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Effectiveness of the chitin synthesis inhibitor, diofenolan, on survival and development of the pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae)

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Abstract
The objective of current investigation was to evaluate the toxicity and developmental effects of diofenolan (10.0, 1.0, 0.1, 0.01 and 0.001 ppm) on this insect pest *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) in Physiology laboratory, department of Zoology and Entomology, Faculty of Science, Al-Azhar University during 1917. LC_{50} values were estimated in 0.028 ppm and 0.036 ppm, after treatment of newly hatched and full grown larvae, respectively. After treatment of the newly hatched or full grown larvae with sublethal concentrations of Diofenolan, larval duration was pronouncedly prolonged and the developmental rate was drastically regressed, in a dose-dependent course. The pupation process was detrimentally prohibited, regardless the larval instar under treatment. Although diofenolan failed to exhibit a disruptive effect on the metamorphosis program (larval-pupal intermediates) after treatment of the newly hatched larvae, such program was impaired after treatment of full grown larvae, especially at the higher three concentrations. Also, the pupal morphogenesis was disturbed (pupal deformities) after treatment of larvae, irrespective of the instar under treatment.

Keywords: Adult, larva, metamorphosis, morphogenesis, mortality, pupa

1. Introduction
The discriminate and intensive uses of many conventionally synthetic pesticides have led to several dramatic problems, such as the environmental pollution, hazards to human and animals, destruction of the pollinators and other non-target insects as well as the natural enemies, like parasites and predators [1-7]. At present, insect growth regulators (IGRs) are considered as the possible alternative agents of the traditional insecticides for controlling insect pests [3-5]. IGRs can be grouped according to their mode of action as chitin synthesis inhibitors (CSIs) and substances that interfere with the action of insect hormone (i.e. juvenile hormone analogues, ecdysteroids) [8]. Diofenolan is a CSI used for the control of several pests, such as some lepidopterous species and scale insects [9, 10], *Papilio demoleus* [11], *Musca domestica* [12], *Rhynchophorus ferrugineus* [13, 14], *Schistocerca gregaria* [15-17] and *Pectinophora gossypiella* [18, 19]. Fortunately, it was found non-toxic for several beneficial parasitoids and predators of some pests, such as *Chrysoperla carnea* [20].
Worldwide, the pink bollworm *Pectinophora gossypiella* is one of the most destructive insect pests that cause terrible damage to the cotton because it is difficult to be controlled by conventional insecticides [21, 22]. Larvae damage the floral outgrowths, flowers, bolls, developing seeds within bolls and deteriorate the staple length and strength of lint. The termination of boll growth results in boll rotting and premature or partial boll opening [23]. In Egypt, this insect causes serious damage to cotton arising to one million kentar annually [24, 25]. Moreover, *P. gossypiella* has been reported to develop resistance against the transgenic cotton varieties in some regions of the world, such as Arizona (USA) [26]. In Egypt, also, this insect has recently developed resistance to several classes of traditional insecticides currently used in cotton fields because of its ability to detoxify these chemicals [27]. Therefore, IGRs have been initiated recently to avoid the environment hazards and to minimize the serious problems of synthetic insecticides to humans and animals, as well as to delay the resistance development in *P. gossypiella* [28-32]. The present study was conducted to evaluate the toxicity of Diofenolan and its disruptive effect on development and metamorphosis of *P. gossypiella*.

2. Materials and Methods

2.1 Insect culture

A culture of the pink bollworm *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) was established under constant conditions (27±2 °C and 75±5% R.H.) at Department of Zoology and Entomology, Faculty of science, Al-Azhar University, Cairo, Egypt. For this purpose, a sample of newly hatched larvae was obtained from the susceptible culture of *P. gossypiella* maintained in Plant Protection Research Institute, Doqqi, Giza, Egypt. Larvae were provided with an artificial diet as described by Abd El-Hafez *et al*. [33]. For rearing details and manipulation of all developmental stages under the previously mentioned laboratory conditions, see Ghoneim *et al*. [34]. The study was conducted during Feb.-Oct. 2017.

2.2 Diofenolan administration

Diofenolan (CGA-59205, Aware®) (2S,4R)-2-Ethyl-4-[(4-phenoxyphenoxy) methyl]-1,3-dioxolane has the molecular formula $C_{18}H_{20}O_4$. It was purchased from Sigma-Aldrich Chemicals. Five concentration levels of Diofenolan were prepared by diluting with distilled water in volumetric flasks, as follows: 10.0, 1.0, 0.1, 0.01 and 0.001 ppm. Two experimental series of larvae were used: newly hatched larvae and full grown (4th instar) larvae. Four replicates (10/replicate) of newly hatched larvae, were separately transferred 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 an atomizer, with each of the prepared concentrations. Control replicates were treated with distilled water only using the same technique. Also, four replicates (10/replicate) of full grown larvae were separately transferred into Petri dishes (one replicate/dish). Each replicate was sprayed with one of the prepared concentrations using an atomizer. 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

Toxicity: All mortalities of treated and control (larvae, pupae and adults) of *P. gossypiella* were recorded every day and corrected according to Abbott's formula [35] as follows:

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

The LC_{50} values were calculated for general mortality by Microsoft® office Excel (2007), according to Finny[36].

Developmental and metamorphic parameters:
Developmental rate: Dempster's equation [37] was applied for calculating the developmental duration, and Richard's equation [38] 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*. [39] as follows:

P.R. = [No. puparated 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

Data obtained were analyzed by the Student's *t*-distribution, and refined by Bessel correction [40] for the test significance of difference between means.

3. Results

3.1 Toxic effects of Diofenolan

Five concentration levels (10.0, 1.0, 0.1, 0.01 and 0.001 ppm) of diofenolan had been applied, *via* the artificial diet, on the newly hatched larvae of *P. gossypiella*. Data of toxic effects on all developmental stages are presented in Table 1. On the basis of these data, the strongest toxic effect of diofenolan was exhibited at its highest concentration level since complete mortality (100%) was recorded among treated larvae. Thus, no pupae had been produced. At other concentration levels, diofenolan displayed various degrees of toxicity among larvae and pupae but failed to exhibit adulticidal effect because no adult mortality was observed. Both larval and pupal mortalities were consecutively correlated with the concentration level. Moreover, the strongest acute toxicity was exhibited on larvae at 1.0 ppm (77.5% *vs*. 10% mortality of control congeners). In an ascending trend of concentration level, the corrected mortalities were determined as 11.1, 41.7, 61.1, 88.9 and 100 %, respectively. LC_{50} was calculated in 0.028 ppm.

In the light of data arranged in Table 2, treatment of full grown larvae of *P. gossypiella* with diofenolan concentrations resulted in different degrees of toxicity because it failed to exhibit lethal effect on larvae, at the lower concentration levels, or adults, at the lowest concentration level. On the other hand, it caused complete pupal mortality (100%) at the highest concentration level. Thus, no adults could emerge. In general, pupal and adult mortalities run in a dose-dependent course and the corrected mortalities were found in a similar trend (10.80, 37.80, 64.90, 78.40 and 100%, at 0.001, 0.01, 0.1, 1.0 and 10.0 ppm, respectively). LC_{50} was calculated in 0.036 ppm.

3.2 Diofenolan effects on Development and Metamorphosis

After treatment of the newly hatched larvae of *P. gossypiella* with diofenolan concentrations, data of the most important developmental criteria were distributed in Table 3. According to these data, the larval duration was significantly prolonged in a dose-dependent course (38.4±3.57, 35.6±1.09, 32.5±1.04 and 29.3±0.72 days, at 1.0, 0.1, 0.01 and 0.001 ppm, respectively, *vs*. 15.1±0.25 days of control larvae). The developmental rate is another estimated parameter substantiating this prolongation since it was considerably regressed proportionally to the ascending concentration. In respect of the inhibitory effect of diofenolan on pupation, the calculated pupation% was severely reduced in a dose-dependent course (22.5, 50.0, 67.5 and 82.50%, at 1.0, 0.1, 0.01 and 0.001 ppm, respectively, *vs*. 90.0% pupation of controls). In addition, the pupal duration was elaborately prolonged in a consecutive trend with the ascending concentration (10.5±1.32, 10.1±0.15, 9.8±0.75 and 9.2±0.29

days, at 1.0, 0.1, 0.01 and 0.001 ppm, respectively, *vs*. 7.3±0.08 days of control pupae).

Because the pupal death may be due to the desiccation caused by diofenolan, loss of body water (%) was estimated. Water loss of pupae was found in diverse percentages since the larger amount of body water was lost by pupae at the highest concentration level while lower water loss% was calculated for pupae at other concentration levels (for detail, see Table 3). Thus, the desiccating action of Diofenolan on pupae depended on the concentration level.

After treatment of the full grown larvae with diofenolan concentration levels, data of the developmental criteria are arranged in Table 4. Depending on these data, the larval duration was remarkably lengthened in a dose-dependent manner (9.0±0.98, 8.3±0.49, 6.2±0.41, 4.6±0.50 and 4.0±0.41 days, at 10.0, 1.0, 0.1, 0.01 and 0.001 ppm, respectively, *vs*. 3.0±0.09 days of control pupae). This prevalent prolongation was reflected on the developmental rate since it was considerably regressed in a dose-dependent trend. Only at the higher concentrations of Diofenolan, the pupation rate was remarkably depressed (70.0, 92.5 and 97.5%, at 10.0, 1.0 and 0.1 ppm, respectively, *vs*. 100% pupation of control insects).

At the highest concentration, all pupae died. So, pupal duration could not be measured. At other concentration levels, the pupal duration was significantly prolonged (9.4±0.52, 9.0±0.16, 8.1±0.21 and 7.4±0.1, at 1.0, 0.1, 0.01 and 0.001 ppm, respectively, *vs*. 7.4±0.17 days of control pupae). The pupal water loss% increased in a dose-dependent course indicating that Diofenolan exerted a predominant desiccating action on the treated pupae.

In respect of the metamorphosis program, diofenolan exhibited no effect since no larval-pupal or pupal-adult intermediates could be produced after treatment of newly hatched larvae (Table 3). On the contrary, treatment of full grown larvae with the higher three concentration levels of diofenolan resulted in impaired program (51.14, 29.73 and 15.38% larval-pupal intermediates, at 10.0, 1.0 and 0.1 ppm, respectively, Table 4). The major features of these intermediates were shown in Figure (1). With regard to the pupal morphogenesis, treatment of newly hatched larvae with diofenolan resulted in the formation of deformed pupae (66.67 and 50.0%, at 1.0 and 0.1 ppm, respectively, Table 3). After treatment of full grown larvae with only the highest concentration (10.0 ppm) resulted in 25.0% pupal deformations (Table 4). Some features of the pupal malformations were demonstrated in Figure 2.

Table 1: Toxicity and lethal effects (%) of diofenolan treatments of newly hatched larvae of *P. gossypiella*.

Conc. (ppm)	Larval mortality	Pupal mortality	Adult mortality	Total mortality	Corrected mortality	LC_{50} (ppm)
10.0	100.0	---	---	100.0	100.0	0.028
1.0	77.5	58.3	00.00	90.00	88.90	
0.1	50.0	30.4	00.00	65.00	61.10	
0.01	32.5	22.1	00.00	47.50	41.70	
0.001	17.5	3.10	00.00	20.00	11.10	
Control	10.0	00.0	00.00	10.00	00.00	

Conc.: Concentration level. ---: no pupae or adults.

Table 2: Toxicity and lethal effects (%) of diofenolan treatments of full grown larvae of *P. gossypiella*.

Conc. (ppm)	Larval mortality	Pupal mortality	Adult mortality	Total mortality	Corrected mortality	LC_{50} (ppm)
10.0	30.00	100.0	---	100.0	100.0	0.036
1.0	07.50	45.80	60.00	80.00	78.40	
0.1	02.50	38.60	45.10	67.50	64.90	
0.01	00.00	25.00	23.70	42.50	37.80	
0.001	00.00	17.50	00.00	17.50	10.80	
Control	00.00	07.50	00.00	07.50	00.00	

Conc.: see footnote of Table (1), ---: no adults.

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

Conc. (ppm)	Larval stage			Pupal stage			
	Duration (mean days ± SD)	Develop. rate (%)	Larval-pupal Inter. (%)	Pupation rate (%)	Deformed pupae (%)	Duration (mean days ± SD)	Water loss (%)
1.0	38.4±3.57 d	2.60	00.00	22.5	66.67	10.5±1.32 c	21.1
0.1	35.6±1.09 d	2.81	00.00	50.0	50.00	10.1±0.15 d	17.1
0.01	32.5±1.04 d	3.07	00.00	67.5	00.00	9.8±0.75 d	15.1
0.001	29.3±0.72 d	3.41	00.00	82.5	00.00	9.2±0.29 d	16.9
Control	15.1±0.25	6.62	00.00	90.0	00.00	7.3±0.08	18.6

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). Develop.: Developmental. Inter.: Intermediates.

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

Conc. (ppm)	Larval stage			Pupal stage			
	Duration (mean days ± SD)	Develop. rate (%)	Larval-pupal Inter. (%)	Pupation rate (%)	Deformed pupae (%)	Duration (mean days ± SD)	Water loss (%)
10.0	9.0±0.98 d	11.11	57.14	70.00	25.00	---	---
1.0	8.3±0.49 d	12.04	29.73	92.50	00.00	9.4±0.52 d	23.5
0.1	6.2±0.41 d	16.12	15.38	97.50	00.00	9.0±0.16 d	21.9
0.01	4.6±0.50 d	21.74	00.00	100.0	00.00	8.1±0.21 c	19.5
0.001	4.0±0.41 c	25.00	00.00	100.0	00.00	7.4±0.10 a	18.1
Control	3.0±0.09	33.33	00.00	100.0	00.00	7.4±0.17	17.7

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

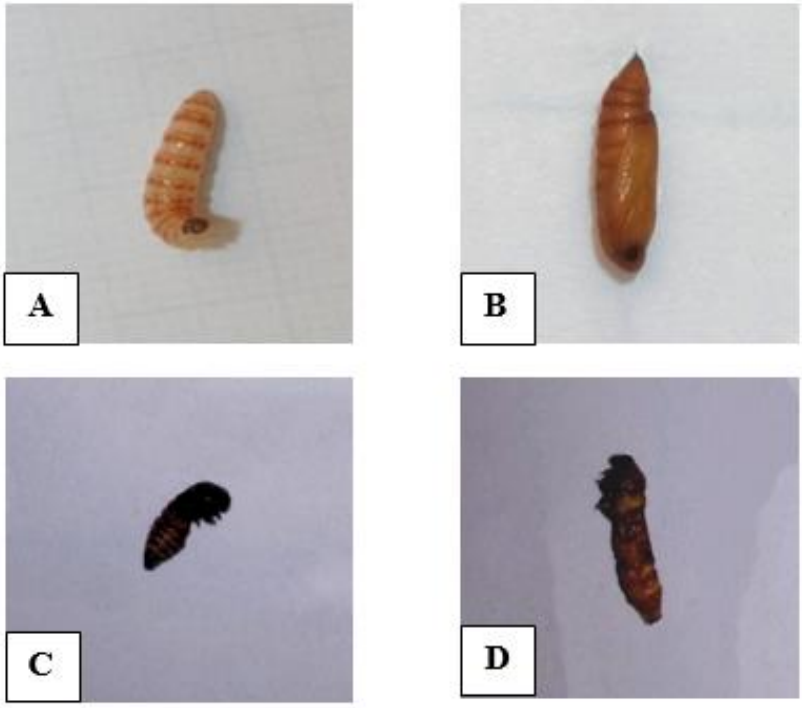

Fig 1: Larval-pupal intermediates of *P. gossypiella* as features of disturbed metamorphosis program after treatment of the full grown larvae with diofenolan larval treatments, regardless the treated instar or concentration level. (A): normal full grown larva. (B): normal pupa. (C & D): various larval-pupal intermediates.

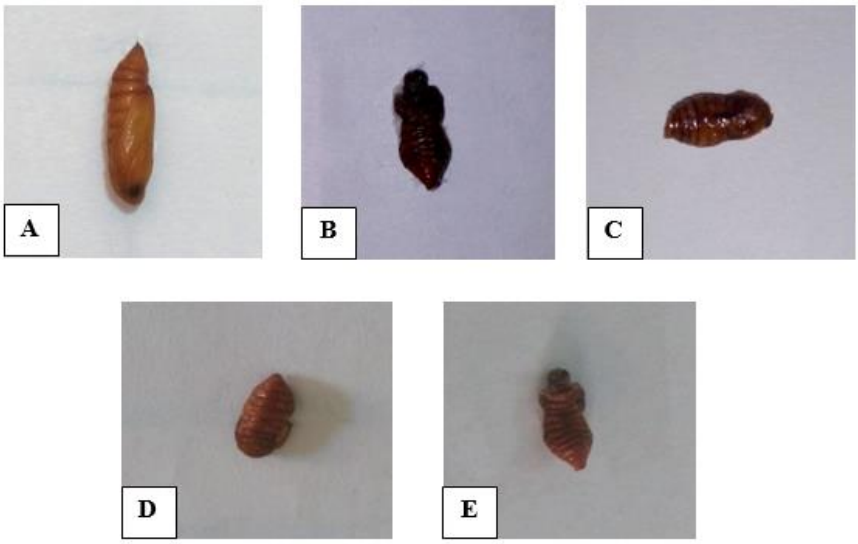

Fig 2: Deformed pupae of *P. gossypiella* by diofenolan. (A): normal pupa. (B, C, D & E): different features of pupal deformations.

4. Discussion

4.1 Affected survival potential of *P. gossypiella* by Diofenolan

Various insect species had been reported to suffer the toxic effects of several IGRs, such as *Spodoptera littoralis* by diflubenzuron [41], triflumuron [42], flufenoxuron [43], lufenuron [44-47], buprofezin [48, 49], methoxyfenozide [50], cyromazine [51]; *Papilio demoleus* by diofenolan [11]; *Eurygaster integriceps* by

Pyriproxyfen [52]; *Dysdercus koenigii* by flufenoxuron [53]; *Halyomorpha halys* by diflubenzuron [54]; *Spodoptera litura* by chlorfluazuron [55]; *Locusta migratoria* by flufenoxuron, RH-5849 and Pyriproxyfen [56]; *Culex pipiens* by kinoprene [57]; *Agrotis ipsilon* by flufenoxuron and methoprene [58] and *Tribolium castaneum* by lufenuron [59]. Recently, IGRs of different categories exhibited varying degrees of toxicity against some insects, such as pyriproxyfen against *Spodoptera mauritia* [60]; lufenuron and methoxyfenozide against *T. castaneum* [61]; methoxyfenozide against *C. pipiens* [62]; RH-5849 and tebufenozide against *Ephestia kuehniella* [63]; lufenuron against *Glyphodes pyloalis* [64] and *Helicoverpa armigera*[65]; Fenoxycarb against *Corcyra cephalonica* [66, 67]; buprofezin against *Paracoccus marginatus* [68]; chlorfluazuron, cyromazine and lufenuron against *Ctenocephalides felis* [69]; methoprene and pyriproxyfen against *Culex quinquefasciatus* and *Aedes albopictus* [70]; cyromazine against *Musca domestica*, *Stomoxys calcitrans* and *Fannia canicularis* [71]; novaluron against *P. gossypiella* [34]; *etc*. Results of the present study on *P. gossypiella* were, to some extent, in agreement with these reported results, since diofenolan displayed various degrees of toxicity on larvae and pupae, after treatment of the newly hatched larvae. The strongest toxic effect was exhibited at the highest concentration level (100% larval mortality, at 10.0 ppm). After treatment of full grown larvae, diofenolan exhibited a dose-dependent toxicity on larvae and pupae but no larval mortality was observed at the lower concentrations. With regard to the adult survival, diofenolan failed to exhibit an adulticidal effect, after treatment of newly hatched larvae. On the other hand, treatment of full grown larvae resulted in adult mortality, in a dose-dependent course, but no adult mortality was recorded at the lowest concentration (0.001 ppm).

Various LC_{50} values of IGRs had been determined against many insects, such as 350.45 and 453.78 ppm of novaluron and lufenuron, respectively against *S. litura* [72]; 0.025% of pyriproxyfen against *S. litura* larvae [73]; 8.47 mg /L of hexaflumuron against *H. armigera* [74]; 0.05 and 0.005 µg/insect of RH-5849 and tebufenozide, respectively against *E. kuehniella* [63]; 24.54 µg/L of methoxyfenozide against *C. pipiens* [62]; 19 ppm of lufenuron against *G. pyloalis* [64]; 0.19, 2.66, and 0.20 ppm of chlorfluazuron, cyromazine and lufenuron, respectively against *C. felis* [69]. Moreover, variation in LC_{50} values was reported for novaluron on *S. littoralis*, since LC_{50} values were 2.71 and 2.65 ppm, after treatment of penultimate instar larvae and last instar larvae, respectively [75]. Also, LC_{50} values of cyromazine against the same lepidopterous insect were 74.44 and 82.91 ppm, after treatment of penultimate instar larvae and last instar larvae, respectively [51].

In the present study on *P. gossypiella*, LC_{50} value of diofenolan varied depending on the larval instar under treatment, since it was estimated in 0.028 ppm, after treatment of newly hatched larvae, but 0.036 ppm after treatment of full grown larvae. As clearly shown, Diofenolan was more toxic than other IGRs against the same lepidopterous insect, since LC_{50} values of tebufenozide, acetamiprid and ethoxazole were determined in 2.41, 6.07 and 31.01 ppm, respectively [76]; 0.042 and 0.196 ppm of diflubenzuron and chlorfluazuron, respectively [77]; 87.5 and 15.1 ppm of buprofezin alone and in combination with piperonyl butoxide, respectively [78]; 61.859 ppm of teflubenzuron [79]; 20.6, 47.4 and 50.8 ppm of pyriproxyfen, methoxyfenozide and lufenuron, respectively [80] as well as 0.187 and 0.765 ppm of novaluron, after treatment of newly hatched and full grown larvae, respectively [34]. Thus, 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 level, method and time of treatment, as well as the experimental conditions.

To explicate the recorded mortalities of larvae, pupae and adults of *P. gossypiella* after treatment with diofenolan, 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 or/and deposition of chitin on the exoskeleton or/and other chitinized internal structures, such as the peritrophic matrix [81, 82]. 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' biosynthesis and inhibition of UDP-N-acetylglucosamine transport through the membrane [83]. Furthermore, it was suggested that Diofenolan interferes with the transport system of UDP-N-acetyl amine across the membrane [84].

In some detail, the larval deaths of *P. gossypiella* by Diofenolan, in the current investigation, may be attributed to the failure of larvae to moult owing to the inhibition of chitin formation [44, 45], to the inability to shed their exocuticle[85], or to swallow sufficient air for splitting the old cuticle and expand the new one [86]. Also, these larval deaths may be due to the prevention of feeding and continuous starvation of the present insect [87]. Although the disturbance of hormonal regulation or the disrupting of normal activity of the endocrine system in insects by IGRs was reported [88, 89] and suggested for some mosquito species [90, 91], the pupal deaths in *P. gossypiella*, in the present work, could not be directly relate to the hormonal activity of diofenolan, but to other causes, such as suffocation, bleeding and desiccation due to imperfect exuvation, failure of vital homeostatic mechanisms, *etc*. [92]. This suggestion can easily be substantiated since diofenolan exerted a predominant desiccating action on pupae of *P. gossypiella*, in a dose-dependent manner, in the present study. In addition, the adult mortality of *P. gossypiella* after treatment of full grown larvae with Diofenolan, 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 the haemolymph to other tissues, and/or by lower detoxification capacity of adults against the tested CSI [93].

4.2 Retarded development of *P. gossypiella* by Diofenolan

Many IGRs (including CSIs) exhibited various inhibitory activities against the development of various insects, such as diflubenzuron [41], methoprene and fenoxycarb [94], lufenuron [46], novaluron [75] and cyromazine [51] against *S. littoralis*; diofenolan against *P. demoleus* [11]; chlorfluazuron against *S. litura*[55]; novaluron against *A. aegypti* [95] and *C. pipiens* [96, 91]; kinoprene against *C. pipiens* [57]; methoprene and flufenoxuron against *A. ipsilon* [58]. Recently, the developmental duration was prolonged indicating regressed developmental rate in some other insects by various IGRs, such as *Plutella xylostella* by Pyriproxyfen [97]; *G. pyloalis* by Lufenuron [64]; *C. pipiens* by methoxyfenozide [62] and N-*tert*-butylphenyl thenoylhydrazide (ecdysteroid derivative) [98]; *C. cephalonica* by fenoxycarb [67]; *etc*.

In agreement with those aforementioned results of retarded development, results of the present investigation recorded a

drastic retarding effect of diofenolan on the development of *P. gossypiella*, since larval and pupal durations were remarkably prolonged after treatment of newly hatched or full grown larvae. In addition, the present results were in accordance with those reported results of retarded development (as expressed in regressed developmental rate) of the same lepidopterous insect after treatment of newly hatched larvae with hexaflumuron [99]; diflubenzuron and chlorfluazuron [77]; buprofezin [78]; teflubenzuron [79]; chromafenozide and diflubenzuron [100]; lufenuron and Pyriproxyfen [80] and novaluron [34]. In contrast, the present results disagreed with those results of enhanced development (shortened larval and/or pupal durations) in *P. gossypiella* after treatment with Methoxyfenozide [80] and other insects, such as *Rhynchophorus ferrugineus* by lufenuron and diofenolan [101], *A. ipsilon* by flufenoxuron[102] and *Schistocerca gregaria* by lufenuron [103]. In addition, diofenolan and lufenuron failed to affect the development of *M. domestica* [12].

In the current study, retarded development of *P. gossypiella* by Diofenolan, as expressed in prolonged larval and pupal durations, may be attributed to the indirect interference of this compound with neuroendocrine organs responsible for the synthesis and release of tropic hormones, such as prothoracicotropic hormone [104]. Also, diofenolan might affect the tissues and cells undergoing mitosis [105] or exhibited a delaying effect on the ecdysis and transformation [86]. 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 the developmental period [91].

4.3. Disrupted metamorphosis and morphogenesis of *P. gossypiella* by Diofenolan

From the practical point of view, the disruptive effects of IGRs on insect metamorphosis may be important because they could lead to various morphogenic defects as well as mortality [106]. It is obvious from various studies that the major symptoms of impaired metamorphosis of an insect, after treatment with various IGRs, 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 [107], triflumuron [43], lufenuron [44, 45], flufenoxuron [42, 43], methoprene and fenoxycarb [94]; novaluron [75] and cyromazine [51]. 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* [108], *Liriomyza trifolii* [109] and *Callosobruchus maculates* [110] by Cyromazine; *H. armigera*[111], *Phlebotomus papatasi* [112], *A. aegypti* [113, 114], *M. domestica* [115] by novaluron; *Lipaphis erysimi* by Pyriproxyfen [116]; *Rh. Ferrugineus* [101] and *P. demoleus*[11] by diofenolan; *Lobesia botrana* by lufenuron [117]; *C. pipiens* by kinoprene [62]; *etc*.

In the current study, diofenolan detrimentally suppressed the pupation rate, after treatment of newly hatched or full grown larvae of *P. gossypiella*, regardless the concentration level. This results was, to a great extent, consistent with those reported results of suppressed pupation rate of some insects by various IGRs, such as *P. xylostella* by hexaflumuron [97], *S. littoralis* by novaluron [75] and cyromazine [51], *G. pyloalis* by lufenuron [64] and fenoxycarb [66] as well as *Encarsia formosa* by pyriproxyfen and fenoxycarb [118].

In addition, the pupal morphogenesis in *P gossypiella* was deranged in the present study, as appeared in different pupal deformities, after treatment of newly hatched or full grown larvae with diofenolan. This result corroborated with similar deranged pupal morphogenesis observed in several insects after treatment with different IGRs, such as *T. castaneum* and *T. confusum* after treatment with cyromazine [108], *C. cephalonica* after topical application of last instar larvae with fenoxycarb [67] and *P. gossypiella* after treatment of the full grown larvae with novaluron [34]. Whatever the mode of action, diofenolan suppressed the chitin synthesis and prevented the normal deposition of new cuticle during apolysis leading to the production of pupal deformities [119].

With regard to the metamorphosis program, in the current investigation on *P. gossypiella*, diofenolan failed to produce larval-pupal intermediates after treatment of the newly hatched larvae, but the program was seriously impaired after treatment of full grown larvae, especially at the higher three concentrations. This feature of impaired metamorphosis was, also, described as abnormal or lethal pupation [120]. Our result was, to some 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 [74], *S. littoralis* by novaluron [75] and cyromazine [51], *C. cephalonica* by fenoxycarb [67] and *P. gossypiella* by novaluron [34]. 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 [121, 92, 122].

The production of larval-pupal intermediates on *P. gossypiella*, in the present study, indicated the disturbance of metamorphosis program by diofenolan. It can be interpreted by the interference of diofenolan with the hormonal regulation of pupation program [88]. For some detail, some conceivable scenarios can be described herein. (1) Diofenolan might inhibit the metamorphosis program *via* an ecdysteroid reduction, interference with the release of eclosion hormone or/and inhibition of the neurosecretion [123]. (2) The production of these larval-pupal intermediates might indicate a juvenile property of diofenolan retarding the perfect larval-pupal transformation. These mosaic creatures are unusual and died soon after formation. (3) The production of intermediate creatures can be explicated by an inhibitory effect of diofenolan on the DNA synthesis [124] or the chitin biosynthesis and chitin synthase [125]. (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 developmental stages [126]. The molt induction during the early phase of last instar produce larval-like individuals, while those formed in the late phase produce pupal-like individuals [127].

5. Conclusion

On the basis of overall findings, it can be concluded that diofenolan is toxic to some developmental stages of *P. gossypiella*, as well as caused various impairing effects on its development, metamorphosis and morphogenesis. Thus, diofenolan may be considered as a leading target compound having the potential to control *P. gossypiella* which has

developed resistance to the majority of synthetic pesticides.

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