Scholars Academic Journal of Biosciences (SAJB) Sch. Acad. J. Biosci., 2017; 5(6):412-424 ©Scholars Academic and Scientific Publisher (An International Publisher for Academic and Scientific Resources) www.saspublishers.com

ISSN 2321-6883 (Online) ISSN 2347-9515 (Print)

DOI: 10.36347/sajb.2017.v05i06.003

Original Research Article

Ovicidal activities and developmental effects of the chitin synthesis inhibitors, Noviflumuron and Novaluron, on the pink bollworm *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae)

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Abstract: The pink bollworm *Pectinophora gossypiella* is one of the most destructive insects attacking cotton fields world-wide. The present study was conducted to evaluate the survival and developmental effects of the novel chitin synthesis inhibitors (CSIs), Noviflumuron and Novaluron, on this insect pest, after treatment of 1-day old eggs. These CSIs exhibited ovicidal activities on eggs, in a dose-dependent course. Complete (100%) mortality of larvae was recorded at the highest concentration (8.0 and 5.0 ppm, respectively) and various degrees of toxicity were recorded on larvae at other concentrations. Both CSIs failed to exhibit pupicidal or adulticidal effects. Noviflumuron exhibited stronger ovicidal activity than Novaluron (LC50 values: 0.188 and 0.274 ppm, respectively) and *vice versa* for larvicidal activity (LC50 values: 0.153 and 0.342 ppm of Novaluron and Noviflumuron, respectively). The larval and pupal durations were slightly or remarkably prolonged. The pupation rate was slightly reduced by Noviflumuron but conspicuously hindered by Novaluron. Noviflumuron and Novaluron could not affect the metamorphosis or morphogenesis of *P. gossypiella*.

Keywords: egg, larva, metamorphosis, morphogenesis, mortality, pupation.

INTRODUCTION

Worldwide, the pink bollworm Pectinophora gossypiella (Saunders) (Lepidoptera: Gelechiidae) is one of the most destructive insect pests that cause terrible damage to the cotton because it is difficult to be controlled by insecticides [1, 2]. Larvae damage the floral outgrowths, flowers, bolls, developing seeds within bolls and deteriorate the staple length and strength of lint. The termination of boll growth results in boll rotting and premature or partial boll opening [3]. In Egypt, this insect pest causes serious damage to cotton arising to one million kentar annually [4, 5]. Moreover, P. gossypiella has been reported to develop resistance against the transgenic cotton varieties in Arizona (USA) [6] and different districts of India [7]. In Egypt, also, this insect has recently developed resistance to several classes of insecticides currently used in cotton fields because of its ability to detoxify these chemicals [8].

As reported by many authors [9-15], the intensive and discriminate uses of many conventional pesticides lead to several drastic problems, such as the environmental pollution, hazards to human and animals like birds, fishes and mammals, destruction of the pollinators and other non-target insects as well as the

natural enemies, like parasites and predators. Therefore, alternative materials have been initiated recently to minimize the environment hazards and the serious toxicological problems to humans and animals [16] as well as to delay the resistance development in P. gossypiella [17-20]. At present, using insect growth regulators (IGRs) is considered as the possible alternative way of synthetic insecticides for controlling this pest. In contrast to the classical chemical insecticides, IGRs are not directly toxic, but act selectively on the development, metamorphosis or reproduction of the target insect species [21, 22]. They are quite selective in their mode of action and potentially act only on the target species [23]. As reviewed by Tunaz and Uygun [24], chitin synthesis inhibitors (CSIs) are usually classified in IGRs interfering with chitin biosynthesis in insects and thus prevent moulting, or produce an imperfect cuticle.

Noviflumuron is a new chemical currently being developed by Dow Agro Sciences, Indianapolis, Indiana, USA, for the structural pest control market [25]. Noviflumuron is a benzoyl phenyl urea IGR that prevents the molting and development of some insects, such as fleas, ants, termites and houseflies [26, 27]. Its suspension concentrate, dust or gel bait can effectively

suppress *Blattella germanica* populations with a pattern of activity similar to that expected from a CSI [28-30]. Also, adult *B. germanica* exposed to Noviflumuron fail to produce viable eggs, hence their reproductive potential is reduced [31].

Novaluron is a relatively new benzovl phenyl urea CSI with low mammalian toxicity [32, 33]. The compound has no appreciable effect on parasitoids and has probably a mild effect on the natural enemies [34, 35]. Its residues tend to dissipate with half-life of 2.08 days and the safe use of it on tomatoes, and possibly on other crops in Egypt was established [36]. Novaluron is a powerful suppressor of the pest populations, such as Bemisia tabaci and Trialeurodes vaporariorum [37]. It acts by ingestion and contact against several insect pests, such as Spodoptera spp., Tuta absoluta, Helicoverpa armigera, and Liriomyza huidobrensis [38]. It exhibited, also, a good activity against the Colorado potato beetle [39-42] and impaired the development [43] and adult performance [44] of Spodoptera littoralis. Recently, treatment of newly hatched and full grown larvae of P. gossypiella with Novaluron led to reduced survival, retarded development, impaired metamorphosis [45], disrupted adult performance, inhibited reproductive potential [46], declined main metabolites [47], and deteriorated larval haemogram [48]. Also, Novaluron adversely affected the survival and development of Palpita unionalis [49]. According to the available literature, few studies had been conducted on the ovicidal effects of insecticides and IGRs on eggs of lepidopterous pests but the majority focused on the treatment of larval stage [50]. Therefore, objectives of the present study were to evaluate the ovicidal action of Noviflumuron and Novaluron, novel CSIs, and to investigate their disruptive effects on the development on P. gossypiella after treatment of 1-day old eggs.

MATERIALS AND METHODS Experimental insect

A culture of the pink bollworm *Pectinophora* gossypiella (Saunders) (Lepidoptera: Gelechiidae) was originated by a sample of newly hatched larvae from the susceptible culture maintained for several generations along some years in Plant Protection Research Institute, Doqqi, Giza, Egypt. It was reared under laboratory controlled conditions $(27\pm2^{\circ}C \text{ and } 75\pm5\% \text{ R.H.})$ at Department of Zoology and Entomology, Faculty of Science, Al-Azhar University, Cairo. Larvae were provided with an artificial diet as described by Abd El-Hafez *et al.;* [51]. For rearing details and manipulation of all developmental stages under the laboratory controlled conditions, see Ghoneim *et al.;* [45].

CSIs and preparation of concentrations

The tested Benzoyl phenyl urea compounds, in the present study, are Noviflumuron and Novaluron.

Noviflumuron (Recruit III, and Recruit III AG) has the chemical name: N{[[3,5-dichloro-2-fluro-4-(1,1,2,3,3,3] hexa fuoro propoxy) phenyl] amino]carbonyl}-2,6difluorobenzamide. molecular Its formula is C17H7C12F9N2O3. It was kindly obtained from Laboratory of Pesticides, Agricultural Research Center, Doqqi, and Giza, Egypt. Novaluron (Rimon) has the chemical name: [1-[chloro-4-(1, 1. 2trifluoromethoxyethoxy) phenyl] 6--3-(2,difluorobenzoyl) urea]. Its molecular formula is C17H9C1F8N2O4. It was purchased from Sigma-Aldrich Chemicals. Five concentrations of each compound were prepared by diluting with distilled water in volumetric flasks as follows: Noviflumuron: 8.0, 4.0, 2.0, 1.0, 0.5 ppm. Novaluron: 5.0, 1.0, 0.5, 0.1, 0.05 ppm.

Egg treatment

Four groups of freshly emerged moths of P. gossypiella, each group 10 pairs $(\stackrel{\frown}{\circ} \stackrel{\frown}{\circ} X \stackrel{\frown}{\circ} \stackrel{\frown}{\circ})$, were confined in a glass chimney cage (17 cm height and 7.12 cm in diameter), inside which a piece of cotton wool previously soaked in 20% sugar solution was suspended to be renewed 48 hr for moths' nutrition [52]. After deposition of eggs on plastic papers at the top and bottom of the cage, these papers had been divided into four pieces in order to obtain four replicates from 1-day old eggs (10 eggs/replicate). Then, each replicate was carefully sprayed with each concentration of each compound using an atomizer. All egg papers were left for 15 min. to allow evaporation of excess water under laboratory conditions. Similar replicates of control eggs were treated with distilled water using the same technique.

Each of the CSI-treated and control replicates was kept separately in a suitable plastic jar for hatching under the controlled conditions $(27\pm2^{\circ}C \text{ and } 75\pm5\%$ R.H.). After the incubation period, each newly hatched larva was transferred into a glass tube (6.0x1.5 cm) containing 2 g of artificial diet. Each larva of treated and control replicates was observed daily for recording all parameters of survival, development, and metamorphosis.

Criteria of study

Ovicidal and lethal effects

The ovicidal effect of each CSI was estimated by the recorded mortality of eggs (%). The successfully hatched larvae of treated and control replicates were observed every day for determining the mortalities of all stages and corrected according to Abbott's formula [53] as follows:

% of test mortality - % of control mortality % of corrected mortality = _____X100 100 - % of control mortality The LC₅₀ values were calculated for the general mortality by Microsoft[®] office Excel (2007), according to Finney [54].

Developmental and metamorphic parameters Developmental rate:

Dempster's equation [55] was applied for calculating the developmental duration, and Richard's equation [56] was used for calculating the developmental rate.

Pupation rate: The pupation rate of the successfully developed pupae was calculated according to Jimenez-Peydro et al. [57] as follows:

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P.R. = [No. pupae / No. treated larvae] \times 100
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Deranged metamorphosis: Deranged metamorphosis program was observed and calculated in larval-pupal or pupal-adult intermediates (%). Also, pupal deformation was calculated in %.

Pupal water loss: Pupal water loss was calculated depending on the data of the initial and final weights of the pupae, as follows:

Water loss % = [initial weight – final weight /initial Weight] × 100

Statistical analysis of data

Data obtained were analyzed by the Student's *t*-distribution, and refined by Bessel correction [58] for the test significance of difference between means.

RESULTS

After treatment of 1-day old eggs of P. gossypiella with five concentrations of Noviflumuron, data of the ovicidal activity and lethal effects on larvae and pupae had been assorted in Table (1). On the basis of these data, Noviflumuron exhibited an ovicidal activity in a dose-dependent course (95.0, 87.5, 82.5, 75.0 and 65.0% of egg mortality, at 8.0, 4.0, 2.0, 1.0 and 0.5 ppm, respectively, vs. 12.5% mortality of control eggs). With regard to Novaluron, data distributed in Table (2) clearly reveal different egg mortalities after treatment of 1-day old eggs with five concentrations, proportional to the ascending concentration (92.5, 84.5, 55.0, 42.5 and 30.0% of egg mortality, at 5.0, 1.0, 0.5, 0.1 and 0.05 ppm, respectively, vs. 10.0% mortality of control eggs). Depending on data assorted in both tables, Noviflumuron exhibited stronger ovicidal activity than Novaluron (LC50 values: 0.188 and 0.274 ppm, respectively).

In the light of data included in Tables (1 and 2), treatment of 1-day old eggs with the highest concentration level of each compound resulted in

complete (100%) mortality of larvae. At other concentrations of Noviflumuron, larvicidal effect was detected in various larval mortalities (66.7, 37.5, 39.6 and 06.3%, at 4.0, 2.0, 1.0 and 0.5 ppm, respectively, in comparison of 05.6% mortality of control larvae, Table 1). Also, Novaluron exhibited a larvicidal effect on the successfully hatched larvae (50.0, 30.0, 22.8 and 18.0% mortality of larvae, at 1.0, 0.5, 0.1 and 0.05 ppm, respectively, compared to 11.2% mortality of control larvae, Table 2). On the other hand, both compounds failed to display pupicidal or adulticidal effects, since no pupal or adult mortality could be observed. LC50 values of Noviflumuron and Novaluron for larvae were estimated in 0.342 and 0.153 ppm, respectively, indicating that Novaluron appeared to be more toxic than Noviflumuron.

After treatment of 1-day old eggs with Noviflumuron concentrations, data of the most important developmental criteria were assorted in Table (3). According to these data, larval duration was insignificantly prolonged, in almost dose-dependent course (15.5±0.71, 15.5±0.5, 15.4±0.75 and 15.4±0.50 days, at 4.0, 2.0, 1.0 and 0.5 ppm, respectively, vs. 15.1±0.48 days of control larvae). Also, the developmental rate was slightly regressed, in a similar trend. In respect of the inhibitory effect of Noviflumuron on pupation, the determined pupation percentage was remarkably reduced, in a dosedependent fashion (33.3, 60.4, 62.5 and 93.7%, at 4.0, 2.0, 1.0 and 0.5 ppm, respectively, vs. 94.4% pupation of control insects). In addition, the pupation duration was slightly prolonged (for detail, see Table 3). Although Noviflumuron failed to exhibit a pupicidal effect, it induced the water loss of pupae, in a dosedependent manner (21.40, 19.40, 19.27 and 18.16%, at 4.0, 2.0, 1.0 and 0.5 ppm, respectively, vs. 10.11% water loss of control pupae). Depending on data included in the aforementioned table, Noviflumuron failed to affect the metamorphosis and morphogenesis programs, since no deformed larvae, deformed pupae, larval-pupal or pupal-adult intermediates had been produced.

With regard to the inhibitory effects of Novaluron on the development of *P. gossypiella*, Table (4) contained data of the most important parameters. Depending on these data, the larval duration was considerably prolonged after treatment with the highest concentration (18.7 ± 0.38 days, compared to 18.0 ± 0.10 days of control larvae) but slightly prolonged after treatment with other concentrations (18.5 ± 0.71 , 18.5 ± 0.69 and 18.0 ± 0.17 days, at 0.5, 0.1 and 0.05 ppm, respectively, *vs.* 18.0 ± 0.10 days of control congeners). Also, the developmental rate was slightly regressed, in no certain trend. In connection with the affected pupation process, treatment of 1-day old eggs with Novaluron resulted in conspicuously blocked pupation,

in a dose-dependent manner (50.0, 70.0, 77.2 and 82.0%, at 1.0, 0.5, 0.1 and 0.05 ppm, respectively, *vs.* 94.4% pupation on control insects). In addition, the pupal duration was slightly prolonged, in no certain trend (7.5 \pm 0.71, 7.6 \pm 0.43, 7.5 \pm 0.24 and 7.5 \pm 0.34 days, at 1.0, 0.5, 0.1 and 0.05 ppm, respectively, *vs.* 7.4 \pm 0.05 days of control pupae). Although Novaluron failed to exhibit a pupicidal action, pupae had been subjected to

a powerful desiccation action of this compound, since water loss was estimated in greater percentage than of control pupae (26.20, 17.45, 18.72 and 20.23%, at 1.0, 0.5, 0.1 and 0.05 ppm, respectively, *vs.* 10.11% from control pupae). As clearly shown in the previously mentioned table, neither metamorphosis nor morphogenesis of *P. gossypiella* had been affected by Novaluron treatments.

Table 1: Ovicidal activity and lethal effect (%) of Noviflumuron after treatment of 1-day old eggs of P.	gossypiella
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Conc. (ppm)	č		Larval mortality	Pupal mortality	Adult mortality	Total mortality	Corrected mortality	LC ₅₀	
	Mortality	Corrected mortality	LC ₅₀						
8.0	95.0	94.3		100.0	*	*	100.0	100.0	
4.0	87.5	85.7		66.7	00.0	00.0	95.0	93.93	
2.0	82.5	80.0	0 100	37.5	00.0	00.0	90.0	87.87	0.342
1.0	75.0	71.4	0.188	39.6	00.0	00.0	82.5	78.78	
0.5	65.0	60		06.3	00.0	00.0	67.5	60.60	
Control	12.5			05.6	00.0	00.0	17.5		

Conc.: Concentration level. *: no developed pupae or adults.

Table 2: Ovicidal activity and lethal effect (%) of Novaluron after treatment of 1-day old eggs of P. gossypiella

Conc. (ppm)	Ovicidal activity		Larval mortality	Pupal mortality	Adult mortality	Total mortality	Corrected mortality	LC ₅₀	
	Mortality	Corrected mortality	LC ₅₀						
5.0	92.5	91.7		100.0	*	*	100.0	100.0	
1.0	84.5	82.8		50.0	00.0	00.0	95.0	93.75	
0.5	55.0	50.0	0.074	30.0	00.0	00.0	67.5	59.37	0.153
0.1	42.5	36.1	0.274	22.8	00.0	00.0	55.0	43.75	
0.05	30.0	22.2		18.0	00.0	00.0	42.5	28.12	
Control	10.0			11.2	00.0	00.0	20.0		

Conc., *: see footnote of Table (1).

Table 3: Effect of Noviflumuron on the development of P. gossypiella after treatment of 1-day old eggs

Conc.	Larval stage			Pupal stage				
(ppm)	Duration(days) (mean ±SD)	Develop. Rate (%)	Larval- pupal Inter. (%)	Pupation (%)	Deformed pupae (%)	Duration (days) (mean ±SD)	Water loss (%)	
4.0	15.5±0.71 a	6.45	00.0	33.3	00.0	7.5±0.71 a	21.40	
2.0	15.5±0.50 a	6.45	00.0	60.4	00.0	7.2±0.28 a	19.40	
1.0	15.4±0.75 a	6.49	00.0	62.5	00.0	7.2±0.17 a	19.27	
0.5	15.4±0.50 a	6.49	00.0	93.7	00.0	7.3±0.46 a	18.16	
Control	15.1±0.48	6.62	00.0	94.4	00.0	7.0±0.37	10.11	

Conc.: see footnote of Table (1). Mean±SD followed with (a): not significantly different (P>0.05). Develop: Developmental, Inter.: Intermediates

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	Larval stage			Pupal stage					
Conc. (ppm)	Duration (days) (mean ±SD)	Develop. Rate (%)	Larval- pupal Inter. (%)	Pupation (%)	Deformed pupae (%)	Duration (days) (mean ±SD)	Water loss (%)		
1.0	18.7±0.38 b	5.35	00.0	50.0	00.0	7.5±0.71 a	26.20		
0.5	18.5±0.71 a	5.41	00.0	70.0	00.0	7.6±0.43 a	17.45		
0.1	18.5±0.69 a	5.41	00.0	77.2	00.0	7.5±0.24 a	18.72		
0.05	18.0±0.17 a	5.56	00.0	82.0	00.0	7.5±0.34 a	20.23		
Control	18.0±0.10	5.56	00.0	94.4	00.0	7.4±0.05	10.11		

Table 4: Effects of Novaluron on the development of *P. gossypiella* after treatment of 1-day old eggs

Conc. see footnote of Table (1). a, Develop., Inter.: see footnote of Table (3), Mean \pm SD followed with (b): significantly different (P<0.05).

DISCUSSION

Direct ovicidal activity of CSIs on P. gossypiella

On the basis of the available literature, some insecticides, insect growth regulators (IGRs) (including chitin synthesis inhibitors, CSIs) and botanicals had been reported to exhibit various ovicidal effects (egg mortality) on different ages of eggs of some insect species, such as Flufenoxuron and Hexaflumuron against Lobesia botrana [59]; LC50 values of abamectin and emamectin benzoate (Bacteria-based product) against Spodoptera littoralis [60]; Lufenuron against Lobesia botrana [61]; pyridalyl and hexaflumuron against Plutella xylostella [62]; Buprofezin and Diflubenzuron against Diaphorina citri [63]; emamectin benzoate and pyridalyl against Tuta absoluta [64]; piperine (extracted from the black pepper *Piper nigrum*) against Spodoptera frugiperda and Diatraea saccharalis [65] and thiodicarb against Spodoptera litura [66]. Recently, treatment of Corcyra cephalonica eggs, of different ages, with Fenoxycarb resulted in considerable egg mortality [67]. After treatment of with Tribolium castaneum eggs Pvriproxvfen. Methoxyfenozide and Lufenuron, the highest ovicidal effect (100% egg mortality) was exhibited by Pyriproxyfen, followed by Methoxyfenozide and Lufenuron, respectively [68]. After treatment of 1- and 3-day old egg masses of S. littoralis with LC50 of the sesame oil, the 1-day-old egg masses are more susceptible than 3-day-old eggs [69]. Ovicidal effect of Prunus mahaleb kernels oil (containing basic fatty acids, heneicosane and β -sitosterol) on *S. littoralis* eggs depended on the age of treated eggs and the method of treatment [70]. With regard to Pectinophora gossypiella, Amer et al.; [64] found that emamectin benzoate was more effective than pyridalyl for the ovicidal action on 1-day old eggs.

Results of the present study were, to a great extent, in agreement with those reported results, since Noviflumuron and Novaluron exhibited ovicidal activities on the treated 1-day old eggs of *P*. *gossypiella*, in a dose-dependent course. Noviflumuron possesses stronger ovicidal property than Novaluron. To explicate the ovicidal effect (egg mortality) of Noviflumuron and Novaluron on *P. gossypiella*, in the present study, some suggestions can be conceivable. Egg mortality may be due to the adverse effects of these CSIs on the chorion of eggs before hatching [71]. The present CSIs penetrated into eggs and might interfere with the embryonic cuticle synthesis and subsequently the malformed muscles could not enable the developed embryos to hatch from the egg wall [72].

Latent toxicity of CSIs on P. gossypiella

The available literature contains many reported results of toxic effects of several insect growth regulators (IGRs)(Juvenoids, ecdysteroids and chitin synthesis inhibitors, CSIs) on various insect species, such as Spodoptera littoralis by Diflubenzuron [73], Triflumuron [74], Flufenoxuron [75], Lufenuron [76,77], Buprofezin [78], Cyromazine [79]; Papilio demoleus by Diofenolan [80]; Eurygaster integriceps Pyriproxyfen [81]; Dysdercus koenigii by bv Flufenoxuron Halvomorpha by [82]; halvs Diflubenzuron [83]; S. litura by Chlorfluazuron [84]; Culex pipiens by Kinoprene [85]; Agrotis ipsilon by Flufenoxuron and Methoprene[86] and Tribolium castaneum by Lufenuron [87]. Recently, IGRs of different categories exhibited varying degrees of toxicity against some insects, such as Pyriproxyfen against Spodoptera mauritia [88]; Lufenuron and Methoxyfenozide against *T. castaneum* [68]; Methoxyfenozide against C. pipiens [89]; RH-5849 and Tebufenozide (RH-5992) against Ephestia kuehniella [90]; Lufenuron against Glyphodes pyloalis [91] and Helicoverpa armigera [92]; Fenoxycarb against Corcyra cephalonica [93, 67]; Buprofezin against Paracoccus marginatus [94]; Chlorfluazuron, Cyromazine, Lufenuron, and Precocene I against Ctenocephalides [95]; Methoprene felis and Pyriproxyfen against *Culex guinguefasciatus* and *Aedes* albopictus [96]; Cyromazine against Musca domestica [97] and Novaluron against P. gossypiella [45].

Results of the current study on *P. gossypiella*, to some extent, are in accordance with those reported

results, since treatment of 1-day old eggs of P. gossypiella with Noviflumuron or Novaluron resulted in complete (100%) larval mortality at the highest concentration level (8.0 and 5.0 ppm, respectively) and various degrees of toxicity on larvae at other concentrations. In contrast, both CSIs failed to exhibit pupicidal or adulticidal effects, since no pupal or adult mortality was observed. The larval deaths of P. gossypiella by Noviflumuron and Novaluron, in the current investigation, may be attributed to the failure of larvae to moult owing to the inhibition of chitin formation [98], to the inability to shed their exocuticle [99], or to swallow volumes if air for splitting the old cuticle and expand the new one during ecdysis [100]. Also, these larval deaths may be due to a prohibition of feeding and continuous starvation of the present insect [101].

According to the available literature, a great variation in the LC50 values of different IGRs had been reported against various insect species, such as 350.45 and 453.78 ppm of Novaluron and lufenuron, respectively, against Spodoptera litura [102]; 8.47 mg /L of Hexaflumuron against H. armigera [103]; 0.05 and 0.005 µg/insect of the ecdysone agonists RH-5849 and RH-5992 (Tebufenozide), respectively, against Ephestia kuehniella [90]; 24.54 µg/L of Methoxyfenozide against C. pipiens [89]; 19 ppm of Lufenuron against G. pyloalis [91]; etc. These reported LC50 values were determined depending on the larval treatment with IGRs.

In addition, treatment of eggs with IGRs or other compounds resulted in various LC50 values as reported for the present experimental insect, P. gossypiella, since LC50 values of Hexaflumuron were found 3.754, 2.863 and 2.004 ppm after treatment of 1-, 2- and pre-hatching day-old eggs [52]. After treatment of 4-day old eggs with Lufenuron, El-Shennawy [104] estimated LC₅₀ in 2.276 ppm. Depending on the LC₅₀ values of soluble liquid formulations of tar oil, 1-day old eggs were the most susceptible (highest egg mortality%) among other ages of eggs [105]. After treatment of 1-day old eggs, LC50 values of Lufenuron, Chlorfluazuron and Chromafenozide were calculated in 3.471, 4.189 and 122.703 ppm, respectively [5]. After treatment of 1-day old eggs, LC50 values of Radiant (Spintoram) and Profenofos were found 3.94 and 0.031 ppm, respectively [106]. In the current investigation on P. gossypiella, Noviflumuron exhibited stronger ovicidal activity than Novaluron (LC50 values: 0.188 and 0.274 ppm, respectively) and vice versa for larvicidal activity (LC50 values: 0.153 and 0.342 ppm of Novaluron and Noviflumuron, respectively). In the light of the present results on P. gossypiella and other reported results of larval or egg treatment with IGRs or other compounds, the estimated LC50 values depend on several factors, such as susceptibility of the insect and

its treated stage or instar, ovicidal or lethal potency of the tested compound and its concentration levels, age of the treated eggs, method and time of treatment, as well as the experimental conditions.

Effects of CSIs on the development of P. gossypiella

Depending on the currently available literature, development of various insects had been retarded (as expressed in prolonged larval and pupal durations) after treatment of larvae with different IGRs, as reported for S. littoralis by Diflubenzuron [73], Methoprene and Fenoxycarb [107], Lufenuron [108], Novaluron [43], Cyromazine [79]; P. demoleus by Diofenolan [80]; S. litura by Chlorfluazuron [84]; A. aegypti [109] and C. *pipiens* [110,111] by Novaluron; C. *pipiens* by Kinoprene [85] and A. ipsilon by Methoprene and Flufenoxuron [86]; Plutella xylostella by Pyriproxyfen [112]; G. pyloalis by Lufenuron [91]; C. pipiens by Methoxyfenozide [89]; Corcyra cephalonica by Fenoxycarb [67]; etc. Also, the development of some insects was retarded after treatment of eggs with different IGRs, as reported for S. littoralis after treatment of 1- and 3-day old egg masses with LC50 of the sesame oil [69] and for P. gossypiella after treatment of 1-, 2- and 3-day old eggs with Hexaflumuron [52] as well as after treatment of 1-day old eggs with LC₅₀ values of Lufenuron, Chlorfluazuron and Chromafenozide [5].

The retarded development of P. gossypiella were found in corroboration with those reported results, since treatment of 1-day old eggs with different concentrations of Noviflumuron and Novaluron, in the present study, resulted in an insignificantly or remarkably prolonged larval and pupal durations, in a dose-dependent course by Noviflumuron but in no certain trend by Novaluron. In a similar course, the developmental rate was slightly regressed. In addition, the present results are in accordance with those reported results of retarded development of the same lepidopterous insect after treatment of newly hatched larvae with Hexaflumuron [113]; Diflubenzuron and Chlorfluazuron [114]; Buprofezin [115]; Teflubenzuron [116]; and Chromafenozide and Diflubenzuron [117]; Lufenuron and Pyriproxyfen [23] and Novaluron [45].

In the current study, retarded development of *P. gossypiella* by egg treatments with Noviflumuron and Novaluron (as expressed in prolonged larval and pupal durations) may be attributed to the indirect interference of these CSIs with neuroendocrine organs responsible for the synthesis and release of tropic hormones, like prothoracicotropic hormone (PTTH) [118]. Also, the tested CSIs may affect the tissues and cells undergoing mitosis [119] or exhibit a delaying effect on the ecdysis and transformation [100]. In particular, the final step of chitin biosynthesis pathway was inhibited by the present CSIs and the precursor was

not converted into chitin leading to a prolongation of larval life [111].

Disrupted metamorphosis of P. gossypiella by CSIs

Depending on the available literature, the symptoms and features of the impaired major metamorphosis of an insect after treatment with various IGRs (including CSIs) had been described as reduction of pupation and adult emergence, production of larvalpupal and/or pupal-adult intermediates, deformed larvae and/or pupae and the production of supernumerary larval instars (superlarvae). However, all or some of these disrupted features were recorded in various insects by some IGRs, such as Chrysoperla rufilabris by Fenoxycarb [120]; H. armigera [121], Phlebotomus papatasi [122], A. aegypti [123], M. domestica and Stomoxys calcitrans [124] by Novaluron; S. littoralis by Chlorfluazuron [125], Triflumuron [75], Lufenuron [76], Flufenoxuron [75], Methoprene and Fenoxycarb [107], Novaluron [43] and Cyromazine [79]; T. castaneum [126], Liriomyza trifolii [127] and Callosobruchus maculates [128] by Cyromazine; Rhynchophorus ferrugineus [129] and P. demoleus [80] by Diofenolan; Lobesia botrana by Lufenuron [130]; C. pipiens by Kinoprene [85]; etc.

In the present study, our results disagree with the previously reported results, because Noviflumuron and Novaluron could not affect the metamorphosis or morphogenesis of P. gossypiella after treatment of 1day old eggs, viz. no deformed larvae, deformed pupae, larval-pupal or pupal-adult intermediates had been produced. The inconsistency of the present results with those reported results may be due to the feeble potency of tested CSIs, different responsiveness of our experimental insect, stage under treatment or other factors. Moreover, few authors reported various disruptive effects of other CSIs on metamorphosis and/or morphogenesis of the same lepidopterous insect after treatment of eggs of different ages with Hexaflumuron [52] or Lufenuron, Chlorfluazuron and Chromafenozide [5].

However, it is important to point out that treatment of 1-day old eggs of P. gossypiella, in the current investigation, with Noviflumuron led to a slight reduction of pupation but it was conspicuously hindered after treatment with Novaluron, in a dose-dependent manner. These results are, to a great extent, consistent with those reported results of reduced pupation rate of some insects by various IGRs, such as P. xylostella by Hexaflumuron [131]; S. littoralis by Novaluron [43] and Cyromazine [79]; G. pyloalis by Lufenuron [91] and Fenoxycarb [93]; Encarsia formosa bv Pyriproxyfen and Fenoxycarb [132]; etc. To understand the hindered pupation process of P. gossypiella, after treatment of 1-day old eggs with Noviflumuron and Novaluron in the present study, these compounds may

interfere with the hormonal regulation of the pupation program by prohibiting the ecdysteroid synthesis or release [133]. Also, these CSIs might block such program *via* the disturbance of chitin synthase which is necessary for the pupal cuticle formation [134].

CONCLUSION

Although Noviflumuron and Novaluron failed to affect the survival of pupae and adults of P*gossypiella*, they exhibited high toxicities on eggs and larvae as well as retarded development and blocked pupation had been recorded after treatment of 1-day old eggs. From the practical point of view, these compounds appeared to be effective for controlling this pest which has developed resistance against the majority of conventional pesticides.

ACKNOWLEDGEMENTS

Authors greatly thank Laboratory of Pesticides, Agricultural Research Center, Doqqi, Giza, Egypt, for kindly providing the Benzoyl phenyl urea Noviflumuron.

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