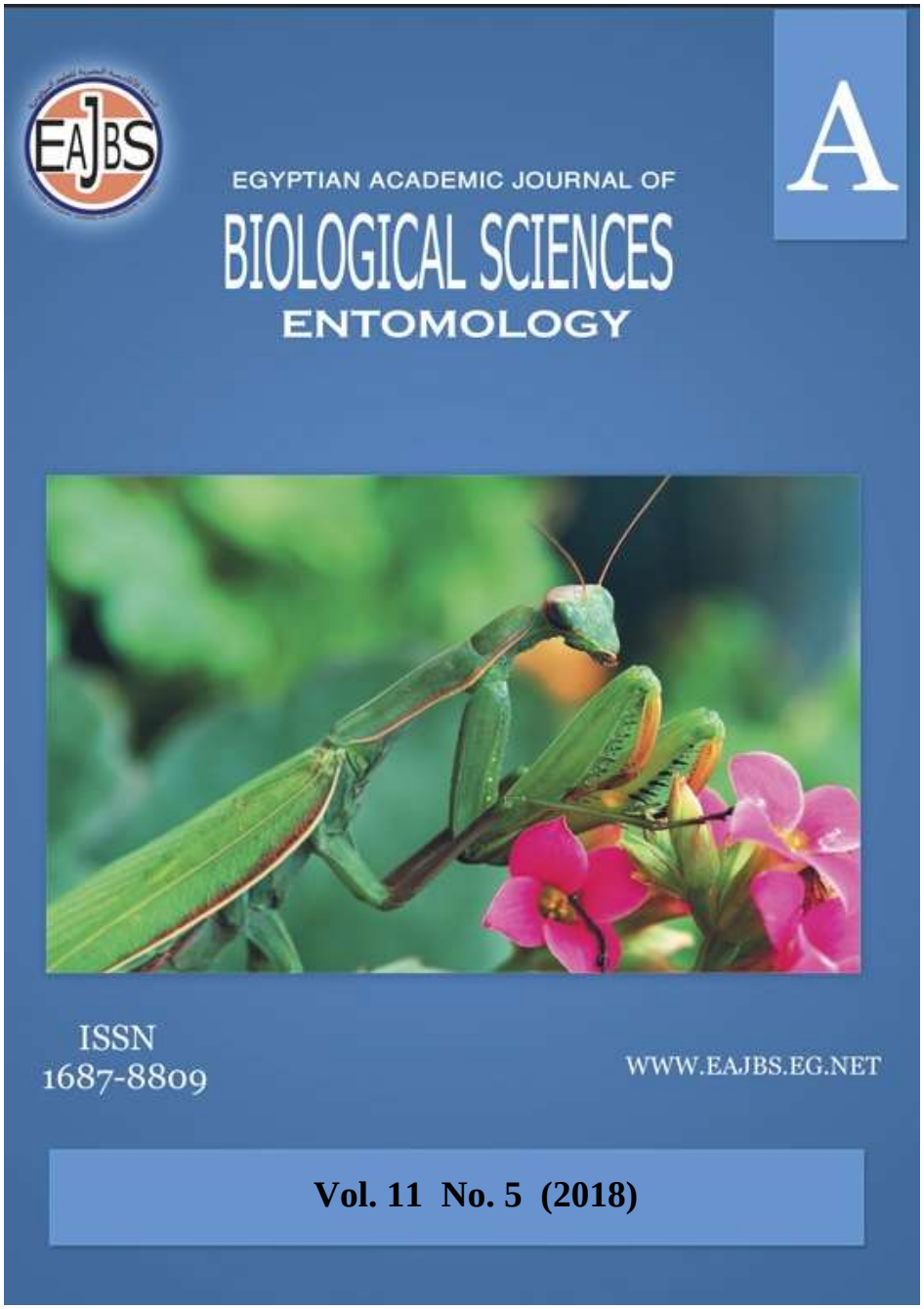

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Disruptive Effects of Certain Chitin Synthesis Inhibitors on Adult Life Parameters and Reproductive Potential of the Pink Bollworm, *Pectinophora gossypiella* (Saunders)(Lepidoptera: Gelechidae).

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ABSTRACT

The pink bollworm, *Pectinophora gossypiella*, is a dangerous pest of cotton worldwide, The objective of the current study was to evaluate the disruptive effects of the chitin synthesis inhibitors, noviflumuron and novaluron, on the adult performance and reproductive potential of this pest after treatment of 1-day old eggs with sublethal concentrations of noviflumuron (4.0, 2.0, 1.0 and 0.5 ppm) and novaluron (1.0, 0.5, 0.1 and 0.05 ppm). The adult emergence was considerably blocked by both compounds but they failed to affect the adult survival or morphogenesis. The total adult longevity was remarkably shortened. Noviflumuron treatment caused a slight prolongation or shortening of pre-oviposition period, depending on the concentration but novaluron treatment led to slightly prolonged period. The oviposition period was remarkably shortened. Prolonged post-oviposition period was recorded by both compounds. The oviposition efficiency was significantly prohibited, regardless the tested compound. Fecundity of the successfully reproducing females and fertility of eggs laid by these females were drastically reduced. The incubation period of eggs was considerably prolonged by both compounds.

INTRODUCTION

The pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae), is one of the destructive insects attacking cotton plant in the world (Patil, 2003; Mohammed, 2013; Kranthi, 2015), and it is a pest difficult to control (Liu *et al*., 2009). This insect pest attacks cotton plants in Egypt during the flowering and fruiting stages causing enormous reduction in quantity and quality of cotton yield (Khidr *et al*., 1996; El-Aswad and Aly, 2007; Khatter and Abuldahb, 2011; Kandil *et al*., 2012). In Egypt**,** also, control of *P. gossypiella* depends mostly on the use of the conventional insecticides (Radwan and El-Malla, 2015). This pest has recently developed high resistance against most of those insecticides used because of its ability to detoxify these chemicals (Abd-Elhady and Abd El-Aal, 2011; Sabry and Abdel-Aziz, 2013). Moreover, it has develops resistance to the transgenic cotton

varieties in USA (Fabrick and Tabashnik, 2012) and India (Monsanto, 2010). In addition to the development of resistance, the intensive use of many conventional pesticides led to several drastic problems, such as the environmental pollution, hazards to human and animals, destruction of the natural enemies (Rose, 2001; Rashad *et al*., 2015). Therefore, alternative control agents have been initiated recently to minimize the pesticide hazards (Derbalah *et al.*, 2014) and to delay the resistance development in *P. gossypiella* (Salama *et al*., 2013).

During the late few decades, a new class of comparatively safe compounds have been developed and known as insect growth regulators (IGRs) (Dhadialla *et al*., 1998; Khan and Qamar, 2012). In contrast to the conventional insecticides, IGRs are not directly toxic, but act selectively on the development, metamorphosis or reproduction of the target insect (Hoffmann and Lorenz, 1998; Martins and Silva, 2004). They are quite selective in their mode of action and potentially act only on the target species (Sabry and Abdou, 2016). Chitin synthesis inhibitors (CSIs) are usually classified in IGRs (Tunaz and Uygun, 2004) interfering with chitin biosynthesis in insects and thus prevents moulting, or produces an imperfect cuticle (Hammock and Quistad, 1981). These compounds affect, also, the hormonal balance in insects, thereby resulting in physiological disturbances (Soltani *et al*., 1984).

Noviflumuron is a new chemistry currently being developed by Dow AgroSciences, Indianapolis, Indiana, USA, for the structural pest control market (Ameen *et al*., 2002). Noviflumuron is a benzoylphenyl urea CSI that prevents the successful molting and development of some insects, such as fleas, ants, termites and houseflies (Sheets and Karr, 2001; Karr *et al*., 2004). 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 (Ameen *et al*., 2005; Smith *et al*., 2002; Wang and Bennett, 2006). Also, adult *B. germanica* exposed to Noviflumuron fail to produce viable eggs, hence their reproductive potential is reduced (King, 2005).

Novaluron is a relatively new benzoylphenyl urea CSI with low mammalian toxicity (Barazani, 2001; Ishaaya and Horowitz, 2002). The compound has no appreciable effect on parasitoids and has probably a mild effect on the natural enemies (Ishaaya *et al*., 2001, 2002). 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 (Malhat *et al*., 2014). Novaluron is a powerful suppressor of the pest populations, such as *Bemisia tabaci* and *Trialeurodes vaporariorum* (Ishaaya *et al*., 2003). It acts by ingestion and contact against several insect pests, such as *Spodoptera* spp., *Tuta absoluta*, *Helicoverpa armigera*, and *Liriomyza huidobrensis* (Kim *et al*., 2000). It exhibited, also, a good activity against the Colorado potato beetle (Cutler *et al*., 2005 a,b, 2007; Alyokhin *et al*., 2009) and impaired the development (Ghoneim *et al*., 2015) and adult performance (Hamadah *et al*., 2015) 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 (Ghoneim *et al*., 2017a), disrupted adult performance, inhibited reproductive potential (Hassan *et al*., 2017), declined main metabolites (Tanani *et al*., 2017), and deteriorated larval haemogram (Ghoneim *et al*., 2017b).

Few studies had been conducted investigating the ovicidal effects of IGRs on eggs of lepidopterous pests but the majority focused on treatment of larval stage (Abd-El-Aziz and Sayed, 2014). Taking all of these considerations into account, the present study was carried out to evaluate the long-term effects of Noviflumuron and Novaluron, novel CSIs, on the adult performance parameters and reproductive potential of *P. gossypiella* after treatment of 1-day old eggs.

MATERIALS AND METHODS

1. 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 in Plant Protection Research Institute, Giza, Egypt. It was reared under constant conditions (27±2°C and 75±5% 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*. (1982). For rearing details and manipulation of all developmental stages under the laboratory controlled conditions, see Ghoneim *et al*. (2017a).

2. CSIs and Preparation of Concentrations:

The tested Benzoylphenylurea compounds, in the present study, were 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 hexafuoropropoxy) phenyl] amino]carbonyl}-2,6-difluorobenzamide with molecular formula $C_{17}H_7Cl_2F_9N_2O_3$. It was kindly obtained from Dr. Heba Hassan, Plant Protection Research Institute, Giza, Egypt. Novaluron (Rimon) has the chemical name: [1-[chloro-4-(1,1,2-trifluoromethoxyethoxy) phenyl] -3- (2,6-difluorobenzoyl) urea] and molecular formula $C_{17}H_9C_lF_8N_2O_4$. It was purchased from Sigma-Aldrich Chemicals. Four concentrations of each CSI were prepared by diluting with distilled water in volumetric flasks as follows: Noviflumuron: 4.0, 2.0, 1.0 and 0.5 ppm. Novaluron: 1.0, 0.5, 0.1 and 0.05 ppm.

3. Egg Treatment:

Four groups of freshly emerged moths of *P. gossypiella*, each group 10 pairs (♂♂ X♀♀), 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 (El-Barkey *et al*. 2009). 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 of 1-day old eggs (10 eggs/replicate). Then, each replicate was carefully sprayed with each concentration of each CSI 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 only using the same technique.

Each of the CSI-treated and control replicates were kept separately in a suitable plastic jar for hatching under the controlled conditions (27±2°C and 75±5% 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. The treated and control larvae and pupae were carefully handled until the adult emergence. Newly emerged moths were sexed and transferred to chimney glass cage (six pairs /cage). The moths were fed on 20% sucrose solution. Cages were examined daily to record all parameters of adult performance and reproductive criteria.

4. Adult Performance Parameters:

Adult Emergence: Number of emerged adults was expressed in % according to Jimenez-Peydro *et al*. (1995) as follows:

[No. of completely emerged adults / No. of pupae] × 100

Adulticidal activity: Adulticidal activity of the tested CSIs was detected by mortalities throughout the adult longevity and calculated in percentage.

Morphogenic Efficiency: The morphogenic efficiency of the tested CSIs was detected by the adult deformities and calculated in percentage as follows:

[No. of deformed adults / No. of emerged adults] × 100

Adult longevity: Total adult longevity of females was measured in mean days±SD. The major compartments of adult longevity are pre-oviposition (ovarian maturation) period, oviposition period (reproductive life-time) and post-oviposition period. All durations were measured in mean days±SD.

5. Reproductive Parameters

Oviposition rate was calculated as follows:

Number of laid eggs per ♀/reproductive lifetime (in days).

Eggs laid by the successfully reproducing adult females were counted for calculating the number of eggs per female (Fecundity). The laid eggs were kept in Petri dishes under the same controlled laboratory conditions as previously mentioned. Just after the oviposition, eggs were observed until hatching elapsing an incubation period (in days). The hatchability (Fertility) was usually expressed in hatching percentage of laid eggs. Sterility index was calculated according to Toppozada *et al.* (1966) as follows:

Sterility Index = 100 – [(a b / A B) × 100]

Where: a: mean number of eggs laid per female in the treatment. b: percentage of hatching in the treatment. A: mean number of eggs laid per female in the controls. B: percentage of hatching in the controls.

6. Statistical Analysis of Data:

Data obtained were analyzed by the Student's *t*-distribution, and refined by Bessel correction (Moroney, 1956) for the test significance of difference between means.

RESULTS

1. Effects of CSIs on Adult Performance of *P. gossypiella*:

After treatment of 1-day old eggs of *P. gossypiella* with four sublethal concentrations (4.0, 2.0, 1.0 and 0.5 ppm) of noviflumuron, data of the adult performance parameters were arranged in Table (1). Depending on these data, the adult emergence was considerably blocked, in a dose-dependent course (90.0, 88.25, 88.17 and 78.44 emergence %, at 0.5, 1.0, 2.0 and 4.0 ppm, respectively, *vs.* 100% emergence of control adult females). Noviflumuron failed to exhibit neither adulticidal nor morphogenic effect, since no mortality or adult deformity had been observed. With regard to the adult longevity and its main compartments, data contained in the same table clearly revealed that the total longevity of adult females was remarkably shortened, in a dose-dependent manner (13.7±0.96, 13.5±0.71, 13.2±0.50 and 10.5±0.80 days, at 0.5, 1.0, 2.0 and 4.0 ppm, respectively, in comparison with 15.0±0.82 days of control adult females). In respect of the pre-oviposition period (ovarian maturation period), treatment of 1-day old eggs with the highest concentration level led to failure of the successfully emerged adult females to lay eggs, thus the pre-oviposition period could not be measured. However, such period was affected after treatment of eggs with other concentrations, in no certain

trend (3.5±1.29, 1.5±0.71 and 2.5±1.80 days, at 0.5, 1.0 and 2.0 ppm, respectively, *vs*. 2.3±0.50 days of control females). Moreover, noviflumuron exerted a tremendously enforcing action on the ovipositing adult females to quickly lay eggs, since their oviposition period (reproductive life-time) was significantly shortened, in a reverse course of concentration (8.7±0.90, 8.5±0.71 and 8.0±1.41 days, at 2.0, 1.0 and 0.5 ppm, respectively, compared to 11.3±0.96 days of control females). The last compartment of adult longevity is post-oviposition period which was pronouncedly prolonged, in no certain trend (3.3±0.77, 3.5±0.71 and 2.2±0.50 days, at 2.0, 1.0 and 0.5 ppm, respectively, *vs*. 1.5±1.29 days of control females).

Table 1. Adult performance of *P. gossypiella* as affected by treatment of 1-day old eggs with noviflumuron.

Conc. (ppm)	Adult emergence (%)	Adult mortality (%)	Adult deformations (%)	Adult longevity (mean days ±SD)			
				Ovarian maturation period	Reproductive lifetime	Post-oviposition period	Total longevity
4.0	78.44	00.0	00.0	---	---	---	10.5±0.80 c
2.0	88.17	00.0	00.0	2.5±1.80 a	8.7±0.90 b	3.3±0.77 b	13.2±0.50 b
1.0	88.25	00.0	00.0	1.5±0.71 a	8.5±0.71 b	3.5±0.71 b	1 b
0.5	90.0	00.0	00.0	3.5±1.29 a	8.0±1.41 c	2.2±0.50 b	13.7±0.96 a
	100.0	00.0	00.0	2.3±0.50	11.3±0.96	1.5±1.29	15.0±0.82

Conc.: concentration level. Mean±SD followed with a: not significantly different (P>0.05), b: significantly different (P<0.05), c: highly significantly different (P<0.01). ---: adult females failed to lay eggs although mating with normal males was done.

Table 2. Adult performance of *P. gossypiella* as affected by treatment of 1-day old eggs with novaluron.

Conc. (ppm)	Adult emergence (%)	Adult mortality (%)	Adult deformations (%)	Adult longevity (mean days ±SD)			
				Ovarian maturation period	Reproductive life-time	Post-oviposition period	Total longevity
1.0	75.50	00.0	00.0	---	---	---	11.8±0.50 b
0.5	75.88	00.0	00.0	2.9±0.96 a	8.5±1.29 b	3.0±1.41 a	14.3±0.96 a
0.1	87.50	00.0	00.0	2.8±0.58 a	9.0±0.82 b	2.3±0.96 a	13.8±0.96 a
0.05	93.80	00.0	00.0	2.5±0.58 a	10.3±1.71 b	2.0±0.82 a	14.8±0.50 a
Control	100.0	00.0	00.0	2.3±0.50	11.0±0.82	1.5±0.58	14.8±0.50

Conc., a, b, ---: see footnote of Table 1.

After treatment of 1-day old eggs of *P. gossypiella* with four sublethal concentrations (1.0, 0.5, 0.1 and 0.05 ppm) of novaluron, data of the adult life parameters were assorted in Table (2). On the basis of these data, the adult emergence was remarkably blocked, in a dose-dependent course (93.80, 87.50, 75.88 and 75.50 emergence %, at 0.05, 0.1, 0.5 and 1.0 ppm, respectively, *vs*. 100% emergence of control adult females). Novaluron failed to affect the survival of successfully emerged adult females or their morphogenesis, since no mortality or adult malformations had been observed. In connection with the adult longevity and its main compartments, data included in the same table exiguously demonstrated that the total longevity was elaborately shortened only at the highest concentration level (11.8±0.50 days, compared to 14.8±0.50 days of controls) but slightly shortened at other concentrations, in no certain trend. After treatment of eggs with the highest concentration level of novaluron, the emerged adult females failed to lay eggs, thus no pre-oviposition period could be estimated. At other concentrations, the pre-oviposition period was unremarkably prolonged, in a dose-dependent fashion

(2.5±0.58, 2.8±0.58 and 2.9±0.96 days, at 0.05, 0.1 and 0.5 ppm, respectively, *vs*. 2.3±0.50 days of control adult females), indicating a slight retarding effect of Novaluron on the ovarian maturation rate. On the other hand, the oviposition period was seriously shortened, in a dose-dependent course (10.3±1.71, 9.0±0.82 and 8.5±1.29 days, at 0.05, 0.1 and 0.5 ppm, respectively, *vs*. 11.0±0.82 days of control congeners) denoting a powerful inducing action of novaluron on the ovipositing females to lay eggs quickly. Also, data of the aforementioned table clearly displayed that the post-oviposition period was slightly prolonged, in a dose-dependent trend 2.0±0.82, 2.3±0.96 and 3.0±1.41 days, at 0.05, 0.1 and 0.5 ppm, respectively, *vs*. 1.5±0.58 days of control congeners). For the comparative purpose, there is no remarkable difference in effect on noviflumuron and novaluron on adult survival, morphogenesis or blocking emergence. However, noviflumuron exhibited a relatively stronger shortening effect than novaluron on the total adult longevity and oviposition period.

2. Effects of CSIs on the Reproductive Potential of *P. gossypiella*:

After treatment of 1-day old eggs of *P. gossypiella* with sublethal concentrations of noviflumuron, data of the most important reproductive criteria had been distributed in Table (3). Depending on these data, the highest concentration level could be described as the extremely anti-reproductive one because the successfully emerged adult females failed to lay eggs in spite of the mating with normal adult males. The oviposition efficiency of treated females was tremendously prohibited by noviflumuron, since the oviposition rate was drastically regressed, in a dose-dependent course (3.4±1.50, 3.1±0.49 and 2.2±1.20, at 0.5, 1.0 and 2.0 ppm, respectively, *vs*. 15.4±1.45 of control congeners). Data of the same table evidently revealed that the fecundity (mean number of eggs/♀) was dramatically reduced, in a consecutive correlation with the concentration (28±14.90, 26±2.12 and 20±2.30 eggs/treated ♀, at 0.5, 1.0 and 2.0 ppm, respectively, *vs*. 174±24.39 eggs/control ♀). Another parameter of the reproductive capacity is fertility (hatching % of laid eggs or egg viability) which was severely reduced, in a dose-dependent manner (38.4, 29.4 and 10.0%, at 0.5, 1.0 and 2.0 ppm, respectively, *vs*. 71.2% of control hatchability). In other words, the sterility index considerably increased as the concentration was increased. With regard to the embryonic development in the laid eggs, it was seriously retarded by noviflumuron, as detected by the remarkably prolonged incubation period (4.8±0.50, 5.5±0.71 and 5.8±0.50 days, at 0.5, 1.0 and 2.0 ppm, respectively, *vs*. 4.3±0.0 days of laid eggs by control females).

Table 3. Reproductive potential of *P. gossypiella* adults as affected by treatments of 1-day old eggs with noviflumuron.

Conc. (ppm)	Oviposition rate	Fecundity (No. of eggs/♀±SD)	Fertility (%)	Sterility index (%)	Incubation period (mean days±SD)
4.0	0.0	---	---	---	---
2.0	2.2±1.20 d	20±2.30 c	10.0	98.38	5.8±0.50 b
1.0	3.1±0.49 d	26±2.12 c	29.4	93.83	5.5±0.71 b
0.5	3.4±1.50 d	28±14.90 d	38.4	91.32	4.8±0.50 b
Control	15.4±1.45	174±24.39	71.2	---	4.3±0.50

Conc., a, b, c, ---: see footnote of Table 1. d: very highly significantly different (P<0.001).

After treatment of 1-day old eggs of *P. gossypiella* with the sublethal concentrations of novaluron, data of the most important reproductive criteria were summarized in Table (4). As obviously shown in this table, no oviposition was observed for the adult females after treatment of 1-day old eggs with the highest concentration. Thus, no reproductive data could be recorded. At other concentrations, the oviposition efficiency was tremendously inhibited by novaluron, since the oviposition rate was severely regressed, in a dose-dependent course (10.2±5.02, 7.3±1.55 and 4.8±1.18, at 0.05, 0.1 and 0.5 ppm, respectively, *vs*. 19.7±4.27 of control congeners). Also, fecundity was drastically reduced, proportional to the concentration (109±57.19, 65±11.34 and 41±12.19 eggs/treated ♀, at 0.05, 0.1 and 0.5 ppm, respectively, *vs*. 218±56.12 eggs/control ♀). Sterility index considerably increased as the concentration was increased. In addition, data of the same table clearly displayed that novaluron exerted a strong retarding action on the embryonic development, since the incubation period was significantly prolonged, in a dose-dependent course (4.8±0.5, 4.8±0.50 and 4.9±0.58 days, at 0.05, 0.1 and 0.5 ppm, respectively, *vs*. 4.3±0.5 days of eggs laid by control females).

Table 4. Reproductive potential of *P. gossypiella* adults as affected by treatments of 1-day old eggs with novaluron.

Conc. (ppm)	Oviposition rate	Fecundity (mean No. of eggs/♀±SD)	Fertility (%)	Sterility index (%)	Incubation period (mean days ±SD)
1.0	0.0	---	---	---	---
0.5	4.8±1.18 d	41±12.19 d	31.1	91.89	4.9±0.58 b
0.1	7.3±1.55 c	65±11.34 c	45.3	81.27	4.8±0.50 b
0.05	10.2±5.02 b	109±57.19 b	49.8	65.46	4.8±0.50 b
Control	19.7±4.27	218±56.12	72.1	---	4.3±0.50

Conc., a, b, c, ---: see footnote of Table 1. d: see footnote of Table 3.

For the comparative purpose, the deteriorating effect of noviflumuron on the oviposition rate, fecundity and fertility appeared to be stronger than that exhibited by novaluron. In respect of the incubation period, and subsequently the embryonic development, there was no considerable difference between the potencies of the tested CSIs.

DISCUSSION

1. Influenced Adult Performance of *P. gossypiella* by CSIs:

1.1. Blocked Adult Emergence:

The majority of reported studies focused on the investigation of the effects of IGRs after treatment of larvae. The adult emergence of different insect species was significantly hindered after treatment of larvae with several IGRs (including CSIs), such as *Plutella xylostella* by hexaflumuron (Mahmoudvand *et al*., 2012); *Drosophila melanogaster* by pyriproxyfen (Benseba *et al*., 2015); *Spodoptera littoralis* by novaluron (Ghoneim *et al*., 2015) or cyromazine (Tanani *et al*., 2015); *Glyphodes pyloalis* by lufenuron (Aliabadi *et al*., 2016); *Culex quinquefasciatus* and *Aedes albopictus* by pyriproxyfen and methoprene (Khan *et al*., 2016); *P. gossypiella* by novaluron (Hassan *et al*., 2017). Moreover, adult emergence was

completely blocked in *Corcyra cephalonica* after treatment of 4th instar larvae with fenoxycarb (Singh and Tiwari, 2016). In addition, treatment of *Encarsia formosa* pupae with Pyriproxyfen resulted in prohibited adult emergence (Wang and Liu, 2016).

On the other hand, very scarce results of blocked adult emergence had been reported after treatment of eggs, such as the blocked adult emergence of *P. gossypiella* after treatment of pre-hatching eggs with LC_{50} of hexaflumuron (El-Barkey *et al*., 2009). In agreement with those reported results, adult emergence of *P. gossypiella*, in the present study, was drastically blocked after treatment of 1-day old eggs with sublethal concentrations of noviflumuron and novaluron, in a dose-dependent course. The present result of blocked adult emergence can be interpreted by the interference of the tested CSIs with some aspects of the hormonal regulation, such as disturbance of release of adult eclosion hormone and/or inhibition of the neurosecretion (prothoracicotropic hormone, PTTH)(Al-Sharook *et al*., 1991; Josephrajkumar *et al*., 1999).

1.2. Affected Adult Survival*:*

A scarce studies focused on the adulticidal effects of IGRs on insects, such as *S. littoralis* after treatment of larvae with the novaluron, especially at the higher concentrations (Hamadah *et al*., 2015), *Delia antique* after treatment of larvae with pyriproxyfen (Zhou *et al*., 2016) and *P. gossypiella* after treatment of the newly hatched larvae with novaluron (Hassan *et al.*, 2017). Results of the present study were inconsistent with those reported results, since neither noviflumuron nor novaluron could exhibit an adulticidal effect on *P. gossypiella*, after treatment of 1-day old eggs. This failure of toxicity on adults can be understood in the light of the following suggestions. Noviflumuron and novaluron may be metabolized of chemically degraded throughout the developmental stages of *P. gossypiella*, and subsequently no retention or distribution could be taken place in the insect body. Also, these CSIs may be denied or challenged by a high detoxification capacity of adults.

1.3. Impaired Adult Morphogenesis*:*

Impaired adult morphogenesis, as expressed in the production of deformed adults, was widely reported after treatment of larvae of various insects with different IGRs (or CSIs), such as *S. littoralis* by tebufenozide and methoxyfenozide (Pineda *et al*., 2004), flufenoxuron (Bakr *et al*., 2010), Novaluron (Hamadah *et al*., 2015); *Rhynchophorus ferrugineus* by diofenolan (Tanani, 2001); *Eurygaster integriceps* by Pyriproxyfen (Mojaver and Bandani, 2010); *Dysdercus koenigii* by flucycloxuron (Khan and Qamar, 2011); *Anagasta kuehniella* by diflubenzuron and hexaflumuron (Ashouri *et al*., 2014); *Helicoverpa armigera* with hexaflumuron (Taleh *et al*., 2015); *C. cephalonica* by fenoxycarb (Begum and Qamar, 2016); *etc*. Results of the current study disagreed with the previously reported results, since both noviflumuron and novaluron failed to affect the adult morphogenesis of *P. gossypiella*, after treatment of 1-day old eggs with sublethal concentrations. Also, the current result was found contradictory to those reported results of the production of deformed adults of the same lepidopterous insect, after treatment of eggs of different ages with hexaflumuron (El-Barkey *et al*., 2009) or after treatment of 1-day old eggs with LC_{50} values of lufenuron, chlorfluazuron and chromafenozide (Kandil *et al*., 2012). On the contrary, the present result coincided with the failure of novaluron to affect the adult morphogenesis of the same lepidopterous insect (Hassan *et al*., 2017). However, the

disappearance of adult deformities of *P. gossypiella*, in the present investigation, may be explained by the failure of noviflumuron and novaluron to disturb the hormonal regulation of the adult metamorphosis program.

1.4. Disturbed adult longevity*:*

***Total adult longevity*:**

After the attainment of sexual maturity, insects often show degenerative changes in some tissues and organs which can be called 'senility' or 'aging'. In insects, the affected adult longevity can be considered an informative indicator for the adult aging, i.e., prolongation of longevity may denote a delay of aging and *vice versa*. In the present study, the total adult longevity of *P. gossypiella* was remarkably shortened after treatment of 1-day old eggs with sublethal concentrations of noviflumuron or novaluron. Comparatively, noviflumuron exhibited a stronger shortening effect than novaluron. Our result was in accordance with those reported results for some insects by different IGRs, such as *S. littoralis* by lufenuron (Sammour *et al.*, 2008), methoxyfenozide (Pineda *et al*., 2009) and novaluron (Hamadah *et al*., 2015); *Agrotis ipsilon* by flufenoxuron (El-Sheikh, 2002); *Grapholita molesta* (Reinke and Barrett, 2007) and *Spodoptera exigua* (Luna *et al*., 2011) by methoxyfenozide and *G. pyloalis* by lufenuron (Aliabadi *et al*., 2016). Also, our result of considerably shortened total adult longevity of *P. gossypiella*, as a response to treatment of 1-day old eggs with noviflumuron and novaluron, corroborated with several reported results for the same lepidopterous insect after treatment of newly hatched larvae with diflubenzuron (Kandil *et al*., 2005; Rashad *et al*., 2006; Salem, 2015), Chlorfluazuron (Kandil *et al*., 2005), chromafenozide (Salem, 2015) and methoxyfenozide (Sabry and Abdou, 2016).

On the contrary, the present result was inconsistent with those reported results of prolonged adult longevity of the same lepidopterous insect after treatment of eggs of different ages with hexaflumuron (El-Barkey *et al*., 2009), after treatment of 1-day old eggs with LC_{50} values of lufenuron, chlorfluazuron and chromafenozide (Kandil *et al*., 2012), as well as after treatment of newly hatched larvae with hexaflumuron and chlorfluazuron (Kandil *et al*., 2013), lufenuron and pyriproxyfen (Sabry and Abdou, 2016). However, no effect was exhibited by some IGRs on the total adult longevity of some insects, such as diofenolan against *Musca domestica* (Hamadah, 2003), tebufenozide or methoxyfenozide against *Cydia pomonella* (Saenz-de-Cabezon *et al*., 2005), buprofezin against *S. littoralis* (Ragaei and Sabry, 2011) and novaluron against *Lygus lineolaris* (Portilla *et al*., 2012).

To explain the predominantly shortened adult longevity of *P. gossypiella*, in the current study, the tested CSIs might exert a general accelerating action on these adult females to quickly pass aging ending in death. Noviflumuron exerted a stronger accelerating action than novaluron. However, this result can be interpreted by the accumulation of xenobiotics in the body which upsets a complicated balance of factors such as absorption, excretion and detoxification (Abdel-Aal, 1996). On the other hand, this shortened longevity of *P. gossypiella* adult females may be attributed to the effect of the tested CSIs on a hormonal activity because there is a close relation between certain hormones and adult longevity, such as representatives of peptide hormone, lipophilic hormones and bioactive amines as reported for *Drosophila* (Simon *et al*., 2003; Broughton *et al.*, 2005; Carbone *et al*., 2006). At least one of the *Drosophila* insulin-linked peptides expressed in the median neurosecretory cells (which produce PTTH) is likely to contribute to the endocrine regulation of longevity (Toivonen and Partridge, 2009). However, the exact mode of

action of the tested CSIs on the biochemical sites in adults of *P. gossypiella* is unknown until now!

***Pre-oviposition period*:**

In most insects, the pre-oviposition period can be called 'ovarian maturation period' and it may be an informative indicator for the ovarian maturation rate, i.e., the shorter period indicates faster rate and *vice versa*. In the present study, treatment of 1-day old eggs of *P. gossypiella* with noviflumuron led to a slight prolongation or shortening of pre-oviposition period, depending on the concentration while treatment of eggs with novaluron led to a slight prolongation of such period, in a dose-dependent manner. The current result of prolongation corroborated with those reported results of prolonged period after treatment of newly hatched larvae of the same lepidopterous insect with diflubenzuron, hexaflumuron and chlorfluazuron (Kandil *et al*., 2005, Kandil *et al*., 2013), LC_{50} values of chromafenozide and diflubenzuron (Salem, 2015), LC_{50} of teflubenzuron (El-Khayat *et al*., 2015) and after treatment of newly hatched or full grown larvae with different concentrations of novaluron (Hassan *et al*., 2017) or after treatment of 1- and 2-day old eggs with LC_{50} of hexaflumuron (El-Barkey *et al*., 2009). Our result was in agreement, also, with those reported prolongation of the pre-oviposition period in other insects, such as *S. littoralis*, after larval treatment with diflubenzuron (Aref *et al*., 2010) and *Ephestia kuehniella*, after larval treatment with tebufenozide (Bouzera and Soltani-Mazouni, 2014).

On the other hand, the shortened pre-oviposition period after egg treatment with certain concentrations of noviflumuron, in the present study on *P. gossypiella*, agreed with those reported results of shortened period in the same insect, after treatment of 1- and 2-day old eggs with LC_{50} of Radiant (Spintoram, bacteria-based product)(El-Barkey *et al*., 2009) and after treatment of newly hatched larvae with diflubenzuron (Rashad *et al*., 2006) as well as after larval treatment of *D. antique* with a dose of 100 mg kg^{-1} of pyriproxyfen (Zhou *et al*., 2016).

Many lepidopterous species have a relatively short, non-feeding adult stage, which requires the adult female to emerge with most of her eggs ready to be fertilized and oviposited within hours. This life style constrains these insects to a program of ovarian organogenesis and follicle development that must occur at stages earlier than in other insects. The determinants required for germ cell formation are similar in moths, but there are spatial differences in their localization within the presumptive germ band (Richard *et al*., 1998). In the light of this information, retarding effect of noviflumuron (at certain concentrations) and novaluron on the ovarian maturation in *P. gossypiella*, in the present study, may be understood by influenced germ band or the number of germ cells formed in the embryo (Hodin and Riddiford, 1998). However, the exact mode of retarding action is unfortunately available right now but interference of the tested CSIs with the hormonal regulation needs further investigation in the foreseeable future.

***Oviposition period*:**

In respect of another important compartment of adult longevity, oviposition period (reproductive life-time), scarcely reported results have been seen in the available literature. According to the few reported results, oviposition period in the adult females of *P. gossypiella* had been shortened after treatment of newly hatched larvae with chlorfluazuron (Kandil *et al*., 2005), diflubenzuron (Rashad *et al*., 2006), hexaflumuron and chlorfluazuron (Kandil *et al*., 2013), LC_{50} of methomyl (El-Khayat *et al*., 2015) and after treatment of newly hatched or full grown larvae with

Novaluron (Hassan *et al*., 2017) as well as after treatment of 1- and 2-day old eggs with Radiant (El-Barkey *et al*., 2009). Result of the current investigation on *P. gossypiella*, was, to a great extent, concomitant to those reported results, since treatment of 1-day old eggs with sublethal concentrations of noviflumuron and novaluron resulted in considerably shortened oviposition period (reproductive life-time). On the contrary, this result disagreed with the reported considerable prolongation of oviposition period in the same lepidopterous insect, after treatment of newly hatched larvae with LC_{50} of chromafenozide or diflubenzuron (Salem, 2015) and teflubenzuron (El-Khayat *et al*., 2015) and after treatment of 1- and 2-day old eggs with LC_{50} of hexaflumuron (El-Barkey *et al*., 2009).

On the basis of shortened oviposition period of *P. gossypiella* after treatment of 1-day old eggs with noviflumuron and novaluron, in the current investigation, these CSIs exerted tremendously enforcing actions on the ovipositing females to lay eggs quickly. This accelerated oviposition may be attributed to a physiological behaviour of the ovipositing adult females to avoid a long time interval under stress of the tested CSIs as xenobiotic factors.

***Post- oviposition period*:**

Depending on the currently available literature, very scarce studies have examined the effects of IGRs on the post-oviposition period, the last compartment of adult life in insects. With regard to the present experimental insect, *P. gossypiella*, the post-oviposition period was significantly prolonged after treatment of larvae with hexaflumuron and chlorfluazuron (Kandil *et al*., 2013) or treatment of 1- and 2-day old eggs with LC_{50} of hexaflumuron (El-Barkey *et al*., 2009). Results of the present study were, to some extent, in agreement with those reported results, since treatment of 1-day old eggs of *P. gossypiella* with noviflumuron resulted in pronouncedly prolonged post-oviposition period but slightly prolonged period was recorded after treatment of similar eggs with by novaluron. In contrast, our result disagreed with those reported results of shortened post-oviposition period of the same insect after treatment of 1- and 2-day old eggs with LC_{50} of Radiant (El-Barkey *et al*., 2009) and diverse effect of novaluron, after treatment of larvae (Hassan *et al*., 2017). Unfortunately, there is no acceptable interpretation for this effect right now!!

2. Disrupted Reproductive Potential of *P. gossypiella* by CSIs:

Reproduction in insects is mainly controlled by corpus allatum hormone (juvenile hormone, JH), which is also responsible for protein metabolism, and is specifically needed for egg maturation. The insect growth regulators (IGRs) have been found to render treated insects either sterile or less fecund (Ghoneim *et al*.**,** 2014). The IGR-treated insects may develop as morphologically deformed adults who would be non-viable or at least their reproductive capacity is reduced (Williams and Amos 1974). However, effects of IGRs on the insect reproduction can be grouped into the following categories: i) reproductive behaviour, ii) oviposition, iii) hatchability of eggs (ovicidal and embryocidal), and iv) sterilization of adults (Mondal and Parween, 2000). On the other hand, ecdysteroids have essential functions in controlling the processes involved in insect reproduction, i.e., vitellogenesis, ovulation of matured eggs and spermatocyte growth (Wigglesworth, 1984; Hagedorn, 1985). In the present study on *P. gossypiella*, treatment of 1-day old eggs with the highest concentration of noviflumuron (4.0 ppm) or novaluron (1.0 ppm) led to a failure of the adult females, in spite of mating with normal males, to lay eggs. On the other hand, various degrees of perturbed reproductive potential

could be recorded at other concentrations of the present CSIs.

2.1. Inhibited Oviposition Efficiency:

In insects, the oviposition rate can be used as an informative indicator for the oviposition efficiency. In the present study on *P. gossypiella*, treatment of 1-day old eggs with sublethal concentrations of noviflumuron or novaluron resulted in drastically prohibited oviposition efficiency, since the oviposition rate was severely regressed, in a dose-dependent course. This result was coincided with the reported inhibition of oviposition efficiency of the same lepidopterous insect after treatment of newly hatched and full grown larvae with novaluron (Hassan *et al*., 2017) as well as reported results for other insects, such as *S. littoralis* by Tebufenozide (Bakr *et al*., 2005), flufenoxuron (Bakr *et al*., 2010) and novaluron (Ghoneim *et al*., 2014); *Schistocerca gregaria* by flufenoxuron and lufenuron (Soltani-Mazouni and Soltani, 1994) or tebufenozide (Al-Dali *et al*., 2008); *Plodia interpunctella* by the ecdysteroid agonist RH-5849 (Smagghe and Degheele, 1994) and *Callosobruchas maculates* by cyromazine (Al-Mekhlafi *et al*., 2011). In contrast, the present result disagreed with the stimulated oviposition of *Gryllus bimaculatus* by some ecdysteroid agonists (Behrens and Hoffmann, 1983).

The prohibited oviposition efficiency, in the current study, may be explained as a result of inhibition of ovarian DNA synthesis or the interference of noviflumuron and Novaluron with vitellogenesis in *P. gossypiella via* certain biochemical processes, as will be mentioned later. However, these CSIs may exert a reverse action to those exerted by the ecdysteroid agonists which stimulate the neurosecretory cells to release a myotropic ovulation hormone (Parween *et al*., 2001).

2.2. Perturbation of the Reproductive Capacity:

The reproductive capacity of an insect can be detected by two major parameters: fecundity (mean number of eggs/female) and fertility (egg hatching % or egg viability).

***Prohibited fecundity*:**

Many studies recorded a prohibited fecundity of several insects after treatment of larvae with various IGRs (and CSIs), such as *S. littoralis* after treatment with diflubenzuron (Aref *et al*., 2010), lufenuron (Gaaboub *et al*., 2012), methoxyfenozide (Pineda *et al*., 2009) and novaluron (Ghoneim *et al*., 2014). Also, fecundity of other insect species was reduced by various IGRs, such as *E. kuehniella* by tebufenozide (Khebbeb *et al*., 2008); *Choristoneura rosaceana* (Sun *et al*., 2000), *Lobesia botrana* (Saenz-de-Cabezon *et al*., 2005) and *S. litura* (Shahout *et al*., 2011) by the ecdysteroid agonist methoxyfenozide; *Leptinotarsa decemlineata* (Farinos *et al*., 1999) and *Tenebrio molitor* (Taibi *et al*., 2003) by the ecdysteroid agonist halofenozide (RH-0345); *S. litura* by chlorfluazuron (Perveen and Miyata, 2000), *M. domestica* by lufenuron (Hamadah 2003), *D. koenigi* by flufenoxuron (Khan and Qamar, 2011); *A. kuehniella* by diflubenzuron and hexaflumuron (Ashouri *et al*., 2014); *P. xylostella* by pyriproxyfen (Mahmoudvand *et al*., 2015); *Callosobruchus chinensis* by terpene compounds (α-pinene and β-caryophyllene) (Chaubey, 2015); *T. castaneum* (Gado *et al*., 2015) and *D. antique* (Zhou *et al*., 2016) by lufenuron and *C. cephalonica* by fenoxycarb (Begum and Qamar, 2016); *etc*.

The present results on *P. gossypiella* corroborated, to a great extent, with the previously reported results, since treatment of 1-day old eggs with sublethal

concentrations of noviflumuron or novaluron resulted in dramatically reduced fecundity of the successfully reproducing females, in a dose-dependent fashion. Comparatively, noviflumuron exhibited a stronger reducing effect than novaluron. These results were, also, in agreement with some of the reported results of dramatically reduced fecundity of the same lepidopterous insect after treatment of 1-day old eggs with Hexaflumuron (El-Barkey *et al*., 2009) and LC_{50} values of lufenuron, chlorfluazuron and chromafenozide (Kandil *et al*., 2012). Also, fecundity of *P. gossypiella* was drastically reduced after treatment of newly hatched larvae with tebufenozide (El-Khayat *et al*., 2015), diflubenzuron (Rashad *et al*., 2006), chlorfluazuron (Kandil *et al*., 2005), buprofezin (Al-Kazafy, 2013), hexaflumuron and chlorfluazuron (Kandil *et al*., 2013), chromafenozide (Salem, 2015), as well as pyriproxyfen, methoxyfenozide and lufenuron (Sabry and Abdou, 2016) and novaluron (Hassan *et al*., 2017).

On the contrary, the recorded results in the current investigation disagree with some reported results of failure of some IGRs to affect the fecundity of various insects, such as fenoxycarb against *Apis mellifera* (Thompson *et al*., 2005), methoxyfenozide against *S. exigua* (Christian-Lius and Pineda, 2010) and Novaluron and diflubenzuron against *Halyomorpha halys* (Kamminga *et al*., 2012). Moreover, feeding of larvae on leaves treated with methoxyfenozide enhanced the fecundity of *S. littoralis* (Ishaaya *et al*., 1995). However, these diverse effects can be attributed to the different modes of action of IGRs, different susceptibilities of the insect species, time of treatment and other factors.

The drastically prohibited fecundity of *P. gossypiella*, after treatment of 1-day old eggs with noviflumuron and novaluron, in the present study, may be due to its interference with one or more processes, from the ovarian follicle development to egg maturation. In some detail, this can be explained by some reasons. First, the tested CSIs may cause some disorders in the ovaries, including cell death in the germarium, resorption of oocytes in the pre-vitellarium and vitellarium, formation of vitellin envelops and undue proliferation of follicle cells sometimes resulting in malformation of the whole ovary (Lucantoni *et al*., 2006; Khan *et al*., 2007). Second, these CSIs may inhibit the development of some ovarioles and/or synthesis and metabolism of proteinaceous constituents during the oogenesis (Salem *et al*., 1997). Third, the tested CSIs exerted an inhibitory action on the ecdysone activity, threshold of which is essential for the normal oogenesis (Terashima *et al*., 2005). Fourth, on the basis of hormonal regulation of insect reproduction, the present CSIs may disturb the production and/or function of the gonadotropic hormone (juvenile hormone, JH) responsible for the synthesis of vitellogenins (yolk precursors) and vitellogenesis (Di Ilio *et al*., 1999). Fifth, eggs may develop normally in ovaries, but they could not be lay, owing to the adversely deformed ovipositor of adult females or to the reduced mechanical strength (Moreno *et al*., 1994) or their rebsorpion before oviposition (Zhou *et al*., 2016). Sixth, it may be acceptable to suggest that the prohibited fecundity of *P. gossypiella*, in the current work, may be due to inhibitory effects of the tested CSIs on synthesis of both DNA and RNA, suboptimal nutrition owing to reduced feeding, altered mating behaviour as a result of sublethal intoxication, or a combination of factors.

***Reduced fertility*:**

Another parameter of the reproductive capacity in insects is fertility (egg viability). In the present study on *P. gossypiella*, treatment of 1-day old eggs with sublethal concentrations of noviflumuron or novaluron led to dangerously reduced

fertility of the eggs laid by reproducing adult females. Comparatively, noviflumuron exhibited a stronger reducing effect than novaluron. This result was in accordance with those reported results of considerably reduced fertility in the same lepidopterous insect after treatment of 1-day old eggs with hexaflumuron (El-Barkey *et al*., 2009), lufenuron, chlorfluazuron and chromafenozide (Kandil *et al*., 2012), LC_{50} of chromafenozide and diflubenzuron (Salem, 2015), chlorfluazuron and hexaflumuron (Kandil *et al*., 2013) or after treatment of newly hatched larvae with novaluron (Hassan *et al*., 2017). Also, our result was, to some extent, in agreement with those reported results of reduced fertility of other insects after treatment of larvae with various IGRs, such as *S. littoralis* by chlorfluazuron (Sammour *et al*., 2008), methoxyfenozide (Pineda *et al*., 2009), diflubenzuron (Aref *et al*., 2010), lufenuron (Gaaboub *et al*., 2012), triflumuron (El-Naggar, 2013) and novaluron (Ghoneim *et al*., 2014); *S. litura* by diofenolan (Perveen and Miyata, 2000) and chromafenozide (Shahout *et al*., 2011); *T. molitor* by halofenozide (Taibi *et al*., 2003); *M. domestica* by diofenolan (Hamadah 2003), *T. castaneum* by novaluron (Kostyukovsky and Trostanelsky, 2004); *E. kuehniella* by tebufenozide (Khebbeb *et al*., 2008); *D. koenigi* by flufenoxuron (Khan and Qamar, 2011), *C. maculates* by cyromazine (Al-Mekhlafi *et al*., 2011), *A. kuehniella* by diflubenzuron and hexaflumuron (Ashouri *et al*., 2014); *etc*.

For explicating the fertility reduction in *P. gossypiella* by noviflumuron and novaluron, in the present study, some suggestions can be provided herein. First, maturation of the insect eggs depends basically on the vitellogenins, precursor materials of vitellins including proteins, lipids and carbohydrates, all of which are necessarily required for the embryonic development (Soltani and Mazouni, 1992; Chapman, 1998). These materials are synthesized primarily by fat body during the immature stages (Telfer, 2009) or by the ovary *in situ* (Indrasith *et al*., 1988). Wherever the site of vitellogenin synthesis, the tested CSIs might disturb their production and/or accumulation in adult females of *P. gossypiella* leading to reduction of fertility. Second, the tested compounds might indirectly affect the fertility *via* the disruption of opening of the intracellular spaces in follicular epithelium or generally inhibition the role of JH (gonadotropic hormone) responsible for the regulation of vitellogenesis (Davey and Gordon, 1996). Third, the fertility reduction may be due to the penetration of residual amounts of the present CSIs into *P. gossypiella* eggs and disturbance of embryonic cuticle synthesis. So, the fully mature embryos had weakened chitinous mouth parts that were insufficiently rigid to perforate the surrounding vitellin membrane for hatching process (Sallam, 1999; Sammour *et al*., 2008). Fourth, fertility reduction in *P. gossypiella*, in the current study, may be due to serious effects of the present CSIs on the survival of developing embryos at certain stages. Fifth, because the molecular studies revealed the effects of some IGRs on insect reproduction owing to the perturbation of gene expression in the hierarchy cascade of vitellogenesis and/or choriogenesis (Sun *et al*., 2003), the tested CSIs may interfere with the gene expression resulting in a reduction of the developed embryos in *P. gossypiella*, in the present study.

2.3. Retarded Embryonic Development*:*

In insects, incubation period can be used as a valuable indicator of the embryonic developmental rate, i.e., longer period usually denotes slower rate and *vice versa*. In the present study on *P. gossypiella*, treatment of 1-day old eggs with sublethal concentrations of noviflumuron or novaluron led to considerably prolonged incubation period of eggs laid by the successfully reproducing females, denoting

severely retarding effect of both CSIs on the embryonic developmental rate. The present result corroborated with the scarcely reported results, concerning a similar retarding action of some IGRs on the embryonic development of the same lepidopterous insect, after treatment of 0-3-day old eggs with LC_{50} of hexaflumuron (El-Barkey *et al*., 2009) and after treatment of larvae with LC_{50} values of lufenuron, chlorfluazuron or chromafenozide (Kandil *et al*., 2012) and novaluron (Hassan *et al*., 2017). Also, such period was prolonged after treatment of larvae of *C. maculates* with cyromazine (Al-Mekhlafi *et al*., 2011) and *S. littoralis* with novaluron (Ghoneim *et al*., 2014) as well as after treatment of 1- and 3-day old egg masses of the latter insect with LC_{50} of sesame oil (Khedr, 2016). The delayed embryonic development in *P. gossypiella* after treatment of 1-day old eggs with noviflumuron and novaluron, in the present study, may be due to their effects on ecdysteroids responsible for the regulation of embryogenesis at certain stages, especially those originating from the ovary *in situ* (Chapman, 1998).

5. Conclusions

In the light of the recoded results, noviflumuron and novaluron exhibited long-term disruptive effects on the adult performance and reproductive capacity of the pink bollworm *P. gossypiella* leading to a considerable reduction of its population. Therefore, the tested compounds, especially noviflumuron, may be potential IGRs being involved in the integrated control program against this worldwide serious pest.

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