

# Toxicity and Disruptive Impacts of Novaluron, A Chitin Synthesis Inhibitor, on Development and Metamorphosis of The Olive Leaf Moth *Palpita unionalis* (Hübner) (Lepidoptera: Pyralidae)

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## ABSTRACT

The olive leaf moth *Palpita unionalis* (Lepidoptera: Pyralidae) is an economic pest of the commercial olive groves in Egypt and different Mediterranean countries. The present study was conducted aiming to assess the effects of Novaluron, a chitin synthesis inhibitor, on survival, growth, development and metamorphosis of this pest. The newly moulted last instar (6th) larvae had been treated with six concentrations (100.0, 10.0, 1.00, 0.10, 0.01 and 0.001 ppm), via the fresh olive leaves, as food. Different degrees of toxicity were recorded on all developmental stages. LC<sub>50</sub> was calculated in 0.97 ppm. The somatic weight gain of larvae was drastically reduced and the larval growth rate was severely regressed, regardless the concentration. The larval duration was generally shortened but the pupal duration was remarkably prolonged, in a dose-dependent manner. The pupation rate was regressed, especially at the higher four concentrations. The metamorphosis program was impaired, since larval-pupal intermediates had been produced at some concentrations. In addition, the pupal morphogenesis was disrupted, since some pupal deformities had been observed at some concentrations.

**Keywords:** growth, larva, morphogenesis, mortality, pupa, toxicity

## I. INTRODUCTION

From the Zoogeographical point of view, the Mediterranean Basin was reported as the original area of the olive leaf moth *Palpita unionalis* (Hübner)(Lepidoptera: Pyralidae). Now it is an international lepidopterous migratory pest in the tropical and subtropical regions of the Old World [1, 2]. *P. unionalis* is one of the most dangerous pests of

olives in Egypt and other Mediterranean countries [3-6]. The most important damage of this pest occurs on young trees, nurseries and shoots of old trees [7, 8]. The control of *P. unionalis* on olive trees has relied upon the use of traditional synthetic insecticides [9]. Different pesticides exhibited a good control when applied on the early larval instars [10]. Insecticide residues have been detected in olive oil and in the environment where olives are grown [11]. In addition, the extensive use of conventional insecticides has caused resistant insect strains to emerge [12, 13] and serious toxicological problems to humans and the environment [14, 15]. Therefore, alternative materials have been initiated recently to minimize the pesticide hazards and introduce of new effective and safer ways and negligible effects on ecosystem.

Over the past four decades, efforts have been made to develop insecticidal compounds with selective properties that act specifically on biochemical sites that are present in particular insect groups but with properties that differ from conventional insecticides[16-18]. Insect Growth Regulators (IGRs) belong to a group of compounds which are not directly toxic, but act selectively on normal growth, development metamorphosis and/or reproduction in insects *via* disrupting the hormonally regulated physiological processes [19-24]. Because of their desirable characteristics, such as low toxicity, less environmental pollution, high selectivity, and low impact on natural enemies and people, IGRs are used to control various insect pests [25-27]. Several IGRs have been extensively studied for investigating their effects on metamorphosis and reproduction in a number of insect species [28, 29]. On the basis of the mode of action, IGRs had been grouped in three categories: (i) Juvenile hormone analogues (JHAs) (also called as Juvenoids), (ii) Ecdysteroid agonists and (iii) Chitin synthesis inhibitors (CSIs) or moult

inhibitors [30, 16, 31]. They had been, also, grouped in CSIs and substances that interfere with the action of insect hormones (i.e. juvenile hormone analogues, and ecdysteroids) [32].

CSIs interfere with chitin biosynthesis in insects and thus prevent moulting, or produce an imperfect cuticle [33]. By affecting the hormonal balance, they disrupt several physiological processes in insect body [33]. Also, CSIs are less toxic compounds to the non-target organisms and beneficial biota and have no residual effects [34]. One of the novel benzoylphenyl ureas is the Novaluron. It inhibits the chitin formation on larvae of various insects of different orders [35, 36] and exhibits a high toxicity against several dipterous species [37-42]. It is, also, a powerful suppressor of lepidopteran larvae [43] and whiteflies [44, 45] as well as some species of Hemiptera [46, 47] and Coleoptera [48-50]. The disruptive effects of Novaluron on survival, growth, development, metamorphosis and/or morphogenesis had been reported in some insects, such as *Helicoverpa armigera* [51], *Musca domestica* [37], *Phlebotomus papatasi* [38], *Aedes aegypti* [52, 39, 53], *Culex pipiens* [42], *Stomoxys calcitrans* [54] and *Spodoptera littoralis* [55], *Pectinophora gossypiella* [56] as well as it disrupted the adult performance and reproductive potential [57], declined the main metabolites [58], and deteriorated the larval haemogram [59] of the latter lepidopteran pest. 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 [60]. The compound has no appreciable effect on natural enemies [44] and low mammalian toxicity [61, 62]. Depending on the currently available literature, no body assessed the effects of Novaluron on *P. unionalis*. Taking all of these considerations into account, the present study was carried out aiming to investigate the effects of Novaluron on the survival, growth, development, metamorphosis of this serious pest.

## II. MATERIALS AND METHODS

### 1. Experimental insect.

A sample of olive leaf moth *Palpita unionalis* (Hubner) (Lepidoptera: Pyralidae) larvae was kindly obtained from the culture of susceptible strain maintained for several generations in Desert Research Center, Cairo, Egypt. A new culture was maintained

in Department of Zoology and Entomology, Faculty of Science, Al-Azhar University, Cairo, Egypt, under laboratory controlled conditions (27±2°C, 65±5% R.H., photoperiod 14 and 10 h L:D) according to Mansour [63]. Larvae were daily provided with fresh olive leaves *Olea europaea* L, as a food. After the larval stage, the developed pupae were collected and transferred to Petri dishes (5.5×1.4cm). The emerged adults were daily collected and released in plastic jars (3L) provided with cotton pieces, soaked in 10% sugar solution, for feeding, as well as olive twigs (20 cm in length) as an oviposition site. After egg deposition, adult males and females were transferred into new plastic jars. The jars of eggs were provided with fresh tender olive twigs fixed in a small bottle containing water, so as to keep the leaves flat and fresh, for feeding of the newly hatched larvae. The fresh tender olive leaves were renewed daily until pupation.

### 2. Bioassay of Novaluron.

Novaluron [1-[chloro-4-(1,1,2-trifluoromethoxyethoxy) phenyl] -3- (2,6-difluorobenzoyl) urea] has the molecular formula: C<sub>17</sub>H<sub>9</sub>ClF<sub>8</sub>N<sub>2</sub>O<sub>4</sub>. It was supplied by Sigma-Aldrich Chemicals. A series of concentration levels of Novaluron was prepared by diluting with distilled water in volumetric flasks as follows: 100.0, 10.0, 1.0, 0.1, 0.01 and 0.001 ppm. Bioassay tests were carried out using the newly moulted last instar (6<sup>th</sup>) larvae. Fresh olive leaves were dipped in each concentration of Novaluron for 5 minutes and air dried before introduction to larvae for feeding. Control larvae were provided with water-treated olive leaves. Ten replicates of treated and control larvae (one larva/replicate) were kept separately in glass vials. The larvae were allowed to feed on treated leaves for 24 hrs. Then, they provided with fresh untreated olive leaves and all biological and physiological parameters were recorded daily.

### 3. Criteria of study.

#### 3.1. Toxicity test.

All mortalities of treated and control (larvae, pupae and adults) were recorded every day and corrected according to Abbott's formula [64] as follows:

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

The LC<sub>50</sub> value was calculated for general mortality by Microsoft office Excel, 2007, according to Finney [65].

#### 3.2. Growth, development and metamorphosis.

**Weight gain:** Each individual larva (treated and control) was carefully weighed every day using a digital balance for calculating the growth as follows:

$$\frac{\text{Initial weight (before the beginning of experiment)}}{\text{final weight (at the end of experiment)}}$$

**Growth rate:** Growth rate (GR) can be calculated according to Waldbauer [66] as follows:

$$\text{GR} = \frac{\text{fresh weight gain during feeding period}}{\text{feeding period} \times \text{mean fresh body weight of larvae during the feeding period}}$$

**Developmental rate:** Dempster's equation [67] was applied for calculating the developmental duration, and Richard's equation [68] was used for calculating the developmental rate.

**Pupation rate:** The pupation rate was expressed in % of the successfully developed pupae.

**Deranged metamorphosis:** different features of impaired metamorphosis program of *P. unionalis* were observed as larval-pupal intermediates, pupal-adult intermediates or extra moult and calculated in (%). Also, impaired pupal morphogenesis was observed as pupal deformations and calculated in %.

Various features of impaired metamorphosis and morphogenesis were recorded in photos.

#### 3.3. Pupal water loss.

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

$$\text{Water loss \%} = \frac{\text{[initial weight} - \text{final weight /initial Weight]} \times 100$$

### 4. Statistical analysis of data.

Data obtained were analyzed by the Student's *t*-distribution, and refined by Bessel correction [69] for the test significance of difference between means.

## III. RESULTS

### 1. Toxicity and lethal effects.

After treatment of the newly moulted last instar (6th) larvae of *P. unionalis* with six concentrations of Novaluron (100.0, 10.0, 1.00, 0.10, 0.01 and 0.001 ppm), via the fresh olive leaves, as food, data of toxicity and lethal effect on all developmental stages were distributed in Table (1). Depending on these data, Novaluron failed to affect the larval survival at the lower two concentrations but it exhibited various degrees of toxicity on larvae, at other concentrations, in no certain trend. The successfully developed pupae suffered a toxic effect of the tested compound, since different mortality %s had been recorded, in a dose-dependent course (20.0, 20.0, 28.5, 40.0, 71.4 and 100% mortality, at 0.001, 0.01, 0.10, 1.00, 10.00 and 100 ppm, respectively). As clearly shown, complete pupal mortality was recorded at the highest concentration of Novaluron. With regard to the adult moths, Novaluron failed to affect their survival except 0.01 ppm at which 12.5% adult mortality was estimated. The corrected mortality was found in a dose-dependent manner (for detail, see Table 1). LC<sub>50</sub> was calculated in 0.97 ppm.

## 2. Effects on Growth, Development and Metamorphosis.

The most important growth, developmental and metamorphic criteria of *P. unionalis*, after treatment of newly moulted last instar larvae with six concentration levels of Novaluron, were summarized in Table (2). According to these data, the somatic weight gain of larvae was drastically reduced, in a dose-dependent course ( $2.44 \pm 3.18$ ,  $2.42 \pm 1.45$ ,  $2.27 \pm 0.50$ ,  $2.10 \pm 1.46$ ,  $1.84 \pm 1.48$  and  $1.42 \pm 0.63$  mg, at 0.001, 0.01, 0.10, 1.00, 10.0 and 100 ppm, respectively, in comparison with  $6.23 \pm 3.34$  mg of control larvae). Also, Novaluron exhibited a strong suppressing effect on the larval growth rate, regardless the concentration ( $0.017 \pm 0.004$ ,  $0.019 \pm 0.002$ ,  $0.019 \pm 0.002$ ,  $0.013 \pm 0.006$ ,  $0.015 \pm 0.001$ ,  $0.014 \pm 0.001$ , at 100, 10.0, 1.00, 0.10, 0.01 and 0.001 ppm, respectively, vs.  $0.034 \pm 0.007$  of control larvae). In addition, the larval duration was generally shortened, in a dose-dependent course ( $3.33 \pm 0.50$ ,  $3.30 \pm 0.48$ ,  $3.28 \pm 0.48$ ,  $2.20 \pm 0.44$ ,  $2.14 \pm 0.37$  and  $1.75 \pm 0.50$  days, at 0.001, 0.01, 0.10, 1.00, 10.00 and 100 ppm, respectively, vs.  $3.60 \pm 0.69$  days of control larvae). Developmental rate of larvae is another parameter indicating an enhancing action of Novaluron, since the treated larvae developed in faster rate than control congeners. As obviously shown in the previously mentioned table, a reversal action of Novaluron was exerted on the developed pupae, since their duration was remarkably prolonged, in a dose-dependent manner ( $9.25 \pm 1.98$ ,  $9.28 \pm 0.75$ ,  $9.60 \pm 0.54$ ,  $10.66 \pm 1.52$  and  $12.50 \pm 0.70$  days, at 0.001, 0.01, 0.10, 1.00 and 10.0 ppm, respectively, vs.  $9.20 \pm 0.78$  days of control pupae). This prolongation of pupal stage was reflected in a retarded development, i.e., pupae developed in slower rate than that of control pupae (for detail, see Table 2).

Because the pupal death may be due to the desiccation caused by Novaluron, loss of body water was estimated in %. In general, the successfully developed pupae from treated larvae lost more body water than control pupae (28.6, 31.0, 31.0, 31.0 and 38.7%, at 0.001, 0.01, 0.10, 1.0 and 10.0 ppm, respectively, compared to 28.2% of control pupae).

With regard to the effects of Novaluron on metamorphosis and morphogenesis of *P. unionalis*, data listed in Table (2) exiguously revealed various disruptive effects such as the regressed pupation rate,

especially at the higher four concentrations (70, 60, 80 and 40%, at 0.10, 1.0, 10.0 and 100 ppm, respectively, vs. 100% pupation on control larvae). The metamorphosis program was impaired, since larval-pupal intermediates had been produced at some concentration levels (10, 30 and 10% at 0.10, 1.00 and 10.0 ppm, respectively). Description of these intermediate creatures was provided in Plate (1). Moreover, 10% of pupal-adult intermediates had been produced only at 0.01 ppm (see Plate 2). In addition, the pupal morphogenesis was disrupted, since some pupal deformities had been observed at some concentration levels (12.5 and 20.0%, at 10.0 and 1.0 ppm, respectively). Some malformed pupae had been observed in non-tanned segmented body or segmented body with tanned part and incompletely tanned part, depending on the concentration level of Novaluron (see Plate 3).

Table 1. Toxicity and lethal effects (%) of Novaluron treatment of newly moulted last instar larvae of *P. unionalis*.

Conc. (ppm)	Larval mortality	Pupal mortality	Adult mortality	Total mortality	Corrected mortality	LC <sub>50</sub>
100	60	100	*	100	100	0.97
10.0	30	71.4	00.0	80	80	
1.00	50	40.0	00.0	70	70	
0.10	30	28.5	00.0	50	50	
0.01	00	20.0	12.5	30	30	
0.001	00	20.0	00.0	20	20	
Control	00	00.0	00.0	0	----	

Conc.: Concentration level. \*: no adults.

Table 2. Growth and developmental effects of Novaluron treatment of newly moulted last instar larvae of *P. unionalis*.

Conc. (ppm)	Larval stage					Pupal stage					
	Weight gain (mg±SD)	Growth rate (Mean ±SD)	Larval duration (Mean days±SD)	Devel op. rate	Larv al-pupal inter. (%)	Pupa tion (%)	Pupal duration (Mean days±SD)	Devel op. rate	Pupal - adult inter. (%)	Pupal defor mities (%)	Wat er loss (%)
100	1.42±0.63 b	0.017±0.004d	1.75±0.50 d	57.1	00	40	*	*	*	*	---
10.0	1.84±1.48 b	0.019±0.002d	2.14±0.37 d	46.7	10	80	12.50±0.70 d	8.00	00	12.5	38.7
1.00	2.10±1.46 b	0.019±0.002d	2.20±0.44 c	45.4	30	60	10.66±1.52 b	9.38	00	20.0	31.0
0.10	2.27±0.50 c	0.013±0.006d	3.28±0.48 a	30.4	10	70	9.60±0.54 a	10.4	00	00.0	31.0
0.010	2.42±1.45 c	0.015±0.001d	3.30±0.48 a	30.3	00	100	9.28±0.75 a	10.7	10	00.0	31.0
0.001	2.44±3.18 b	0.014±0.001d	3.33±0.50 a	30.0	00	100	9.25±1.98 a	10.7	00	00.0	28.6
Control	6.23±3.34	0.034±0.007	3.60±0.69	27.7	00	100	9.20±0.78	10.8	00	00.0	28.2

Conc.: See footnote of Table (1). Develop. rate: Developmental rate. inter.: intermediates. Mean ± SD followed with the 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 ( $p < 0.001$ ). \*: died pupae.

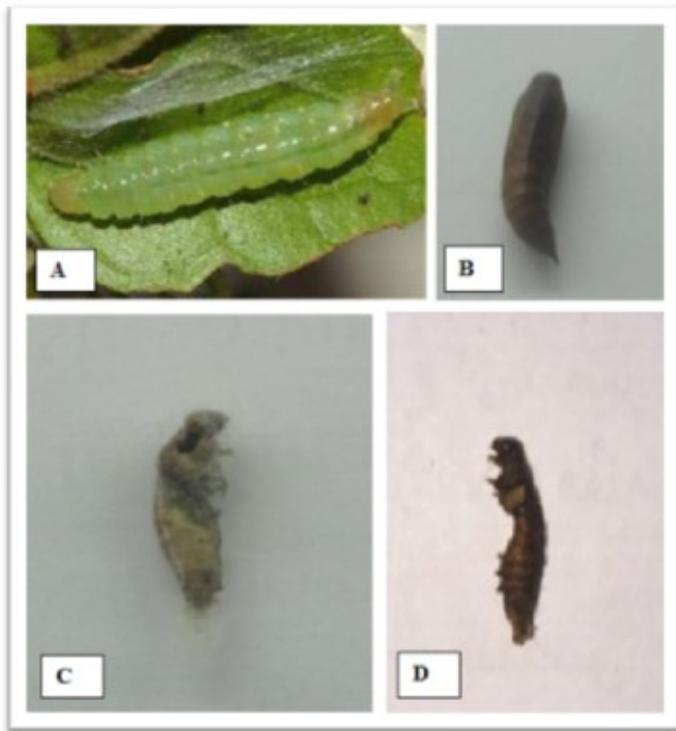


Plate 1. Larval-pupal intermediates of *P. unionalis* as a feature of disturbed metamorphosis program after treatment of newly moulted last instar larvae with Novaluron. (A): Control larva. (B): Control pupa. (C): Larval-pupal intermediate (larval head and thorax with pupal abdomen: 10.0 ppm and 1.0 ppm). (D): Larval-pupal intermediate (pupated dorsal part with larval legs and head: 0.1 ppm and 1.0 ppm).

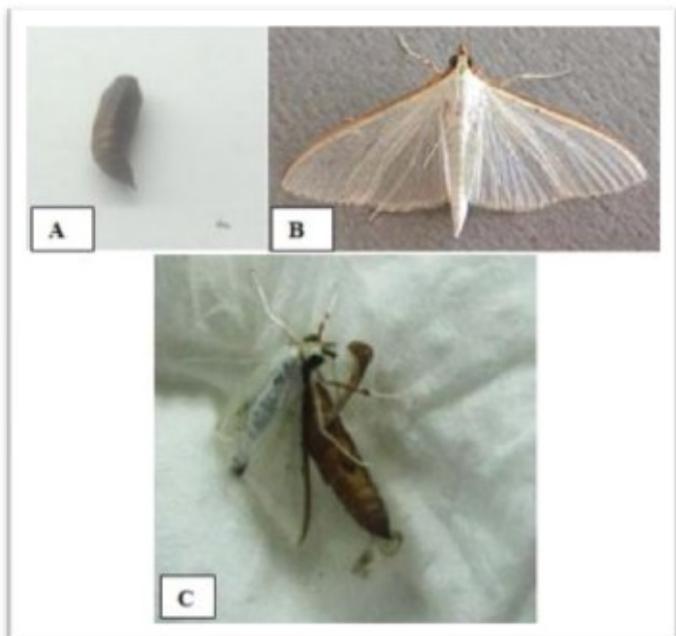


Plate 2. Pupal-adult intermediate of *P. unionalis* as a feature of disturbed metamorphosis program after treatment of newly moulted last instar larvae with Novaluron. (A): Control pupa. (B): Control adult. (C): Pupal-adult intermediate (0.01 ppm).



Plate 3. Deteriorated pupal morphogenesis of *P. unionalis* by Novaluron. (A) Control pupa (B): deformed pupa: non-tanned segmented body (1ppm). (C): deformed pupa: segmented pupa with tanned part and incompletely tanned part (10 ppm).

#### IV. DISCUSSION

##### I. Affected survival potential of *P. unionalis* by Novaluron.

The currently available literature contains many reported results of toxic effects of several insect growth regulators (IGRs)(Juvenoids, ecdysteroids and chitin synthesis inhibitors, CSIs) on various insect species, such as *Spodoptera littoralis* by Diflubenzuron [70], Triflumuron [71], Flufenoxuron [72], Lufenuron [73,74], Buprofezin [75,76], Tebufenozide and Methoxyfenozide [77], Cyromazine [78]; *Papilio demoleus* by Diofenolan [79]; *Eurygaster integriceps* by Pyriproxyfen [80]; *Dysdercus koenigii* by Flufenoxuron [81]; *Halyomorpha halys* by Diflubenzuron [46]; *Spodoptera litura* by Chlorfluazuron [82]; *Locusta migratoria* var. *manilensis* by Flufenoxuron, RH-5849 and Pyriproxyfen [83]; *Culex pipiens* by Kinoprene [84]; *Agrotis ipsilon* by Flufenoxuron and Methoprene [85] or Pyriproxyfen [86] and *Tribolium castaneum* by Lufenuron [87]. Recently, IGRs of different categories exhibited varying degrees of toxicity against some insects, such as Pyriproxyfen against *Spodoptera mauritia* [29]; Lufenuron and Methoxyfenozide against *T. castaneum* [88];

Methoxyfenozide against *C. pipiens* [89]; RH-5849 and Tebufenozide (RH-5992) against *Ephestia kuehniella* [90]; Lufenuron against *Glyphodes pyloalis* [91] and *Helicoverpa armigera* [92]; Fenoxycarb against *Corcyra cephalonica* [93,94]; Buprofezin against *Paracoccus marginatus* [95]; Chlorfluazuron, Cyromazine, Lufenuron, and Precocene I against *Ctenocephalides felis* [96]; Methoprene and Pyriproxyfen against *Culex quinquefasciatus* and *Aedes albopictus* [97]; Cyromazine against *Musca domestica*, *Stomoxys calcitrans* and *Fannia canicularis* [98], as well as Pyriproxyfen and Methoxyfenozide [99] and Novaluron [56] against *Pectinophora gossypiella*.

Results of the present study on *P. unionalis* were, to some extent, in agreement with the previously reported results of toxicity, since Novaluron (a chitin synthesis inhibitor, CSI) exhibited various degrees of toxicity on larvae, at all concentrations, except the lower two concentrations. Also, different pupal mortality %s had been recorded, in a dose-dependent course. Only at 0.01 ppm of Novaluron, 12.5% of adult mortality was estimated.

As reported in the available literature, LC<sub>50</sub> values of Novaluron and lufenuron against *S. litura* were determined as 350.45 and 453.78 ppm, respectively [100]; LC<sub>50</sub> of Pyriproxyfen was found to be 0.025% against *S. litura* larvae [86]; LC<sub>50</sub> of Hexaflumuron against *H. armigera* was 8.47 mg /L [101]; LD<sub>50</sub> values of RH-5849 and Tebufenozide against *E. kuehniella* were 0.05 and 0.005 µg/insect, respectively [90]; LC<sub>50</sub> of Methoxyfenozide against *Culex pipiens* was calculated in 24.54 µg/L [89]; LC<sub>50</sub> of Lufenuron against *G. pyloalis* was 19 ppm [91] and LC<sub>50</sub> values of Chlorfluazuron, Cyromazine, Lufenuron and Precocene I against *C. felis* were 0.19, 2.66, 0.20, and 10.97 ppm, respectively [96]. Also, a variation in LC<sub>50</sub> values was reported for Novaluron on *S. littoralis*, since LC<sub>50</sub> values were 2.71 and 2.65 ppm, after treatment of penultimate instar larvae and last instar larvae, respectively [55]. In the current investigation on *P. unionalis*, LC<sub>50</sub> of Novaluron was calculated in 0.97 ppm. Thus, LC<sub>50</sub> 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.

To explicate the recorded toxic effect of Novaluron on larvae, pupae and adults of *P. unionalis*, in the present study, IGRs exhibit their toxic effects on insects with a mode of action other than that of conventional insecticides. Furthermore, CSIs interfere with the synthesis and/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 [102,103]. Furthermore, it was suggested that the tested CSI interferes with the transport system of UDP-N-acetyl amine across the membrane [104].

For some detail, the larval deaths of *P. unionalis* by Novaluron, in the current study, may be attributed to the failure of larvae to moult (lethal moult) owing to the inhibition of chitin formation [105,106], to the inability to shed their exocuticle [107], or to swallow volumes of air for splitting the old cuticle and expand the new one during ecdysis [108]. Also, these larval deaths may be due to the prevented feeding and continuous starvation of the present insect [109].

Although the disturbance of hormonal regulation or the disrupting of normal activity of the endocrine system in insects by IGRs was reported [110,111] and suggested for some mosquito species [35,112], the pupal deaths in *P. unionalis*, in the present investigation, could not be directly relate to the hormonal activity of Novaluron, but to other causes, such as suffocation, bleeding and desiccation due to imperfect exuvation, failure of vital homeostatic mechanisms, etc. [113]. This suggestion can easily be substantiated since Novaluron exerted a predominant desiccating action on the successfully developed pupae of *P. unionalis* to lose more body water than control pupae, in the present study.

In addition, the adult mortality of *P. unionalis* after treatment of newly moulted last instar larvae only with 0.01 ppm of Novaluron, in the current 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, by the direct and rapid transport *via* the haemolymph to other tissues, and/or by lower detoxification capacity of adults against the tested CSI [114].

## 2. Disturbance of growth and development of *P. unionalis* by Novaluron.

Depending on the currently available literature, some authors have taken into account the body weight gain by the insect larvae as a valuable indicator for growth [115]. In the present study, both larval weight gain and growth rate had been determined after treatment of newly moulted last (6<sup>th</sup>) instar larvae of *P. unionalis* with different concentrations of Novaluron. The somatic weight gain of larvae was drastically reduced and the larval growth rate was severely regressed, regardless the concentration. Also, larval duration was generally shortened and the developmental rate of these larvae was enhanced.

However, the inhibited growth of *P. unionalis* by Novaluron, in the present study, was in accordance with those reported results of inhibited growth of some insects by various IGRs, such as *Spodoptera littoralis* by Tebufenozide [116], Flufenoxuron [71], Lufenuron [106], Triflumuron [72] and Novaluron [117, 55]; *Cirratitis capitata* by Cyromazine [118], *P. demoleus* by Diofenolan [79], *S. litura* by Chlorfluazuron [82], *Aedes aegypti* [53] and *Culex pipiens* [42,112] by Novaluron, *C. pipiens* by Kinoprene [84] and *A. ipsilon* by Methoprene and Flufenoxuron [85]. Likewise, some IGRs failed to affect the growth of various insects, such as *M. domestica* [119], *Periplaneta americana* and *Oncopeltus fasciatus* [120], *Spodoptera exempta*, *Spodoptera exigua*, and *Leptinotarsa decemlineata* [113].

On the other hand, the present results of shortened larval duration and enhanced developmental rate of *P. unionalis* larvae were in agreement with the reported results of shortened larval duration of *P. gossypiella* after treatment of newly hatched larvae with Methoxyfenozide [99] and other insects, such as *Rhynchophorus ferrugineus* by Lufenuron and Diofenolan [121], *A. ipsilon* by Flufenoxuron [122] and *Schistocerca gregaria* by Lufenuron [123]. On the contrary, the present results disagreed with the reported results of prolonged larval duration of *S. littoralis* larvae after treatment of penultimate or last instar larvae with by Novaluron [55] and Cyromazine [78]; prolonged larval duration after treatment of 5<sup>th</sup> instar larvae of *Spodoptera frugiperda* with LC<sub>10</sub> and LC<sub>25</sub> of Methoxyfenozide [124] and prolonged larval duration in *P. gossypiella* after treatment of the first instar larvae with Pyriproxyfen [99].

Lepidoptera belong to the most sensitive groups of insects regarding the growth regulating effects of IGRs. The inhibited growth of *P. unionalis* by some concentrations of Novaluron, in the current study, may be a result of the blocked release of morphogenic peptides, causing alteration in the ecdysteroid and juvenoid titers [125]. Also, Novaluron may affect the tissues and cells undergoing mitosis [126].

As reported in the available literature, many IGRs (including CSIs) exhibited some inhibitory effects on the general development of various insects, such as *S. littoralis* by Diflubenzuron [70], Methoprene and Fenoxycarb [127], Lufenuron [73], Novaluron [55] and Cyromazine [78]; *P. demoleus* by Diofenolan [79]; *S. litura* by Chlorfluazuron [82]; *A. aegypti* [53] and *C. pipiens* [42, 112] by Novaluron; *C. pipiens* by Kinoprene [84]; *A. ipsilon* by Methoprene and Flufenoxuron [85]; *P. gossypiella* by Diflubenzuron and Chlorfluazuron [128], Buprofezin [129]; Teflubenzuron [130] and Chromafenozide [131]. Recently, the developmental duration was prolonged indicating retarded development in some other insects by various IGRs, such as *G. pyralis* by Lufenuron [91]; *C. pipiens* by Methoxyfenozide [89] and N-tert-butylphenyl thenoylhydrazide (ecdysteroid derivative)

[132]; *C. cephalonica* by Fenoxycarb [94]; *P. gossypiella* by Lufenuron and Pyriproxyfen [99] and Novaluron [56]; etc. In agreement with those reported results of retarded development, the present study recorded a powerful retarding effect of Novaluron on the development of *P. unionalis*, since the pupal duration was remarkably prolonged and the developmental rate of pupae was considerably regressed.

In the current study, retarded development of *P. unionalis* by Novaluron, as expressed in prolonged pupal duration and regressed developmental rate, 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)[133]. The prolongation of larval or pupal duration may be due to the persistence of juvenile hormone (JH) in the haemolymph where it is only in the absence of JH that ecdysone could be activated and lead to the formation of the next stage [134]. Also, Novaluron may exhibit a delaying effect on the ecdysis and transformation [108]. 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 developmental duration [112].

### 3. Impaired metamorphosis and morphogenesis of *P. unionalis* by Novaluron.

The effects exhibited by IGRs on insect metamorphosis may be important from the practical stand-point because they could result in various morphogenic defects as well as mortality [135]. Depending on the available literature, the major symptoms and features of the impaired metamorphosis of an insect after treatment with various IGRs (including 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 as responses to the

disruptive effects of different IGRs, such as *S. littoralis* by Chlorfluazuron [136], Triflumuron [72], Lufenuron [105,106], Flufenoxuron [71,72], Methoprene and Fenoxycarb [127]; Novaluron [55] and Cyromazine [78]. Also, some or all of these symptoms of the impaired metamorphosis were recorded after treatment of different insects with several IGRs, such as *T. castaneum* and *T. confusum* [137], *Liriomyza trifolii* [138] and *Callosobruchus maculatus* [139] by Cyromazine; *H. armigera* [51], *Phlebotomus papatasi* [38], *A. aegypti* [52, 39], *M. domestica* [54] by Novaluron; *Lipaphis erysimi* by Pyriproxyfen [140]; *Rh. ferrugineus* [121] and *P. demoleus* [79] by Diofenolan; *Lobesia botrana* by Lufenuron [141]; *C. pipiens* by Kinoprene [84]; etc.

In the present study on *P. unionalis*, Novaluron detrimentally prohibited the pupation process, since pupation % considerably decreased, especially at the higher four concentrations. This results was, to a great extent, consistent with those reported results of reduced pupation rate of some insects by various IGRs, such as *P. xylostella* by Hexaflumuron [142], *S. littoralis* by Novaluron [55] and Cyromazine [78], *G. pyloalis* by Lufenuron [91] and Fenoxycarb [93] as well as *Encarsia formosa* by Pyriproxyfen and Fenoxycarb [24].

In the present study on *P. unionalis*, the pupal morphogenesis was deranged, since different pupal deformities had been observed, at some concentrations of Novaluron. Some malformed pupae appeared in non-tanned segmented body or segmented body with tanned part and incompletely tanned part, depending on the concentration level of Novaluron. To some extent, similar deranged pupal morphogenesis had been reported for *T. castaneum* and *T. confusum* after treatment with Cyromazine [137], *Spodoptera frugiperda* after feeding of 5<sup>th</sup> instar larvae on a diet treated with LC<sub>10</sub> and LC<sub>25</sub> of Methoxyfenozide [124], *C. cephalonica* after topical application of last instar larvae with Fenoxycarb [94] and *P. gossypiella* after treatment of the full grown larvae with Novaluron [56]. 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 deformities [19].

In the current investigation on *P. unionalis*, Novaluron exhibited a disruptive effect on the metamorphosis program, since larval-pupal intermediates had been produced, after treatment of newly moulted last instar larvae with some concentrations. This feature of impaired metamorphosis was, also, described as abnormal or lethal pupation [124]. Our result was, to a great extent, 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 [101], *S. littoralis* by Novaluron [55] and Cyromazine [78], *C. cephalonica* by Fenoxycarb [94] and *P. gossypiella* by Novaluron [56]. Also, the larval-pupal intermediates were observed after topical treatment of last instar larvae of *Spodoptera exempta*, *Spodoptera exigua*, *S. littoralis*, *Mamestra brassicae*, *Galleria mellonella*, *Mythimna unipuncta* and *Spodoptera frugiperda* with RH-5849, Tebufenozide or Methoxyfenozide [143, 113, 116]. Moreover, some pupal-adult intermediates of *P. unionalis* had been produced only at 0.01 ppm of Novaluron, in the current investigation, as a feature of impaired metamorphosis program. As far as our literature survey could ascertain, no information was available on the production of pupal-adult intermediates.

The production of larval-pupal and pupal-adult intermediates, in the present study on *P. unionalis*, indicated the disturbance of metamorphosis program by Novaluron. It can be interpreted by the interference of Novaluron with the hormonal regulation of pupation program [110]. For some detail, some conceivable scenarios can be described herein. (1) Novaluron may inhibit the metamorphosis program via an ecdysteroid reduction, interference with the release of eclosion hormone or/and inhibition of the neurosecretion (PTTH) [144]. (2) The production of these intermediates may indicate a juvenile property

of Novaluron retarding the perfect larval-pupal or/and pupal-adult transformation. These mosaic creatures are unusual and died soon after formation. (3) The production of intermediate creatures in *P. unionalis* can be explicated by an inhibitory effect of Novaluron on the DNA synthesis [145] or the chitin biosynthesis and chitin synthase [146]. (4) The molt induction had lethal consequences because the induction of a rapid molt did not provide enough time for the completion of larval-pupal transformation. Thus, the insects molted to nonviable forms between the life stages [147]. Molts induced during the early phase of the last instar produce larval-like individuals, while those formed in the late phase generate pupal-like individuals [148].

## CONCLUSION

Depending on results of the present study, it can be concluded that Novaluron exhibited various degrees of toxicity against all developmental stages of *P. unionalis*, as well as it displayed some disruptive effects on development, metamorphosis and pupal morphogenesis. Therefore, Novaluron may be considered as a promising control agent against this economic pest of the commercial olive groves in Egypt and other olive producing countries as a potential alternative to the conventional pesticides.

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