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RESEARCH ARTICLE

DERANGED ADULT PERFORMANCE AND REPRODUCTIVE POTENTIAL OF THE OLIVE LEAF MOTH *PALPITA UNIONALIS* (HÜBNER) (LEPIDOPTERA: PYRALIDAE) BY THE NON-STEROIDAL ECDYSONE AGONIST, METHOXYFENOZIDE

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ARTICLE INFO	ABSTRACT
Article History:	The olive leaf moth <i>Palpita unionalis</i> (Lepidoptera: Pyralidae) is an economic pest of the olive groves
Received 02 nd March, 2017 Received in revised form 27 th April, 2017 Accepted 09 th May, 2017 Published online 30 th June, 2017	in Egypt and other olive producing countries in the world. The present study was conducted aiming t assess the effects of Methoxyfenozide, on adult performance and reproductive potential after treatmer of newly moulted last (6 th) instar larvae with sublethal concentrations (0.001, 0.01, 0.10, 1.00 and 10. ppm) of the tested ecdysteroid agonist.Methoxyfenozide could not affect the adult morphogenesis be displayed adulticidal effects (10% mortality) only at 0.10 and 1.00 ppm and arrested the adu
Keywords:	— emergence, especially at the higher four concentrations. The total adult longevity, pre-oviposition period andpost-oviposition period had been pronouncedly prolonged, in a dose-dependent course, but
Emergence, Fecundity, Fertility, Incubation, Longevity, Morphogenesis, Oviposition.	the oviposition period was slightly shortened. Oviposition efficiency of the successfully emerged females was pronouncedly inhibited, in a dose-dependent trend. Fecundity and fertility were tremendously reduced, in a dose-dependent course. The embryonic development was drastically retarded, since the incubation period was significantly prolonged, proportional to the concentration.
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INTRODUCTION

Olive (Olea europaea L.) is one of the economically important crops in the Mediterranean Basin.Olive tree is subjected to attack by several insect pests causing considerable yield losses in quality and quantity (Spooner-Hart et al., 2007). The olive leaf moth Palpita unionalis (Hübner) (Lepidoptera: Pyralidae) is one of the most dangerous pests of olives in Egypt and other Mediterranean countries (Broumas et al., 2002; Shehata et al., 2003; Yilmaz and Genc, 2012). The most important damage of the pest occurs on young trees, nurseries and shoots of old trees (Pinto and Salemo, 1995; Grossley, 2000). The control of P. unionalis on olive trees has relied upon the use of traditional insecticides (Fodaet al., 1976). Different pesticides exhibited a good control when applied on the early larval instars (Fodale and Mule, 1990). Insecticide residues have been detected in olive oil and in the environment where olives are grown (Montiel and Jones, 2002). In addition, the intensive and discriminate uses of many broad-spectrum conventional insecticides led to several drastic problems, such as the environmental hazards, destruction of the natural enemies, like parasites, predators, birds, fishes and mammals, serious

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toxicological problems to humans, as well as the development of insect resistance toward different insecticides (Rose, 2001; Davies et al., 2007; Costa et al., 2008; Mosallanejad and Smagghe, 2009). Therefore, alternative control agents have been searched recently to minimize the pesticide hazards. As a result, scientists have looked for new target sites beyond the nervous system on which the conventional insecticides have act. During the last few decades, a new class of comparatively safe compounds have been developed and known as insect growth regulators (IGRs) (Dhadialla et al., 1998; Khan and Qamar, 2012). In contrast to the classical chemical (neurotoxic) insecticides, IGRs are not directly toxic, but act selectively on the development, metamorphosis and/or reproduction of the target insect pests (Biddinger and Hull, 1995; Hoffmann and Lorenz, 1998; Nicholas et al., 1999; Martins and Silva, 2004) owing to their disruptive effects on the normal activity of endocrine system of insects (Wang and Liu, 2016). On the basis of the mode of action, IGRs had been grouped in three categories: (i) Juvenile hormone analogues (JHAs) (also called as Juvenoids), (ii) Ecdysteroids or ecdysone agonists and (iii) Chitin synthesis inhibitors (CSIs) or moult inhibitors (Wing and Aller, 1990; Dhadialla et al., 1998; Oberlander and Silhacek, 2000). Latter, Tunaz and Uygun (2004) classified IGRs into CSIs and substances that interfere with the action of insect hormones (i.e. JHAs, and ecdysteroids).

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Ecdysone agonists are harmless to vertebrates (Carlson et al., 2001) with little or no adverse effects on the beneficial insects (Retnakaran et al., 2003). They have narrow spectrum of activity, positive ecotoxicological profile, and short persistence in the environment (Sundaram et al., 2002, Osorio et al., 2008).Like other IGRs, these compounds act more slowly than neurotoxic insecticides disrupting the hormonal regulation or the physiological development of insects rather than directly killing them (Biddinger et al., 2006). In some detail, uses of ecdysone agonists lead to a premature and lethal larval molt by binding directly to the ecdysteroid receptors (Smagghe et al., 2004), delayed or accelerated developmental rates (Biddinger et al., 2006), weight loss of immature stages (Pineda et al., 2007), pupal deformations (Pineda et al., 2004), adult deformities (Sundaram et al., 2002), disturbed diapause (Eizaguirre et al., 2007), impaired reproductive parameters (Palli and Retnakaran, 2001; Yanagi et al., 2006; Pineda et al., 2009) and changes in the adult sex ratio (Biddinger et al., 2006). Although these compounds are used for controlling lepidopterous and coleopterous pests (Ishaayaet al., 2001; Palli and Retnakaran, 2001; Yanagi et al., 2006) and orthopterous pests (Al-Dali et al, 2008), they are highly selective against lepidopterous larvae (Schneider et al., 2008). Nowadays, ecdysone agonists are being commercialized as selective biorational insecticides to be used in combinations with other control strategies to develop integrated pest management programs in agricultural ecosystems.

Several substituted dibenzovl hydrazines that act as nonsteroidal ecdysone agonists have been synthesized, such as RH-5849 (the prototype compound), Tebufenozide(RH-5992), Methoxyfenozide (RH-2485) and Halofenozide (RH-0345) and Chromafenozide (ANS-118).Methoxyfenozide is a potent synthetic non-steroidal ecdysteroid agonist discovered by Rohm and Haas (Spring House, PA, USA)(Dhadialla et al., 2005). It 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). Its high efficacy against lepidopterous larvae (including many species in families Pyralidae, Pieridae, Tortricidae and Noctuidae) has been widely recognized (Saenz-de-Cabezon et al., 2005; Pineda et al., 2007). For some detail, toxic effects of Methoxyfenozide had been reported on some insect species, such as Choristoneura fumiferana (Sundaram et al., 2002) and Pectinophora gossypiella (Sabry and Abdou, 2016). It caused morphological aberrations during moulting/ some metamorphosis of Leptinotarsa decemlineata (Smagghe and Degheele, 1994), Spodoptera littoralis (Gobbi et al., 2000, Pineda et al., 2004), Lymantria dispar (Ouakid et al., 2016) and Culex pipiens (Hamaidia and Soltani, 2016). After larval treatment with Methoxyfenozide, fecundity and fertilityin several insects were inhibited, such as S. littoralis (Pineda et al., 2009), Choristoneura rosaceana (Sun et al., 2000), Tribolium castaneum (Ali et al., 2016), Culex pipiens (Hamaidia and Soltani, 2016), P. gossypiella (Sabry and Abdou, 2016) and L. dispar (Ouakid et al., 2016). In addition, Methoxyfenozide interfered with the ability of Argyrotaenia velutinana and Choristoneura rosaceana adult males to respond to the pheromonesof sexually mature females (Hoelscher and Barret, 2003). Objective of the current study was to determine the effects of the ecdysteroid agonist, Methoxyfenozide, on the most important parameters of adult performance and reproduction of P. unionalis.

MATERIALS AND METHODS

Experimental insect

A sample of olive leaf moth Palpita unionalis (Hubner) (Lepidoptera: Pyralidae) larvae was kindly obtained from the culture of susceptible strain maintained for several generations in Desert Research Center, Cairo, Egypt.A new culture was maintained in Department of Zoology and Entomology, Faculty of Science, Al-Azhar University, Cairo, Egypt, under laboratory controlled conditions (27±2°C, 65±5% R.H., photoperiod 14 and 10 h L:D) according to 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 to Petri dishes $(5.5 \times 1.4 \text{ cm})$. The emerged adults were daily collected and released in plastic jars (3L) provided with cotton pieces, soaked in 10% sugar solution, for feeding, as well as olive twigs (20 cm in length) as an oviposition site. After egg deposition, adult males and females were transferred into new plastic jars. The jars of eggs were provided with fresh tender olive twigs fixed in a small bottle containing water, so as to keep the leaves flat and fresh, for feeding of the newly hatched larvae. The fresh tender olive leaves were renewed daily until pupation.

Methoxyfenozide and larval treatment

The ecdysone agonist, Methoxyfenozide:3-methoxy-2methylbenzoic acid 2-(3,5-dimethylbenzoyl)-2-(1,1dimethylethyl) hydrazide has the molecular formula: C22H28N2O3. It was kindly obtained from Plant Protection Research Institute, Agricultural Research Center, Doqqi, Giza, series sublethal Egypt. А of concentrations of Methoxyfenozide was prepared by diluting with distilled water in volumetric flasks as follows: 0.001, 0.01, 0.10, 1.00 and 10.0 ppm). Fresh olive leaves were dipped in each concentration for 5 minutes and air dried 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 treated and control larvae (one larva/replicate) were kept separately in glass vials. The larvae were allowed to feed on treated leaves for 24 hrs. Then, they provided with fresh untreated olive leaves.Just after the adult emergence, all parameters of adult performance and reproductive potential were recorded.

Adult performance parameters

Adult emergence: Number of successfully metamorphosed adults was expressed in % according to Jimenez-Peydro *et al.* (1995) as follows:

[No. of completely emerged adults / No. of pupae] \times 100

Adulticidal activity: The adulticidal activity of the IGR was determined by observing the adult mortality.

Morphogenic efficiency: It was determined by the impaired adult morphogenesis as appeared in deformed adult females and recorded in %. It was calculated in percentage as follows:

[No. of deformed adults / No. of emerged adults] ×100

Adult longevity: Total longevity of adult females and its major compartments were measured in mean days±SD: pre-oviposition (ovarian maturation) period, oviposition period (reproductive life-time) and post-oviposition period.

Criteria of the reproductive potential

The emerged adults of *P. unionalis* were daily collected and released in plastic jars (3L) provided with sterilized cotton pieces, soaked in 10% sugar solution, for feeding, as well as olive twigs (20 cm in length) as an oviposition site. The treated adult females were coupled with normal adult males (1:2) of the same age obtained from the main culture. The eggs were collected daily, and carefully transferred to Petri dishes to count eggs.

Oviposition efficiency

Oviposition efficiency could be detected by the oviposition rate which was calculated as follows:

Number of laid eggs per \mathcal{Q} /reproductive lifetime (in days).

Reproductive capacity

Fecundity: The laid eggs were counted for calculating the number of eggs per female.

Fertility: Thehatchability was usually expressed in hatching percentage of laid eggs.

Sterility index: It was calculated according to Toppozada *et al.*(1966) as follows:

Sterility Index = $100 - [(a b / A B) \times 100]$

Where: a: mean number of eggs laid per female in the treatment. b: percentage of hatching in the treatment. A: mean number of eggs laid per female in the controls. B: percentage of hatching in the controls.

Incubation period

The laid eggs were kept in Petri dishes under the same controlled laboratory conditions, as previously mentioned. Just after the oviposition, eggs were observed until hatching elapsing an incubation period (in days).

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

Effects of Methoxyfenozide on adult performance of *P. unionalis*

Data of the most important parameters of the adult performance of *P. unionalis*, after treatment of newly moulted last (6^{th}) instar larvae with sublethal concentrations of

Methoxyfenozide (0.001, 0.01, 0.10, 1.00 and 10.0 ppm), were distributed in Table (1). As clearly shown in this table, Methoxyfenozide could not exhibit morphogenic efficiency on the emerged moths, since no adult malformation was observed. On the other hand, it displayed an adulticidal effect (10% mortality) only at 0.10 and 1.00 ppm. Moreover, the tested ecdysteroid agonist considerably arrested the adult emergence, especially at the higher four concentrations, in a dosedependent manner (70, 50, 40 and 10% adult emergence, at 0.01, 0.10, 1.00 and 10.0 ppm, respectively, vs. 100% emergence of control adults). In respect of the adult longevity, data assorted in the aforementioned table obviously demonstrated that only one adult female could reproduce after larval treatment with the highest concentration level of Methoxyfenozide. Its total longevity and major compartments had been remarkably prolonged. At other concentrations, the total adult longevity was significantly prolonged, in a dosedependent course (10.60±1.14, 11.25±0.95, 12.33±0.57 and 13.50±2.12 days, at 0.001, 0.01, 0.10 and 1.00 ppm, respectively, vs. 10.00±1.41 days of control females). In addition, the larval treatment with Methoxyfenozide resulted in pronouncedly prolonged pre-oviposition period, indicating a serious retarding action of the tested compound on the ovarian maturation rate (3.2±0.83, 4.2±1.25, 4.6±0.57 and 5.5±0.70 days, respectively, vs. 3.0±0.89 days of control females). Not only the pre-oviposition period was prolonged but the postoviposition period was, also, considerably prolonged. In contrast, the oviposition period (reproductive life-time) was insignificantly shortened, in no certain trend, indicating a slight promoting action of Methoxyfenozide on the ovipositing females to lay eggs quickly (4.40±0.54, 4.00±0.01, 3.66±0.57 and 3.50±0.70 days, at 0.001, 0.01, 0.10 and 1.00 ppm, respectively, vs. 4.5±0.83 days of control females).

Effects of Methoxyfenozide on reproductive potential of *P. unionalis*

After treatment of the newly moulted last instar larvae of P. unionalis with Methoxyfenozide, data of the most important reproductive criteria were summarized in Table (2). On the basis of these data, only one adult female could reproduce at the highest concentration level, therefore the statistical analysis could not be applied. The oviposition efficiency of females was pronouncedly prohibited, since the oviposition rate was seriously regressed, in a dose-dependent trend (36.06±4.63, 32.68±4.30, 28.04±1.00, 27.97±7.52 and 10 eggs/Q/day, at 0.001, 0.01, 0.10, 1.00 and 10.0 ppm, respectively, vs. eggs/control Q/day). Dealing with the 48.48±10.13 reproductive capacity, data arranged in the previously mentioned table exiguously revealed that fecundity (mean number of eggs/ $\stackrel{\bigcirc}{_+}$) was tremendously reduced, in a dosedependent course (157.8±19.95, 130.7±17.21, 99.6±8.96, 98.5±23.33 and 30 eggs/♀, at 0.001, 0.01, 0.10, 1.00 and 10.0 ppm, respectively, vs. 213.1 \pm 29.7 eggs/control $\stackrel{\bigcirc}{\downarrow}$). Another major parameter of the reproductive capacity is fertility (hatching % of laid eggs, or egg viability) which was considerably inhibited, proportional to the ascending concentration (51.1, 27.5, 26.4, 21.6 and 6.66%, at 0.001, 0.01, 0.10, 1.00 and 10.0 ppm, respectively, vs. 71.6% of eggs laid by control females). Sterility was increasingly induced by increasing concentration level of Methoxyfenozide. Incubation period of the laid eggs is good indicator of the embryonic developmental rate in insects. As evidently shown in the same table, the embryonic development was drastically retarded by Methoxyfenozide, since the incubation period was significantly prolonged in accordance with the concentration $(3.6\pm0.89, 5.0\pm0.81, 6.0\pm1.00, 6.5\pm0.70 \text{ and } 7.0\pm0.72 \text{ days, at } 0.001, 0.01, 0.10, 1.00 \text{ and } 10.0 \text{ ppm, respectively, } vs. 3.2\pm0.83 \text{ days of eggs laid by control females}).$

the interference of Methoxyfenozide with some aspects of the hormonal regulation such as disturbance of adult eclosion hormonerelease and/or inhibition of the neurosecretion (prothoracicotropic hormone, PTTH)(Al-Sharook *et al.*, 1991; Josephrajkumar *et al.*, 1999).

 Table 1. Adult performance of P. unionalis as affected by treatment of newly moulted last instar larvae with sublethal concentrations of Methoxyfenozide

Conc.	Adult emergence	Adultmortality	Adultdeformities	Longevity (mean days±SD)				
(ppm)	(%)	(%)	(%)	Ovarian maturation period	Reproductiv e lifetime	Post-oviposition period	Total Longevity	
10.00	10	00	00	6*	5*	5*	16*	
1.000	40	10	00	5.5±0.70b	3.50±0.70a	4.50±0.70b	13.50±2.12b	
0.100	50	10	00	4.6±0.57b	3.66±0.57a	4.00±1.0 b	12.33±0.57b	
0.010	70	00	00	4.2±1.25b	4.00±0.01a	3.20±0.50b	11.25±0.95b	
0.001	100	00	00	3.2±0.83a	4.40±0.54a	3.00±0.70a	10.60±1.14a	
Control	100	00	00	3.0±0.89	4.50±0.83	2.33±1.03	10.00±1.41	

Conc.: concentration level. Mean \pm SD followed with the letter a: not significantly different (p>0.05), b: significantly different (p<0.05). *: Only one adult female.

 Table 2. Affected reproductive potential of P. unionalis adults after treatment of the newly moulted last instar larvae with sublethal concentrations of Methoxyfenozide

Conc. (ppm)	Oviposition rate (Mean±SD)	Fecundity (Mean no. of eggs/♀)	Fertility (%)	Sterility index	Incubation period (Mean days±SD)
10.00	10*	30*	6.66	99.81	7.00±0.72 d
1.00	27.97±7.52 b	098.5±23.33 c	21.6	93.50	6.50±0.70 c
0.10	28.04±1.00 b	099.6±8.96 d	26.4	91.97	6.00±1.00 c
0.01	32.68±4.30 b	130.7±17.21 c	27.5	85.57	5.00±0.81 c
0.001	36.06±4.63 b	157.8±19.95 c	51.1	58.88	3.60±0.89 a
Control	48.48±10.13	213.1±29.7	71.6		3.20±0.83

Conc.: See footnote of Table (1). Mean \pm 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). *: only one adult female.

DISCUSSION

Influenced adult life parameters of *P. unionalis* by Methoxyfenozide

Blocked adult emergence

As reported in the available literature, the adult emergence of many insect species was significantly blocked after larval treatment with various IGRs, such as Plutella xylostella after larval treatment with Hexaflumuron (Mahmoudvand et al., 2012), Drosophila melanogaster after topical application of 3rd instar larvae with Pyriproxyfen (Benseba et al., 2015), Spodoptera littoralis after treatment of penultimate or last instar larvae with Novaluron (Ghoneim et al., 2015) or Cyromazine (Tanani et al., 2015), Glyphodes pyloalis after treatment of the 4th instar larvae with LC₃₀ of Lufenuron (Aliabadi et al., 2016), Culex quinquefasciatus and Aedes albopictus after larval treatments with Pyriproxyfenand Methoprene (Khan et al., 2016) and Pectinophora gossypiella after treatment of newly hatched or full grown larvae with Novaluron (Hassan et al., 2017). Pupal treatment of Encarsia formosa with Pyriproxyfen resulted in prohibited adult emergence (Wang and Liu, 2016). Moreover, adult emergence was completely blocked in Corcyra cephalonica after treatment of 4^{th} instar larvae with Fenoxycarb (Singh and Tiwari, 2016). In agreement with those reported results, adult emergence of *P. unionalis*, in the present study, was drastically blocked after treatment of newly moulted last (6^{th}) instar larvae with Methoxyfenozide, in a dose-dependent course. The present result of blocked adult emergence can be interpreted by

Reduced adult survival

In the current study, Methoxyfenozide exhibited an adulticidal effect (10% mortality) on P. unionalis, after treatment of newly moulted last instar larvae with only 0.10 and 1.00 ppm. This result of the extended toxic action of Methoxyfenozide on adults of P. unionalis was, to some extent, in accordance with those very scarcely reported toxicities of some IGRs (including CSIs) on adults of some insect species, such as S. littoralis after treatment of larvae with Novaluron, especially at the higher concentrations (Hamadah et al., 2015) and Delia antique after larval treatment with Pyriproxyfen (Zhou et al., 2016). Also, reduced adult survival was reported for P. gossypiella after treatment of larvae, especially the newly hatched larvae, with Novaluron (Hassan et al., 2017). The reduced adult survival of P. unionalis by Methoxyfenozide (at some concentrations), in the present 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, and then into different tissues of the successfully emerged adults, and/or may be due to a lower detoxification capacity of adults against the tested IGR (Osman et al., 1984; Smagghe and Degheele, 1992). Also, a latent lethal effect of Methoxyfenozide may be due to the disturbance of enzymatic pattern and hormonal hierarchy in adults of P. unionalis (Kartal et al., 2003). However, the adult life in insects depends on healthy immature stages. Digestive disorders such as starvation, metabolism disturbance, degeneration of peritrophic membranes and accumulation of faecal materials at the hind gut may be the cause of untimely adult mortality, as a result of IGR treatment (Soltani, 1984).

Affected adult morphogenesis

Impaired adult morphogenesis, as expressed in the production of deformed adults, was widely reported in the available literature, after treatment of various insects with different IGRs (or CSIs), such as S. littoralis after treatment with Tebufenozide and Methoxyfenozide (Pineda et al., 2004), Flufenoxuron (Bakr et al., 2010) or Novaluron (Hamadah et al., 2015); Rhynchophorus ferrugineus after treatment with Diofenolan (Tanani, 2001); Choristoneura fumiferana after treatment with Tebufenozide and Methoxyfenozide (Sundaram et al., 2002); Tribolium castaneum and Tribolium confusum after treatment with Cyromazine (Kamaruzzaman et al., 2006); Eurygaster integriceps after treatment with Pyriproxyfen (Mojaver and Bandani, 2010); Dysdercus koenigii after treatment with Flucycloxuron (Khan and Qamar, 2011); Spodoptera frugiperda after treatment with Methoxyfenozide (Zarate et al., 2011); Anagasta kuehniella after treatment with Diflubenzuron and hexaflumuron (Ashouriet al., 2014); Helicoverpa armigeraafter treatment with Hexaflumuron (Taleh et al., 2015); C. cephalonicaafter treatment with Fenoxycarb (Begum and Qamar, 2016); etc. Results of the present study on P. unionalis disagreed with the previously reported results because Methoxyfenozide had no morphogenic efficiency on adults, since no adult deformities had been caused after treatment of the newly moulted last instar larvae with different concentrations of this ecdysone agonist. On the other hand, the present result coincided with the result of failure of Novaluron to affect the adult morphogenesis of P. gossypiella (Hassan et al., 2017).

Disturbed adult longevity

Total adult longevity

After the attainment of sexual maturity, insects often show degenerative changes in some tissues and organs which can be called 'senility' or 'aging'. In insects, the affected adult longevity can be considered an informative indicator for the adult aging, i.e., prolongation of longevity may denote a delay of aging and vice versa, although the death is usually the destiny of all creatures. In the current investigation, the total adult longevity of P. unionalis was remarkably prolonged, in a dose-dependent course, after treatment of newly moulted last instar larvae with Methoxyfenozide, indicating a delaying effect of this compound on the adult aging. The present result was in agreement with those reported results of prolonged adult longevity in some insects by delaying action of different IGRs, such as P. gossypiella by Lufenuron, Chlorfluazuron and Chromafenozide(Kandil et al., 2012), Hexaflumuron (Kandilet al., 2013), Pyriproxyfen(Sabry and Abdou, 2016) and the lowest concentration of Novaluron (Hassan et al., 2017); as well as Lipaphis erysimi by pyriproxyfen (Liu and Chen, 2001).

On the contrary, the present result is inconsistent with those reported results of shortened adult longevity of other insects by various IGRs, such as *Spodoptera litura* by the ecdysone agonist RH-5849 (Seth *et al.*, 2004); *S. littoralis* by Lufenuron (Sammour *et al.*, 2008), Methoxyfenozide (Pineda *et al.*, 2009) and Novaluron (Hamadah *et al.*, 2015); *Agrotis ipsilon* by Flufenoxuron (El-Sheikh, 2002); *Grapholita molesta* (Reinke and Barrett, 2007) and *Spodoptera exigua* (Luna *et al.*, 2011)

by Methoxyfenozide; G. pyloalis by Lufenuron (Aliabadi et al., 2016); P. gossypiella by Diflubenzuron and Chlorfluazuron (Kandil et al., 2005; Salem, 2015); etc. Furthermore, no effect was exhibited by some IGRs on the adult longevity of a number of insects, such as Diofenolan against Musca 2003), domestica (Hamadah, Tebufenozide or methoxyfenozide against Cvdia pomonella (Saenz-de-Cabezon et al., 2005), Buprofezin against S. littoralis (Ragaei and Sabry, 2011), Methoxyfenozide against S. frugiperda (Zarate et al., 2011) and Novaluron against Lygus lineolaris (Portilla et al., 2012). In the current study, the prolongation of female adult longevity of P. unionalis, after treatment of newly moulted last instar larvae with Methoxyfenozide, may be attributed to its interference with the hormonal regulation of adult longevity because a close relation between certain hormones and adult longevity was reported in other insects, such as Drosophila(Broughton et al., 2005; Clancy et al., 2001; Simon et al., 2003; Carbone et al., 2006). At least one of the Drosophila insulin-linked peptides expressed in the median neurosecretory cells (which produce PTTH) is likely to contribute to the endocrine regulation of longevity (Toivonen and Partridge, 2009). However, the exact mode of action of the tested IGR on the biochemical sites in adults of P. unionalis is unknown until now. Also, more information on the adult endocrine system of P. unionalisis required to clarify the mechanism by which ecdysone agonists can affect the adult longevity.

Pre-oviposition period

In most insects, the pre-oviposition period can be called 'ovarian maturation period' and it may be an informative indicator for the ovarian maturation rate, i.e., the shorter period indicates faster rate and vice versa. In the present study, the pre-oviposition period was considerably prolonged, indicating drastically retarding effect of Methoxyfenozide on the ovarian maturation rate. The present result corroborated with those reported results of prolonged period after treatment of newly hatched larvae of P. gossypiella with Diflubenzuron, Hexaflumuron or Chlorfluazuron (Kandil et al., 2005, 2013), LC50 values of Chromafenozideand Diflubenzuron (Salem, 2015), LC₅₀ of Teflubenzuron (El-Khayat et al., 2015) and after treatment of newly hatched or full grown larvae with Novaluron (Hassan et al., 2017). It was in agreement, also, with those reported prolongation of the pre-oviposition period in other insects, such as S. littoralis, after larval treatment with Diflubenzuron (Aref et al., 2010) and Ephestia kuehniella, after larval treatment with Tebufenozide (Bouzera and Soltani-Mazouni, 2014). On the other hand, the present result of prolonged pre-oviposition period in P. unionalis contradictory to those reported results of shortened period in *P. gossypiella*, after treatment of newly hatched larvae with Diflubenzuron (Rashadet al., 2006) and D. antique, after larval treatment with a dose of 100 mg kg⁻¹ of Pyriproxyfen (Zhou et al., 2016). Moreover, this period was unaffected in S. litura, after larval treatment with Chlorfluazuron and Methoxyfenozide (Shahout et al., 2011) and in D. antique, after larval treatment with high doses of Pyriproxyfen (Zhou et al., 2016). The retarding effect of Methoxyfenozide on the ovarian maturation rate (prolonged pre-oviposition period) in P. unionalis may be understood by influenced germ band or the number of germ cells formed in the embryo (Hodin and Riddiford, 1998). However, the exact mode of retarding action of the tested IGR on pre-oviposition period is unfortunately available right now but its interference with the hormonal regulation needs further investigation in the foreseeable future.

Oviposition period

In respect of the oviposition period (reproductive life-time), scarcely reported results have been seen in the available literature. For instances, the oviposition period in S. litura was significantly shortened after treatment of 2^{nd} instar larvae with LC_{30} of Methoxyfenozide (Shahout *et al.*, 2011). The oviposition period in P. gossypiella had been shortened after treatment of newly hatched larvae with Chlorfluazuron (Kandil 2006), et al., 2005), Diflubenzuron (Rashadet al., Hexaflumuron and Chlorfluazuron (Kandilet al., 2013) and LC₅₀ of Methomyl(El-Khayat et al., 2015) as well as after treatment of newly hatched or full grown larvae with Novaluron (Hassan et al., 2017). The oviposition period in Plutella xylostella was significantly shortened by Pyriproxyfen (Mahmoudvand et al., 2015). Result of the present study on P. unionalis was, to a great extent, concomitant to those reported results, since the oviposition period was conspicuously shortenedafter treatment of newly moulted last instar larvae with Methoxyfenozide. On the contrary, this result disagreed with the reported considerable prolongation of oviposition period in P. gossypiella, after treatment of newly hatched larvae with LC₅₀ of Chromafenozide or Diflubenzuron (Salem, 2015) and Teflubenzuron (El-Khayat et al., 2015). In the current study. Methoxyfenozide exhibited an enforcing effect on the adult females of P. unionalis to quickly lay eggs during a very short time interval. The exact mechanism of this enforcing action of Methoxyfenozide on P. unionalis adult females is still unknown. However, these females may be enforced to lay their eggs quickly to avoid this toxic xenobiotic factor.

Post- oviposition period

Depending on the currently available literature, very scarce studies have examined the effects of IGRs on post-oviposition period. After treatment of newly moulted last instar larvae of *P. unionalis* with Methoxyfenozide, in the present study, the post-oviposition period was remarkably prolonged. This result was in accordance with those reported results of prolonged post-oviposition period of *P. gossypiella* after larval treatment with Hexaflumuron or Chlorfluazuron (Kandil*et al.*, 2013) but diversely affected by Novaluron, depending on the concentration (Hassan *et al.*, 2017). Unfortunately, we have no acceptable interpretation for this prolongation right now!!

Disrupted reproductive potential of *P. unionalis* by Methoxyfenozide

Reproduction in insects is mainly controlled by the juvenile hormone (JH), which is also responsible for protein metabolism, and is specifically needed for egg maturation. The insect growth regulators (IGRs) have been reported to cause sterility of insects or prohibited their fecundity (Ghoneim *et al.*, 2014). However, effects of IGRs on the insect reproduction can be grouped into: i) reproductive behaviour, ii) oviposition, iii) egg hatchability (ovicidal and embryocidal), and iv) sterilization of adults (Mondal and Parween, 2000). On the other hand, ecdysteroids have essential functions in controlling the processes involved in insect reproduction, i.e., vitellogenesis, ovulation of matured eggs and spermatocyte growth (Wigglesworth, 1984; Hagedorn, 1985).

Inhibited oviposition efficiency of *P. unionalis* by Methoxyfenozide

In insects, the oviposition rate can be used as an informative indicator for the oviposition efficiency (Ghoneim et al., 2014). In the present study on P. unionalis, treatment of the newly moulted last instar larvae with Methoxyfenozide resulted in drastically prohibited oviposition efficiency, since the oviposition rate was conspicuously regressed, in a dosedependent manner. This result was in conformity with the reported results of inhibited oviposition efficiency of some insects by various IGRs, such as S. littoralis by Tebufenozide (Bakr et al., 2005), Flufenoxuron (Bakr et al., 2010) and Novaluron (Ghoneim et al., 2014); Schistocerca gregaria by Flufenoxuron and lufenuron (Soltani-Mazouni and Soltani, Tebufenozide (Al-Dali 1994) or et al., 2008); Plodiainterpunctella by the ecdysteroid agonist RH-5849 (Smagghe and Degheele, 1994); Callosobruchas maculates by Cyromazine (Al-Mekhlafi et al., 2011) and P. gossypiella by Novaluron (Hassan et al., 2017). In contrast, the present result disagreed with the stimulated oviposition of Gryllus bimaculatus by some ecdysteroid agonists (Behrens and Hoffmann, 1983). The prohibited oviposition efficiency, in the current study, may be explained as a result of the inhibition of ovarian DNA synthesis or the interference of Methoxyfenozide with vitellogenesis in P. unionalisvia certain biochemical processes. However, the tested compound may exert a reverse action to those exerted by the ecdysteroid agonists which stimulate the neurosecretory cells to release a myotropic ovulation hormone (Parween et al., 2001).

Perturbation of the reproductive capacity of *P. unionalis* by Methoxyfenozide

Prohibited fecundity

The available literature contains many reported results of prohibited fecundity (mean number of eggs/female) of several insects after treatment of their larvae with various IGRs, such as S. littoralis after treatment with Diflubenzuron (Aref et al., 2010), Lufenuron (Gaaboub et al., 2012), Methoxyfenozide (Pineda et al., 2009) and Novaluron (Ghoneim et al., 2014). Also, fecundity of other insect species was reduced by various IGRs, such as E. kuehniella by Tebufenozide (Khebbeb et al., 2008); Choristoneura rosaceana (Sun et al., 2000), Lobesia botrana(Saenz-de-Cabezon et al., 2005) and S. litura (Shahout et al., 2011) by Methoxyfenozide; Leptinotarsa decemlineata (Farinos et al., 1999) and Tenebrio molitor(Taibi et al., 2003) by Halofenozide (RH-0345); S. litura by Chlorfluazuron (Perveen and Miyata, 2000); Argyrotaenia velutinana (Sun et al., 2000), T. castaneum (Ali et al., 2016) and Lymantria dispar (Ouakidet al., 2016) by Methoxyfenozide; M. domestica by Lufenuron (Hamadah, 2003), D. koenigi by Flufenoxuron (Khan and Qamar, 2011); A. kuehniella by Diflubenzuron and Hexaflumuron (Ashouri et al.. 2014); P. xylostella by Pyriproxyfen (Mahmoudvand et al., 2015); Callosobruchus chinensis by some terpene compounds (Chaubey, 2015); T. castaneum (Gado et al, 2015) and D. antique (Zhou et al., 2016) by Lufenuron and C. cephalonica by Fenoxycarb(Begum

and Qamar, 2016); *etc.* In the present study on *P. unionalis*, our result corroborated with the previously reported results, since treatment of newly moulted last instar larvae with Methoxyfenozide resulted in tremendously reduced fecundity, in a dose-dependent manner.

This result was, also, in agreement with some of the reported results of considerably reduced fecundity in some insects, such as P. gossvpiella after treatment of newly hatched larvae with Tebufenozide(Zidan et al., 1998; El-Khayat et al., 2015), Diflubenzuron (Kandil et al., 2005; Rashadet al., 2006; Salem, 2015), Chlorfluazuron (Kandil et al., 2005), Buprofezin (Al-Kazafy, 2013), Hexaflumuron and Chlorfluazuron (Kandilet Chromafenozide (Salem, 2015), as well as al., 2013), Pyriproxyfen, Methoxyfenozide and Lufenuron (Sabry and Abdou, 2016) and Novaluron (Hassan et al., 2017). On the contrary, recorded result in the current investigation disagree with some reported results of failure of some IGRs to affect the fecundity in various insects, such as Fenoxycarb against Apis mellifera (Thompson et al., 2005), Methoxyfenozide against S. exigua (Christian-Lius and Pineda, 2010) as well as Novaluron and Diflubenzuron against Halyomorpha halys (Kamminga et al., 2012). Moreover, feeding of larvae on leaves treated with Methoxyfenozide enhanced the fecundity of S. littoralis (Ishaaya et al., 1995).

However, these diverse effects can be attributed to the different modes of action of IGRs, different susceptibilities of the insect species, time of treatment and other factors. The drastically prohibited fecundity of P. unionalis, after treatment of the newly moulted last instar larvae with Methoxyfenozide, in the present study, may be due to the interference of this compound with one or more processes, from the ovarian follicle development to egg maturation. In some detail, this can be explained by some reasons, as follows. (1) The tested IGR may cause some disorders in the ovaries, including cell death in the germarium, resorption of oocytes in the pre-vitellarium and vitellarium, formation of vitellin envelops and undue proliferation of follicle cells sometimes resulting in malformation of the whole ovary (Lucantoni et al., 2006; Khan et al., 2007). (2) The tested IGR may inhibit the development of some ovarioles and/or synthesis and metabolism of proteinaceous constituents during the oogenesis (Salem et al., 1997). (3)

The tested IGR exerted an inhibitory action on the ecdysone activity, threshold of which is essential for the normal oogenesis (Terashima et al., 2005). (4) On the basis of hormonal regulation of insect reproduction, the present IGRmay disturb the production and/or function of the gonadotropic hormone (juvenile hormone, JH) responsible for the synthesis of vitellogenins (yolk precursors) and vitellogenesis (Di Ilio et al., 1999). (5) Eggs may develop normally in ovaries, but they could not be lay, owing to the adversely deformed ovipositor of adult females or to the reduced mechanical strength (Moreno et al., 1994) or their rebsorpion before oviposition (Zhou et al., 2016). (6) It may be acceptable to suggest that the prohibited fecundity of P. unionalis, in the current work, may be due to inhibitory effects of the tested IGR on synthesis of both DNA and RNA, suboptimal nutrition owing to reduced feeding, altered mating behaviour as a result of sublethal intoxication, or a combination of factors.

Reduced fertility

Fertility (egg hatching % or egg viability) is another informative parameter of the reproductive capacity in insects. In the present study, fertility of the eggs laid by P. unionalis had been drastically reduced after treatment of newly moulted last instar larvae with Methoxyfenozide, in a dose-dependent course. This result was in accordance with those reported results of reduced fertility in P. gossypiella after treatment of newly hatched larvae with some IGRs, such as Lufenuron, methoxyfenozide, Chromafenozide and Chlorfluazuron (Kandil et al., 2012) and Novaluron (Hassan et al., 2017); as well as some of other insects, such as S. littoralis by Chlorfluazuron (Sammour et al., 2008), Methoxyfenozide (Pineda et al., 2009), Diflubenzuron (Aref et al., 2010), Lufenuron (Adel, 2012; Gaaboub et al., 2012), Triflumuron (El-Naggar, 2013) and Novaluron (Ghoneim et al., 2014); S. litura by Diofenolan (Perveen and Miyata, 2000) and Chromafenozide (Shahout et al., 2011); T. molitorby Halofenozide (Taibi et al., 2003); M. domestica by Diofenolan (Hamadah, 2003), T. castaneum by Novaluron (Kostyukovsky and Trostanelsky, 2004); E. kuehniellaby Tebufenozide (Khebbeb et al., 2008); D. koenigi by Flufenoxuron (Khan and Qamar, 2011), C. maculates by Cyromazine (Al-Mekhlafi et al., 2011), A. kuehniella by Diflubenzuron and Hexaflumuron (Ashouri et al., 2014); A. velutinana and Ch. rosaceana (Sun et al., 2000), T. castaneum (Ali et al., 2016), P. gossypiella (Sabry and Abdou, 2016), Culex pipiens (Hamaidia and Soltani, 2016) and L. dispar (Ouakidet al., 2016) by Methoxyfenozide; etc.

For explicating the fertility reduction in P. unionalis by Methoxyfenozide, in the present study, some suggestions can be provided herein. (1) Maturation of the insect eggs depends basically on the vitellogenins, precursor materials of vitellins including proteins, lipids and carbohydrates, all of which are necessarily required for the embryonic development (Soltani and Mazouni, 1992; Chapman, 1998). These materials are synthesized primarily by fat body during the immature stages (Telfer, 2009) or by the ovary in situ (Indrasith et al., 1988). Wherever the site of synthesis of these materials, the tested IGR may disturb their production and/or accumulation in adult females of *P. unionalis* leading to the reduction of fertility. (2) The tested IGR may indirectly affect the fertility via its disruptive effect on opening of the intracellular spaces in follicular epithelium or generally inhibited the role of JH (gonadotropic hormone) responsible for the regulation of vitellogenin deposition into oocytes (Davey and Gordon, 1996). (3) The reduction in fertility may be due to the penetration of residual amounts of Methoxyfenozide in P. unionalis mothers into their eggs and disturbance of embryonic cuticle synthesis. So, the fully mature embryos had weakened chitinous mouth parts that were insufficiently rigid to perforate the surrounding vitellin membrane and free from the eggs (Sallam, 1999; Sammour et al., 2008). (4) The reduced fertility of P. unionalis, in the current study, may be due to serious effect of the tested IGR on survival of the developing embryos at certain stages as recorded in decreasing hatching percentage. (5) Because some molecular studies revealed the effects of some IGRs on insect reproduction owing to the perturbation of gene expression in the hierarchy cascade of vitellogenesis and/or choriogenesis (Sun et al., 2003), Methoxyfenozide may interfere with the gene expression resulting in a reduction of the developed embryos in *P. unionalis*, in the present study.

Retarded embryonic development of *P. unionalis* by Methoxyfenozide

In insects, incubation period can be used as a valuable indicator of the embryonic developmental rate, i.e., longer period usually denotes slower rate and vice versa. In the present study, the embryonic development of P. unionalis was considerably retarded, since the incubation period of laid eggs was significantly prolonged, in a dose-dependent manner, after treatment of newly moulted last instar larvae with Methoxyfenozide. The present result corroborated with the scarcely reported results, in the available literature, concerning a similar retarding action of some IGRs on the embryonic development of P. gossypiella, after larval treatment with LC₅₀ of lufenuron, chlorfluazuron or chromafenozide (Kandil et al., 2012) and after larval treatment with different concentrations of Novaluron (Hassan et al., 2017), as well as C. maculates after treatment with Cyromazine (Al-Mekhlafi et al., 2011) and S. littoralis after treatment with Novaluron (Ghoneim et al., 2014). The delayed embryonic development in P. unionalis after treatment of larvae with Methoxyfenozide, in the present study, may be due to its disturbing effect on the ecdysteroid level responsible for the regulation of embryogenesis at certain stages, especially those originating from the ovary in situ (Chapman, 1998).

Conclusion

Depending on results of the present study, Methoxyfenozide disruptively affected the adult emergence, survival, ovarian maturation rate, reproductive life-time and longevity of the olive leaf moth*P. unionalis*, as well as it drastically prohibited the oviposition efficiency, reproductive capacity and impaired the embryonic development leading to a reduction of the pest population. Therefore, Methoxyfenozide may be a potential IGR being involved in the integrated control program against this worldwide insect which was reported as an economic pest of the olive groves in Egypt and other olive producing countries in the world.

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