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Impaired adult performance and reproductive potential of the pink bollworm *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) by the chitin synthesis inhibitor, Novaluron

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Abstract
The present study was conducted to investigate the disruptive effects of Novaluron on the adult performance and reproductive potential of the pink bollworm *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae). The newly hatched larvae were treated with four sublethal concentrations (1.0, 0.5, 0.1 & 0.05 ppm) and full grown larvae were treated with five sublethal concentrations (5.0, 1.0, 0.5, 0.1 & 0.05 ppm) of Novaluron. The adult emergence was drastically blocked, after treatment of full grown larvae and Novaluron exhibited, also, a considerable adulticidal effect. The adult morphogenesis had not been affected, regardless the larval instar under treatment. The pre-oviposition period was prolonged but the oviposition period was shortened, regardless the larval instar under treatment. The post-oviposition period was diversely affected, depending on the concentration. The total adult longevity was slightly prolonged, at the lowest concentration, but slightly shortened, at the higher concentrations. Regardless the larval instar under treatment, Novaluron drastically prohibited the oviposition efficiency. Also, both fecundity and fertility had been tremendously reduced. The incubation period was slightly or remarkably prolonged. Therefore, Novaluron may be a potential IGR being involved in the integrated pest management program against this insect pest which has developed resistance to the majority of conventional insecticides.

Keywords: *Pectinophora gossypiella*, fecundity, fertility, incubation, longevity, oviposition

1. Introduction
Cotton growing and production in Egypt have been faced with several infestations, especially lepidopterous insects, most of them being the pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae). This insect is considered as one of the major economic pests of cotton worldwide bacuase larvae cause an enormous damage to cotton bolls and consequently considerable losses in yield and reducing the quality of lint [1-4].
The control of *P. gossypiella* in Egypt depends mostly on the use of various synthetic insecticides. Often, the excessive use of these chemicals has been accompanied by increased resistance [5]. In addition, the release of these chemicals pollutes the environment and affects non-target organisms [5]. For these reasons, relatively safe alternative compounds should be searched. At present, using insect growth regulators (IGRs) have been considered as the possible alternative way of synthetic insecticides for controlling this pest [6]. IGRs differ widely from the commonly used insecticides, as they exert their insecticidal effects through their influence on development, metamorphosis and reproduction of the target insects by disrupting the normal activity of the endocrine system [7, 8]. Their comprehensive effects and high selectivity as well as lower toxicity to non-target animals and the environment provide new tools for integrated pest management [9, 10]. In addition, latent effects of IGRs appear on the longevity, fecundity and fertility of adult stage of lepidopterous insects, such as *P. gossypiella* [11].
Chitin synthesis inhibitors (CSIs) are usually classified in IGRs interfering with chitin biosynthesis in insects and thus prevent moulting, or produces an imperfect cuticle. These compounds are effective suppresors of development for the entire life cycle of insect pests [12]. Novaluron is a relatively new benzoylphenyl urea CSI with low mammalian toxicity [13, 14]. The compound has no appreciable effect on parasitoids and has probably a mild effect on the natural enemies [15, 16]. 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 [17].

Novaluron is a powerful suppressor of the pest populations, such as *Bemisia tabaci* and *Trialeurodes vaporariorum* [18]. It acts by ingestion and contact against several insect pests, such as *Spodoptera* spp., *Tuta absoluta, Helicoverpa armigera* and *Liriomyza huidobrensis* [19]. It exhibited, also, a good activity against the Colorado potato beetle [20-23]. Ghoneim *et al*. [24] recorded various degrees of inhibited growth and retarded development of *Spodoptera littoralis* by Novaluron. Treatment of last instar larvae of the same insect with Novaluron resulted in some features of impaired adult morphogenesis [25]. Therefore, the current work was undertaken to assess the impairing effects of Novaluron on the adult performance and reproductive potential of the destructive insect *P. gossypiella*.

2. Materials and Methods

2.1 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 along some years in Plant Protection Research Institute, Doqqi, 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*. [26]. Ten pairs of freshly emerged male and female moths were confined in plastic jars (10 X 25 cm) as cages. Inside each jar, a piece of cotton wool soaked in 10% sucrose solution was suspended from the top by a thread and renewed every 48 hrs for feeding moths. After adult mating, females deposited eggs through screening meshes on pieces of paper placed at top and bottom. Then, the collected eggs were kept in glass vials (5 X 12.5 cm) covered with muslin and kept under the same constant conditions until hatching. Thereafter, the newly hatched larvae were transferred using a soft brush into glass vials (2 X 7 cm) containing 5 gm of the artificial diet until pupation under the controlled conditions. The pupae were kept in clean glass vials without diet (one pupa/vial) which plugged with cotton until moth emergence.

2.2 Novaluron and larval treatments

Novaluron (Rimon) [1-[chloro-4-(1,1,2-trifluoromethoxyethoxy) phenyl] -3-(2,6-difluorobenzoyl) urea] was supplied by Sigma-Aldrich Chemicals. Its molecular formula is $C_{17}H_9ClF_8N_2O_4$. Different concentration levels of Novaluron were prepared by diluting with distilled water in volumetric flasks.

In a preliminary experiment, a wide range on Novaluron concentrations (5.0-0.05 ppm) had been applied on the newly hatched larvae of *P. gossypiella* through the artificial diet. Depending on the insect metamorphosis into adults, four sublethal concentrations (1.0, 0.5, 0.1 & 0.05 ppm) after treatment of newly hatched larvae and five sublethal concentrations (5.0, 1.0, 0.5, 0.1 & 0.05 ppm) after treatment of full grown (4^{th} instar) larvae were ascertained.

Four replicates (10/replicate) of newly hatched larvae, were transferred separately from the culture into test tube (1.0 X 6.0 cm) (one larva/tube) containing 3 gm of the artificial diet and sprayed (1 spray/tube) with each of the prepared concentrations. A similar number of replicates of full grown larvae were transferred from the culture into Petri dishes (one replicate/dish). Each replicate was sprayed with one of the prepared concentrations. Similar replicates of control larvae were treated with distilled water only using the same technique. The control and treated larvae were carefully handled until the adult emergence just after which all parameters of adult performance and reproductive criteria were recorded.

2.3 Adult performance parameters

2.3.1 Adult emergence: Number of successfully metamorphosed adults was expressed in% according to Jimenez-Peydro *et al*. [27] as follows:

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

2.3.2 Adulticidal activity: The adulticidal activity of Novaluron was detected by the adult mortalities throughout the total longevity and calculated in percentage.

2.3.3 Morphogenic efficiency: The morphogenic efficiency of Novaluron was detected by the adult deformities and calculated in percentage as follows:

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

2.3.4 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.

2.4 Criteria of the reproductive potential

2.4.1 Oviposition rate was calculated as follows:

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

The laid eggs 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.* [28] 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.

2.5 Statistical analysis of data

Data obtained were analyzed by the Student's *t*-distribution, and refined by Bessel correction [29] for the test significance of difference between means.

3. Results

3.1 Effects of Novaluron on Adult performance

After treatment of newly hatched larvae of *P. gossypiella* with the sublethal concentrations of Novaluron, performance data of the successfully metamorphosed adult females (♀♀) had been assorted in Table (1). Depending on these data, Novaluron failed to affect the adult emergence, survival or morphogenesis, since all adults completely emerged and survived with normal appearance (no deformed adults). As clearly in the same table, adults died before the reproduction process at the highest concentration level. Thus, total longevity and its major compartments could not be measured. The total adult longevity was slightly prolonged at the lowest concentration level but slightly shortened at the higher two concentration levels (14.0±1.41 and 13.0±3.4 days, at 0.5 and 0.1 ppm, respectively, *vs*. 15.0±1.63 days of control ♀♀).

According to data assorted in the same table, the pre-oviposition period was unremarkably prolonged, in a dose-dependent course, indicating a slight retarding effect of Novaluron on the ovarian maturation rate. In contrast, the oviposition period (reproductive life-time) was insignificantly shortened indicating a slight enhancing action of Novaluron on the ovipositing adult ♀♀, but in no certain trend (9.0±1.41, 8.7±2.51 & 10.3±0.58 days, at 0.5, 0.1 & 0.05 ppm, respectively, compared to 11.5±1.00 days of control adults). The post-oviposition period was diversely affected by Novaluron, depending on the concentration, since it was slightly lengthened at the lowest concentration (2.7±0.58 days) but slightly shortened at 0.1 ppm (1.3±0.58 days) *vs*. 1.5±0.58 days of control adults.

As obviously shown in Table (2), treatment of full grown larvae with the sublethal concentrations of Novaluron resulted in various degrees of effect on different criteria of the adult performance. In the light of these data, the adult emergence was blocked in a dose-dependent manner. In other words, the emergence% decreased with the increasing concentration. Novaluron exhibited, also, an adulticidal effect on the successfully emerged adult females proportional to the ascending concentration (2.5, 03.6, 13.4, 17.5 & 25.0% mortality, at 0.05, 0.1, 0.5, 1.0 & 5.0 ppm, respectively, *vs*. 00.0% of control adults). With regard to the adult morphogenesis program, no effect was recorded because no adult deformities could be observed, regardless the concentration.

In respect of the adult longevity and its compartments, data of the same table clearly revealed that only one adult male and one adult female could reproduce after larval treatment with the highest concentration level, hence the statistical analysis could not be applied at this concentration level. The pre-oviposition period was prolonged at other concentration levels (6.3±1.53, 6.3±1.53, 5.0±1.73, 7.0±1.4 & 4 days, at 0.05, 0.1, 0.5, 1.0 & 5.0 ppm, respectively, *vs*. 3.0±1.00 days of control adult females) indicating a delaying effect of Novaluron on the ovarian maturation rate. Novaluron exerted a strong promoting action on the ovipositing females to lay eggs during remarkably shortened period, in a dose-dependent course (5.3±1.16, 4.0±1.00, 3.0±2.00, 1.5±0.71 & 1 days, at 0.05, 0.1, 0.5, 1.0 & 5.0 ppm, respectively, *vs*. 9.3±0.58 days of control adult females). Novaluron failed to affect the total adult longevity except at the higher two concentration levels because a slight shortage was recorded (14 & 14.0±1.41 days, at 5.0 & 1.0 ppm, respectively, *vs*. 14.3±1.16 days of control adult females).

3.2 Effects of Novaluron on Reproductive potential

After treatment of newly hatched larvae, data of the most important criteria of reproductive potential were arranged in Table (3). As clearly shown by these data, the oviposition rate, which can be used as an informative indicator of the oviposition efficiency in insects, was severely regressed, in a dose-dependent course (2.7±0.99, 7.2±1.58 & 12.0±0.3, at 0.5, 0.1 & 0.05 ppm, respectively, *vs*. 14.9±0.64 of control adult females) denoting a strong prohibiting action of Novaluron on this efficiency.

The reproductive capacity can be detected by the determination of two parameters, fecundity (mean number of eggs/♀) and fertility (egg hatching% or egg viability) of an insect. Data assorted in the same table obviously revealed that fecundity of *P. gossypiella* was drastically reduced by Novaluron, in a dose-dependent manner (24.02±4.95, 62.10±19.60 and 124.15±9.64 eggs/ treated ♀, at 0.5, 0.1 and 0.05 ppm, respectively, compared to 172.28±18.67 eggs/control ♀). In the light of data listed the same table, Novaluron exerted a dramatic action on fertility which was unexceptionally reduced, but in no certain trend (50.7, 31.1 and 59.4% fertility of treated females, at 0.5, 0.1 and 0.05 ppm, respectively, *vs*. 69.4% fertility of control females). Moreover, the sterility index was calculated in a dose-dependent manner.

The incubation period of eggs can be used as a good informative indicator of the embryonic developmental rate, i.e., longer period usually denotes slower rate and *vice versa*. As obviously seen the previously mentioned table, incubation period was slightly prolonged, proportional to the concentration level (4.5±0.58, 4.7±0.58 and 5.5±0.71 days, at 0.05, 0.1 and 0.5 ppm, respectively, compared to 4.3±0.50 days of control eggs). These data indicated that an extended inhibitory effect was exhibited by Novaluron on the embryonic development in the treated *P. gossypiella*.

After treatment of full grown larvae with the sublethal concentrations of Novaluron, data of the oviposition efficiency and reproductive capacity parameters were assorted in Table (4). According to these data, the oviposition efficiency was drastically prohibited since the oviposition rate was severely regressed, in a dose-dependent course (6.1±4.11, 2.9±0.93, 1.5±1.04, 1.0±0.90 and 0.5±0.38, at 0.05, 0.1, 0.5, 1.0 and 5.0 ppm, respectively, *vs*. 12.4±1.70 of control adult females). Fecundity, as a parameter of the reproductive capacity, was seriously reduced, almost in a dose-dependent manner (29.72±2.34, 11.58±2.65, 4.58±3.51, 1.20±0.30 and 1.10±0.58 mean number of eggs/treated ♀, at 0.05, 0.1, 0.5, 1.0 and 5.0 ppm, respectively, *vs*. 115.90±9.54 mean number of eggs/control ♀). Also, fertility was dramatically reduced, proportional to the concentration 57.4, 34.1, 20.8, 00.0 & 00.0%, at 0.05, 0.1, 0.5, 1.0 & 5.0 ppm, respectively, *vs*. 73.2% fertility of controls). As obviously seen, no eggs hatched at the higher two concentration levels indicating complete sterility was caused by Novaluron at these concentrations.

In addition, the embryonic development was pronouncedly retarded by Novaluron, since the incubation period was remarkably prolonged (5.3±0.58, 5.5±0.58 & 5.8±0.71 days, at 0.05, 0.1 & 0.5 ppm, respectively, *vs*. 4.3±0.50 days of controls).

Table 1: Adult performance of *P. gossypiella* as affected by treatment of newly hatched larvae with Novaluron

Conc. (ppm)	Adult emergence (%)	Adult mortality (%)	Adult deformations (%)	Longevity (mean days± SD)			
				Ovarian maturation period	Reproductive lifetime	Post-oviposition period	Total Longevity
1.0	100.0	00.0	00.0	---	---	---	---
0.5	100.0	00.0	00.0	3.5±0.71a	9.0±1.41a	1.5±0.71a	14.0±1.41a
0.1	100.0	00.0	00.0	3.0±1.00a	8.7±2.51a	1.3±0.58a	13.0±3.46a
0.05	100.0	00.0	00.0	2.3±0.58a	10.3±0.58a	2.7±0.58b	15.3±0.58a
Control	100.0	00.0	00.0	2.0±0.81	11.5±1.00	1.5±0.58	15.0±1.63

Conc.: concentration level. Mean±SD followed by letter (a): not significantly different (P>0.05), (b): significantly different (P<0.05), (c): highly significantly different (P<0.01), (d): very highly significantly different (P<0.001). ---: no reproduction

Table 2: Adult performance of *P. gossypiella* as affected by treatment of full grown larvae with Novaluron

Conc. (ppm)	Adult emergence (%)	Adult mortality (%)	Adult deformations (%)	Longevity (mean days ± SD)			
				Ovarian maturation period	Reproductive lifetime	Post-oviposition period	Total Longevity
5.0	45.8	25.0	00.0	4*	1*	9*	14*
1.0	66.2	17.5	00.0	7.0±1.40 b	1.5±0.71 d	5.5±0.71 b	14.0±1.41 a
0.5	79.9	13.4	00.0	5.0±1.73 a	3.0±2.00 c	6.3±2.08 b	14.3±1.16 a
0.1	82.2	03.6	00.0	6.3±1.53 b	4.0±1.00 c	4.0±1.00 a	14.3±1.53 a
0.05	90.0	02.5	00.0	6.3±1.53 b	5.3±1.16 c	2.7±0.58 a	14.3±1.16 a
Control	92.5	00.0	00.0	3.0±1.00	9.3±0.58	2.0±1.00	14.3±1.16

Conc., a, b, c and d: see footnote of Table (**1**). *: Only one adult male and one adult female.

Table 3: Reproductive potential of *P. gossypiella* adults as affected by treatments of newly hatched larvae with Novaluron

Conc. (ppm)	Oviposition rate	Fecundity (mean no. of eggs/♀±SD)	Fertility (%)	Sterility index (%)	Incubation period (mean days ±SD)
0.5	2.7±0.99 d	24.02±4.95 d	50.7	89.81	5.5±0.71a
0.1	7.2±1.58 d	62.10±19.60 d	31.1	83.85	4.7±0.58 a
0.05	12.0±0.3 d	124.15±9.64 b	59.4	38.29	4.5±0.58 a
Control	14.9±0.64	172.28±18.67	69.4	---	4.3±0.50

Conc., a, b and c: see footnote of Table (1).

Table 4: Reproductive potential of *P. gossypiella* adults as affected by treatments of full grown larvae with Novaluron

Conc. (ppm)	Oviposition rate	Fecundity (mean no. of eggs/♀±SD)	Fertility (%)	Sterility index (%)	Incubation period (days) (mean ± SD)
5.0	0.5±0.38 d	1.10±0.58 d	00.0	100.0	---
1.0	1.0±0.90 d	1.20±0.30 d	00.0	100.0	---
0.5	1.5±1.04 d	4.58±3.51 d	20.8	99.15	5.8±0.71 b
0.1	2.9±0.93 c	11.58±2.65 d	34.1	96.17	5.5±0.58 b
0.05	6.1±4.11 b	29.72±2.34 d	57.4	83.03	5.3±0.58 b
Control	12.4±1.70	115.90±9.54	73.2	---	4.3±0.50

Conc., a, b, c: see footnote of Table (1).

4. Discussion

4.1 Affected adult performance of *P. gossypiella* by Novaluron

4.1.1 Blocked adult emergence

According to the reported results in the available literature, the adult emergence rate of *Plutella xylostella* was significantly regressed after larval treatment with Hexaflumuron[30]. The adult emergence of *Drosophila melanogaster* was inhibited after topical application of 3rd instar larvae with Pyriproxyfen[31]. Treatment of penultimate or last instar larvae of *Spodoptera littoralis* with Novaluron [24] and Cyromazine[32] resulted in different degrees of blocked adult emergence. After treating the 4th instar larvae of *Glyphodes pyloalis* with LC_{30} of Lufenuron, the adult emergence was blocked [33]. Pyriproxyfen was stronger than Methoprene for inhibition of the adult emergence of *Culex quinquefasciatus* and *Aedes albopictus*[34]. Pupal treatment of *Encarsia formosa* with Pyriproxyfen resulted in prohibited adult emergence [35]. Moreover, adult emergence was completely blocked in *Spodoptera litura* by 50 ppm of Diflubenzuron [36] and in *Corcyra cephalonica* after treatment of 4th instar larvae with Fenoxycarb[37]. To a great extent, the adult emergence of *P. gossypiella* was drastically blocked after treatment of full grown larvae with five sublethal concentrations (5.0-0.05 ppm) of Novaluron but no blockage was recorded after treatment of newly hatched larvae with three concentrations (1.0-0.05 ppm) of this compound. The present result of blocked adult emergence can be interpreted by the interference of Novaluron 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) [38, 39].

4.1.2 Reduced adult survival

In the current study, Novaluron failed to affect the adult survival of *P. gossypiella* after treatment of newly hatched larvae but it had been considerably reduced after treatment of full grown larvae. This result of latent lethal action of Novaluron on adults of *P. gossypiella* is, to some extent, in agreement with those very scarcely reported toxicities of some IGRs (and CSIs) on a number of insect species, such as *S. littoralis* after treatment of penultimate or last instar larvae with the same CSI, especially at the higher concentrations [25] and the onion fly *Delia antique* after larval treatment with Pyriproxyfen [40]. The adult mortality of *P. gossypiella* by Novaluron, in the present study, can be explained by the retention and distribution of this compound in the insect body as a result of rapid transport from the gut of treated larvae into other tissues, the direct and rapid transport of haemolymph to other tissues, and/or to lower detoxification capacity against the tested CSI [41]. Also, an extended or chronic lethal effect of Novaluron may be due to disturbed adult enzymatic pattern and hormonal hierarchy [42]. However, the adult life in insects depends on healthy immature stages. Digestive disorders such as starvation, disturbance in metabolism, degeneration of peritrophic membranes and accumulation of faecal materials at the hind gut may be the cause of untimely adult mortality as a result of CSIs exposure [43].

4.1.3 Impaired adult morphogenesis

Impaired adult morphogenesis, as expressed in the production of deformed adults, was widely reported in the literature after treatment of various insects with different IGRs (or CSIs), such as *S. littoralis* by Tebufenozide and methoxyfenozide [44, 45], Flufenoxuron [46], Novaluron [25]; *Rhynchophorus ferrugineus* by Diofenolan [47]; *Choristoneura fumiferana* by

Tebufenozide and Methoxyfenozide [48]; *Tribolium castaneum* and *Tribolium confusum* by Cyromazine [49]; *Eurygaster integriceps* by Pyriproxyfen [50]; *Dysdercus koenigii* by Flucycloxuron [51]; *Anagasta kuehniella* by Diflubenzuron and hexaflumuron [52]; *Helicoverpa armigera* with Hexaflumuron [53]; *C. cephalonica* by Fenoxycarb (at concentrations 0.05% and 0.025%) [54]; *etc*. Results of the present study disagree with those reported results, since Novaluron failed to affect the adult morphogenesis of *P. gossypiella*, regardless the treatment of newly hatched or full grown larvae.

4.1.4 Influenced adult longevity

4.1.4.1 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 current investigation, the total adult longevity of *P. gossypiella* was insignificantly shortened after treatment of newly hatched or full grown larvae with sublethal concentrations, especially the higher ones. The present result is in accordance with those reported results for some insects by different IGRs, such as *S. littoralis* by Lufenuron[55], Methoxyfenozide [56] and Novaluron [25]; *Agrotis ipsilon* by Flufenoxuron[57]; *Grapholita molesta*[58] and *Spodoptera exigua* [59] by Methoxyfenozide and *G. pyloalis* by Lufenuron [33]. Also, the present result of shortened total adult longevity of *P. gossypiella* corroborated with several reported results for the same insect after treatment of newly hatched larvae with Diflubenzuron [11, 60-62], Chlorfluazuron [11], Chromafenozide [62] and Methoxyfenozide [63].

It may be fruitful to pay an attention to the exceptional case of prolonged total adult longevity of *P. gossypiella*, in the present study, which could be recorded after treatment of newly hatched larvae with the lowest concentration level of Novaluron. This exceptionally prolonged longevity is, to a great extent, consistent with those reported results for the same insect after treatment of 1-day old eggs with LC_{50} values of Lufenuron, Chlorfluazuron and Chromafenozide [64] and after treatment of newly hatched larvae with Hexaflumuron more than Chlorfluazuron [65] and Lufenuron and Pyriproxyfen [63] as well as those reported results for other insects, such as *Lipaphis erysimi* by pyriproxyfen [66]. However, no effect was exhibited by some IGRs on the adult longevity of some insects, such as Diofenolan against *Musca domestica* [67], Tebufenozide or methoxyfenozide against *Cydia pomonella* [68], Buprofezin against *S. littoralis* [69] and Novaluron against *Lygus lineolaris* [70].

To understand the predominantly shortened adult longevity of *P. gossypiella*, in the current work, Novaluron might exert a general accelerating action on these adult females to quickly pass aging ending in death. However, this result can be interpreted by the accumulation of toxic xenobiotics in the body which upsets a complicated balance of factors such as absorption, excretion and detoxification [71]. On the other hand, this shortened longevity of *P. gossypiella* adult females may be attributed to the effect of tested CSI on a hormonal activity because there is a close relation between certain hormones and adult longevity. This suggestion can be appreciated in the light of reported results for *Drosophila*. In this fly, representatives of peptide hormone, lipophilic hormones and bioactive amines have been shown to modulate longevity by manipulations that directly decrease hormone production [72], through inactivating mutations in hormone receptors or their downstream targets[73, 74] or by polymorphic alterations in the genes required for hormone biosynthesis [75]. 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 [76]. However, the exact mode of action of the tested CSI on the biochemical sites in adults of *P. gossypiella* is unknown until now. Therefore, more information on the adult endocrine system is needed before a general interpretation can be formulated on the susceptibility of the life stage toward Novaluron.

4.1.4.2 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, the pre-oviposition period of the successfully emerged adult females of *P. gossypiella* was prolonged, regardless the treated instar larvae with Novaluron. The present result corroborated with those reported results of prolonged period after treatment of newly hatched larvae of the same insect with Diflubenzuron, Hexaflumuron and Chlorfluazuron[11,65], LC_{50} values of Chromafenozide and Diflubenzuron[62] and LC_{50} of Teflubenzuron [77]. It is in agreement, also, with those reported prolongation of the pre-oviposition period in other insects, such as *S. littoralis*, after larval treatment with Diflubenzuron [78] and *Ephestia kuehniella*, after larval treatment with Tebufenozide [79]. On the other hand, the present result of prolonged pre-oviposition period in *P. gossypiella*, as a response to the delaying action of Novaluron, contradictory to those reported results of shortened period in the same insect, after treatment of newly hatched larvae with Diflubenzuron [60, 61] and *D. antique*, after larval treatment with a dose of 100 mg kg^{-1} of Pyriproxyfen [40] or unaffected period in *S. litura*, after larval treatment with Chlorfluazuron and Methoxyfenozide [102] and in *D. antique*, after larval treatment with high doses of Pyriproxyfen [40].

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 [80]. In the light of this information, delaying or retarding effect of Novaluron on the ovarian maturation in *P. gossypiella* may be understood by influenced germ band or the number of germ cells formed in the embryo [81]. However, the exact mode of retarding action of Novaluron on pre-oviposition period, as a compartment of *P. gossypiella* adult life, is unfortunately available right now but its interference with the hormonal regulation of this physiological process needs further investigation.

4.1.4.3 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 reported results, oviposition period in the adult females of *P. gossypiella* had been shortened after treatment of newly hatched larvae with Chlorfluazuron[11], Diflubenzuron [60, 61], Hexaflumuron and

Chlorfluazuron [65] and LC_{50} of Methomyl [77]. Result of the present study is, to a great extent, concomitant to those reported results, since the oviposition period of the same insect was shortened after treatment of newly hatched or full grown larvae with Novaluron. On the contrary, this result disagrees 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 [62] and Teflubenzuron [77].

In the current work, Novaluron exhibited a prevalent enforcing effect on the adult females of *P. gossypiella* since they quickly laid their eggs during a very short time interval, regardless the larval instar under treatment. The exact mechanism of this enforcing action is still unknown. However, these females may be enforced to lay their eggs quickly to avoid this toxic xenobiotic factor.

4.1.4.4 Post- oviposition period

Depending on the currently available literature, very scarce studies have examined the effects of IGRs on post-oviposition period, the last compartment of adult life in insects. After treatment of newly hatched or full grown larvae of *P. gossypiella* with sublethal concentrations of Novaluron, in the present study, the post-oviposition period was diversely affected, depending on the concentration. However, such period was prolonged after treatment of *P. gossypiella* larvae with Hexaflumuron or Chlorfluazuron, as reported by Kandil *et al*. [65]. Unfortunately, we have no acceptable interpretation for this effect right now!!

4.2 Disrupted reproductive potential of *P. gossypiella* by Novaluron

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 [82, 83]. The IGR-treated insects may develop as morphologically deformed adults who would be non-viable or at least their reproductive capacity is reduced [84]. 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 [85]. 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 [86, 87].

4.2.1 Inhibited oviposition efficiency of *P. gossypiella* by Novaluron

In insects, the oviposition rate can be used as an informative indicator for the oviposition efficiency. After treatment of newly hatched or full grown larvae of *P. gossypiella* with sublethal concentrations of Novaluron, in the present study, the oviposition efficiency was seriously prohibited since the oviposition rate was drastically regressed. This result is in agreement with those reported results of regressed oviposition rate (or index) of *S. littoralis* by Tebufenozide [88], Flufenoxuron[46] and Novaluron [83]. It also agrees, to a great extent, with the inhibited oviposition of *Schistocerca gregaria* by Flufenoxuron and lufenuron [89] or Tebufenozide [90], *Plodia interpunctella* by the ecdysteroid agonist RH-5849 [91] and *Callosobruchas maculates* by Cyromazine [92]. In contrast, the present result disagrees with the stimulated oviposition of *Gryllus bimaculatus* by some ecdysteroid agonists [93]. The prohibited oviposition efficiency, in the current work, may be explained as a result of inhibition of ovarian DNA synthesis or the interference of Novaluron with vitellogenesis in *P. gossypiella via* certain biochemical processes, as will be mentioned later. However, this CSI may exert a reverse action to those exerted by the ecdysteroid agonists which stimulate the neurosecretory cells to release a myotropic ovulation hormone [94, 95].

4.2.2 Perturbation of the reproductive capacity of *P. gossypiella* by Novaluron

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).

4.2.2.1 Prohibited fecundity

The available literature contains a lot of results of prohibited fecundity of several insects after treatment of their larvae with various IGRs (and CSIs), such as *S. littoralis* after treatment with Diflubenzuron [78], Lufenuron [96], Methoxyfenozide [56] and Novaluron [83]. Also, fecundity of other insect species was reduced by various IGRs, such as *Rhyzopertha dominica* by Methoprene [97]; *Helicoverpa zea* and *C. pomonella* [98, 99] and *E. kuehniella* [100] by Tebufenozide; *Choristoneura rosaceana* [101], *Lobesia botrana* [68] and *S. litura* [102] by the ecdysteroid agonist methoxyfenozide; *Leptinotarsa decemlineata* [103] and *Tenebrio molitor*[104] by the ecdysteroid agonist Halofenozide (RH-0345); *S. litura* by Chlorfluazuron [105], *M. domestica* by Lufenuron [67], *D. koenigi* by Flufenoxuron [51]; *A. kuehniella* by Diflubenzuron and Hexaflumuron [52]; *P. xylostella* by Pyriproxyfen [106]; *Callosobruchus chinensis* by terpene compounds (α-pinene and β-caryophyllene) [107]; *T. castaneum* [108] and *D. antique* [40] by Lufenuron and *C. cephalonica* by Fenoxycarb [54]; *etc*.

The present results on *P. gossypiella* corroborated with the previously reported results, since treatment of newly hatched or full grown larvae with sublethal concentrations of Novaluron resulted in tremendously prohibited fecundity of the successfully reproducing adult females. These results are, also, in agreement with some of the reported results of considerably reduced fecundity of the same lepidopterous insect after treatment of newly hatched larvae with Tebufenozide [77,109], Diflubenzuron [11, 60-62], Chlorfluazuron [11], Buprofezin [110], Hexaflumuron and Chlorfluazuron [65], Chromafenozide [62], as well as Pyriproxyfen, Methoxyfenozide and Lufenuron [63].

On the contrary, 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* [111], Methoxyfenozide against *S. exigua* [112] and Novaluron and Diflubenzuron against *Halyomorpha halys* [113]. Moreover, feeding of larvae on leaves treated with Methoxyfenozide enhanced the fecundity of *S. littoralis* [114]. 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 prohibited fecundity of *P. gossypiella*, after treatment of larvae with 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, the present prohibited fecundity can be explained by some reasons. First, Novaluron may inhibit the development of some ovarioles and/or synthesis and metabolism of proteinaceous constituents during the oogenesis [94, 115]. Second, eggs may develop normally in ovaries, but they could

not be laid, may be due to the adversely deranged morphogenesis of ovipositor of adult females. This may be due, also, to the reduced mechanical strength, as reported for *Anastrepha ludens* [116]. Third, eggs had been formed in the female ovaries, but they were resorbed before being laid, as suggested for *D. antique* [40]. Fourth, Novaluron exerted an inhibitory action on the ecdysone activity, threshold of which is essential for normal oogenesis[117, 118]. Fifth, Novaluron 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 [119, 120]. Sixth, On the basis of hormonal regulation of insect reproduction, Novaluron may disturb the production and/or function of the gonadotropic hormone (juvenile hormone, JH) responsible for the synthesis of vitellogenins (yolk precursors) and vitellogenesis [121]. Seventh, it may be acceptable to suggest that the prohibited fecundity of *P. gossypiella*, in the current work, may be due to an inhibitory effect of Novaluron 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.

4.2.2.2 Reduced fertility

Another parameter of the reproductive capacity in insects is fertility (egg viability). In the present study, treatment of newly hatched or full grown larvae of *P. gossypiella* with sublethal concentrations of Novaluron resulted in dramatically reduced fertility of eggs laid by the successfully reproducing adult females. This result is in accordance with those reported results of reduced fertility in the same insect after treatment of newly hatched larvae with some IGRs, such as Lufenuron, methoxyfenozide, Chromafenozide and Chlorfluazuron [64]; as well as some of other insects, such as *S. littoralis* by Chlorfluazuron [55], Methoxyfenozide [56], Diflubenzuron [78], Lufenuron [96, 122], Triflumuron[123] and Novaluron [83]; *S. litura* by Diofenolan [105] and Chromafenozide [102]; *T. molitor* by Halofenozide [104]; *M. domestica* by Diofenolan [67], *T. castaneum* by Novaluron [124]; *E. kuehniella* by Tebufenozide [100]; *D. koenigi* by Flufenoxuron [51], *C. maculates* by Cyromazine [92], *A. kuehniella* by Diflubenzuron and Hexaflumuron [52]; *etc*.

For explicating the fertility reduction in *P. gossypiella* by 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 [125, 126]. These materials are synthesized primarily by fat body during the immature stages [127] or by the ovary *in situ* [128]. Wherever the site of synthesis of these materials, Novaluron may disturb their production and/or accumulation in adult females of *P. gossypiella* leading to reduction of fertility. Second, Novaluron may indirectly affect the fertility *via* its disruptive effect on opening of the intracellular spaces in follicular epithelium or generally inhibited the role of JH (gonadotropic hormone) responsible for the regulation of vitellogenin deposition into oocytes [129]. Third, the reduction in fertility may be due to the penetration of residual amounts of Novaluron in *P. gossypiella* mothers into their 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 and free from the eggs [55, 130]. Fourth, reduced fertility of *P. gossypiella*, in the current study, may be due to serious effect of Novaluron on the survival of developing embryos at certain stages as recorded in decreasing hatching percentage. 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 [131], Novaluron may interfere with the gene expression resulting in a reduction of the developed embryos in *P. gossypiella*, in the present study.

4.2.3 Retarded embryonic development of *P. gossypiella* by Novaluron

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 current study, treatment of newly hatched or full grown larvae of *P. gossypiella* with sublethal concentrations of Novaluron resulted in insignificantly or remarkably prolonged incubation period of eggs laid by the successfully reproducing adult females. This indicated a slight or drastic retarding effect was exhibited by Novaluron on the developing embryos. The present result corroborates with the scarcely reported results in the available literature concerning a similar retarding action of some IGRs on the embryonic development of some insects, such as *P. gossypiella* after treatment with LC_{50} of lufenuron, chlorfluazuron and chromafenozide [64], *C. maculates* after treatment with Cyromazine [92] and *S. littoralis* after treatment with Novaluron [83]. The delayed embryonic development in *P. gossypiella* after treatment of larvae with Novaluron, in the present study, may be due to its effect on ecdysteroids responsible for the regulation of embryogenesis at certain stages, especially those originating from the ovary *in situ* [126].

5. Conclusion

In the light of the present results, Novaluron disruptively affected the adult emergence, survival, ovarian maturation rate, reproductive life-time and longevity of the pink bollworm *P. gossypiella*, as well as it drastically prohibited the oviposition efficiency, reproductive capacity and impaired the embryonic development leading to a reduction of the pest population. Therefore, Novaluron may be a potential IGR being involved in the integrated pest management program against this insect pest which has developed resistance to the majority of conventional insecticides.

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7. References

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