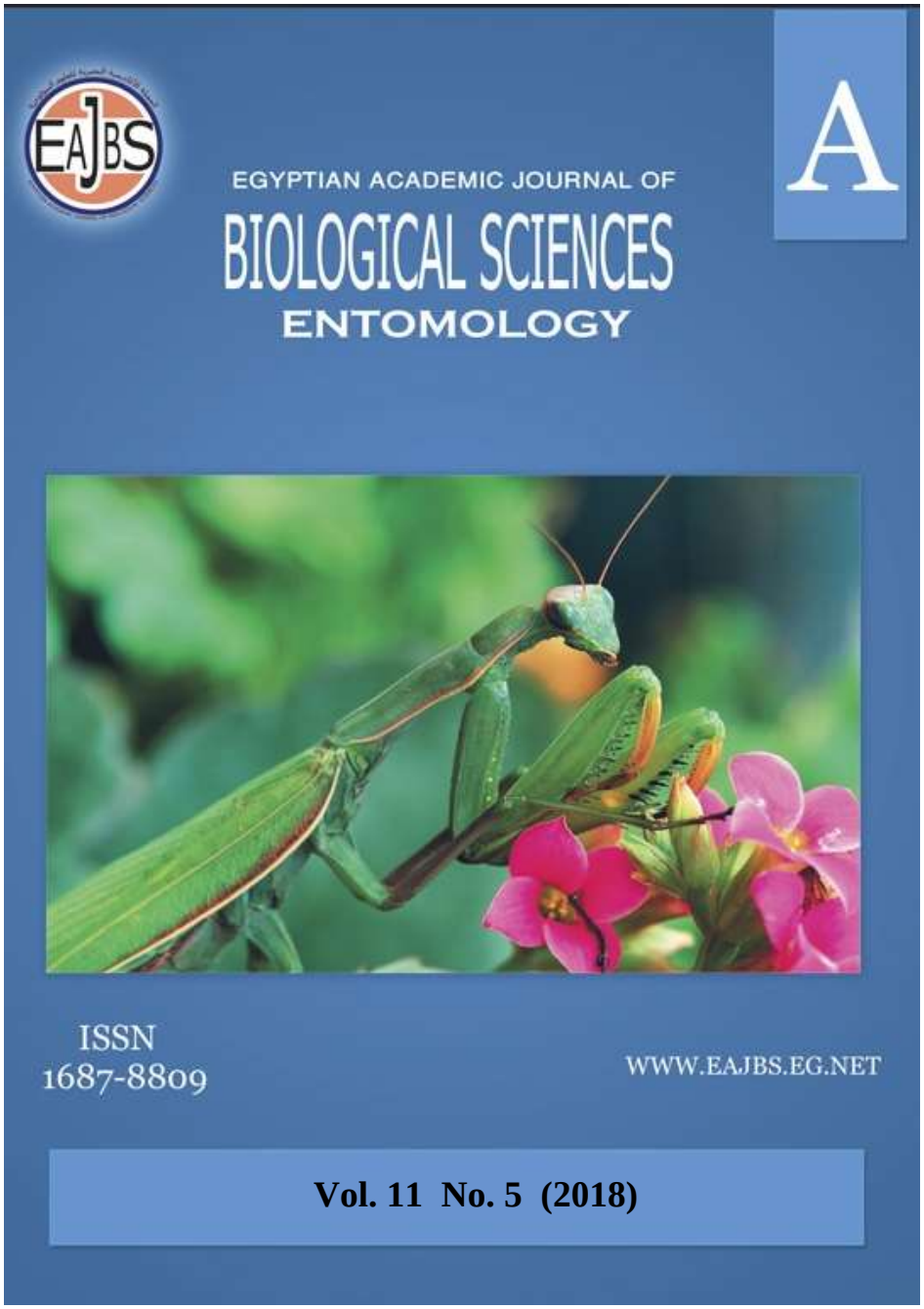

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Deteriorating Effects of Methoxyfenozide on Survival, Development and Metamorphosis of the Olive Leaf Moth, *Palpita unionalis* (Hübner) (Lepidoptera: Pyralidae).

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ABSTRACT

The olive leaf moth *Palpita unionalis* is one of the serious olive pests in Egypt and several countries. The objective of the current study was to evaluate the effects of methoxyfenozide, an ecdysteroid agonist, on survival, growth, development and metamorphosis of this pest. The newly moulted last instar (6^{th}) larvae were treated with six concentrations (100, 10.0, 1.00, 0.10, 0.01 and 0.001 ppm) *via* the fresh olive leaves, as food. The strongest acute toxicity (100% mortality) was exhibited against larvae at the highest concentration, but no mortality was observed at the lowest one. The developed pupae had been subjected to the toxic effect only at the higher three concentrations. Increasing adult mortality% was recorded at concentrations other than the highest or lowest one. LC_{50} was calculated in 0.176 ppm. The somatic weight gain of larvae, growth rate, larval duration and developmental rate of larvae had been pronouncedly reduced. The pupal duration was non-significantly prolonged and the pupal developmental rate was slightly regressed. The successfully developed pupae suffered a desiccation action of methoxyfenozide. The metamorphosis program was impaired, since some larval-pupal intermediates had been produced. In addition, the pupal morphogenesis was deteriorated, since some pupal deformations had been produced after larval treatment only with 0.10 ppm.

INTRODUCTION

The olive leaf moth, *Palpita unionalis* (Hübner) (Lepidoptera: Pyralidae) attracted a considerable attention of some researchers during the last few decades because of its serious attack against young olive trees in nurseries (Solaiman, 1997; Hegazi *et al*., 2007; Ghoneim, 2015). At the high population, it destroys a remarkable portion of the olive crop (Hegazi *et al*., 2012; Mahmoud, 2014). Different losses had been reported in Greece (Vassilaina-Alexopoulou and Santorini, 1973), Italy (Fodale and Mule, 1990; Antonelli and Rossi, 2004) and Egypt (El-Kifl *et al*., 1974; El-Hakim and El-Helmy, 1982). The most economic damage of this pest occurs on the young trees and nurseries as well as the shoots of old trees (Pinto and Salemo, 1995; Grossley, 2000).

The traditional insecticides have usually been used to control *P. unionalis* on olive trees (Foda *et al*., 1976). In Sicily, insecticides exhibited a good control when

applied on 1st and 2nd instar larvae (Fodale and Mule, 1990). On the other hand, insecticidal residues have been detected in the olive oil and in the environment where olives are grown (Montiel and Jones, 2002). In addition, the intensive use of many conventional insecticides led to several dramatic problems, such as destruction of the natural enemies, environmental hazards and serious toxicological problems to humans, as well as the development of insect resistance toward different insecticides (Davies *et al*., 2007; Costa *et al*., 2008; Mosallanejad and Smagghe, 2009; Sharifian *et al*., 2012). Therefore, alternative control agents have been initiated recently to minimize the insecticide hazards and introduce of new effective control tools with negligible effects on the ecosystem (Korrat *et al*., 2012; Derbalah *et al*., 2014). During the last few decades, a new class of comparatively safe compounds has been developed and known as insect growth regulators (IGRs)(Dhadialla *et al*., 1998; Khan and Qamar, 2012). IGRs are not directly toxic, but act selectively on the development, metamorphosis and/or reproduction of the target pest (Nicholas *et al*., 1999; Martins and Silva, 2004) owing to their disruptive effects on the normal activity of endocrine or hormone system of insects (Wang and Liu, 2016). Because of their desirable characteristics, such as potential action of the target pest, low toxicity to non-target organisms, less environmental pollution, high selectivity, and low impact on natural enemies, domestic animals and people, IGRs are used to control various insect pests and can assist in the development of sustainable agriculture (Wang and Wang, 2007; Taleh *et al*., 2015; Sabry and Abdou, 2016). Many IGRs have shown potentiality against different lepidopterous insects (Talikoti *et al*., 2012; El-Aasar *et al*., 2013; Awad *et al*., 2014; Ghoneim *et al*., 2017a; Hassan *et al*., 2017; Tanani *et al*., 2017).

From the classification point of view, IGRs had been grouped in three categories: (i) Juvenile hormone analogues, (ii) Ecdysteroid agonists and (iii) Chitin synthesis inhibitors (Dhadialla *et al*., 1998; Oberlander and Silhacek, 2000; Tunaz and Uygun, 2004). Methoxyfenozide (RH-2485) is a potent non-steroidal ecdysteroid agonist; a new class of IGRs discovered by Rohm and Haas (Spring House, PA). Methoxyfenozide is significantly more active than Tebufenozide (Ishaaya *et al*., 1995). Its high efficacy against eggs and/or larvae of many lepidopterous insects has been widely reported (Gobbi *et al*., 2000; Carlson *et al*., 2001; Sundaram *et al*., 2002; Pineda *et al*., 2004; Saenz-de-Cabezon *et al*., 2005; Pineda *et al*., 2007; Pineda *et al*., 2009; Ouakid *et al*., 2016; Sabry and Abdou, 2016). Hamadah *et al*. (2017) recorded deranging effects of Methoxyfenozide on the adult performance and reproductivity of *P unionalis*. In addition, methoxyfenozide was reported as an efficient control agent for several dipterous insects (Hamaidia and Soltani, 2016) and coleopterans (Smagghe and Degheele, 1994; Ali *et al*., 2016). Methoxyfenozide has an excellent margin of safety to non-target organisms, including a wide range of beneficial insects (Medina *et al*., 2004; Schneider *et al*., 2008). The objective of the present study was to evaluate the disruptive effects of methoxyfenozide on survival, growth, development, metamorphosis and morphogenesis of *P. unionalis*.

MATERIALS AND METHODS

1. Experimental Insect:

A sample of olive leaf moth *Palpita unionalis* 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 the procedure described by Mansour (2012). 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 into 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 the feeding of the newly hatched larvae. The fresh tender olive leaves were renewed daily until pupation.

2. Methoxyfenozide Administration:

The ecdysone agonist, methoxyfenozide: 3-methoxy-2-methylbenzoic acid 2-(3,5-dimethylbenzoyl)-2-(1,1-dimethylethyl)hydrazide has the molecular formula: $C_{22}H_{28}N_2O_3$. It was kindly obtained from Plant Protection Research Institute, Giza, Egypt. A series of six concentrations of was prepared by diluting it with distilled water in volumetric flasks as follows: 100.0, 10.0, 1.00, 0.10, 0.01 and 0.001 ppm. Fresh olive leaves were dipped in each concentration for 5 minutes and dried in air before introducing to the newly moulted last instar (6^{th}) larvae of *P. unionalis* for feeding. Control larvae were provided with water-treated olive leaves. Ten replicates of both treated and control larvae (one larva/replicate) were kept separately in glass vials. The larvae were allowed to feed on the 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:

Toxicity test:

All mortalities of treated and control (larvae, pupae and adults) were recorded every day and corrected according to Abbott's formula (Abbott, 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).

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:

Initial weight (before the beginning of experiment) - final weight (at the end of experiment).

Growth rate: Growth rate (GR) can be calculated according to (Waldbauer, 1968) as follows:

Fresh weight gain during feeding period / feeding period X mean fresh body weight of larvae during the feeding period.

Developmental rate: Dempster's equation (1957) was applied for calculating the developmental duration, and Richard's equation (1957) 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.

Pupal Water Loss: Pupal water loss (%) was calculated depending on the data of the initial and final weights of the pupae, as follows:

Initial weight – (final weight /initial Weight) × 100

4. Statistical Analysis of Data:

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

RESULTS

1. Toxicity and Lethal Effects of Methoxyfenozide on *P. unionalis*:

After treatment of the newly moulted last instar (6^{th}) larvae of *P. unionalis* with methoxyfenozide (100, 10.0, 1.00, 0.10, 0.01 and 0.001 ppm) *via* the fresh olive leaves, as food, data of toxicity against all developmental stages were assorted in Table (1). In the light of these data, the strongest acute toxicity (100% mortality) was exhibited against larvae at the highest concentration, but no mortality was observed at the lowest concentration. At other concentration levels, the toxic action was exerted proportional to the concentration (20, 20, 30 and 80% mortality, at 0.01, 0.10, 1.00 and 10.00 ppm, respectively). The successfully developed pupae had been subjected to the toxic effect of methoxyfenozide only at the higher three concentrations (25, 28 and 50% mortality, at 0.10, 1.00 and 10.0 ppm, respectively). At the lower two concentrations, no pupal mortality was observed. In respect of the adulticidal effect of the tested compound, no lethal effect was exhibited at the highest or lowest concentration level, but an increasing mortality % was determined at other concentrations (12.5, 16.0 and 20.0% mortality, at 0.01, 0.10 and 1.00 ppm, respectively). As clearly seen in the same table, the corrected mortality was found in a dose-dependent course, with an exception of the lowest concentration. LC_{50} was calculated in 0.176 ppm.

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

Conc. (ppm)	Larval mortality	Pupal mortality	Adult mortality	Total mortality	Corrected mortality	LC_{50}
100	100	*	*	100	100	0.176
10.0	80	50	00.0	90	90	
1.00	30	28	20.0	60	60	
0.10	20	25	16.0	50	50	
0.01	20	00	12.5	30	30	
0.001	00	00	00.0	00	00	
Control	00	00	00.0	00	---	

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

2. Effects of methoxyfenozide on growth, development and metamorphosis of *P. unionalis*:

Table (2) contains data of the most important growth, development and metamorphosis criteria of *P. unionalis* as disturbed by the treatment of newly moulted last instar larvae with five concentration levels of methoxyfenozide. Depending on these data, the somatic weight gain of larvae was unremarkably reduced (4.03±0.96, 3.62±1.26, 3.26±1.56, 3.21±2.79 and 2.15±0.07 gm, at 0.001, 0.01, 0.10, 1.00 and 10.00 ppm, respectively, in comparison with 4.45±1.48 gm of control larvae). Also, methoxyfenozide exerted a strong suppressing action on the larval growth rate, in a dose-dependent course (for detail, see Table 2). In addition, the larval duration was insignificantly prolonged proportional to the ascending concentration (3.61±0.69, 3.62±0.91, 3.90±0.74, 4.0±1.29 and 4.50±1.41 days, at 0.001, 0.01, 0.10, 1.00 and 10.0 ppm, respectively, *vs*. 3.60±0.51 days of control larvae). The developmental rate was slightly regressed, almost in dose-dependent fashion. To a great extent, a similar effect of methoxyfenozide was exhibited on the successfully developed pupae, since their duration was non-significantly prolonged (9.15±0.81, 9.14±0.69, 9.40±1.34, 10.25±1.25 and 12.01±1.10 days, at 0.001, 0.01, 0.10, 1.00 and 10.0 ppm, respectively, *vs*. 9.13±1.05 days of control pupae) and developmental rate was slightly regressed.

Because the pupal death may be due to the desiccation caused by methoxyfenozide, water loss (%) of pupal body was taken into consideration. As obviously seen in the aforementioned table, water loss of treated pupae was generally greater than that of control pupae (37.3, 42.0, 39.2, 49.1 and 59.9%, at 0.001, 0.010, 0.10, 1.00 and 10.00 ppm, respectively, *vs*. 30.40% of control pupae).

In respect of the disruptive effect of methoxyfenozide on metamorphosis and morphogenesis programs of *P. unionalis*, the distributed data in Table (2) exiguously demonstrated that the pupation was severely hindered, since decreasing pupation % was determined (90, 80, 80, 70 and 20% pupation of the treated larvae, at 0.001, 0.01, 0.10, 1.0 and 10.0 ppm, respectively, *vs*. 100% pupation of control congeners).

Table 2. Growth and developmental effects of methoxyfenozide 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)	Develop. rate	Larval-pupal inter. (%)	Pupation (%)	Pupal duration (Mean days±SD)	Develop. rate	Pupal deformities (%)	Water loss (%)
10.00	2.15±0.07 a	0.010±0.007d	4.5±1.41 a	22.2	00	20	12.01±1.10 a	8.33	00	59.9
1.000	3.21±2.79 a	0.017±0.001d	4.0±1.29 a	25.0	10	70	10.25±1.25 a	9.75	10	49.1
0.100	3.26±1.56 a	0.017±0.001d	3.90±0.74 a	25.6	10	80	9.40±1.34 a	10.63	10	39.2
0.010	3.62±1.26 a	0.020±0.001d	3.62±0.91 a	27.6	10	80	9.14±0.69 a	10.94	00	42.0
0.001	4.03±0.96 a	0.022±0.001c	3.61±0.69 a	27.6	00	90	9.15±0.81 a	10.94	00	37.3
Control	4.45±1.48	0.024±0.002	3.60±0.51	27.7	00	100	9.13±1.05	10.95	00	30.4

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 ($p < 0.001$).

The metamorphosis program was seriously affected by methoxyfenozide, since some larval-pupal intermediates (10%) had been produced after larval treatment with 0.01, 0.10 and 1.0 ppm. As evidently shown in Fig (1), these intermediates were observed in various features, such as pupal head and thorax with larval abdomen, or pupal dorsal part with larval ventral part. In addition, the pupal morphogenesis was deteriorated by methoxyfenozide, some pupal deformations had been produced after larval treatment only with 0.10 ppm. Such deformations were observed as non-tanned bodies (see Fig. 2).

Fig. 1. Larval-pupal intermediates of *P. unionalis* as features of disturbed metamorphosis program after treatment of newly moulted last instar larvae with methoxyfenozide. (A): Control larva, (B): Control pupa. (C): Larval-pupal intermediate (pupal head and thorax with larval abdomen: 1 ppm), (D): Larval-pupal intermediat (pupal dorsal part and larval ventral part: 0.1 ppm). (E): Larval-pupal intermediate (0.01 ppm).

Fig. 2.: Deteriorated pupal morphogenesis of *P. unionalis* by methoxyfenozide. (A): Control pupa. (B): Deformed pupa: non-tanned body (1.0 & 0.1 ppm).

DISCUSSION

1. Toxicity of Methoxyfenozide Against Different Developmental Stages of *P. unionalis*:

Toxic effects of several IGRs of different categories against various insect species had been reported, such as flufenoxuron (El-Naggar, 2013), lufenuron (Bakr *et al*., 2013), buprofezin (Nasr *et al*., 2010), methoxyfenozide (Pineda *et al*., 2004) and cyromazine (Tanani *et al*., 2015) against *Spodoptera littoralis*; pyriproxyfen against *Eurygaster integriceps* (Mojaver and Bandani, 2010); diofenolan against *Papilio demoleus* (Singh and Kumar, 2011); diflubenzuron against *Halyomorpha halys* (Kamminga *et al*., 2012); chlorfluazuron against *Spodoptera litura* (Perveen, 2012); flufenoxuron and pyriproxyfen against *Locusta migratoria* (Hu *et al*., 2012); kinoprene against *Culex pipiens* (Hamaidia and Soltani, 2014); flufenoxuron and methoprene against *Agrotis ipsilon* (Khatter, 2014) and lufenuron against *Tribolium castaneum* (Gado *et al*., 2015). Recently, IGRs exhibited various toxicities against some insects, such as pyriproxyfen against *Spodoptera mauritia* (Resmitha and Meethal, 2016); lufenuron and methoxyfenozide against *T. castaneum* (Ali *et al*., 2016); methoxyfenozide against *C. pipiens* (Hamaidia and Soltani, 2016); tebufenozide against *Ephestia kuehniella* (Tazir *et al*., 2016); lufenuron against *Glyphodes pyloalis* (Aliabadi *et al*., 2016) and *Helicoverpa armigera* (Vivan *et al*., 2016); fenoxycarb against *Corcyra cephalonica* (Begum and Qamar, 2016); methoprene and pyriproxyfen against *Culex quinquefasciatus* and *Aedes albopictus* (Khan *et al*., 2016); cyromazine against *Musca domestica*, *Stomoxys calcitrans* and *Fannia canicularis* (Donahue *et al*., 2017); novaluron against *Pectinophora gossypiella* (Ghoneim *et al*., 2017a) and *P. unionalis* (Ghoneim *et al*., 2017b). Results of the present study on *P. unionalis* were, to some extent, in agreement with the previously reported results of toxicity, since methoxyfenozide exhibited toxic effects on larvae, proportional to the concentration, except at the lowest one. The developed pupae had been subjected to the toxic effect only at the higher three concentrations. No lethal effect was exhibited by methoxyfenozide on adults at the highest or lowest concentration, but increasing mortality% was recorded at other concentrations.

LC_{50} of methoxyfenozide against *P. unionalis*, in the current investigation, was calculated in 0.176 ppm. As reported by many studies, 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. For examples, LC_{50} values of novaluron and lufenuron against *S. litura* were determined as 350.45 and 453.78 ppm, respectively (Sharma and Pathania, 2014); LC_{50} of pyriproxyfen against *S. litura* larvae was found to be 0.025% (Kaur and Chandi, 2015); LC_{50} of hexaflumuron against *H. armigera* was 8.47 mg /L (Taleh *et al*., 2015); 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} of methoxyfenozide against *Culex pipiens* was calculated in 24.54 µg/L (Hamaidia and Soltani, 2016); LC_{50} of lufenuron against *G. pyloalis* was 19 ppm (Aliabadi *et al*., 2016); LC_{50} values of chlorfluazuron, cyromazine, lufenuron and precocene I against *C. felis* were 0.19, 2.66, 0.20, and 10.97 ppm, respectively (Rust and Hemsarth, 2017) and 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)

To explicate the toxic effect of methoxyfenozide 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 the conventional insecticides. Furthermore, it was suggested that the tested IGR interferes with the transport system of UDP-N-acetyl amine across the membrane (Eto, 1990). For some detail, the larval deaths of *P. unionalis* by methoxyfenozide, in the current study, might be attributed to the prevention of moulting larvae to swallow volumes of air for splitting the old cuticle and expand the new one during ecdysis (Linton *et al*., 1997). Also, these larval deaths might be due to the prevented feeding and continuous starvation of the present insect (Ghoneim *et al*., 2000). Although the disturbance of hormonal regulation or the disruption of normal activity of the endocrine system in insects by IGRs was reported (Djeghader *et al.*, 2014), the pupal deaths in *P. unionalis*, in the present investigation, could not be directly relate to the hormonal activity of methoxyfenozide, but to other causes, such as suffocation, bleeding and desiccation due to imperfect exuvation, failure of vital homeostatic mechanisms, *etc*. (Smagghe and Degheele, 1994). This suggestion can easily be substantiated since methoxyfenozide exerted a predominant desiccating action on the 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 with methoxyfenozide concentrations, other than the highest and lowest ones, in the current study, could be explained by the retention and distribution of this IGR 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 compound (Osman *et al*., 1984).

2. Inhibited Growth and Retarded Development of *P. unionalis* by Methoxyfenozide:

In the present study, both larval weight gain and growth rate had been determined after treatment of newly moulted last (6^{th}) instar larvae of *P. unionalis* with different concentrations of methoxyfenozide. The somatic weight gain of larvae was slightly inhibited and the larval growth rate was slightly regressed, in a dose-dependent course. Also, larval duration was generally prolonged and the developmental rate of these larvae was regressed, in almost dose-dependent manner. This inhibited growth of *P. unionalis* by methoxyfenozide was in accordance with those reported results of inhibited 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), *Aedes aegypti* (Farnesi *et al*., 2012), *Culex pipiens* by novaluron (Djeghader *et al*., 2014) and kinoprene (Hamaidia and Soltani, 2014). Likewise, some IGRs failed to affect the growth of different insects, such as *M. domestica* (Ghoneim *et al*., 1991), *Periplaneta americana* and *Oncopeltus fasciatus* (Darvas *et al*., 1992), *Spodoptera exempta, Spodoptera exigua*, and *Leptinotarsa decemlineata* (Smagghe and Degheele, 1994).

Lepidoptera belongs to the most sensitive groups of insects regarding the growth regulating effects of IGRs. The inhibited growth of *P. unionalis* by methoxyfenozide, in the current study, might be a result of the blocked release of morphogenic peptides, causing alteration in the ecdysteroid and juvenoid titre (Barnby and Klocke, 1990). Also, methoxyfenozide might affect the tissues and cells undergoing mitosis (Nasiruddin and Mordue, 1994).

In addition, the present results of prolonged larval duration and regressed

developmental rate of *P. unionalis*, after treatment of newly moulted last instar larvae with methoxyfenozide corroborated with the reported results of prolonged larval duration in some insect species by various IGRs, such as *S. littoralis* larvae after treatment of penultimate or last instar larvae with by novaluron (Ghoneim *et al*., 2015) and cyromazine (Tanani et al., 2015); *Spodoptera frugiperda* by Methoxyfenozide (Zarate *et al*., 2011) and *P. gossypiella* by pyriproxyfen (Sabry and Abdou, 2016) or noviflumuron and novaluron (Hamadah and Ghoneim, 2017). On the contrary, the present results disagreed with the reported results of shortened larval duration of some insects after treatment with different IGRs, such as *Rhynchophorus ferrugineus* by lufenuron and diofenolan (Tanani, 2001**)**, *A. ipsilon* by flufenoxuron (El-Sheikh, 2002), *Schistocerca gregaria* by lufenuron (Bakr *et al*., 2008), *P. gossypiella* by methoxyfenozide (Sabry and Abdou, 2016) and *P. unionalis* by novaluron (Ghoneim *et al*., 2017b)

As reported in the available literature, many IGRs exhibited some inhibitory Diflubenzuron (Aref *et al*., 2010), lufenuron (Gaaboub *et al*., 2012), novaluron (Ghoneim *et al*., 2015) and Cyromazine (Tanani *et al*., 2015); *C. pipiens* by kinoprene (Hamaidia and Soltani, 2014); *A. ipsilon* by methoprene and flufenoxuron (Khatter, 2014); *P. gossypiella* by buprofezin (Al-Kazafy, 2013); teflubenzuron (El-Khayat *et al*., 2015) and chromafenozide (Salem, 2015). Recently, the developmental duration was prolonged indicating a retarded development in some other insects by various IGRs, such as *G. pyloalis* by lufenuron (Aliabadi *et al*., 2016); *C. pipiens* by methoxyfenozide (Hamaidia and Soltani, 2016); *C. cephalonica* by fenoxycarb (Begum and Qamar, 2016); *P. gossypiella* by lufenuron and Pyriproxyfen (Sabry and Abdou, 2016), noviflumuron and novaluron (Hamadah and Ghoneim, 2017) and Novaluron (Ghoneim *et al*., 2017a); and *P. unionalis* by novaluron (Ghoneim *et al*., 2017b); *etc*. In agreement with those previously reported results of retarded development, the present study recorded a slight retarding effect of methoxyfenozide on the development of *P. unionalis*, since the pupal duration was non-significantly prolonged and the developmental rate of pupae was slightly regressed.

In the current study, retarded development of *P. unionalis* by methoxyfenozide, as expressed in prolonged pupal duration, might be attributed to the indirect interference of this IGR with neuroendocrine organs responsible for the synthesis and release of tropic hormones, like prothoracicotropic hormone (PTTH)(Subrahmanyam *et al.*, 1989). In general, 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 (Kuwano *et al*., 2008). Also, methoxyfenozide may exhibit a delaying effect on the ecdysis and transformation (Linton *et al*., 1997). In particular, the final step of chitin biosynthesis pathway was inhibited by this IGR and the precursor was not converted into chitin leading to a prolongation of developmental duration (Djeghader *et al*., 2014).

3. Deranged Metamorphosis And Morphogenesis of *P. unionalis* by Methoxyfenozide:

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 (Pineda *et al*., 2009). The major symptoms and features of the 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 Flufenoxuron (El-Naggar, 2013), novaluron (Ghoneim *et al*., 2015) and cyromazine (Tanani *et al*., 2015). Also, some or all of these symptoms of the impaired metamorphosis were recorded after treatment of different insects with several IGRs, such as *Liriomyza trifolii* (Saryazdi *et al*., 2012) and *Callosobruchus maculates* (Al-Mekhlafi *et al*., 2012) by cyromazine; *H. armigera* (Murthy and Ram, 2002), *A. aegypti* (Nwankwo *et al*., 2011) and *M. domestica* (Lohmeyer *et al*., 2014) by novaluron; *Lipaphis erysimi* by pyriproxyfen (Liu and Chen, 2001); *Rh. ferrugineus* (Tanani, 2001) and *P. demoleus* (Singh and Kumar, 2011) by diofenolan; *Lobesia botrana* by lufenuron (Saenz-de-Cabezon *et al*., 2005); *C. pipiens* by kinoprene (Hamaidia and Soltani, 2014); *P. gossypiella* (Ghoneim *et al*., 2017a) and *P. unionalis* (Ghoneim *et al*., 2017b) by novaluron; *etc*.

In respect of the pupation process of *P. unionalis*, in the present study, methoxyfenozide severely hindered it, since pupation % considerably decreased, regardless the concentration. This result 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 (Mahmoudvand *et al*., 2012); *S. littoralis* by novaluron (Ghoneim *et al*., 2015) and cyromazine (Tanani *et al*., 2015); *G. pyloalis* by lufenuron (Aliabadi *et al*., 2016) and fenoxycarb (Singh and Tiwari, 2016); *Encarsia formosa* by pyriproxyfen and fenoxycarb (Wang and Liu, 2016); *P. gossypiella* (Ghoneim et al., 2017a) and *P. unionalis* (Ghoneim *et al*., 2017b) by novaluron as well as *P. gossypiella* after treatment of 1-day old eggs with noviflumuron or novaluron (Hamadah and Ghoneim, 2017).

With regard to the pupal morphogenesis of *P. unionalis*, in the present study, it was deranged, since different pupal deformities had been observed, only after larval treatment with 0.10 ppm of methoxyfenozide. To some extent, this result was in partial resemblance with the reported results of deranged pupal morphogenesis in *T. castaneum* and *T. confusum* after treatment with cyromazine (Kamaruzzaman *et al*., 2006), *S. frugiperda* after feeding of 5th instar larvae on a diet treated with LC_{10} and LC_{25} of methoxyfenozide (Zarate *et al*., 2011), *C. cephalonica* after topical application of last instar larvae with fenoxycarb (Begum and Qamar, 2016), *P. gossypiella* after treatment of the 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). In contrast, the present result disagreed with some reported results of IGR failure to affect the morphogenesis of some insect species, such as *P. gossypiella* after treatment of the 1-day old eggs with noviflumuron or novaluron (Hamadah and Ghoneim, 2017). Whatever the mode of action, methoxyfenozide suppressed 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 connection with the metamorphosis program of *P. unionalis*, in the current investigation, methoxyfenozide exhibited a disruptive effect on it, since some larval-pupal intermediates had been produced after larval treatment with some concentrations. These intermediates were observed in various features, such as pupal head and thorax with larval abdomen, or pupal dorsal part with larval ventral part. 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 (Taleh *et al*., 2015), *S. littoralis* by novaluron (Ghoneim *et al*., 2015) and cyromazine (Tanani *et al*., 2015), *C. cephalonica* by fenoxycarb

(Begum and Qamar, 2016) and *P. gossypiella* (Ghoneim *et al*., 2017a) and *P. unionalis* (Ghoneim *et al*., 2017b) by novaluron.

The production of larval-pupal intermediates, in the present study, indicated the disturbance of metamorphosis program of *P. unionalis* by methoxyfenozide. It can be interpreted by the interference of this IGR with the hormonal regulation of pupation program (Al-Sharook *et al*., 1991). For some detail, some conceivable scenarios can be described herein. (1) Methoxyfenozide may inhibit the metamorphosis program *via* an ecdysteroid reduction, interference with the release of eclosion hormone or/and inhibition of the neurosecretion (PTTH) (Josephrajkumar *et al*., 1999). (2) The production of these intermediates may indicate a juvenile property of methoxyfenozide retarding the perfect larval-pupal 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 methoxyfenozide on the DNA synthesis (Mitlin *et al*., 1977) or the chitin biosynthesis and chitin synthase (Mayer *et al*., 1980). (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 (Tateishi *et al*., 1993). 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 (Eizaguirre *et al*., 2007).

Conclusions:

According to the obtained results of the present study, it can be concluded that methoxyfenozide 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, Methoxyfenozide may be considered as an effective control agent against this economic pest which attacks the commercial olive groves in Egypt and other olive producing countries, and can be considered as a potential alternative to the conventional pesticides used for controlling this pest.

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