INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 13, No. 1, p. 42-64, 2018

RESEARCH PAPER OPEN ACCESS

Assessment of toxic and developmental impacts of methoprene on the grey flesh fly *Parasarcophaga argyrostoma* (Diptera: Sarcophagidae)

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Key words: Development, Growth, Metamorphosis, Morphogenesis, Pupation

http://dx.doi.org/10.12692/ijb/13.1.42-64 Article published on July 15, 2018

Abstract

Beside its role in human cutaneous wounds and eye myiasis, the grey flesh fly *Parasarcophaga argyrostoma* (Robineau-Desvoidy) (Diptera: Sarcophagidae) is known as parasitoid of various animals. Objective of the current study was the evaluation of methoprene impacts on survival, development and metamorphosis of this fly. Five doses (10.0, 5.0, 1.0, 0.1 and 0.01µg/larva) were prepared and topically applied onto the early last (3rd) instar larvae and prepupae.Thirty replicates (one larva/replicate) of healthy larvae or prepupae were topically treated, individually using Hamilton microapplicator (NHN 737). Similar number of replicates of larvae and prepupae had been topically treated with 1µ acetone only as controls.The most important results can be summarized as follows. Methoprene exhibited larvicidal, pupicidal and adulticidal activities. LD_{50} values were found 0.155 and 0.258 µg/insect after treatment of larvae and prepupae, respectively. The maximal body weight of treated larvae was remarkably reduced. The duration of treated larvae was prolonged. The growth coefficient of treated larvae was depressed. The pupal duration was considerably prolonged. Only at the higher two doses, some larval-pupal intermediates had been observed. After treatment of prepupae, only with the lower two doses, a state of 'permanent prepupae' was recorded. The pupation rate was slightly regressed after treatment of prepupae, only with the lower two doses. The adult emergence was completely blocked after topical treatment of either larvae or prepupae with the highest dose of methoprene. At other dose levels, the adult eclosion was partially blocked. Different percentages of deformed pupaeand adults were recorded.

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Introduction

Conventional insecticides have been extensively used in agriculture and medicine since World War II. The intensive and indiscriminate application of these insecticides cause several drastic problems, such as environmental hazards, destruction of the natural enemies, serious toxicological problems to humans, as well as the development of insect resistance to different insecticides (Davies *et al.*, 2007; Costa *et al.*, 2008; Mosallanejad and Smagghe, 2009). Therefore, alternative materials have been initiated recently to minimize these hazards and introduce of new effective and safer ways with negligible effects on the ecosystem (Derbalah *et al.*, 2014).

In insects, moulting, growth, development and metamorphosis are controlled by prothoracicotropic hormone (PTTH), ecdysone or moulting hormone (MH), and juvenile hormone (JH) (Xiang *et al.*, 2005). Balance in levels of MH and JH has define the outcome of each developmental transition (for detail, see Riddiford *et al.*, 2003; Dubrovsky, 2005). In addition, JHs control several aspects of physiology and behavior in insects (Mitsuoka *et al.*, 2001; Goodman and Granger, 2005; Raikhel *et al.*, 2005; Truman and Riddiford, 2007; Riddiford, 2008; Flatt *et al.*, 2008; Zhan *et al.*, 2011; Denlinger *et al.*, 2012; Amsalem *et al.*, 2014).

Screening new targets involved in JH-biosynthesis within the CA has been an area of study during the last few decades (Bede *et al.*, 2001). Therefore, compounds that interact with JH, stimulate JH-biosynthesis, inhibit JH-biosynthesis or interfere with its catabolism can be utilized as new safe insecticides against insect pests (Nandi and Chakravarty, 2011). These compounds are collectively named 'insect growth regulators' (IGRs) (Khan and Qamar, 2012) which are not directly toxic, but act selectively on normal growth, development, metamorphosis and/or reproduction in insects (Martins and Silva, 2004; Wang and Liu, 2016).

On the basis of the mode of action, IGRs has been classified into three categories: (i) Juvenile hormone analogues (JHAs)(or Juvenoids), (ii) Ecdysteroids or ecdysone agonists and (iii) Chitin synthesis inhibitors (CSIs)(or moult inhibitors) (for detail, see Oberlander and Silhacek, 2000; Tunaz and Uygun, 2004**)**. Because of their desirable characteristics, such as low toxicity, almost no apparent side-effects on non-target organisms, less environmental pollution, high selectivity, and low impact on natural enemies and human health, IGRs are used to control various insect pests (Wang and Wang, 2007; Ghasemi *et al.*, 2010; Talikoti *et al.*, 2012; Taleh *et al.*, 2015; Resmitha and Meethal, 2016; Ghoneim *et al.*, 2017a; Hassan *et al.*, 2017; Tanani *et al.*, 2017).

Methoprene is a molecule that closely resembles the insect JH (Crosby and Minyard, 1991). The insect growth regulating properties of this JHA were first described in 1973 and registered as a biological pesticide by the EPA in 1975 (Crosby and Minyard, 1991). It was later re-classified by the EPA as a biochemical pesticide (Glare and O'Callaghan, 1999). Methoprene is a highly effective JHA for regulation of growth and development as well as many of the physiological and behavioral processes controlled by JH in insects (e.g., Wyatt and Davey, 1996; Zera and Zhao, 2004; Struger *et al.*, 2007). This JHA has been successfully used to control some species of mosquitoes (Ross *et al.*, 1994a,b; Ali *et al.*, 1995; Ritchie, 1997; Pinkney *et al.*, 2000;Nishiura et al.,2003), but is effective against a range of insects, including the orders Diptera, Lepidoptera and Coleoptera (Glare and O'Callaghan, 1999).

From the sanitary point of view, flesh flies (Diptera: Sarcophagidae) are of relevant importance. Their impact on human and animal health is well known for their potential ability as myiasis producers (Guimarães *et al.*, 1983) and for their role as vector of pathogens (Greenberg, 1971). On the other hand, they are among the most useful insects for forensic investigations (Wells *et al.*, 2001).

The grey flesh fly *Parasarcophaga argyrostoma* is worldwide in distribution, including Europe, North America, Chile, Africa, India, Argentina, the Hawaiian Islands, and the Marshall Islands (Lopes, 1961).

The adult flies visit decaying substances, faeces and also feed on flowers. Larvae normally develop in decaying meat but are also known as parasitoids of various animals (Povolny and Verves, 1997). *P. argyrostoma* has received much research attention owing to its role in human cutaneous wounds and eye myiasis (Razmjou *et al.*, 2007; Gómez-Hoyos *et al.*, 2012). Also, interest in the study of *P. argyrostoma* maggots has increased with the step forward in forensic entomology (Wells *et al.*, 2001; Buenaventura *et al.*, 2009). Chemical control of *P. argyrostoma* by conventional insecticides is difficult because of the larvae being protected inside wounds or bodies, and the high mobility of the adults. Searching for alternative pest management agents is necessary. Therefore, the current investigation was conducted as a contribution in searching for some control agents alternative to the conventional insecticides against *P. argyrostoma*. In the present study, efficacy of methoprene was assessed on survival, development and metamorphosis of this medically dangerous fly.

Materials and methods

Insect of study

A culture of *P. argyrostoma* was established under controlled laboratory conditions (28±0.1°C, 65±5% R.H.). It was originated by a sample of susceptible strain pupae obtained from the continuously maintained culture for several years at the Department of Entomology, Faculty of Science, Cairo University. The rearing routine work and daily manipulation were carried out according to Zohdy and Morsy (1982a, b). Larvae (maggots) and pupae (puparia) were confined in plastic vials covered with muslin and supplied with a small piece of red meat mixed with a suitable amount of bran dust. The food was renewed daily. Adult flies were confined in wooden cages (30×30×30 cm) with wire gauze sides.

Methoprene administration

Methoprene of 98.5% purity was purchased from Sigma-Aldrich Co., Egypt. Common trade names include Altosid®, Apex®, Diacon®, Dianex®, Precor®, and Z-515®.Its chemical name is 1, isopropyl 2E, 4E-11 methoxy-3,7, 11-trimethyl-2, 4-dodecadienoates, with the molecular formula: $C_{19}H_{34}O_3$.

Using acetone, this JHA was diluted to prepare five dose levels: 10.0, 5.0, 1.0, 0.1 and 0.01µg/larva. Thirty replicates (one larva/ replicate) of healthy larvae of the early last (3rd) instar and similar number of prepupae were topically treated, individually, with each dose using Hamilton microapplicator (NHN 737). Similar number of replicates of early last instar larvae and prepupae had been topically treated with 1µ acetone only as controls. Treated and control insects were kept under the previously mentioned laboratory conditions. All treated and control insects were checked daily for feeding of larvae and recording all criteria of study.

Criteria of investigation

Methoprene toxicity

Toxicity was determined by observed mortality. All mortalities of treated and control (larvae, pupae and adults) were recorded every day and total mortality was corrected according to Abbott's formula (1925) as follows:

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

The LC_{50} value was calculated for general mortality by Microsoft office Excel, 2007, according to Finny (1971).

Larval growth

Coefficient of growth (mean±SD) was calculated according to El-Ibrashy and Aref (1985) as follows: maximal body weight (mg) of full grown larvae/ duration (in days).

Developmental and metamorphic parameters

Developmental durations had been calculated(mean days±SD) using Dempster's equation (1957). Pupation rate was expressed in % of the developed pupae. Adult emergence was determined in %. All of the possible aberrations of metamorphosis and morphogenesis, such as larval-pupal or pupal-adult intermediates, permanent insects, and malformed pupae, were calculated in %.

Statistical analysis of data

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

Results

Toxic effect of methoprene

After topical application of methoprene (once) onto the early last (3rd) instar larvae, data of the lethal effect was expressed as mortalities of larvae (maggots), pupae (puparia) and adult flies and assorted in Table (1).

Table 1. Toxic effect (%) of methoprene on *P. argyrostoma* after topical treatment of the early last instar larvae.

Dose (µg/larva)	Larval mortality	Pupal mortality	Adult mortality	Total mortality	Corrected mortality	LD_{50} (µg/larva)
10.0	45	100.0	--	100	100	0.155
5.0	40	16.7	50.0	75	72.2	
1.0	35	07.7	41.7	65	61.1	
0.1	15	05.9	31.3	45	38.9	
0.01	10	11.1	25.0	40	33.3	
Control	05	05.3	00.0	10	---	

-:Noadult mortality could be calculated because no adult flies emerged.

After topical application of methoprene (once) onto prepupae, data of mortalities were arranged in Table (2).

*Methoprene toxicity on larvae***:** Depending on data of Table (1), treatment of last instar larvae with methoprene caused different percentages of larval mortality in a dose-dependent course (10, 15, 35, 40 and 45% mortality, at 0.01, 0.1, 1.0, 5.0 and 10.0 µg/larva, respectively, *vs*. 05% mortality of control larvae). Methoprene had high initial killing power, since the majority of larval mortality was observed during the first 24 h post-treatment.

Table 2. Toxic effect (%) of methoprene on *P. argyrostoma* after topical treatment of the prepupae.

Dose (µg/larva)	Pupal mortality	Adult mortality	Total mortality	Corrected mortality	LD_{50} (µg/larva)
10.0	100	---	100	100	0.258
5.0	60	37.5	75	73.7	
1.0	30	42.9	60	57.9	
0.1	15	29.4	40	36.8	
0.01	10	16.7	25	21.1	
Control	05	00.0	05	---	

-: See footnote of Table (1).

Methoprene toxicity on pupae:Depending to data assorted in Table (1), a latent toxic action of methoprene was exerted on pupae, since different pupal mortalities were recorded, in no certain trend, after treatment of last instar larvae. The strongest toxic effect was exhibited at the highest dose (100% pupal mortality, *vs*. 5.3% mortality of control pupae). After topical application of prepupae with methoprene, the pupal mortalities were recorded in a dose-dependent manner.

The extreme mortal potency of methoprene was exhibited at the highest dose level (10, 15, 30, 60 and 100% pupal mortality, at 0.01, 0.1, 1.0, 5.0 and 10.0 µg/prepupa, respectively, *vs*. 5.0% mortality of control pupae).

Methoprene toxicity on adult flies:As clearly shown in Tables (1 and 2), no adult mortality could be recorded at the highest dose level of methoprene because no adults emerged, may be due to the complete death of pupae, regardless the time of treatment. The tested compound exhibited increasing adulticidal effect by the increasing dose level applied onto the early last instar larvae (25.0, 31.3, 41.7 and 50.0% adult mortality, at 0.01, 0.1, 1.0 and 5.0 µg/larva, respectively, *vs.* 0% mortality of control adult flies, Table 1). According to data distributed in Table (2), the extended toxic effect of methoprene on adult flies appeared in no certain trend (16.7, 29.4. 42.9 and 37.5% adult mortality, at 0.01, 0.1, 1.0 and 5.0 µg/larva, respectively, *vs.* 0% mortality of control adult flies).

Table 3. Larval growth of *P. argyrostoma* after topical application of methoprene onto the early last instar larvae.

Dose (µg/larva)	Maximal body weight (mean mg±SD)*	Duration (mean days±SD)	Coefficient of growth (mean±SD)
10.0	068.7±4.42 d	2.85±0.66 c	33.02±19.06 a
5.0	094.2±16.09 d	3.73±0.62 a	26.18±04.67 b
1.0	102.9±14.80 c	3.62±0.63 a	29.16±04.46 b
0.1	103.7±12.26 b	3.60±0.50 a	32.64±12.03 a
0.01	116.5±3.51 a	4.00±0.43 b	29.91±05.32 b
Control	116.5±6.13	3.55±0.50	37.3±13.04

Mean±SD followed with the same letter a: insignificantly 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).

According to the corrected mortality, after treatment of last instar larvae, methoprene exerted lethal potency against *P. argyrostoma* parallel to the dose level (33.3, 38.9, 61.1, 72.2 and 100% corrected mortality, at 0.01, 0.1, 1.0, 5.0 and 10.0 µg/larva, respectively, Table 1). In a similar trend, the lethal potency of methoprene increased by increasing dose level, after treatment of prepupae (21.1, 36.8, 57.9, 73.7 and 100% corrected mortality, at 0.01, 0.1, 1.0, 5.0 and 10.0 µg/prepupa, respectively). The calculated LD_{50}values of methoprene were found 0.155 and 0.258 µg/insect after topical treatment of last instar larvae and prepupae, respectively. Therefore, the early last instar larvae were more sensitive to the toxicity of methoprene than prepupae.

Table 4. Development and metamorphosis of *P. argyrostoma* after topical application of methoprene onto the early last instar larvae.

Dose (µg/larva)	Larval-pupal inter. (%)	Pupation rate (%)	Pupal Duration (mean days±SD)	Deformed pupae (%)	Adult emergence (%)	Deformed adults (%)
10.0	20	92.31	--- *	3.33	00.00	---
5.0	10	78.57	14.33±0.81 c	13.33	26.66	6.7
1.0	00	92.86	15.13±0.45 c	10.0	17.69	6.7
0.1	00	64.29	15.66±0.47 c	10.0	33.33	0.0
0.01	00	78.57	12.60±0.80 b	3.33	45.47	0.0
Control	00	100	11.36±0.48	00.00	94.95	0.0

a, c: See footnote of Table (3). Larval-pupal inter.: Larval-pupal intermediates, they perished without pupation. *: The pupal duration could not be measured because no adults emerged.

Effect of methoprene on the larval growth

After topical application of methoprene doses (once) onto the early last instar larvae, data of the maximal body weight (max. wt), duration and coefficient of growth (CG) of the treated and control larvae were assorted in Table (3).

In the light of these data, max.wt considerably decreased, almost in a dose-dependent course (116.5±3.51, 103.7±12.26, 102.9±14.80, 094.2±16.09 and 068.7±4.42 mg of treated larvae, at 0.01, 0.1, 1.0, 5.0 and 10.0 µg/larva, respectively, in comparison with 116.5±6.13 mg of control larvae). With regard to the larval duration, data of the same table clearly show a slightly or remarkably prolongation, depending on the dose of methoprene (4.00±0.43, 3.60±0.50, 3.62±0.63 and 3.73±0.62 days of treated larvae, at 0.01, 0.1, 1.0 and 5.0 µg/larva, respectively, *vs*. 3.55±0.50 days of control larvae). An exceptional case of significantly shortened larval duration was recorded at the highest dose of methoprene (2.85±0.66 days of treated larvae, *vs*. 3.55±0.50 days of control larvae).

Table 5. Development and metamorphosis of *P. argyrostoma* after topical application of methoprene onto the prepupae.

Dose (µg/larva)	Permanent prepupae (%)*	Pupation rate (%)	Pupal Duration (mean days±SD)	Deformed pupae (%)	Adult emergence (%)	Deformed adults (%)
10.0	00.0	100	--- **	0	00.00	---
5.0	00.0	100	14.50±0.50 c	0	44.29	20.5
1.0	00.0	100	14.47±0.93 c	0	48.62	20.5
0.1	10.5	92.86	15.08±0.27 c	0	55.39	00.0
0.01	10.0	92.86	13.00±1. 04 b	0	100	00.0
Control	00.0	100	12.08±0.73	0	100	00.0

b, c: See footnote of Table (3). *: Permanent prepupae perished without pupation. **: The pupal duration could not be measured because no adults emerged.

As obviously shown in the same table, CG of the treated larvae was slightly or pronouncedly depressed, depending on the dose level of methoprene. The potent inhibitory action was exerted on the larval growth at the doses 0.01, 1.0 and 5.0 µg/larva (29.91±5.32, 29.16±4.46 and 26.18±4.67, respectively, *vs*. 37.3±13.04 CG of control larvae).

Effect of methoprene on the development

After treatment of the early last instar larvae with methoprene, data of the affected development was listed in Table (4).

After treatment of prepupae with methoprene, data of the affected development was summarized in Table (5).

The pupal duration can be used as a good indicator of the pupal development, i.e., shorter duration may denote faster rate and *vice versa*. At the highest dose of methoprene, no pupal duration could be measured because no adults emerged, irrespective of the time of treatment.

According to data of Table (4), the pupal duration was remarkably prolonged after application of methoprene onto the last instar larvae (12.60±0.80, 15.66±0.47, 15.13±0.45 and 14.33±0.81 days of treated pupae (puparia) , at 0.01, 0.1, 1.0 and 5.0 µg/larva, respectively, *vs*. 11.36±0.48 days of control pupae). Depending on data listed in Table (5), the pupal duration was considerably prolonged after treatment of the prepupae with methoprene (13.00±1.04, 15.08±0.27, 14.47±0.93 and 14.50±0.50 days of treated pupae, at 0.01, 0.1, 1.0 and 5.0 µg/larva, respectively, *vs*. 12.08±0.73 days of control pupae). Depending on these data, methoprene exerted an inhibitory effect on the successfully formed pupae, since they developed in slower rate than that of the control pupae, regardless the time of treatment.

Disrupted developmental program

*Larval-pupal intermediates:*As distinctly shown in Table (4), treatment of the last instar larvae with the higher two dose levels of methoprene impaired the process of larval-pupal transformation, because some larval-pupal intermediates had been produced (20 and 10% intermediates, at 10.0 and 5.0 µg/larva, respectively). These intermediates died just after production.

Permanent prepupae

As obviously shown in Table (5), topical treatment of prepupae only with the lower two doses of methoprene induced a state of suspended development, as expressed in 'permanent prepupae' (10.5 and 10.0% permanent prepupae, at 0.1 and 0.01 µg/prepupa, respectively). These permanent prepupae suffered the adverse action of methoprene along 12 days and eventually perished without external feature of puparium formation.

Effect of methoprene on metamorphosis and morphogenesis

*Pupation process:*On the basis of data assorted in Table (4), the methoprene-treated last instar larvae pupated in a slightly or considerably regressed rate, depending on the dose level (78.57, 64.29, 92.86, 78.57 and 92.31% pupation (pupariation), at 0.01, 0.1, 1.0, 5.0 and 10.0 µg/larva, respectively, *vs*. 100% pupation of control larvae). The pupation inhibition could be calculated as 21.43, 35.71, 7.14, 21.43 and 7.69%, at 0.01, 0.1, 1.0, 5.0 and 10.0 µg/larva, respectively). On the other hand, the pupation rate was slightly regressed after topical treatment of prepupae only with the lower two doses of methoprene (92.86 and 92.86% pupation of treated larvae, at 0.01 and 0.1 µg/prepupa, *vs*. 100% pupation of control prepupae).

Adult emergence

Depending on data summarized in Tables (4 & 5), the adult emergence of flies was completely blocked after topical treatment of either the early last instar larvae or prepupae with the highest dose of methoprene. Also, the adult eclosion of flies was drastically blocked, in no certain trend, after treatment of last instar larvae with other methoprene doses (26.66, 17.69, 33.33 and 45.47%, at 5.0, 1.0, 0.1 and 0.01 µg/larva, respectively, *vs*. 94.95% emergence of control adult flies) (Table 4). After topical treatment of prepupae with other doses of methoprene, the adult eclosion was correlated directly to the dose level, i.e., the inhibitory action of methoprene increased as the dose level was elevated (100, 55.39, 48.62 and 44.29% emergence, at 0.01, 0.1, 1.0 and 5.0 µg/prepupa, respectively, *vs*. 100% emergence of control adult flies, Table 5).

*Morphogenesis:*As unambiguously shown by data of Table (4), methoprene displayed anti-morphogenic efficiency on the developed pupae, since different %s of deformed pupaewere produced after treatment of last instar larvae, but in no certain trend (3.33, 10.0, 10.0, 13.33 and 3.33% deformed pupae, at 0.01, 0.1, 1.0, 5.0 and 10.0 µg/larva, respectively,*vs*. 0% malformation of control pupae). In contrast, methoprene failed to exhibit similar efficiency on the pupal morphogenesis, after treatment of prepupae, since no deformed pupae were observed, Table 5). In respect of the disruptive effect of methoprene on the adult morphogenesis, data of Table (4) revealed that topical treatment of last instar larvae only with the doses 5.0 and 1.0 µg/larva of methoprene deranged the morphogenesis of the emerged adult flies as recorded in 6.7 and 6.7% deformed adults, respectively (vs. 0% deformation of control adult flies). Also, topical treatment of the prepupae with the same two doses led to 20.5% malformed adult flies (compared to 0% deformity of control adult flies, Table 5).

Discussion

Reduced survival of P. argyrostoma by methoprene

Lethal impacts of various insect growth regulators (IGRs) on many insects had been reported, such as lethal impact of Fenoxycarb on the parasitoid *Phanerotoma ocularis* (Moreno *et al*., 1993a), therice meal moth*Corcyra cephalonica* (Begum and Qamar, 2016) and the desert locust *Schistocerca gregaria* (Ghoneim and Ismail, 1995a). Toxic effects were reported against the Egyptian cotton leafworm *Spodoptera littoralis* by different IGRs, such as Flufenoxuron (El-Naggar, 2013), Lufenuron (Bakr *et al*., 2013), Buprofezin (Nasr *et al*., 2010) and Cyromazine (Tanani *et al*., 2015). Pyriproxyfen exhibited toxic effects on both *Eurygaster integriceps* (Mojaver and Bandani, 2010) and *Spodoptera mauritia* (Resmitha and Meethal, 2016). In addition, various IGRs were reported to exhibit toxic effects on insects, such as Kinoprene on the common house mosquito *Culex pipiens* (Hamaidia and Soltani, 2014); Flufenoxuron and Methoprene on the black cutworm *Agrotis ipsilon* (Khatter, 2014); Lufenuron on the red flour beetle *Tribolium castaneum*(Gado *et al*., 2015); the lesser mulberry snout moth *Glyphodes pyloalis* (Aliabadi *et al*., 2016) and

thecorn earworm *Helicoverpa armigera* (Vivan *et al*., 2017); Tebufenozide (RH-5992) onthe Mediterranean flour moth *Ephestia kuehniella* (Tazir *et al*., 2016); Cyromazine on the flies *Musca domestica*, *Stomoxys calcitrans* and *Fannia canicularis* (Donahue *et al*., 2017); Novaluron on the pink bollworm *Pectinophora gossypiella* (Ghoneim *et al*., 2017a) and olive leaf moth *Palpita unionalis* (Ghoneim *et al*., 2017b).

To some extent, the current results were in agreement with the previously mentioned results, since the tested IGR exhibited lethal impacts on larvae (maggots), pupae (puparia) and adult flies of *P. argyrostoma*. Moreover, the larval mortality was observed in a dose-dependent course. Also, methoprene appeared to posses high initial killing power, since the majority of larval mortality was observed during the first 24 h post-treatment. In addition, different pupal mortalities and a chronic toxicity of methoprene against the adult flies were recorded. Such effect increased by increasing applied dose level onto the last instar larvae but appeared in no certain trend after prepupal treatment.

In mortal activity of methoprene against larvae of *P. argyrostoma*, in the current investigation, was in corroboration with many reported results of methoprene larvicidal activity against a number of insects, such as *Culex pipiens* (Gelbic *et al*., 2002), *Aedes albopictus* (Khan *et al*., 2016), *Agrotis ipsilon* (Khatter, 2014) and *C. cephalonica* (Tripathi and Tiwari, 2006). Also, the current result of methoprene lethality against *P. argyrostoma* agreed with the pupicidal activity of methoprene against some insects, such as *Aedes aegypti* (Braga *et al*., 2005) and *C. cephalonica* (Tripathi and Tiwari, 2006).

In the present investigation, larval deaths of *P. argyrostoma* may be attributed to the impediment of larvae to swallow air for splitting the old cuticle and expand the new one during moulting (Linton *et al*., 1997). In addition, the larval deaths may be due to the permanent starvation of larvae (Ghoneim *et al*., 2000). On the other hand, the pupal deaths of *P. argyrostoma* can be directly related to the hormonal activity of the tested methoprene or may be due to secondary factors like bleeding, suffocation and desiccation, as well as for failure of some homeostatic mechanisms (Smagghe and Degheele, 1994). With regard to the adult mortalities of *P. argyrostoma*, the retention and distribution of the tested IGR in the body as a result of direct and rapid transport *via* haemolymph to different tissues, and/or by lower detoxification capacity of adults against this IGR (Osman *et al*., 1984).

Variable LC_{50} (or LD_{50}) values of several IGRs were recorded against various insects. For examples, LC_{50} values of Novaluron and Lufenuron against *Spodoptera litura* were calculated in 350.45 and 453.78 ppm, respectively (Sharma and Pathania, 2014); LC_{50} of Hexaflumuron against *H. armigera* was 8.47 mg /L (Taleh *et al*., 2015); LC_{50} of Methoxyfenozide against *C. pipiens* was determined as 24.54 µg/L (Hamaidia and Soltani, 2016); LD_{50} values of RH-5849 and Tebufenozide against *E. kuehniella* were 0.05 and 0.005 µg/insect, respectively (Tazir *et al*., 2016); LC_{50} values of Noviflumuron and Novaluron were 0.153 and 0.342 ppm after treatment of 1-day old eggs of *P. gossypiella* (Hamadah and Ghoneim, 2017); etc. In the current study, LD_{50} values of methoprene against *P. argyrostoma* were measured as 0.155 and 0.258 µg/insect, after treatment of last instar larvae and prepupae, respectively. In insects, however, LC_{50} (or LD_{50}) value of a compound depends on different factors, such as susceptibility of the insect itself, toxic potency of the compound and its concentration, method and time of application, and the experimental conditions. In addition, the last instar larvae of *P. argyrostoma* were more sensitive to methoprene than prepupae, in the present investigation. This observation coincided with some results revealing that the early larval instars of different flies were more susceptible than the later ones to IGRs, like *Musca domestica* (Fouda *et al*., 1991), *Lucilia cuprina* (Friedel and McDonell, 1985), *Fannia* spp. (Meyer *et al*., 1987) and *Ceratitis capitata* (Vinuela *et al*., 1993).

Inhibited growth of P. argyrostoma by methoprene

The decreased body weight of methoprene-treated larvae of *P. argyrostoma*, in the current study, disagreed with the increasing weight gain of *Bombyx*

mori 5th instar larvae after treatment of 4th instar larvae with the same IGR (Miranda *et al.*, 2002). On the other hand, the present result was, to some extent, in conformity with the reduced larval body weight in *C. capitata* after treatment with Cyromazine (Vinuela *et al.*, 1993), *P. argyrostoma* after treatment of 3rd instar larvae with Pyriproxyfen (Ismail, 1995) or chlorfluazuron (Ghoneim and Ismail, 1995b). Additionally, inhibited growth of *P. argyrostoma*, after treatment of last instar larvae with methoprene, in the current study, was in accordance with the reported inhibition of larval growth of some insects by various IGRs, such as *S. littoralis* by Flufenoxuron (Bakr *et al.*, 2010), Lufenuron (Adel, 2012), and Novaluron (Ghoneim *et al.*, 2015); *P. demoleus* by Diofenolan (Singh and Kumar, 2011); *S. litura* by Chlorfluazuron (Perveen, 2012); *Ae. aegypti* and *C. pipiens* (Farnesi *et al.*, 2012; Djeghader *et al.*, 2014) and *A. ipsilon* by methoprene (Khatter, 2014). The inhibited growth of *P. argyrostoma* by methoprene, in the current investigation, may be a result of the hindered release of morphogenic peptides, leading to alteration in ecdysteroid and juvenoid levels (Barnby and Klocke, 1990). Also, methoprene might affect the tissues and cells undergoing mitosis (Nasiruddin and Mordue, 1994).

Deranged development of P. argyrostoma by methoprene

Influenced developmental durations

Larval and/or pupal periods of many insects had been prolonged after treatment with methoprene. For examples, larval duration of *C. cephalonica* was prolonged after topical application ofmethoprene (Tripathi and Tiwari, 2006). Duration of *B. mori* 5th (last) larval instar was prolonged after topical application of methoprene onto 4th instar larvae (Miranda *et al.*, 2002). Topical application of different doses of methoprene (0.1-5.0 µg/insect) onto newly moulted 5th instar larvae of *S. litura* led to slightly changed 5th instar duration, but application onto newly moulted 6th (last) larvae resulted in a dose-dependent prolongation (Yoshiga and Tojo, 2001). The pupal duration was dose-dependently prolonged after treatment of *Ae. aegypti* larvae with methoprene (Braga *et al.*, 2005).

To a great extent, results of the current study on *P. argyrostoma* were concomitant to the aforementioned results, since the methoprene-treated larvae spent slightly or considerably prolonged time interval, depending on the dose. Moreover, the tested IGR exerted a retarding action on the pupal development, because the treated pupae developed in a slower rate than that of control congeners.

Also, the present results of extended duration of *P. argyrostoma* larvae corroborated with the results of prolonged larval duration in some insects after treatment with various IGRs, such as *S. littoralis* after treatment of 5th instar or last instar larvae with Novaluron (Ghoneim *et al.*, 2015) and Cyromazine (Tanani *et al.*, 2015); *Spodoptera frugiperda* by Methoxyfenozide (Zarate *et al.*, 2011); *P. gossypiella* by Pyriproxyfen (Sabry and Abdou, 2016) and Noviflumuron or Novaluron (Hamadah and Ghoneim, 2017). In addition, the current results of delayed development of *P. argyrostoma* were in agreement with several results of retarded development of other insects by different IGRs, such as *S. littoralis* by Lufenuron (Gaaboub *et al.*, 2012), Diflubenzuron (Aref *et al.*, 2010), Cyromazine (Tanani *et al.*, 2015) and Novaluron (Ghoneim *et al.*, 2015); *A. ipsilon* by methoprene and Flufenoxuron (Khatter, 2014); *C. pipiens* by Kinoprene (Hamaidia and Soltani, 2014); *P. gossypiella* by Teflubenzuron (El-Khayat *et al.*, 2015), Buprofezin (Al-Kazafy, 2013) and Chromafenozide (Salem, 2015). Recently, the developmental period was prolonged denoting a delayed development in some of other insects by IGRs, such as *P. unionalis* by Novaluron (Ghoneim *et al.*, 2017b); *P. gossypiella* by Pyriproxyfen or Lufenuron (Sabry and Abdou, 2016) and Novaluron (Ghoneim *et al.*, 2017a); etc. On the contrary, results of the present study disagreed with the reported results of shortened larval period in some insects by different IGRs, such as *Rhynchophorus ferrugineus* by *A. ipsilon* by Flufenoxuron (El-Sheikh, 2002), *S. gregaria* by Lufenuron (Bakr et al., 2008), Lufenuron and Diofenolan (Tanani, 2001), *P. unionalis* by Novaluron (Ghoneim *et al.*, 2017b) and *P. gossypiella* by Methoxyfenozide (Sabry and Abdou, 2016).

In the current study, retarded development of *P. argyrostoma* by methoprene may be attributed to the intervention of this IGR in the functions of endocrine organs responsible for the synthesis and release of tropic hormones, such as prothoracicotropic hormone (Subrahmanyam *et al.*, 1989). In general, the lengthening of larval or pupal period may be due to the elevated level of juvenile hormone (JH) in haemolymph since only in the declination or absence of this hormone, ecdysone could be activated and lead to the production of next stage (Kuwano *et al.*, 2008). Also, methoprenemight exhibit a retarding effect on the pupariation of prepupae of *P. argyrostoma*. In addition, last step of the pathway of chitin biosynthesis might be inhibited by this IGR and the precursor was not converted into chitin, leading to a prolongation of developmental period (Djeghader *et al.*, 2014).

Disturbed developmental program

*Formation of larval-pupal intermediates:*Treatment of the last instar larvae of *P. argyrostoma*, in the present study, with methoprene resulted in impairment of the larval-pupal transformation; since some larval-pupal intermediates were observed, only at the higher two doses. These mosaic intermediates perished soon after formation. To a great extent, this result was in agreement with some of the larval-pupal intermediates reported in other insect species after treatment with some IGRs, such as *C. cephalonica* after treatment with Fenoxycarb (Begum and Qamar, 2016); *S. littoralis* after treatment with Cyromazine (Tanani *et al.*, 2015) or Novaluron (Ghoneim *et al.*, 2015); as well as *P. gossypiella* (Ghoneim *et al.*, 2017a) and *P. unionalis* (Ghoneim *et al.*, 2017b) after treatment with Novaluron. In some fly species, larval-pupal forms were produced after treatment of last instar maggots of *P. argyrostoma* with 150 µg/larva of chlorfluazuron (Ghoneim and Ismail, 1995b); *Stomoxys calcitrans* and *Sarcophaga bullata* with some juvenoids (Weaver and Begley, 1982).

The production of larval-pupal intermediates, in the current study, denoted a disturbing activity of the tested IGR against the developmental program of *P. argyrostoma*.

The formation of these ill-developed creatures can be explained, generally, by the interference of this IGR with the hormonal regulation of pupation (Al-Sharook *et al.*, 1991). On the other hand, a number of conceivable scenarios can be provided herein. The tested IGR might impair the developmental program *via* an ecdysteroid reduction and/or intervention in the neurosecretion release (Josephrajkumar *et al.*, 1999). The formation of these intermediate forms indicated a juvenile activity of methoprene disturbing the larval-pupal transformation. Formation of these mosaic creatures in*P. argyrostoma* may be explained by derangement of DNA synthesis, chitin biosynthesis and chitin synthase owing to the inhibitory action of methoprene (Mayer *et al.*, 1980). In addition, molts induced during the early phase of the last instar produce larval-like individuals, while those formed in the late phase produce pupal-like individuals (Eizaguirre *et al.*, 2007).

*Induction of permanent prepupae:*In insects, the suspended development is usually expressed as 'permanent larvae'.

The case of permanent larvae was recorded in some insect species as a response to various botanicals or IGRs. For examples, permanent nymphs of *S. gregaria* (Orthoptera) had been induced by certain IGRs (Salem *et al.*, 1985; Abou El-Ela, 1993). Permanent larvae of the *Ostrinia nubilalis* (Lepidoptera) were induced by Fenoxycarb (JHA), depending upon the dose and application timing (Gadenne *et al.*, 1990). Permanent larvae of *P. argyrostoma* (Diptera) were induced by chlorfluazuron (CSI)(Ghoneim and Ismail, 1995b).

In addition, some plant extracts or isolated plant products had been reported to induce permanent larvae or nymphs in different insect species, such as *Oncopeltus fasciatus* (Hemiptera) by azadirachtin (Dorn *et al.*, 1986); *O. fasciatus* and *Dysdercus peruvianus* (Hemiptera) by *Manilkara subsericea* extracts (Fernandes *et al.*, 2013); *S. litura* (Lepidoptera) by acetone leaf extract of *Withania somnifera* (Gaur and Kumar, 2010); and

Tribolium confusum (Coleoptera) by Andrographolide (a terpenoid isolated from the leaves of *Andrographis paniculata*)(Lingampally *et al*., 2013). Also, feeding of larvae of *Galleria mellonella* (Lepidoptera) on a diet treated with the JH analogue [methyl 2,7 dimethyl-9-(2-oxolanyl) 2,4 nonadienoate] resulted in the induction of permanent larvae(Slama and Lukas, 2013).

In the present investigation, topical treatment of *P. argyrostoma* prepupae only with the lower two doses of methoprene induced a suspended development in some prepupae.

These permanent prepupae suffered the methoprene action along 12 days and eventually died without pupariation. For understanding this case of the interrupted metamorphosis in *P. argyrostoma*, it is important to mention that the pupariation in dipterous Cyclorrhapha considerably differs from tanning that occurs after pupal ecdysis in other suborders of Diptera and different holometabolous insects. Puparium formation occurs in between the prepupa and pupal apolysis (Zdarek, 1985; Raabe, 1989). However, induction of the 'permanent prepupae', in thepresent study,may be explained by the inhibitory actionofmethoprene on the prothoracic gland and hence the ecdysone could not be synthesized and/or released leading to failure of ecdysis (Gaur and Kumar, 2010; Gibbens *et al*., 2011).

Perturbation of metamorphosis in P. argyrostoma by methoprene

*Impaired pupation:*The methoprene-treated last instar larvae or prepupae of *P. argyrostoma*, n the present investigation, pupated in a slightly or drastically regressed rate, depending on the dose. To a great extent, this result was consistent with the reported results for some insects, since the pupation rate was regressed by the action of some IGRs, such as *Plutella xylostella* by Hexaflumuron (Mahmoudvand *et al*., 2012); *G. pyloalis* by Lufenuron (Aliabadi *et al*., 2016) and Fenoxycarb (Singh and Tiwari, 2016);*P. argyrostoma* by Pyriproxyfen (Ismail, 1995) and Chlorfluazuron (Ghoneim and Ismail, 1995b); *S. littoralis* by Novaluron (Ghoneim *et al*., 2015); *Encarsia formosa* by Pyriproxyfen and Fenoxycarb (Wang and Liu, 2016); *P. unionalis* (Ghoneim *et al*., 2017b) and *P. gossypiella* (Ghoneim *et al*., 2017a) by Novaluron. In the present investigation, the regressed pupation rate of *P. argyrostoma* after larval treatment with methoprene, might be attributed to an inhibitory action of this IGR on the fat body which responsible for synthesis of specific storage proteins during the last instar and their deposition at the pupariation time (Gupta,1985).

*Blocked adult emergence:*The adult emergence of *P. argyrostoma* was reported to be completely or partially blocked after larval treatment with certain doses of different IGRs, such as Pyriproxyfen (Ismail, 1995) and Chlorfluazuron (Ghoneim and Ismail, 1995b). On the other hand, methoprene was reported to inhibit the adult emergence after larval treatment of other insect species, such as *Ae. aegypti* (Braga *et al*., 2005), *C. cephalonica* (Tripathi and Tiwari, 2006), *Culex quinquefasciatus* and *Ae. albopictus* (Khan *et al*., 2016; Bibbs *et al*., 2017). In addition, the adult emergence was slightly or detrimentally blocked in different insects after larval treatment of various IGRs, such as *P. xylostella* after treatment with Hexaflumuron (Mahmoudvand *et al*., 2012); *D. melanogaster* after treatment with Pyriproxyfen (Benseba *et al*., 2015); *S. littoralis* after larval treatment with Novaluron (Ghoneim *et al*., 2015); *G. pyloalis* after treatment with Lufenuron (Aliabadi *et al*., 2016);*C. quinquefasciatus* and *Ae. albopictus* after treatment with Pyriproxyfen(Khan *et al*., 2016); *P. gossypiella* after treatment with Novaluron (Hassan *et al*., 2017) and *P. unionalis* after treatment with Methoxyfenozide (Hamadah *et al*.,2017). Also, the adult emergence in the F1 generation of the fly *Sarcophaga ruficornis* was blocked after pyriproxyfen application onto the parental generation (Singh and Kumar, 2015). To a great extent, results of the current study on *P. argyrostoma* were in agreement with the previously reported results, since the emergence of adult flies was completely prevented after treatment of either the last instar larvae or prepupae with the highest methoprene dose.

After treatment of last instar larvae with other doses, adult eclosion was detrimentally blocked. After topical treatment of prepupae with other doses, however, the adult eclosion was inversely correlated to the dose.

In this context, it is important to emphasize that adult eclosion in insects is a developmental process that be regulated by eclosion hormone. The disturbance of this hormone partially or completely arrests the adults to emerge. The current result of blocked adult emergence of *P. argyrostoma* can be explicated by the disturbing action of methoprene on the adult eclosion hormone release and/or inhibition of the neurosecretion (Al-Sharook *et al*., 1991; Joseph rajkumar *et al*., 1999). On the molecular basis, JHAs and anti-JH compounds may cause misexpression of certain genes, particularly the *brood* complex (*br*-C) transcription factor gene, leading to the impairment of metamorphosis, like blocking of adult emergence (Wilson, 2004; Nandi and Chakravarty, 2011).

Deterioration of morphogenesis

*Deranged pupal morphogenesis:*In the current study, methoprene displayed an anti-morphogenic activity against the developed pupae of *P. argyrostoma*, since various percentages of malformed pupaewere observed after treatment of lastinstar larvae. On the contrary, a similar activity did not recorded for methoprene, after treatment of prepupae. This result was in a partial agreement with the impaired pupal morphogenesis in *Ph. ocularis* after treatment with fenoxycarb (Moreno *et al*., 1993b); *T. castaneum* and *T. confusum* after treatment with Cyromazine (Kamaruzzaman *et al*., 2006), *S. frugiperda* after treatment with Methoxyfenozide (Zarate *et al*., 2011), *C. cephalonica*afterFenoxycarb application ontolast instar larvae (Begum and Qamar, 2016), *P. gossypiella* after treatment of full grown larvae with Novaluron (Ghoneim *et al*., 2017a) and *P. unionalis* after treatment of newly moulted last instar larvae with Novaluron (Ghoneim *et al*., 2017b).

For understanding the anti-morphogenic action of methoprene on pupae of *P. argyrostoma*, as expressed in pupal deformities, after treatment of last instar larvae, in the present investigation, the tested IGR might exert suppressive effect on the chitin synthesis and prevented the normal deposition of new cuticle during apolysis leading to the production of pupal deformities (Retnakaran *et al*., 1985). In addition,methoprene might block the release of morphogenic peptides, causing alteration in titers of ecdysteroids and juvenoids (Barnby and Klocke, 1990). However, the methoprene failure to impair the pupal morphogenesis after treatment of prepupae, in the present study, but after treatment of last instar larvae, revealed that the pupal morphogenesis program of *P. argyrostoma* usually takes place during the first half of last instar.

*Deranged adult morphogenesis:*The deranged adult morphogenesis, as expressed in the appearance of deformed adults, was widely reported in various insects after treatment with different IGRs, such as *Rh. ferrugineus* after treatment with Diofenolan (Tanani, 2001); *S. littoralis* after treatment with Flufenoxuron (Bakr *et al*., 2010), Methoxyfenozide (Pineda *et al*., 2004) and Novaluron (Hamadah *et al*., 2015); *T. castaneum* and *T. confusum* after treatment with Cyromazine (Kamaruzzaman *et al*.,2006); *Choristoneura fumiferana* after treatment with Tebufenozide (Sundaram *et al*., 2002); *S. frugiperda* after treatment with Methoxyfenozide (Zarate *et al*., 2011); *E. integriceps* after treatment with Pyriproxyfen (Mojaver and Bandani, 2010); *A. kuehniella* after treatment with Hexaflumuron (Ashouri *et al*., 2014); *C. cephalonica*after treatment with Fenoxycarb (Begum and Qamar, 2016); *H. armigera* after treatment with Hexaflumuron (Taleh *et al*., 2015); etc. As reported by Singh and Kumar (2015), deformed adults in F1 generation of *S. ruficornis* were observed after pyriproxyfen application onto the parental generation. In addition, treatment of *C. quinquefasciatus* larvae with Fenoxycarb led to mosquito adults incapable to fly (Schaefer *et al*., 1987). In the present investigation on *P. argyrostoma*, obtained results were compatible with the previously reported results of arrested adults, since methoprene exerted an anti-morphogenic action on adults. After treatment of either last instar larvae or prepupae only with doses

5.0 and 1.0 µg/larva, some of the emerged adult flies appeared with anomalous morphology. These deformed adult flies had a poor ability to fly. This result agreed, also, with the reported anti-morphogenic action of methoprene on some insect species, such as *T. confusum* (Smet *et al*., 1989) and *Corcyra cephalonica* (Tripathi and Tiwari, 2006). For interpretation of methoprene anti-morphogenic activity against adult flies of *P. argyrostoma*, in the present investigation, this IGR might exibit a disturbing effect on the hormonal balance during the adult transformation, in particular the disturbance of ecdysteroid titer which led to changes in lysosomal enzyme activity causing overt adult deformation (Joseph rajkumar *et al*., 1999). Also, some suggestions can be conceivable herein. The exogenous application of JHA has lead to increasing JH titer and subsequently imbalanced ecdysteroids. In addition, the observed adult deformities, in the current study, might be due to the disruption of chitin synthase by metabolites of the tested IGR (Cohen and Casida, 1980) and/or inhibition of facilitated diffusion and active transport across cell membranes of nucleosides and amino acids (Mayer *et al*., 1988).

Conclusion

On the basis of the obtained results in the current study, methoprene exerted both acute and chronic toxic actions on different stages of *P. argyrostoma*. Also, the tested IGR exhibited disruptive effects on growth and development of pupae and adult flies. Therefore, this IGR may be an potential compound for remedial control of *P. argyrostoma*.

Acknowledgement

The authors gratefully acknowledgeDr. Karem Ghoneim, Prof. of Insect Physiology, Faculty of Science, Al-Azhar University, for his criticizing reading of the manuscript of the present paper.

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