9±0.27^{ab}	75.63^{c}	11.98±0.3^{ab}	60.76^{c}
	Emacte	17.21^{b}	15.85±0.69^{bc}	83.0^{b}	9.76±0.53^{bc}	70.92^{b}
	Control	4.30^{c}	14.95±0.53^{c}	95.7^{a}	8.3±0.66^{c}	98.20^{a}
P		.0000 ***	.0066 **	.0000 ***	.0125 *	.0000 ***
LSD (0.05)		2.54	2.39	5.18	2.41	4.41
(2-3) Days old eggs	Dimilin	28.53^{a}	20.2±0.42^{a}	69.0^{c}	13.5±0.2^{a}	47.96^{c}
	Nomolt	22.31^{b}	18.8±0.3^{a}	79.33^{b}	11.8±0.2^{a}	52.88^{c}
	Emacte	21.25^{b}	16.9±0.18^{ab}	77.5^{b}	10.35±0.2^{ab}	60.96^{b}
	Control	4.10^{c}	14.55±0.2^{b}	96.0^{a}	8.03±0.1^{b}	97.71^{a}
P		.0000 ***	.0399 *	.0001 ***	.0299 *	.0000 ***
LSD (0.05)		3.959	3.8	6.217	3.36	7.64

Numbers followed by the same letters were statistically not significant

3-Physiological parameters:

Physiological factors play a critical role in the development, reproduction, fertility, and susceptibility to insecticides for all living organisms. Thus, it is necessary to study and understand the effect of tested compounds on some physiological parameters of tested eggs as a result of treatments compared to the untreated check.

3.1The total soluble protein and carbohydrates:

Data in Table (4) cleared that the main value of total soluble protein for *P. gossypiella* untreated eggs was 10 mg/g.b.wt, for (0-1) day-old and decreased by increasing the eggs' age to 7.1 mg/g.b.wt for (2-3) days-old eggs. This value was reduced to 7.21, 8.6, and 7.24 mg/g.b.wt when (0-1) day-old eggs were treated with LC_{50} of Dimilin, Nomolt, and Emacte Pesticides, respectively. The effect was more indicated in the older aged eggs whereas the values were 4.21, 5.1, and 5.9 mg/g.b.wt (with great reductions of 57.9, 28.17, and 16.9%) for (2-3) days-old eggs treated with tested compounds respectively, (Table 4). The real theory of protein level in insect eggs is reduced by the increase in their age which is associated with the developmental process.

Treated (0-1) day-old *P.gossypiella* eggs significantly affect their carbohydrate content. The main value of total soluble carbohydrates was 4.8 mg/g.b.wt for the untreated eggs. This value was significantly reduced to 3.2 and 2.9 mg/g.b.wt in Dimilin and Nomolt treatments, respectively. In contrast, a significant increase was recorded in Emacte treatment (7.4 mg/g.b.wt) (Table 4).

Conversely, no significant difference was found in the total carbohydrate content for the older eggs treated with LC_{50} of Dimilin, Nomolt, and Emacte (5.3, 4.9, and 5.0 mg/g.b.wt, respectively) in comparison to the untreated (.49 mg/g.b.wt).

Table 4. Chemical composition of *Pectinophora gossypiella* eggs treated with LC_{50} of tested compounds.

Treatments		Total soluble protein (mg/g.b.wt)		Total soluble carbohydrate (mg/g.b.wt)		Chitin content (µg NAGA x103/min/g.b.wt /egg)		Chitinase enzyme IU/L	
		Content	C%	Content	C%	Content	C%	SP	RA%
(0-1) Day-old eggs	Dimilin	7.21± 0.647^{b}	-27.9	3.2± 0.529^{c}	-33.3	37.0± 1.732^{a}	+16.4	2.9± 0.306^{a}	-27.5
	Nomolt	8.6± 1.027^{ab}	-14	2.9± 0.100^{c}	-39.6	28.0± 0.577^{b}	-12	3.0± 0.289^{a}	-25
	Emacte	7.24± 0.954^{b}	-25.8	7.4± 0.400^{a}	+54.2	30.9± 1.054^{b}	-2.8	4.0± 0.289^{a}	0
Control		10.0± 0.5^{a}	--	4.8± 0.416^{b}	--	31.8± 1.039^{b}	--	4.0± 0.5^{a}	--
P		.1180 ns	--	.0002 ***		.0042 **	--	.1695 ns	--
LSD (0.05)		2.646	--	1.287	--	3.832	--	1.165	--
(2-3) Days old eggs	Dimilin	4.21± 0.121^{b}	-57.9	5.3± 0.404^{a}	+8.2	81.8± 1.848^{c}	-32.4	60.0±2.887^{a}	+131.7
	Nomolt	5.1± 0.520^{ab}	-28.2	4.9± 0.058^{a}	0	106.7± 3.724^{b}	-11.8	64.0± 2.309^{a}	+147.1
	Emacte	5.9±0.322a^{b}	-16.9	5.0± 0.513^{a}	+2	88.0± 3.215^{c}	-27.3	27.9± 0.416^{b}	+7.7
Control		7.1± 1.097^{a}	--	.49± 0.346^{a}	--	121± 2.309^{a}	--	25.9± 0.833^{b}	--
P		.0586 ns	--	.8519 ns	--	.0000 ***	--	.0000 ***	--
LSD (0.05)		2.057	--	1.209	--	9.360	--	6.664	--

Numbers followed by the same letters were statistically not significant
Content expressed as (mg/g.b.wt)
Gram/body weight (g.b.wt)
Micro gram (µg)
N-acetyl glucosamine (NAGA)
SA (Specific activity) as (µg pyruvate /ml)

$$.\,C\ (\text{Change}\%) = \frac{\text{Treatment}-\text{Control}}{\text{Control}} X100 \qquad .\,RA\ (\text{Relative activity}\ \%) = \frac{\text{Treatment}-\text{Control}}{\text{Control}} X100$$

On the other hand, both Nomolt and Emacte treatments relatively reduced the chitin content to 28.0 and 30.0 (µg NAGA x103/min/g.b.wt /egg, respectively for (0-1) day-old treated eggs, opposite to the increase for Dimilin treatment (37.0 µg NAGA x103/min/g.b.wt /egg), compared to 31.8 (µg NAGA x103/min/g.b.wt /egg) for the untreated check. In addition, a reduction of 27.5 and 25% in chitinase activity was also reported as a result of Dimilin and Nomolt treatments, respectively. Also, a remarkable decrease in chitin content for (2-3) days-old eggs treated with LC_{50} of the tested compounds where it reached (81.8, 106.7, and 88.0 (µg NAGA x103/min/g.b.wt /egg) compared to (121 µg NAGA x103/min/g.b.wt /egg for control) and combined with a high increase of its enzyme activity estimated by 60.0, 64.0, and 27.9 IU, respectively, compared to 25.9 IU for control, (Table 4).

DISCUSSION

Toxicity of tested Pesticides:

Results revealed that older eggs of *P. gossypiella* appeared to be more susceptible to pesticides than the younger ones, this may be due to the lower penetration of insecticide through the chorine of newly deposited eggs, (Smith and Salkeld, 1966 and Said; Abdelaal 2020). These confirmed the results of El-Barkey *et al*. (2009) who demonstrated less susceptibility of one-day-old eggs of *Pectinophora gossypiella* treated by Radiant and Hexaflumuron than the older and pre-hatching ones. Likewise, Peterson *et al.* (1998) indicated that the newly laid eggs of *Heliothis zea* and *H. virescens* were slightly less susceptible to the spinosad compound than older eggs.

The present results cleared that the LC_{50} values of tested compounds indicated remarkably high toxicity of Emacte (E*mamectin benzoate)* against both ages of treated eggs followed by the other IGR compounds, this was

confirmed by the study of Zidan, *et al* (2013) who reported markedly high curative ovicidal effectiveness of *Emamectin benzoate* against *S.littoralis* and whitefly eggs of 24 hrs old whereas a moderate curative ovicidal activity was reported for Lufenuron and Chlorfluazuron IGR compounds

Ovicidal influences:

The presented results displayed a significant increase in the egg incubation periods for both egg groups treated by the LC_{50} of Dimilin, Nomolt, and Emacte compounds in comparison to the untreated check. These results are in accordance with that of Kandil *et al,* (2013) who reported that most of IGR's compounds greatly affect the incubation periods and the hatchability percent of insect-treated eggs as well as larval and pupal durations. Also, El-Barkey *et al*, (2009) reported that the incubation period of one-day-old eggs treated with Hexaflumuron was longer than pre-hatching ones by 1.2-1.5 times. Similarly, Adly (2020) reported ovicidal activities of two IGR compounds; Triflumuron and Hexaflumuron against Earias insulana (Boisd.) eggs. Moreover, Said and Abdelaal (2020) indicated that the incubation period was significantly increased when 1 and 3 days' ages of pink bollworm eggs were treated by the LC_{50} of Emamectin compound and this increase reached approximately 1.5 to 2.5 times than control in 1-day old eggs treatment.

According to our results, the three tested compounds have a strong negative effect on eggs' hatchability percentages that leads to the death of the partially or fully developed embryos (larvae) inside the eggshell. In this sphere, the most effective was Emacte followed by Dimilin then Nomolt for younger and older eggs, respectively. The diffusion of these compounds into the eggs may cause a failure of a key regulatory process and functions to operate throughout embryogenesis. This is confirmed by the results of Marco and Vinuela (1994) and Mass *et al*., (1980) who elucidated that inhibition of egg hatchability may be due to the penetration of these compounds into the eggs, preventing hatching by interfering with embryonic cuticle synthesis, so the new hatch probably cannot use its muscles to exit from the egg shell. This is consistent with the interpretation that treating eggs with different insecticides leads to the killing of a large number and failure in the hatching process. This may be due to interference in the formation of embryonic chitin, which causes growth failure and weak structure of the exoskeleton and muscles, along with an inability to tolerate the high blood pressure required to emerge from the egg (Flint *et al*., 1978).

Our outcomes are in agreement with that of Said and Abdelaal, (2020) who found that at the tested level LC_{50} *Emamectin benzoate* reduced the percent of hatchability to 51 and 53 % for one and three-day-old eggs, respectively. Likewise, Zidan, *et al* (2013) distinguished a high preventive effect of *Emamectin benzoate*, Lufenuron, and Chlorfluazuron on reducing hatchability percentages of *S.littoralis* and whitefly treated eggs. In addition, Said *et al,* (2019) recorded that, IGR compounds namely, Teflubenzuron, Lufenuron, and Flufenoxuron) caused long-term inhibition effects with a defect in the molting process at different stages of *P. gossypiella* insect. Similarly, Taha and Radwan (2023) approved the susceptibility of pink bollworms to insect growth regulator insecticides.

Effects on resulted stages:

Additionally, the results indicated the effect of the tested treatments on the subsequent stages of *P. gossipiella*, as they caused a developmental disturbance represented by an increase in the duration and rates of mortality in the immature stages, with a decrease in the proportions of the emerged adults. These findings are in accordance with some authors who tested different IGRs against lepidopterous insects, e.g., *P. gossypiella*, El-Shennawy (2009); *Spodoptera littoralis*), Abdel-Aal, (2003) and Said *et al* 2017; and *Earias insulana*, El-Shennawy and Kandil *et al* (2017) as they recorded a prolonging in larval and pupal durations with negative latent effect on the emerged adults. In the same trend, Moustafa, *et al* (2019) demonstrated an increase in the time required for all developmental stages of *P. gossypiella* resulting from *Emamectin benzoate* and Lufenuron-treated adults. Similarly in the study of Adly (2020), he declared an increase in the developmental periods of larval and pupal stages of *E. insulana* resulted from treated eggs. Also, a reduction in the adult emergence percentages as a result of treating eggs with tested compounds was previously confirmed by (Tanani and Ghoneim, 2018).

Physiological parameter:

The present results clearly indicate the effect of tested compounds on some biochemical parameters of treated eggs. The total protein and carbohydrate content are the necessary components for growth and development during embryogenesis (García-Guerrero *et al.,* 2003). For the untreated eggs, the main value of total soluble protein was decreased by increasing the eggs' age with a slight flux in carbohydrates. This fluctuation reflects the retardation in embryonic development that may lead to the death of larvae inside eggs. Also; the reduction in protein may be due to its utilization in chitin synthesis which is necessary for cuticle formation in produced larvae. Moreover, protein can be utilized as a significant energy source for hatching insect larvae, (Diss *et al.*, 1996). Also, Pant and Nautiyal, (1974) declared a relatively low glycogen level at the tail-end embryogenesis of *Philasamia ricini* and they added that could be attributed to its participation in cuticular chitin synthesis. Treatments decreased the main value of total soluble protein with a fluctuating effect on the

carbohydrate content. The (2-3) days-old eggs were more sensitive to treatments than the younger ones. These results are in harmony with that of Perveen (2006) who reported a reduction in the amounts of egg constituents and egg hatch after treating *Spodoptera lituralis* with a sub-lethal dose of chlorfluazuron.

According to our results, increasing the age of eggs enhanced a remarkable increase in chitin content as well as chitinase activity. This was previously confirmed by Radha *et al* (1979) who recorded that the cuticular chitin formation occurs just before hatching.

CONCLUSION

Generally, it can be concluded that the tested pesticides; namely Dimilin, Nomolt, and Emacte, exhibited remarkably high curative ovicidal efficiency against the two aged groups of *P. gossypiella-treated* eggs. The LC values indicated much more susceptibility of *P. gossypiella* 0-1 day-old eggs to the tested pesticides than the older ones. The three treatments caused developmental disturbances including an increase in the duration and the mortality percentages of different stages. In addition, significant physiological disruptions at the level of the total soluble protein, carbohydrate, chitin content, and chitinase activity as a result of treatments were also reported.

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تأثير بعض المبيدات الحشرية على التطور الجنيني لبيض دودة اللوز القرنفلية والمراحل اللاحقة

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تعتبر دودة اللوز القرنفلية من الآفات الهامة التي تسبب خسائر اقتصادية كبيرة لمحصول القطن . تمت دراسة تاثيرثلاثة من المركبات : ديميلين ونومولت واكسلنت ضد عمرين مختلفين من بيض البكتينوفورا (0-1& 2-3 يوم) تحت الظروف المعملية (27 ± 1 درجة مئوية و 70 ± 5٪ رطوبة نسبية) . أوضحت النتائج حساسية الأعمار الأكبر من البيض للمبيدات عنها من الأعمارالأصغر حيث كانت قيم LC_{50} 4 .155 و 185.85 و 2.3 جزء في المليون للبيض عمر (0-1) يوم ، بينما انخفضت إلى 81.42 و 103.42 و 1.95 جزء في المليون للبيض عمر (2-3) أيام في معاملات كل من ديميلين ونومولت واكسلنت على التوالي. كما زادت فترات حضانة البيض نتيجة المعاملات مقارنة بمثيلاتها من عينات المقارنة. أيضا زادت أعمارالأطوارغير الناضجة إلى 19.6 و 17.9 و 15.85 يوم لليرقات و 12.5 و 11.8 و 9.76 يوم للعذارى الناتجه من البيض عمر (0-1) يوم بينما وصلت إلي 20.2 و 18.8 و 16.9 يوم لليرقات و 13.6 و 11.98 و 10.36 يوم للعذارى الناتجه من البيض عمر (2-3) أيام نتيجة المعاملات سالفة الذكر على التوالي ، مقارنة بـ 14.55 يوم / يرقة و 8.03 يوم / للعذراء في ا عينات المقارنة. كما تشير الدراسة الحالية أيضًا" إلى حدوث تغيرات في مستويات البروتين الكلي والكربوهيدرات وكذلك تكوين الكيتين أثناء التطور الجنيني لبيض ا دودة اللوز القرنفلية كنتيجة المعاملات مقارنة بالبيض غير المعامل.

الكلمات المفتاحية: دودة اللوز القرنفلية - البيض- دايفلوبنزيرون - تيفلوبنزيرون - ايمامكتين بنزوات