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# Toxic and disruptive effects of Novaluron, a chitin synthesis inhibitor, on development of the pink bollworm *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae)

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**Abstract**
The pink bollworm *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) is one of the most destructive insects attacking cotton fields world-wide. It acquired resistance against most of the conventional pesticides. Therefore, the present study was conducted to evaluate the toxic and developmental effects of Novaluron (concentration range: 5.0-0.05 ppm) on this insect pest. $LC_{50}$ values were estimated in 0.187 ppm and 0.765 ppm, after treatment of newly hatched and full grown larvae, respectively. Novaluron exhibited a retarding effect on the development, especially after treatment of full grown larvae, since larval and pupal durations had been remarkably prolonged, in a dose-dependent manner. Novaluron failed to affect the metamorphosis after treatment of the newly hatched larvae but disrupted it after treatment of full grown larvae (larval-pupal intermediates). The pupation was considerably hindered, especially after treatment of full grown larvae. The pupal morphogenesis was deranged (deformed pupae) after treatment of only newly hatched larvae. Therefore, Novaluron forms an important component in the integrated pest management program for this insect pest which has developed resistance to the majority of conventional insecticides.

**Keywords:** adult, desiccation, larva, metamorphosis, morphogenesis, mortality, pupa, toxicity

## 1. Introduction
Cotton, *Gossypium hirsutum* L. is one of the most important economic crops in Egypt and the world since cotton is cultivated in over 100 countries. In Egypt, cotton represents more than half the income of two million small-scale farmers (Zidan *et al.*, 2012) [1]. The pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) is one of the most serious lepidopterous pests of cotton worldwide, and a particularly pest difficult to control (Liu *et al*., 2009) [2]. It causes serious damage in bolls resulting in high reduction in quantity and quality of cotton yield (Kandil *et al*., 2005, 2012; El-Khayat *et al*., 2015) [3, 4, 5]. To control the attacks of this pest, several types of insecticides have been used. The extensive use of these insecticides caused serious toxicological problems to humans and the environment (Costa *et al*., 2008; Relyea, 2009) [6, 7]. As a result of indiscriminate and continued use of insecticides, insects began to develop high levels of resistance to insecticides (Davies *et al*., 2007; Abd-Elhady and Abd El-Aal, 2011) [8, 9]. Therefore, effective alternatives should be assessed and then used through the rotation program and in the integrated pest management, in general, for controlling this pest.
During the last 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) [10, 11]. In contrast to the classical chemical insecticides, IGRs are not directly toxic, but act selectively on the development, metamorphosis or reproduction of the target insect species (Hoffmann and Lorenz, 1998; Martins and Silva, 2004) [12, 13]. They are quite selective in their mode of action and potentially act only on the target species (Sabry and Abdou, 2016) [14]. Chitin synthesis inhibitors (CSIs) are usually classified in IGRs interfering with chitin biosynthesis in insects and thus prevents moulting, or produces an imperfect cuticle. These compounds are effective suppresors of development for the entire life cycle of insect pests (Hammock and Quistad, 1981) [15].
Novaluron is a relatively new benzoylphenyl urea CSI with low mammalian toxicity (Barazani, 2001; Ishaaya and Horowitz, 2002) [16, 17]. The compound has no appreciable effect on parasitoids and has probably a mild effect on the natural enemies (Ishaaya *et al*., 2001, 2002) [18, 19]. 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 (Malhata *et al*., 2014) [20]. Novaluron is a powerful suppressor of the pest populations, such as *Bemisia tabaci* and *Trialeurodes vaporariorum* (Ishaaya *et al*., 2003) [21]. 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) [22]. It exhibited, also, a good activity against the Colorado potato beetle (Cutler *et al*., 2005 a, b, 2007; Alyokhin *et al*., 2009) [23-26]. Ghoneim *et al*. (2015) [27] 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 (Hamadah *et al*., 2015) [28]. The present study was conducted aiming to investigate the toxicity and disruptive effects of Novaluron on the development of *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*. (1982) [29]. Ten pairs of freshly emerged male and female moths were confined in plastic jars (10X 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 Bioassay of Novaluron

Novaluron (Rimon) [1- [chloro-4-(1, 1, 2-trifluoromethoxyethoxy) phenyl] -3- (2, 6-difluorobenzoyl) urea] was supplied by Sigma-Aldrich Chemicals (https://www.sigmaaldrich.com). Its molecular formula is $C_{17}H_9ClF_8N_2O_4$. Five concentration levels of Novaluron were prepared by diluting with distilled water in volumetric flasks, as follows: 5.0, 1.0, 0.5, 0.1, 0.05 ppm.

For carrying out the bioassay work, two experimental series of larvae were used: newly hatched larvae and full grown ($4^{th}$ instar) larvae. Forty newly hatched larvae, in four replicates (10/replicate), were transferred separately into test tube (1.0 X 6.0 cm) (one larva/tube) containing 3 gm of the artificial diet and sprayed (1 spray/tube), using a microspray pump, with each of the prepared concentrations. Control replicates were treated with distilled water only using the same technique. Also, forty full grown larvae in a similar number of replicates were transferred into Petri dishes (one replicate/dish). Each replicate was sprayed with one of the prepared concentrations. Control replicates were treated with distilled water only using the same technique. The treated and control larvae were left until pupation and all observations were recorded daily.

### 2.3. Criteria of study

#### 2.3.1 Toxicity and lethal effects

All mortalities of treated and control (larvae, pupae and adults) of *P. gossypiella* were recorded every day and corrected according to Abbott's formula (Abbott, 1925) [30] as follows:

$$\% \text{ of corrected mortality} = \frac{\% \text{ of test mortality} - \% \text{ of control mortality}}{100 - \% \text{ of control mortality}} \text{ X100}$$

The $LC_{50}$ values were calculated for general mortality by Microsoft® office Excel (2007), according to Finny (1971) [31].

### 2. Developmental and metamorphic parameters 2.3.

- ***Developmental rate*:** Dempster's equation [32] was applied for calculating the developmental duration, and Richard's equation [33] was used for calculating the developmental rate.
- ***Pupation rate*:** The pupation rate of the successfully developed pupae was calculated according to Jimenez-Peydro *et al*. [34] as follows:

  P.R. = [No. pupated larvae / No. treated larvae] × 100
- ***Deranged metamorphosis***: Deranged metamorphosis program was observed and calculated in larval-pupal or pupal-adult intermediates (%). Also, pupal deformation was calculated in %. Features of impaired development were recorded in photos.
- ***Pupal water loss*:** Pupal water loss was calculated depending on the data of the initial and final weights of the pupae, as follows:

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

### 2.4 Statistical analysis of data

Data obtained were analyzed by the Student's *t*-distribution, and refined by Bessel correction (Moroney, 1956) [35] for the test significance of difference between means.

## 3. Results

### 3.1 Lethal effects of Novaluron

After treatment of the newly hatched larvae of *P. gossypiella* with five concentration levels (5.0-0.05 ppm) of Novaluron through the artificial diet, data of toxic effect on all developmental stages were arranged in Table (1). As obviously shown in this table, complete mortality (100%) was observed among larvae at the highest concentration level. Therefore, the total mortality was recorded in a dose-dependent course (95.0, 75.0, 40.0 & 22.5%, at 1.0, 0.5, 0.1 & 0.05 ppm, respectively, vs. 10.0% mortality of control congeners). Thus, the corrected mortality was calculated in 100%. Novaluron had no chronic lethal effect on the metamorphosed pupae and adults since no mortality was observed. $LC_{50}$ was calculated in 0.187 ppm.

Table (**2**) contains data of survival potential of *P. gossypiella* after treatment of the full grown larvae with different concentration levels of Novaluron. As clearly shown in this table, all larvae, pupae and adult mortalities run proportionally to the ascending concentration levels. Novaluron exhibited the most toxic potency at its highest concentration level (50.0, 54.2 & 25.0% of larvae, pupae and adults, respectively). However, no larval mortality was caused at the lowest concentration level of this CSI. The corrected mortalities were calculated in a dose-dependent manner (81.08, 64.86, 35.13, 16.21 & 5.41%, at 5.0, 1.0, 0.5, 0.1 & 0.05 ppm, respectively). $LC_{50}$ was calculated in 0.765 ppm.

### 3.2 Effects of Novaluron on development and metamorphosis

In the present study, it was very difficult to remove the feeding larvae every day from the artificial diet for weighing. Therefore, no growth rate could be determined for larvae of *P. gossypiella*.

The most important developmental criteria of *P. gossypiella*, after treatment of the newly hatched larvae with four sublethal concentration levels (1.0-0.05 ppm) of Novaluron, were assorted in Table (3). As clearly seen in this table, the larval duration was insignificantly prolonged and the longest duration was measured after treatment with 0.5 ppm Novaluron (18.6±1.37 *vs.* 17.4±0.27 days of control larvae). Also, the developmental rate was slightly regressed except that rate of the single larva, at the highest concentration level, which developed in a rate of 6.25%. The pupation of treated larvae

was partially prevented (5.0, 25.0, 60.0 & 77.5 *vs*. 90.0% of control congeners). In addition, the pupal duration was unremarkably affected by the action of Novaluron (7.4±0.20, 7.4±0.29 & 7.6±0.29 days, at 0.05, 0.1 & 0.5 ppm, respectively, *vs*. 7.3±0.14 days of control pupae).

Because the pupal death may be due to the desiccation caused by Novaluron, loss of body water (%) was estimated. Unexpectedly, the pupal water loss slightly decreased at the lower three concentration levels (18.07, 18.83 & 18.15, compared to 20.64% of control pupae). The exceptional single pupa, which survived after treatment with the highest concentration level, excessively lost body water. Thus, Novaluron failed to exhibit a general desiccating action on pupae.

After treatment of full grown larvae of *P. gossypiella* with five sublethal concentration levels (5.0-0.05 ppm) of Novaluron, the developmental data were summarized in Table (4). Depending on these data, the larval duration was remarkably prolonged in a dose-dependent course (3.5±0.28, 4.1±0.12, 4.2±0.21, 5.5±0.42 & 5.8±0.24 days, at 0.05, 0.1, 0.5, 1.0 & 5.0 ppm, respectively, *vs*. 2.8±0.05 days of controls). These data were confirmed by the calculated developmental rate since these larvae had been prohibited to develop proportionally to the increasing concentration. With regard to the pupation, a considerable prohibition was recorded in a dose-dependent manner, exceptionally at the lowest concentration since all treated larvae had not been affected by Novaluron but could pupate as their control congeners. The pupal period was generally prolonged, in a dose-dependent course. The slightly longer periods were measured at the higher two concentration levels (8.6±0.1 & 8.2±0.29 days, at 5.0 & 1.0 ppm, respectively, *vs*. 7.7±0.17 days of control pupae). On the other hand, the body water loss increased but in no certain trend.

In respect of the metamorphosis program, Novaluron could not exhibit a disruptive effect since no larval-pupal had been formed after treatment of newly hatched larvae of *P. gossypiella* (Table 3) while such program was impaired after treatment of full grown larvae with the higher two concentration levels. This impaired program could be detected in 45. & 20.83% larval-pupal intermediates, at 5.0 & 1.0 ppm, respectively, Table 4). Various features of larval-pupal intermediates had been shown in Fig (1). On the other hand, Novaluron exerted a deteriorating action on the pupal morphogenesis program only after treatment of the newly hatched larvae with the higher three concentration levels (100, 30.0 & 20.83% deformed pupae, at 1.0, 0.5 & 0.1 ppm, respectively, Table 3). The major forms of pupal deformations had been obviously demonstrated in Fig (2).

**Table 1:** Toxicity and lethal effects (%) of Novaluron on *P. gossypiella* after treatment of newly hatched larvae.

| Conc. (ppm) | Larval mortality | Pupal mortality | Adult mortality | Total mortality | Corrected mortality | $LC_{50}$ (ppm) |
|---|---|---|---|---|---|---|
| 5.0 | 100.0 | --- | --- | 100.0 | 100.0 | 0.187 |
| 1.0 | 95.0 | 00.0 | 00.0 | 95.0 | 94.44 | |
| 0.5 | 75.0 | 00.0 | 00.0 | 75.0 | 72.22 | |
| 0.1 | 40.0 | 00.0 | 00.0 | 40.0 | 33.33 | |
| 0.05 | 22.5 | 00.0 | 00.0 | 22.5 | 13.88 | |
| Control | 10.0 | 00.0 | 00.0 | 10.0 | 00.00 | |

Conc.: Concentration level.

**Table 2:** Toxicity and lethal effects (%) of Novaluron on *P. gossypiella* after treatment of full grown larvae.

| Conc. (ppm) | Larval mortality | Pupal mortality | Adult mortality | Total mortality | Corrected mortality | $LC_{50}$ (ppm) |
|---|---|---|---|---|---|---|
| 5.0 | 50.0 | 54.2 | 25.0 | 82.5 | 81.08 | 0.765 |
| 1.0 | 40.0 | 33.8 | 17.5 | 67.5 | 64.86 | |
| 0.5 | 12.5 | 20.1 | 13.4 | 40.0 | 35.13 | |
| 0.1 | 02.5 | 17.8 | 03.6 | 22.5 | 16.21 | |
| 0.05 | 00.0 | 10.0 | 02.5 | 12.5 | 05.41 | |
| Control | 00.0 | 07.5 | 00.0 | 07.5 | 00.00 | |

Conc.: see footnote of Table (1).

**Table 3:** Developmental effects of Novaluron treatments of newly hatched larvae of *P. gossypiella*

| Conc. (ppm) | Larval stage | | | Pupal stage | | | |
|---|---|---|---|---|---|---|---|
| | Duration (days) (mean ± SD) | Develop. rate (%) | Larval-pupal Inter. (%) | Pupation rate (%) | Deformed pupae (%) | Duration (days) (mean ± SD) | Water loss (%) |
| 1.0 | 16.0* | 6.25 | 00.00 | 05.0 | 100.0 | 7.0* | 21.12 |
| 0.5 | 18.6±1.37a | 5.38 | 00.00 | 25.0 | 30.00 | 7.6±0.29a | 18.15 |
| 0.1 | 17.6±0.33a | 5.68 | 00.00 | 60.0 | 20.83 | 7.4±0.29a | 18.83 |
| 0.05 | 17.9±0.59a | 5.59 | 00.00 | 77.5 | 00.00 | 7.4±0.20a | 18.07 |
| Control | 17.4±0.27 | 5.75 | 00.00 | 90.0 | 00.00 | 7.3±0.14 | 20.64 |

Conc.: see footnote of Table (1). 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). *: one individual only. Develop: Developmental. Inter.: Intermediates.

**Table 4:** Developmental effects of Novaluron treatments of full grown larvae of *P. gossypiella*

| Conc. (ppm) | Larval stage | | | Pupal stage | | | |
|---|---|---|---|---|---|---|---|
| | Duration (days) (mean ± SD) | Develop. rate (%) | Larval-pupal Inter. (%) | Pupation (%) | Deformed pupae (%) | Duration (days) (mean ± SD) | Water loss (%) |
| 5.0 | 5.8± 0.24 d | 18.23 | 45.00 | 50.00 | 00.00 | 8.6± 0.10 b | 21.24 |
| 1.0 | 5.5± 0.42 d | 18.18 | 20.83 | 60.00 | 00.00 | 8.2± 0.29 b | 19.47 |
| 0.5 | 4.2± 0.21 d | 23.81 | 00.00 | 87.80 | 00.00 | 8.0± 0.48 a | 19.86 |
| 0.1 | 4.1± 0.12 d | 24.39 | 00.00 | 97.50 | 00.00 | 7.9± 0.34 a | 19.43 |
| 0.05 | 3.5± 0.28 c | 28.57 | 00.00 | 100.0 | 00.00 | 7.7± 0.15 a | 19.90 |
| Control | 2.8± 0.05 | 35.71 | 00.00 | 100.0 | 00.00 | 7.7± 0.17 | 19.29 |

Conc.: see footnote of Table (1). a, b, c, d, Develop., Inter. : see footnote of Table (3).


**Fig 1:** Larval-pupal intermediates of *P. gossypiella* as features of disturbed metamorphosis program after treatment of the full grown larvae with Novaluron, regardless the time of larval treatment and concentration level. (A): normal full grown larva. (B): normal pupa. (C): larval-pupal intermediate.

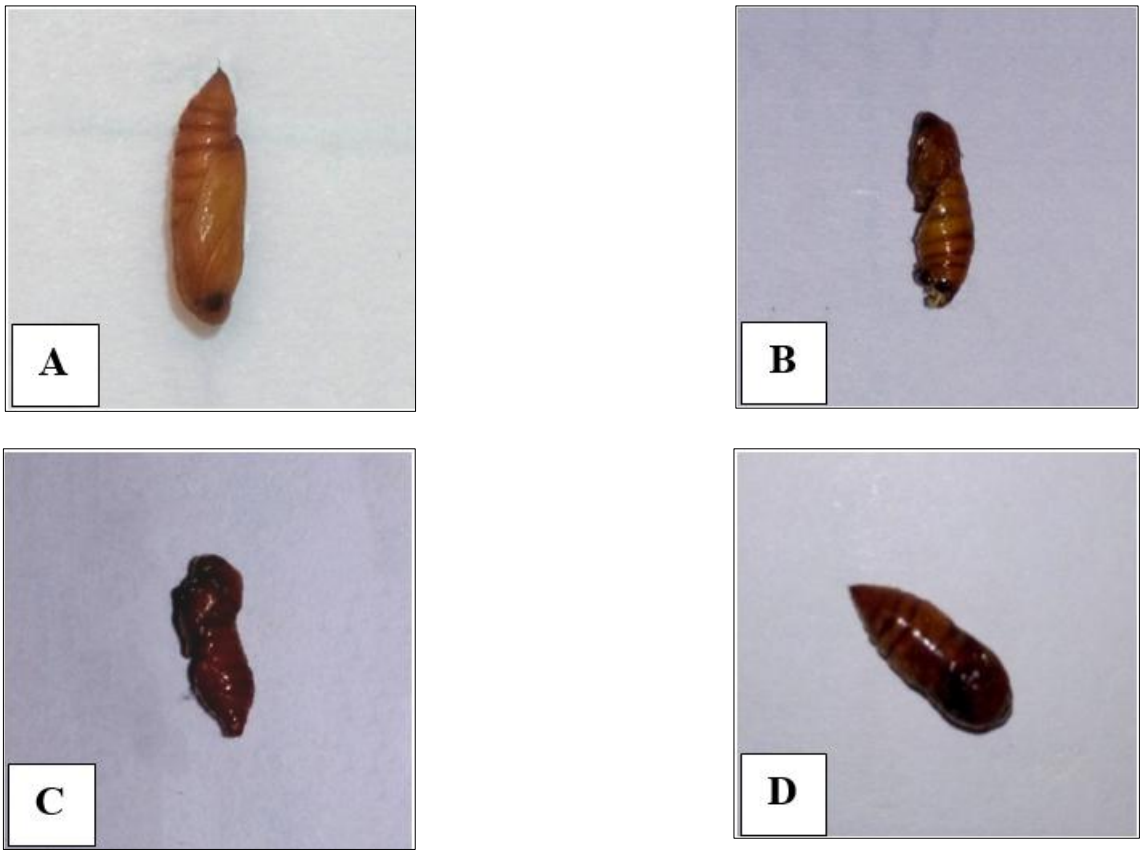


**Fig 2:** Deformed pupae of *P. gossypiella* by Novaluron. (A): normal pupa. (B, C & D): various features of pupal deformations.

## 4. Discussion

### 4.1 Affected survival potential of *P. gossypiella* by Novaluron

The available literature contains many reported results of toxic effects of several IGRs (Juvenoids, ecdysteroids and chitin synthesis inhibitors, CSIs) on various insect species, such as *Spodoptera littoralis* by Diflubenzuron (Aref *et al*., 2010) [36], Triflumuron (Bakr *et al*., 2010; El-Sheikh and Ashour, 2011) [37,38], Chlorfluazuron (Bayoumi *et al*., 1998) [39], Flufenoxuron (El-Naggar, 2013) [40], Lufenuron (Abdel Rahman *et al*., 2007; Adel, 2012; Gaaboub *et al*., 2012; Bakr *et al*., 2013) [41-44], Buprofezin (Nasr *et al*., 2010; Ragaei and Sabry, 2011) [45,46], Tebufenozide and Methoxyfenozide (Pineda *et al*., 2004) [47], Cyromazine (Tanani *et al*., 2015) [48], *etc*. Also, various IGRs exhibited toxic effects against other insects, such as Tebufenozide and Methoxyfenozide against *Choristoneura fumiferana* (Sundaram *et al*., 2002) [49]; Diofenolan against *Musca domestica* (Hamadah, 2003) [50] and *Papilio demoleus* (Singh and Kumar, 2011) [51]; Pyriproxyfen against *Eurygaster integriceps* (Mojaver and Bandani, 2010) [52]; Flufenoxuron against *Dysdercus koenigii* (Khan and Qamar, 2011) [53]; Diflubenzuron against *Halyomorpha halys* (Kamminga *et al*., 2012) [54]; Chlorfluazuron against *Spodoptera litura* (Perveen, 2012) [55]; Flufenoxuron, RH-5849 and Pyriproxyfen against *Locusta migratoria* var. *manilensis* (Hu *et al*., 2012) [56]; Kinoprene against *Culex pipiens* (Hamaidia and Soltani, 2014) [57]; Flufenoxuron and Methoprene against *Agrotis ipsilon* (Khatter, 2014) [58] and Lufenuron against *Tribolium castaneum* (Gado *et al*., 2015) [59]. Recently, IGRs of different categories exhibited varying degrees of toxicity against larvae of some insects, such as Pyriproxyfen against *Spodoptera mauritia* (Resmitha and Meethal, 2016) [60]; Lufenuron and Methoxyfenozide against *T. castaneum* (Ali *et al*., 2016) [61]; Methoxyfenozide against *Culex pipiens* (Hamaidia and Soltani, 2016) [62]; RH-5849 and Tebufenozide (RH-5992) against *Ephestia kuehniella* (Tazir *et al*., 2016) [63]; Lufenuron against *Glyphodes pyloalis* (Aliabadi *et al.*, 2016) [64] and *Helicoverpa armigera* (Vivan *et al*., 2016) [65]; Fenoxycarb against *Corcyra cephalonica* (Singh and Tiwari, 2016; Begum and Qamar, 2016) [66, 67]; Carbaryl and Buprofezin against *Paracoccus marginatus* (Khan, 2016) [68]; Chlorfluazuron, Cyromazine, Lufenuron, and Precocene I against *Ctenocephalides felis* (Rust and Hemsarth, 2016) [69]; Methoprene and Pyriproxyfen against *Culex quinquefasciatus* and *Aedes albopictus* (Khan *et al*., 2016) [70]; Cyromazine appeared to be effective tool for controlling the muscid flies (*M. domestica*, *Stomoxys calcitrans* and *Fannia canicularis*) since considerable mortalities were recorded (Donahue *et al*., 2017) [71].

Results of the present study on *P. gossypiella* were, to a great extent, consistent with those reported results, since treatment of full grown larvae with five concentrations (5.0-0.05 ppm) of Novaluron resulted in remarkable mortalities among all developmental stages, in a dose-dependent course. On the other hand, Novaluron failed to exhibit lethal effects on pupae or adults after treatment of newly hatched larvae. Also, the present results may be agreed with the toxic effects of Novaluron on other insect pests, such as *H. armigera* (Murthy and Ram, 2002) [72], *Aedes aegypti* (Mulla *et al*., 2003; Nwankwo *et al*., 2011) [73,74], *M. domestica* (Cetin *et al*., 2006) [75], *Bombus terrestris* (Mommaerts *et al*., 2006) [76], *Phlebotomus papatasi* (Mascari *et al*., 2007) [77], *Conotrachelus nenuphar* (Hoffmann *et al*., 2008) [78], *Culex quinquefasciatus* (Jambulingam *et al*., 2009) [79], *Megachile rotundata* (Hodgson *et al*., 2011) [80], *Culiseta longiareolata* (Bouaziz *et al*., 2011) [81], *Grapholita molesta* (Batista *et al*., 2011) [82], *Haematobia irritans* and *S. calcitrans* (Lohmeyer and Pound, 2012) [83], *H. halys* (Kamminga *et al*., 2012) [54], *C. pipiens* (Djeghader *et al*., 2013, 2014) [84, 85] and *S. littoralis* (Barrania 2013; Ghoneim *et al*., 2015) [86, 27].

$LC_{50}$ value depends on several factors, such as susceptibility of the insect and its treated stage or instar, lethal potency of the tested compound and its concentration levels, method and time of treatment, as well as the experimental conditions. As reported in the available literature, $LC_{50}$ values of Novaluron and lufenuron against *S. litura* were determined as 350.45 and 453.78 ppm, respectively (Sharma and Pathania, 2014) [87]; $LC_{50}$ of Pyriproxyfen was found to be 0.025% against *S. litura* larvae (Kaur and Chandi, 2015) [88]; $LC_{50}$ of Hexaflumuron against *H. armigera* was 8.47 mg /L (Taleh *et al*., 2015) [89]; $LD_{50}$ values of the ecdysone agonists RH-5849 and RH-5992 (Tebufenozide) against *E. kuehniella* were 0.05 and 0.005 µg/insect, respectively (Tazir *et al*., 2016) [63]; $LC_{50}$ of the ecdysone agonist Methoxyfenozide against *Culex pipiens* was calculated in 24.54 µg/L (Hamaidia and Soltani, 2016) [62]; $LC_{50}$ of Lufenuron against *G. pyloalis* was 19 ppm (Aliabadi *et al*., 2016) [64]; $LC_{50}$ values of Chlorfluazuron, Cyromazine, Lufenuron and Precocene I against *C. felis* were 0.19, 2.66, 0.20, and 10.97 ppm, respectively (Rust and Hemsarth, 2016) [69]; *etc*.

With regard to *P. gossypiella*, the experimental insect in the present study, various $LC_{50}$ values of different IGRs, after treatment of newly hatched larvae, were determined, such as 2.41, 6.07 and 31.01 ppm of Tebufenozide, Acetamiprid and Ethoxazole, respectively (Zidan *et al*., 1998) [90]; 0.0423 and 0.1961 ppm of Diflubenzuron and Chlorfluazuron, respectively (Kandil *et al*., 2005) [3]; 87.5 and 15.1 ppm of Buprofezin alone and in combination with piperonyl butoxide, respectively (Al-Kazafy, 2013) [91]; 61.859 ppm of Teflubenzuron (El-Khayat *et al*., 2015) [5] as well as 20.6, 47.4 and 50.8 ppm of Pyriproxyfen, Methoxyfenozide and Lufenuron, respectively (Sabry and Abdou, 2016) [14]. In the present study, $LC_{50}$ values were calculated in 0.187 and 0.765 ppm, after treatment of newly hatched and full grown larvae of *P. gossypiella* with Novaluron. It was clearly shown that $LC_{50}$ varied depending on the larval instar under treatment. Similar variation was reported for Novaluron on *S. littoralis* against which $LC_{50}$ values were 2.71 and 2.65 ppm, after treatment of penultimate instar larvae and last instar larvae, respectively (Ghoneim *et al*., 2015) [27]. Also, $LC_{50}$ values of Cyromazine against the same lepidopterous insect were 74.44 and 82.91 ppm, after treatment of these larval instars, respectively (Tanani *et al*., 2015) [48].

To understand the recorded mortalities of larvae, pupae and adults of *P. gossypiella* by the toxic effect of Novaluron, in the present study, IGRs exhibit their toxic effects on insects with a mode of action other than that of synthetic insecticides. Furthermore, CSIs interfere with the synthesis or deposition of chitin on the exoskeleton or other chitinized internal structures, such as the peritrophic matrix, hindering the role of peritrophic membrane in protecting the secreting cells from damage (Merzendorf and Zimoch, 2003; Merzendorf, 2005) [92,93]. In other words, three sites have been proposed for describing the mode of action of CSIs namely: inhibition of chitin synthetase

(or its biosynthesis), inhibition of proteases (or its biosynthesis) and inhibition of UDP-N-acetylglucosamine transport through the membrane (Miyamoto *et al*., 1993) [94]. Further, it was suggested that the CSI interferes with the transport system of UDP-N-acetyl amine across the membrane (Eto, 1990) [95].

The larval deaths of *P. gossypiella* by Novaluron, in the current work, may be attributed to the failure of larvae to moult owing to the inhibition of chitin formation (Abdel Rahman *et al*., 2007; Adel, 2012) [41, 42] or to the inability to shed their exocuticle (Zorzetti *et al*., 2015) [96] or to swallow volumes if air for splitting the old cuticle and expand the new one during ecdysis (Linton *et al*., 1997) [97]. Also, the larval deaths of *P. gossypiella* by Novaluron may be due to a prohibition of feeding and continuous starvation (Ghoneim *et al*., 2000) [98].

Although the disturbance of hormonal regulation or the disrupting of normal activity of the endocrine system in insects by IGRs was reported (Al-Sharook *et al*., 1991; Oberlander *et al*., 1997) [ 99,100] and suggested for some mosquito species (Mulla *et al*., 2003; Djeghader *et al.*, 2014) [73,85], the pupal deaths in *P. gossypiella*, in the present investigation, could not be directly relate to the hormonal activity of Novaluron, but other factors or causes, such as suffocation, bleeding and desiccation due to imperfect exuvation, failure of vital homeostatic mechanisms, etc. (Smagghe and Degheele, 1994) [101]. This suggestion can easily be substantiated since Novaluron exerted a general desiccating action on pupae after treatment of full grown larvae of *P. gossypiella*, in the present study, albeit it failed to exert similar action of them after treatment of newly hatched larvae.

In addition, the adult mortality of *P. gossypiella* after treatment of full grown larvae with Novaluron, in the current study, can be explained by the retention and distribution of it in the insect body as a result of rapid transport from the gut of treated larvae into other tissues, the direct and rapid transport the haemolymph to other tissues, and/or to lower detoxification capacity against the tested CSI (Osman *et al*., 1984) [102].

### 4.2. Retarded development of *P. gossypiella* by Novaluron

From the pest control view of point, it is not necessary to use a highly toxic compound, since several IGRs exhibit low toxicity against in insect pest but exhibit disruptive effects on its development, metamorphosis, reproductive potential, general or specific metabolic processes and/or larval haemogram. Therefore, an IGR should be assessed against some or all of these criteria to be recommended for pest control.

As reported in the available literature, many IGRs (and CSIs) exhibited some inhibitory effects on the development of various insects, such as *S. littoralis* by Diflubenzuron (Aref *et al*., 2010) [36], Methoprene and Fenoxycarb (Karam, 2000) [103], Lufenuron (Gaaboub *et al*., 2012) [43], Novaluron (Ghoneim *et al*., 2015) [27], Cyromazine (Tanani *et al*., 2015) [48]; *P. demoleus* by Diofenolan (Singh and Kumar, 2011) [51]; *S. litura* by Chlorfluazuron (Perveen, 2012) [55]; *A. aegypti* (Farnesi *et al*., 2012) [104] and *C. pipiens* (Djeghader *et al*., 2013, 2014) [84, 85] by Novaluron; *C. pipiens* by Kinoprene (Hamaidia and Soltani, 2014) [57] and *A. ipsilon* by Methoprene and Flufenoxuron (Khatter, 2014) [58]. Recently, the developmental duration was prolonged indicating regressed developmental rate in some other insects by various IGRs, such as *Plutella xylostella* by Pyriproxyfen (Mahmoudvand *et al*., 2015) [105]; *G. pyloalis* by Lufenuron (Aliabadi *et al*., 2016) [64]; *C. pipiens* by Methoxyfenozide (Hamaidia and Soltani, 2016) [62] and some novel N-tert-butylphenyl thenoylhydrazide (ecdysteroid agonists) derivatives (Song *et al*., 2016) [106]; *C. cephalonica* by Fenoxycarb (Begum and Qamar, 2016) [67]; *etc*.

In agreement with those reported results of retarded development, the present study recorded a slight or drastic retarding effect of Novaluron on the development of *P. gossypiella*, since larval duration was insignificantly prolonged after treatment of newly hatched larvae, as well as larval and pupal durations had been remarkably prolonged after treatment of full grown larvae. In addition, the present results are in accordance with the reported results of retarded development of the same insect after treatment of newly hatched larvae with Hexaflumuron (Moawad and Khidr, 1982) [107]; Diflubenzuron and Chlorfluazuron (Kandil *et al*., 2005) [3]; Buprofezin (Al-Kazafy, 2013) [91]; Teflubenzuron (El-Khayat *et al*., 2015) [5] and Chromafenozide and Diflubenzuron (Salem, 2015) [108]; Lufenuron and Pyriproxyfen (Sabry and Abdou, 2016) [14]; *etc*.

In contrast, the present results disagree with those reported results of enhanced development (shortened larval and/or pupal durations) in *P. gossypiella* after treatment with Methoxyfenozide (Sabry and Abdou, 2016) [14] and other insects, such as *Rhynchophorus ferrugineus* by lufenuron and Diofenolan (Tanani, 2001) [109], *A. ipsilon* by Flufenoxuron (El-Sheikh, 2002) [110] and *Schistocerca gregaria* by lufenuron (Bakr *et al*., 2008) [111]. Also, Diofenolan and lufenuron failed to affect the development of *M. domestica* (Ghoneim *et al*., 2004) [112].

The retarded development, as expressed in prolonged larval and/or pupal durations of *P. gossypiella* by Novaluron, in the current study, may be attributed to the indirect interference of this CSI with neuroendocrine organs responsible for the synthesis and release of tropic hormones, like prothoracicotropic hormone (PTTH) (Subrahmanyam *et al*., 1989) [113]. Also, Novaluron may affect the tissues and cells undergoing mitosis (Nasiruddin and Mordue, 1994) [114] or exhibit a delaying effect on the ecdysis and transformation (Linton *et al*., 1997) [97]. In particular, the final step of chitin biosynthesis pathway was inhibited by this CSI and the precursor was not converted into chitin leading to a prolongation of larval life (Djeghader *et al*., 2014) [85].

### 4.3. Disrupted metamorphosis and morphogenesis of *P. gossypiella* by Novaluron

Depending on the available literature, the major symptoms and features of the impaired metamorphosis of an insect after treatment with various IGRs (or CSIs) had been described as reduction of pupation and adult emergence, production of larval-pupal and/or pupal-adult intermediates, deformed larvae and/or pupae and the production of supernumerary larval instars (superlarvae). However, all or some of these features were observed in various insects by the disruptive effects of IGRs (or CSIs), such as *S. littoralis* by Chlorfluazuron (Shaaban, 1993; Sammour *et al*., 2008) [115,116], Triflumuron (Radwan, 1992; El-Naggar, 2013) [117, 40], Lufenuron (Abdel Rahman *et al*., 2007; Adel, 2012) [41,42], Flufenoxuron (Bakr *et al*., 2010; El-Naggar, 2013) [37, 40], Methoprene and Fenoxycarb (Karam, 2000) [103]; Novaluron (Ghoneim *et al*., 2015) [27] and Cyromazine (Tanani *et al*., 2015) [48]. Also, some or all of these symptoms of the impaired metamorphosis were recorded after treatment of different insects with several IGRs (or CSIs), such as *Ceratitis capitata* (Viñuela *et al*., 1993) [118]; *T. castaneum*

and *T. confusum* (Kamaruzzaman *et al*., 2006) [119], *Liriomyza trifolii* (Saryazdi *et al*., 2012) [120] and *Callosobruchus maculates* (Al-Makhlafi *et al*., 2012) [121] by Cyromazine; *H. armigera* (Murthy and Ram, 2002) [72], *Phlebotomus papatasi* (Mascari *et al*., 2007) [77], *A. aegypti* (Martins *et al*., 2008; Nwankwo *et al*., 2011) [122,74], *M. domestica* and *S. calcitrans* (Lohmeyer *et al*., 2014) [123] by Novaluron; *Lipaphis erysimi* by Pyriproxyfen (Liu and Chen, 2001) [124]; *Rh. ferrugineus* (Tanani, 2001) [109] and *P. demoleus* (Singh and Kumar, 2011) [51] by Diofenolan; *Lobesia botrana* by Lufenuron (Saenz-de-Cabezon *et al*., 2005) [125]; *C. pipiens* by Kinoprene (Hamaidia and Soltani, 2014) [57]; *etc*.

In the present study on *P. gossypiella*, treatment of full grown larvae with Novaluron concentrations resulted in drastic reduction of the pupation rate, in a dose-dependent manner but slightly reduced after treatment of newly hatched larvae may be due to a gradual declination of the potency of Novaluron. These results are, to some extent, consistent with the reported results of reduced pupation rate of some insects by various IGRs, such as *P. xylostella* by Hexaflumuron (Mahmoudvand *et al*., 2012) [126], *S. littoralis* by Novaluron (Ghoneim *et al*., 2015) [27] and Cyromazine (Tanani *et al*., 2015) [48], *G. pyloalis* by Lufenuron (Aliabadi *et al*., 2016) [64] or Fenoxycarb (Singh and Tiwari, 2016) [66] and *Encarsia formosa* by Pyriproxyfen and Fenoxycarb (Wang and Liu, 2016) [127].

Novaluron failed to affect the metamorphosis program after treatment of newly hatched larvae of *P. gossypiella*, in the current work, while such program was disturbed after treatment of full grown larvae, since some larval-pupal intermediates had been produced, at certain concentrations. These results are in agreement with some of those reported results of disturbed metamorphosis of a number of insect pests by various IGRs, such as *H. armigera* by Hexaflumuron (Taleh *et al*., 2015) [89], *S. littoralis* by Novaluron (Ghoneim *et al*., 2015) [27] and Cyromazine (Tanani *et al*., 2015) [48] and *C. cephalonica* by Fenoxycarb (Begum and Qamar, 2016) [67].

Only after treatment of newly hatched larvae of *P. gossypiella* with Novaluron, in the present investigation, the pupal morphogenesis was deranged (as observed in pupal deformities). No deformed pupae were recorded after treatment of full grown larvae may be due to the finish of major steps of metamorphosis into the pupal stage. Also, no malformed larvae were observed, regardless the time of treatment. Similar deranged pupal morphogenesis had been reported for *T. castaneum* and *T. confusum* after treatment with Cyromazine (Kamaruzzaman *et al*., 2006) [119] and *C. cephalonica* after topical application of last instar larvae with Fenoxycarb (Begum and Qamar, 2016) [67].

As reported by Pineda *et al*. (2009) [128], the effects caused by IGRs on the metamorphosis of insects may be important from a practical stand-point because they could result in various morphogenic defects as well as mortality. Lepidoptera belong to the most sensitive groups of insects regarding the growth regulating effects of these compounds. Disturbed metamorphosis of *P. gossypiella* by Novaluron, in the present study, can be interpreted by its interference with the hormonal regulation of pupation program since its disturbance by IGRs was reported (Al-Sharook *et al*., 1991) [99]. In other words, Novaluron may affect these programs leading to an inhibition of metamorphosis *via* an ecdysteroid reduction, interference with the release of eclosion hormone or/and inhibition of the neurosecretion (PTTH) (Josephrajkumar *et al*., 1999) [129]. In addition, production of larval-pupal intermediates in *P. gossypiella* can be explicated by an inhibitory effect of Novaluron on the chitin biosynthesis, chitin synthase (Mayer *et al*., 1980) [130] and DNA synthesis (Mitlin *et al*., 1977) [131]. Whatever the mode of action, Novaluron suppressed the chitin synthesis and prevented the normal deposition of new cuticle during apolysis leading to the production of pupal abnormalities (Retnakaran *et al*., 1985) [132].

## 5. Conclusion

On the basis of overall findings, it can be concluded that Novaluron is toxic to some developmental stages of *P. gossypiella*, as well as caused various impairing effects on its development, metamorphosis and morphogenesis. Thus, Novaluron may be considered as a leading target compound having the potential to control *P. gossypiella* and can form an important component of integrated pest management program for this insect pest which has developed resistance to the majority of conventional insecticides.

## 6. References


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