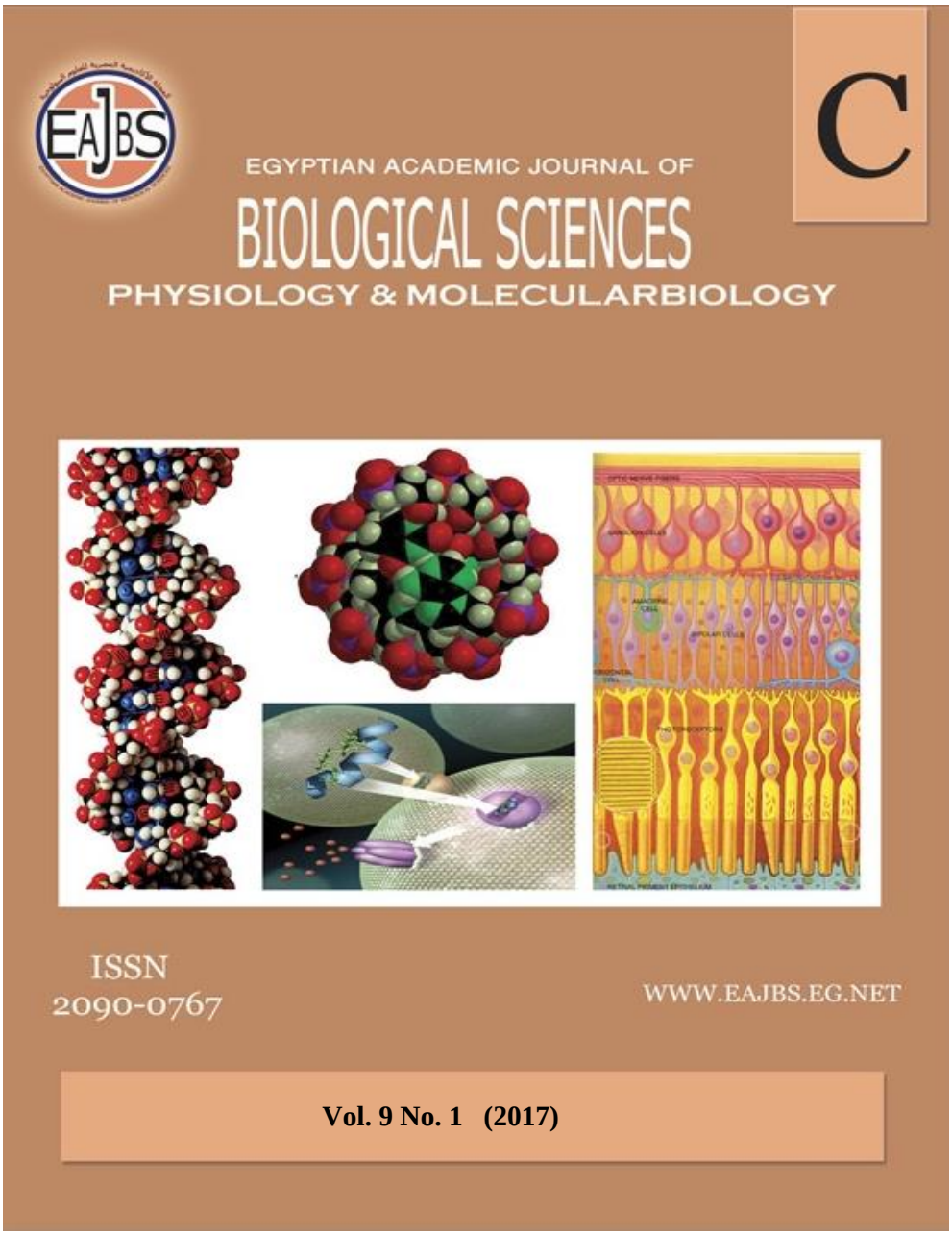

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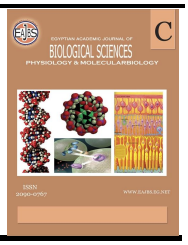

Disturbing Effects of Three Insect Growth Regulators on General Body Metabolism of the Olive Leaf Moth, *Palpita unionalis* (Hübner) (Lepidoptera: Pyralidae)

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ABSTRACT

The olive leaf moth *Palpita unionalis* (Lepidoptera: Pyralidae) is an economic pest of the commercial olive groves in Egypt and different Mediterranean countries. The present study was conducted aiming to assess the disturbing effects of three novel IGRs, *viz*. Novaluron, Methoxyfenozide and Pyriproxyfen (LC_{50} values: 0.97, 0.176 and 0.00009 ppm, respectively) on the main metabolites (proteins, carbohydrates and lipids) in haemolymph and fat bodies of larvae (24 h-, 48 h- and 72 h-post-treatment) as well as in the developed pupae (3-day, 6-day and 9-day old pupae). Both Novaluron and Pyriproxyfen prevalently enhanced the treated larvae to gain increasing protein content in haemolymph, but Methoxyfenozide enhanced only the 48 h-old larvae. In the fat bodies, all IGRs predominantly prohibited the treated larvae to attain normal protein content. All of the tested IGRs profoundly prevented the developed pupae to attain normal protein level, regardless of the age. The carbohydrate content in haemolymph of larvae was dramatically declined, regardless the age. In larval fat bodies, all of the tested IGRs exerted suppressing actions on the carbohydrate content. In the developed pupae, carbohydrate content had been decreased, regardless of the tested IGR or the pupal age. The total lipid content in the haemolymph of treated larvae was elaborately declined, regardless the larval age. In fat bodies, all IGRs induced the larvae to gain more lipids. In the developed pupae, the lipid content was pronouncedly increased by Novaluron and Methoxyfenozide but dramatically reduced by Pyriproxyfen.

INTRODUCTION

The intensive and discriminate uses of many broad-spectrum synthetic insecticides led to several drastic problems, such as the environmental hazards, destruction of the natural enemies, like parasites, predators, birds, fishes and mammals, 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). Development of resistance to conventionally synthetic insecticides is a slow process; however numerous studies confirmed the occurrence of resistance to them in insect pests (Sharifian *et al.*, 2012).

Therefore, alternative materials have been initiated recently to minimize the insecticide hazards and introduce of new effective and safer ways 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 have been developed and known as insect growth regulators (IGRs)(Dhadialla *et al.,* 1998; Khan and Qamar, 2012). In contrast to the classical insecticides, IGRs are not directly toxic, but act selectively on the development, metamorphosis and/or reproduction of the target insect pests (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). 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).

One of the recent IGRs is the benzoylphenyl urea Novaluron (Ishaaya et al., 2007). It was reported to exhibit a high toxicity and effectiveness against several dipterous species (Cetin *et al.,* 2006; Mascari *et al.,* 2007; Martins *et al.,* 2008; Bouaziz et al., 2011; Fontoura *et al.,* 2012; Djeghader *et al.,* 2013; Lohmeyer *et al.,* 2014). It was, also, reported as a powerful suppressor of lepidopterous larvae (Ishaaya *et al.,* 2001; Murthy and Ram, 2002; Ghoneim *et al.,* 2015) and whiteflies attacking cotton, corn and vegetables (Ishaaya et al., 2002, 2003) as well as some species of Hemiptera (Kamminga *et al.,* 2012) and Coleoptera (Cutler *et al.,* 2007; Alyokhin *et al*., 2009; Arthur and Fontenot, 2012). Recently, Novaluron reduced the survival, retarded development, impaired metamorphosis (Ghoneim *et al*., 2017a), disrupted the adult performance and reproductive potential (Hassan *et al*., 2017), declined the main metabolites (Tanani *et al*., 2017), and deteriorated the larval haemogram (Ghoneim *et al*., 2017b) of *Pectinophora gossypiella*. This compound has no appreciable effects on parasitoids and has probably a mild effect on other natural enemies (Ishaaya *et al*., 2001, 2002).

Methoxyfenozide (RH-2485) is a potent synthetic 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 lepidopterous eggs and/or larvae, including many species in families Pyralidae, Pieridae, Tortricidae and Noctuidae, has been widely recognized (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). Methoxyfenozide was reported, also, 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).

Pyriproxyfen was first synthesized by Sumitomo Chemical Co., Japan in 1991 for controlling public health pests (Yokoyama and Miller, 1991). Thereafter, it was reported as a potent JHA disturbing the hormonal balance in insects of several orders resulting thereby in a strong suppression of embryogenesis, metamorphosis, adult formation, oviposition, fecundity and egg viability (Ishaaya and Horowitz, 1995; Aribi *et al.,* 2006; Ghasemi *et al.,* 2010; Hatakoshi, 2012; Ohba *et al.,* 2013; Sabry and Abdou, 2016). Pyriproxyfen has been reported as a broad-spectrum IGR with insecticidal activity against agricultural, horticultural and public health insect pests (Korrat *et al.,* 2012), and has been successfully used to control important pests of many agricultural crops all over the world (Sazo *et al.,* 2008; Moadeli *et al.,* 2014). It was found safe for a variety of predatory arthropods (Naranjo *et al.,* 2003) and compatible with natural enemy conservation (Liu and Stansly, 2004) as well as much less toxic to the ecosystem (Korrat *et al.,* 2012), mammals (Mohandass *et al.,* 2006), some aquatic organisms and is nontoxic to bees (Dhadialla *et al.,* 2005).

As reported by many authors (Hassan, 2002; Chapman, 2012; Cohen, 2010; Sugumaran, 2010), proteins perform a vast array of functions within living organisms, including catalyzing metabolic reactions, replicating DNA, responding to stimuli, and transporting molecules from one location to another. In addition, proteins in all viable cells, as nucleoproteins, are essential to the cell division, enzymes and hormones controlling many chemical reactions in the cell metabolism. Carbohydrates play an important role in the structure and function of all tissues during insect life. Carbohydrates, as energy elements, play a crucial role in the physiology of those insects subjected to IGRs (Kaufmann and Brown, 2008). Lipids represent an important source of energy for insects and are transported from their synthesis site of storage *via* the haemolymph towards the user organs, in particular the vitellogenesis (Zhou and Miesfeld, 2009) and cuticular synthesis (Dapporto *et al*., 2008). Lipid turnover in insects is regulated by neuroendocrine-controlled feed-back loops (Downer, 1985).

As reported by Rodriguez-Ortega *et al.* (2003), the exposure of an organism to the xenobiotic products can modify the synthesis of certain metabolite and disturb the functionality of the organisms. In insects, the use of haemolymph as a medium for controlling insect pests has been made because the changes occurring in the haemolymph are quickly transferred to other portions of the insect's body (Pugazhvendan and Soundararajan, 2009). On the other hand, the insect fat body is an organ analogous which carries out a variety of different metabolic activities comparable to the mammalian liver. It is the main site for protein synthesis as well as the intermediating metabolism of amino acids, which are utilized for the production of hormones and enzymes

and the composition of protein in the body as a whole may be greatly modified (Arrese and Soulages, 2010). An equally important function of the fat body is the storage site of food reserves (Staurengoda-Cunha, and Cruz-Landim, 1983). Thus, the fat body is an important organ that synthesize and stores energy reserve, in addition to regulating the metabolic activities and reproduction (Vivekananthan *et al.*, 2010).

The olive leaf moth, *Palpita unionalis* (Hubner)(Lepidoptera: Pyralidae) had received considerable concern in the last few decades (Solaiman, 1997; Hegazi *et al*., 2007; Ghoneim, 2015) owing to its dangerous attack against young olive trees in nurseries. At the high population, it destroys a significant part 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 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 (Foda et al., 1976). Different insecticides exhibited good control when applied on 1st and 2nd instar larvae of *P. unionalis* in Sicily (Fodale and Mule, 1990). Insecticidal residues have been detected in the olive oil and in the environment where olives are grown (Montiel and Jones, 2002). The objective of the present study was to investigate the disturbances of the main body metabolites (proteins, carbohydrates and lipids) in larvae and pupae of *P. unionalis* after treatment of newly moulted last instar larvae with LC_{50} values of Novaluron, Methoxyfenozide and Pyriproxyfen.

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 the 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 (5.5×1.4cm). The emerged adults were daily collected and released in plastic jars (3L) provided with cotton pieces, soaked in 10% sugar solution, for feeding, as well as olive twigs (20 cm in length) as an oviposition site. After egg deposition, adult males and females were transferred into new plastic jars. The jars of eggs were provided with fresh tender olive twigs fixed in a small bottle containing water, so as to keep the leaves flat and fresh, for feeding of the newly hatched larvae. The fresh tender olive leaves were renewed daily until pupation.

IGRs and Larval Treatment:

The tested compounds in the present study were IGRs, Novaluron, Methoxyfenozide and Pyriproxyfen. The CSI Novaluron (Rimon EC-10) [1-[chloro-4-(1,1,2-trifluoromethoxyethoxy) phenyl] -3-(2,6-difluorobenzoyl) urea] has the molecular formula $C_{17}H_9ClF_8N_2O_4$. It was purchased from Sigma-Aldrich Chemicals. The ecdysteroid agonist

Methoxyfenozide (RH-2485) [3-methoxy-2-methylbenzoic acid 2-(3,5-dimethylbenzoyl)-2-(1,1-dimethylethyl)hydrazide] has the molecular formula $C_{22}H_{28}N_2O_3$. The juvenile hormone analogue Pyriproxyfen Admiral 10% SC: 2-[1-methyl-2-(4-phenoxyphenoxy) ethoxy]pyridine or 4-Phenoxyphenyl (*R/S*)-2-(2-pyridyloxy)propyl ether 2-[1-(4-Phenoxyphenoxy)propan-2-yloxy]pyridine has the molecular formula $C_{20}H_{19}NO_3$ Methoxyfenozide and Pyriproxyfen had been kindly obtained from Plant Protection Research Institute, Agricultural Research Center, Doqqi, Giza, Egypt.

In a preliminary experiment on the newly moulted last (6^{th}) instar larvae of the olive leaf moth *P. unionalis*, LC_{50} values of the IGRs Novaluron, Methoxyfenozide and Pyriproxyfen had been calculated in 0.97, 0.176 and 0.00009 ppm, respectively. Fresh olive leaves were dipped in LC_{50} concentration of each IGR for 5 minutes and air-dried before introduction to larvae for feeding. Control larvae were provided with water-treated olive leaves. Ten replicates of treated and control larvae (one larva/replicate) were kept separately in glass vials. The larvae were allowed to feed on treated leaves for 24 hrs. Then, they provided with fresh untreated olive leaves.

Tissue Sampling:

1. Larval tissues:

For the determination of the main body metabolites, haemolymph was collected from treated and control last instar larvae (24, 48, and 72 hrs post-treatment). The haemolymph was obtained by the amputation of one or two prothoracic legs of the larva with fine scissors. Gentle pressure was done on the thorax until a drop of haemolymph appeared at the point of amputation. Haemolymph was drawn into Eppendorff Pipetman containing few milligrams of phenoloxidase inhibitor (Phenylthiourea) to prevent tanning or darkening and then diluted 5× with saline solution 0.7%. The diluted haemolymph was frozen for 20 s to rupture the haemocytes. Collected haemolymph samples were then centrifuged at 2000 r.p.m. for 5 min, and only the supernatant fractions were used for assay directly or frozen until use. Three replicates were used and the haemolymph of two individuals was never mixed.

Larvae (treated and control), from which the haemolymph samples were obtained, were used also to obtain fat body (parietal and visceral) samples (24, 48, and 72 hrs post-treatment). Collected samples of fat bodies were weighed and then homogenized in a saline solution (the fat body of one insect / 1 ml saline solution 0.7 %) using a fine electric homogenizer, tissue grinder for 2 min. Homogenates were centrifuged at 4000 r.p.m. for 15 min. The supernatant was used directly or frozen until use. Three replicates were used and the fat bodies from two individuals were avoided to be mixed.

2. Pupal Homogenate:

For the determination of the main metabolites and enzyme activities, healthy treated and control pupae (of different ages: 3-, 6-, and 9-day old) were weighed and then homogenized in a saline solution (one pupa / 1 ml saline solution 0.7 %) using a fine electric homogenizer, tissue grinder for 2 min. Homogenates were centrifuged at 4000 r.p.m. for 15 min. The supernatant was used directly or frozen until use. Three replicates were used and homogenates of two individuals were avoided to be mixed.

Determination of the Main Body Metabolites:

Quantitative determination of the total protein content was conducted in the larval tissues and pupal homogenate according to the method

of Weichselbaum (1946) and using the kit of Biomed. The method depended on the protein forms a violet complex with cupric ions in alkaline medium, and then measured the absorbance at 546 nm using a spectrophotometer.

Quantitative determination of the total carbohydrate (as glycogen) content was conducted in the larval tissues and pupal homogenate using the anthrone reagent according to Singh and Sinha (1977) and utilizing the Spectrophotometer at 620 nm.

Quantitative determination of the total lipid content was conducted in the larval tissues and pupal homogenate according to the technique of Folch *et al.* (1957) and lipid estimation was taken place by phosphovanillin reagent depending on Knight *et al.* (1972) using the kit of Biodiagnostic and using the Spectrophotometer at 545 nm.

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 the difference between means.

RESULTS

In a preliminary experiment on *P. unionalis*, LC_{50} values of some of the novel IGRs, *viz*. Novaluron, Methoxyfenozide and Pyriproxyfen, were calculated, after treatment of newly moulted last instar larvae, in 0.97, 0.176 and 0.00009 ppm, respectively. After larval treatments with these LC_{50} values, total contents of the main body metabolites (proteins, carbohydrates and lipids) were determined in haemolymph and fat bodies of treated larvae (24 h-, 48 h- and 72 h-post-treatment) as well as in homogenates of the successfully developed pupae of 3-day old (early-aged), 6-day old (mid-aged) and 9-day old (late-aged).

Effects of IGRs on the Protein Content in Larvae and Pupae:

Depending on the data assorted in Table (1), the total protein content in haemolymph of control larvae of *P. unionalis* gradually decreased with the age (3.40±0.10, 2.48±0.03 and 2.10±0.10 g/dL in haemolymph of 24 h-, 48 h- and 72h-aged larvae, respectively). A reverse trend was recorded for proteins in fat bodies of the same larvae 6.53±0.15, 6.66±0.15 and 6.80±0.10 mg/dL in fat bodies of 24 h-, 48 h- and 72h-aged larvae, respectively).

In the view of data contained in the same table, both Novaluron and Pyriproxyfen prevalently enhanced the treated larvae to gain increasing protein content in haemolymph, but Methoxyfenozide enhanced them only at 48 h-post treatment. On the basis of comparison, the strongest enhancing potency was recorded for Pyriproxyfen in haemolymph of 72 h-aged larvae (3.86±0.25 *vs*. 2.10±0.10 g/dL of control larvae, with 83.80% increment) while the least enhancing potency was exhibited by Methoxyfenozide (2.76±0.03, *vs*. 2.48±0.03 g/dL of control larvae, with 11.29% increment at 48 h-post-treatment). On the other hand, protein content was remarkably declined in haemolymph of Methoxyfenozide-treated larvae at 24 and 72 h (3.13±0.05, *vs*. 3.40±0.10 g/dL in control larvae, at 24 h-post-treatment, and 1.33±0.057, *vs*. 2.10±0.10 g/dL in control larvae, at 72 h-post-treatment).

With regard to the disturbance of protein content in larval fat bodies, after treatment with LC_{50} values of the tested IGRs, data of the previously mentioned table exiguously revealed that Novaluron, Methoxyfenozide and Pyriproxyfen predominantly prohibited the treated larvae to attain the normal level of proteins, since drastically reduced amounts were determined at all larval ages. Comparatively, the most powerful suppressing effect on

protein content in fat bodies was exhibited by Pyriproxyfen at 72 h-post-treatment (76.02% reduction) but the least reducing action was exerted by Methoxyfenozide at the same developmental time (10.88% reduction).

In respect of the protein disturbance in the successfully developed pupae, data arranged in Table (2) obviously demonstrated that all of the tested IGRs profoundly prevented these pupae to attain normal protein level, regardless the age. For some detail, the strongest reducing effect was exhibited by Pyriproxyfen on the late-aged pupae to attain the normal level of proteins (43.3% reduction) while the least reducing effect was exhibited by Novaluron on the mid-aged pupae (3.41% reduction). However, the protein content in control pupae gradually decreased with the age (5.98±0.10, 5.85±0.60 and 5.72±0.11 mg/g, in early-, mid- and late-aged pupae, respectively).

Table 1: Disturbed total protein content in larval haemolymph (g/dL) and fat bodies (mg/dL) after treatment of last instar larvae of *P. unionalis* with LC_{50} values of IGRs.

IGR	Tissue		Larval age		
			24 h	48 h	72 h
Novaluron	Haemolymph	Mean±SD	4.36±0.46 b	4.02±0.08 d	3.06±0.15 d
		Change (%)	+28.23	+62.09	+45.71
	Fat body	Mean±SD	2.20±0.10 d	2.14±0.05 d	2.70±0.52 d
		Change (%)	-66.30	-67.86	-60.29
Methoxyfenozide	Haemolymph	Mean±SD	3.13±0.05 b	2.76±0.03 d	1.33±0.057 d
		Change (%)	-7.94	+11.29	-57.89
	Fat body	Mean±SD	4.53±0.30 d	5.60±0.20 c	6.06±0.057 d
		Change (%)	-30.62	-15.91	-10.88
Pyriproxyfen	Haemolymph	Mean±SD	4.93±0.05 d	4.09±0.64 b	3.86±0.25 d
		Change (%)	+45.00	+64.91	+83.80
	Fat body	Mean±SD	2.03±0.15 d	1.86±0.06 d	1.63±0.05 d
		Change (%)	-68.91	-72.07	-76.02
Control	Haemolymph	Mean±SD	3.40±0.10	2.48±0.03	2.10±0.10
	Fat body	Mean±SD	6.53±0.15	6.66±0.15	6.80±0.10

Mean±SD followed with the letter b: significantly different ($P<0.05$), c: highly significantly different ($P<0.01$), d: very highly significantly different ($P<0.001$).

Table (2): Disturbed total protein content (mg/g±SD) in pupae of *P. unionalis* after treatment of last instar larvae with LC_{50} values of IGRs.

IGR	Homogenate	Pupal age		
		3-day old	6-day old	9-day old
Novaluron	Mean	5.57±0.14 b	5.65±0.0057 c	4.55±0.50 d
	Change (%)	-6.85	-3.41	-20.4
Methoxyfenozide	Mean	5.12±0.37 d	4.71±0.011 d	4.50±0.026 d
	Change (%)	-14.3	-19.4	-20.7
Pyriproxyfen	Mean	4.16±0.035 d	3.39±0.015 d	3.24±0.068 d
	Change (%)	-30.4	-32.8	-43.3
Control	Mean	5.98±0.10	5.85±0.60	5.72±0.11

b, c, d: See footnote of Table (1).

Effects of IGRs on the Carbohydrate Content in Larvae and Pupae:

According to the data distributed in Table (**3**), the carbohydrate content in haemolymph of control larvae gradually elevated with the age (0.16±0.001, 0.17±0.005 and 0.22±0.02 g/dL in larval haemolymph at 24-, 48- and 72-post treatment, respectively). The reverse trend was detected in the fat bodies of control larvae, since carbohydrate content gradually depleted with the age (3.20±0.30, 2.20±0.20 and 1.83±0.15 mg/dL, at 24-, 48- and 72 h-post-treatment, respectively).

In connection with the disturbed carbohydrate content in haemolymph of larvae, after treatment with Novaluron, Methoxyfenozide and Pyriproxyfen, data of the same table obviously showed that the level of this metabolite was dramatically declined, regardless the tested IGR or the larval age. Comparatively, Novaluron exerted the strongest reducing action on larvae 24 h-post-treatment (50.0% reduction) but both Methoxyfenozide and Pyriproxyfen exerted the least reducing action at 24 h-post-treatment (29.41% reduction).

Depending on the data assorted in the same table, all of the tested IGRs exerted suppressing actions on carbohydrate content in the fat bodies of treated larvae. For comparison of effectiveness, Pyriproxyfen exhibited the strongest reducing effect on this metabolite in larvae at 24 h-post-treatment (1.26±0.51, compared to 3.20±0.30 mg/dL of control larvae) while Novaluron exhibited the least reducing effect on it at 72 h-post-treatment (1.40±0.10, compared to 1.83±0.15 mg/dL of control larvae).

After treatment on newly moulted last instar larvae with LC_{50} values of the tested IGRs, data of the carbohydrate content in the developed pupae were distributed in Table (4). In the light of these data, carbohydrate content in pupae, of all ages, had been slightly or considerably decreased, regardless of the tested IGR. The most powerful reducing effect on this metabolite was exhibited by Pyriproxyfen, as estimated in 61.7% reduction in the mid-aged pupae, while Methoxyfenozide exhibited the least

reducing effect, as expressed in 14.2% reduction of carbohydrates in the early-aged pupae (for detail, see the aforementioned table). However, carbohydrate content gradually decreased in the control pupae, with the age (1.90±0.10, 1.70±0.10 and 1.50±0.10 mg/g, in early-, mid- and late-aged pupae, respectively).

Table (3): Disturbed total carbohydrate content in larval haemolymph (g/dL) and fat bodies (mg/dL) after treatment of last instar larvae of *P. unionalis* with LC_{50} values of IGRs.

IGR	Tissue		Larval age		
			24 h	48 h	72 h
Novaluron	Haemolymph	Mean±SD	0.08±0.01 d	0.11±0.005d	0.13±0.001 c
		Change (%)	-50.0	-35.2	-40.9
	Fat body	Mean±SD	1.73±0.05 c	1.60±0.10 c	1.40±0.10 b
		Change (%)	-45.9	-27.27	-23.49
Methoxyfenozide	Haemolymph	Mean±SD	0.11±0.01 d	0.12±0.005 d	0.12±0.10 a
		Change (%)	-31.25	-29.41	-45.45
	Fat body	Mean±SD	1.70±0.01 c	1.46±0.05 c	1.16±0.15 c
		Change (%)	-46.87	-33.63	-36.61
Pyriproxyfen	Haemolymph	Mean±SD	0.11±0.05 d	0.12±0.005 d	0.12±0.010 a
		Change (%)	-31.25	-29.41	-45.45
	Fat body	Mean±SD	1.26±0.51 d	1.13±0.057 d	1.0±0.17 c
		Change (%)	-60.62	-48.63	-45.35
Control	Haemolymph	Mean±SD	0.16±0.001	0.17±0.005	0.22±0.02
	Fat body	Mean±SD	3.20±0.30	2.20±0.20	1.83±0.15

Mean ± SD followed with the letter a: insignificantly different (P >0.05). b, c, d: see footnote of Table (1).

Table (4): Disturbed total carbohydrate content (mg/g±SD) in pupae of *P. unionalis* after treatment of last instar larvae with LC_{50} values of IGRs.

IGR	Homogenate	Pupal age		
		3-day old	6-day old	9-day old
Novaluron	Mean	1.36±0.25 b	1.08±0.12 d	1.13±0.20 b
	Change (%)	-28.42	-36.47	-24.66
Methoxyfenozide	Mean	1.93±0.30 a	1.16±0.20 b	1.02±0.072 c
	Change (%)	-14.2	-31.7	-23.0
Pyriproxyfen	Mean	1.13±0.062a	0.65±0.05 d	0.64±0.052 d
	Change (%)	-40.5	-61.7	-57.3
Control	Mean	1.90±0.10	1.70±0.10	1.50±0.10

a: See footnote of Table (3). b, c, d: See footnote of Table (1).

Effects of IGRs on the Lipid Content in Larvae and Pupae:

After treatment on newly moulted last instar larvae with LC_{50} values of the tested IGRs, data of the lipid content in haemolymph and fat bodies of control and treated larvae were arranged in Table (5). Depending on these data, the lipid content gradually increased in the haemolymph of control larvae, with the age (3.62±0.01, 4.77±0.09 and 5.07±0.05 g/dL, in 24 h-, 48 h- and 72h-aged larvae, respectively). In a similar trend, lipid content gradually increased in the fat bodies of larvae (0.13±0.05, 0.37±0.04 and 0.91±0.10 mg/dL, in 24 h-, 48 h- and 72h-aged larvae, respectively).

Dealing with the disturbed lipids in haemolymph of the treated larvae, data of the same table exiguously revealed that all of the tested IGRs conspicuously prohibited the larvae to attain normal level of lipids, since the total lipid content was elaborately declined, irrespective of the tested IGR of the larval age. Comparatively, the strongest declining effect was exhibited by Pyriproxyfen, since 75.68% reduction of lipids was determined in 48 h-aged larvae, but the least declining effect was recorded for Novaluron, since 58.28% reduction of lipids was determined in 24 h-aged larvae.

In connection with the disturbance of lipids in fat bodies of IGR-treated larvae, data of the same table obviously displayed that all IGRs induced the larvae to gain more lipids than the control congeners, since the lipid content was prevalently raised. Comparatively, the most potent IGR for inducing larvae to gain the highest lipid content was Novaluron (1230.76% lipid increment) but the least inducing effect was exhibited by Methoxyfenozide (24.17% lipid increment). For more detail, see Table (5).

As clearly shown in Table (6), a diverse effect was exhibited on the lipid content in the successfully developed pupae, depending on the potency of the tested IGRs. After treatment of larvae with LC_{50} values of Novaluron and Methoxyfenozide, the lipid content pronouncedly increased in pupae, regardless their age, while Pyriproxyfen exerted a suppressing action on pupae because dramatically reduced lipids had been determined. The most potent IGR for enhancing pupae to gain the highest level of lipids was Novaluron (185.24% increment in 3-day old pupae) but the least promoting action was exerted by Methoxyfenozide (50.54% increment in 6-day old pupae). Nevertheless, the strongest reducing action of Pyriproxyfen was exerted on 5-day old pupae (74.72% lipid reduction).

However, lipids in control pupae run in the gradual elevating course, with the age (0.61±0.01, 0.91±0.14 and 1.06±0.11 mg/g, in 3-, 6- and 9-day old pupae, respectively).

Table (5): Disturbed total lipid content in larval haemolymph (g/dL) and fat bodies (mg/dL) after treatment of last instar larvae of *P. unionalis* with LC_{50} values of IGRs.

IGR	Tissue		Larval age		
			24 h	48 h	72 h
Novaluron	Haemolymph	Mean±SD	1.51±0.01 d	1.94±0.02 d	2.06±0.035 d
		Change (%)	-58.28	-59.32	-59.36
	Fat body	Mean±SD	1.73±0.03 d	2.07±0.01 d	2.38±0.05 d
		Change (%)	+1230.76	+459.45	+150.54
Methoxyfenozide	Haemolymph	Mean±SD	1.41±0.02 d	1.72±0.02 d	1.91±0.01 d
		Change (%)	-61.04	-63.94	-62.32
	Fat body	Mean±SD	0.76±0.01 d	0.99±0.009d	1.13±0.01 d
		Change (%)	+484.61	+167.56	+24.17
Pyriproxyfen	Haemolymph	Mean±SD	1.02±0.01 d	1.16±0.01d	1.31±0.01 d
		Change (%)	-71.82	-75.68	-74.16
	Fat body	Mean±SD	0.51±0.01 d	0.88±0.08 d	2.003±0.02 d
		Change (%)	+292.30	+137.83	+119.81
Control	Haemolymph	Mean±SD	3.62±0.01	4.77±0.09	5.07±0.05
	Fat body	Mean±SD	0.13±0.05	0.37±0.04	0.91±0.10

d: See footnote of Table (1).

Table (6): Disturbed total lipid content (mg/g±SD) in pupae of *P. unionalis* after treatment of last instar larvae with LC_{50} values of IGRs.

IGR	Homogenate	Pupal age		
		3-day old	6-day old	9-day old
Novaluron	Mean	1.74±0.05 d	2.22±0.02 d	2.52±0.01 d
	Change (%)	+185.24	+143.75	+137.73
Methoxyfenozide	Mean	1.18±0.01 d	1.37±0.06 c	1.88±0.01 d
	Change (%)	+93.44	+50.54	+77.35
Pyriproxyfen	Mean	0.18±0.01 d	0.23±0.02 c	0.40±0.01 d
	Change (%)	-70.49	-74.72	-62.26
Control	Mean	0.61±0.01	0.91±0.14	1.06±0.11

c, d: See footnote of Table (1).

DISCUSSIONS

Disturbed Protein Content in *P. unionalis* by IGRs:

The content of macromolecules (i.e. protein, carbohydrates and lipids) is a good indicator of the level of metabolism in insects treated with chemicals (Zhu *et al*., 2012). It is very important to point out that protein synthesis is necessary for insect development and reproduction. As reported by Resmitha *et al.* (**2014**), protein metabolism in insects plays a key role in rebuilding adult structures during

the transformation of larvae/pupae into adults.

Depending on the currently available literature, larval treatment of many insect species with various insect growth regulators (IGRs) resulted in considerable reduction of proteins in haemolymph and/or other tissues of larvae and/or other developmental stages, such as *Pectinophora gossypiella* after treatment with Chlorfluazuron (Kandil *et al*., 2005), Diflubenzuron (Rashad *et al*., 2006), Lufenuron (Kandil *et al*., 2012), Pyriproxyfen (Derbalah *et al*., 2014), Teflubenzuron (Rashad *et al*., 2015), Chromafenozide (Salem, 2015) and Diofenolan (Tanani *et al*., 2017). Also, reduction of the protein content in larvae and/or pupae of other insects had been caused by different IGRs, such as *Spodoptera littoralis* by Pyriproxyfen (Mostafa, 1993), Chlorfluazuron (Ghoneim, 1994), Pyriproxyfen and Diflubenzuron (Ahmed, 2001), Flufenoxuron and Chlorfluazuron (Abdel-Aal, 2003, 2006), Teflubenzuron (El-Sheikh *et al*., 2013) and Novaluron (Basiouny *et al*., 2016); *Schistocerca gregaria* by Pyriproxyfen (Ghoneim *et al*., 2012) and Flufenoxuron (Hamadah, 2014); *Musca domestica* by Diflubenzuron, Triflumuron and Methoprene (Bakr *et al*., 1991) or Methoxyfenozide (Assar and Abo-Shaeshae, 2004); *Leptinotarsa decemlineata* by the ecdysteroid agonists RH-5849 and tebufenozide (Smagghe *et al*., 1999); *Spodoptera litura* by Pyriproxyfen (Perveen and Miyata, 2000); *Tenebrio molitor* by Halofenozide (Soltani *et al*., 2002); *Cephalopina titillator* by Pyriproxyfen and Chlorfluazuron (El-Bassiony *et al*., 2005); *Bombyx mori* (Etebari *et al*., 2007) and *Eurygaster integriceps* (Zibaee *et al*., 2011; Perveen, 2012) by Pyriproxyfen; *Culiseta longiareolata* (Bouaziz *et al*., 2011) and *Culex pipiens* (Djeghader *et al*., 2013) by Novaluron; *Glyphodes pyloalis* by Lufenuron (Aliabadi *et al*., 2016); *Cyphoderus javanus* by Buprofezin, Novaluron and Flubendiamide (Saha and Joy, 2016) and *C. pipiens* by Spiromesifen (Bouabida *et al*., 2017). Results of the present study on the olive leaf moth, *Palpita unionalis*, were in agreement with those previously reported results, since the protein content was remarkably depleted in haemolymph of Methoxyfenozide-treated larvae at 24 and 72 h-post-treatment. In the fat bodies, all of the tested IGRs, *viz*., Novaluron, Methoxyfenozide and Pyriproxyfen, predominantly prohibited the treated larvae to attain normal protein content. The strongest reducing effect was exhibited by Pyriproxyfen but the least reducing effect was exhibited by Methoxyfenozide. Also, all of the tested IGRs profoundly affected the successfully developed pupae to attain declined protein level, regardless of the age.

On the contrary, the treatment of newly moulted last instar larvae of *P. unionalis*, with LC_{50} values of Novaluron or Pyriproxyfen, in the present study, resulted in prevalently increasing proteins in haemolymph of larvae of all ages, while Methoxyfenozide enhanced it in only the 48 h-old larvae. These results corroborated, to some extent, with those results of increasing proteins in some insects by various IGRs, such as *S. littoralis* by Pyriproxyfen and Chlorfluazuron (Farag, 2001; Abdel-Aal, 2003), Novaluron, Cyromazine and Diofenolan (Basiouny *et al*., 2016), *M. domestica* by Methoprene and Triflumuron (Bakr, 1986); *Muscina stabulans* by Chlorfluazuron and Hexaflumuron (Basiouny, 2000); *S. gregaria* by Chlorfluazuron and Pyriproxyfen (El-Sokkary, 2003); *Bactrocera cucurbitae* by Methoprene (ul Haq *et al*., 2010) and *P. gossypiella* pupae by Novaluron (Tanani *et al*., 2017).

In the current investigation, the remarkably declined protein level in

haemolymph of Methoxyfenozide-treated larvae and in the fat bodies of larvae treated with all tested IGRs, as well as in the successfully developed pupae of all ages can be interpreted in the light of some conceivable suggestions, as follows. (1) Proteins are the known biological metabolites which regulate and integrate several physiological and metabolic processes in the body through hormones, enzymes and nucleoproteins. As suggested by Kyung and Kim (1990), protein plays a major role in the synthesis of the microsomal detoxifying enzymes and helps to detoxify the toxicants (foreign compounds) when entering into the insects. In other words, proteins can bind with the toxicants and therefore the decrease of proteins, in the present study, may reflect the decrease in activity of the detoxifying enzymes or may be reflected on the insects' detoxification capability. (2) The IGRs' stress can inhibit the total proteins owing to the breakdown of protein into amino acids, so with the entrance of these amino acids to TCA cycle as a keto acid (Schoonhoven, 1982), they will help to supply energy for the insect (Etebari and Matindoost, 2004). So, protein depletion in tissues may constitute a physiological mechanism and might play a role in compensatory mechanisms under insecticidal (or IGR) stress, to provide intermediates to the Krebs cycle, by retaining free amino acid content in haemolymph (Nath, *et al*., 1997). (3) The protein reduction in the current study may, also, be due to the interference of tested IGRs with the insect endocrine system causing a hormonal imbalance (Hajjar and Casida, 1979) and affecting the metabolism (De Mark and Bennett, 1989) or protein synthesis in insects (Padmaja and Rao, 2000). However, an extensive research should been carried out in future to determine how various toxic agents affect protein synthesis in the present insect, *P. unionalis*.

On the other hand, Novaluron and Pyriproxyfen prevalently enhanced the treated larvae of *P. unionalis* to gain increasing proteins in haemolymph but Methoxyfenozide enhanced only the 48 h-old larvae to gain more haemolymph proteins, in the present study. This result can be acceptable, since proteins could be used to as a biomarker of exposure which is the response to an interaction between a xenobiotic agent (such as IGRs, in the present study) and a molecule or target cell (Owa *et al*., 2010; Sugumaran, 2010). As affected by the tested IGRs, *P. unionalis* failed to uptake the produced and released proteins which accumulated particularly in haemolymph or through the affected enzymes since some authors (Saleem and Shakoori, 1996; Saleem *et al*., 1998) reported that raised level of soluble protein may be related increased activities of various enzymatic activities. In addition, the enhanced proteins may explain the increase or accumulation of proteins and amino acids in larvae as a preparation for the synthesis of cuticular proteins and associated tanning under stress of insecticides or IGRs (Nath *et al*., 1997). Also, some authors (Ahmed *et al*., 1993; Rawi *et al*., 1995) reported that protein leakage during intoxication may arise from reduced body weight, conversion of protein to amino acids, degradation of protein to release energy or the direct effect of the toxic agents on the amino acid transport of the cell. For understanding the mode of action, the tested IGRs, in the present investigation, may either act on the hormonal level in the haemolymph to announce the synthesis, degradation and inhibition of proteins or on the neurosecretory cells which control endocrine organs (Bouaziz *et al*., 2011; Djeghader *et al*., 2013, 2014).

Disturbed Carbohydrate Content in *P. unionalis* by IGRs:

The carbohydrates, as energy elements, play a crucial role in the

physiology of insects (Kaufmann and Brown, 2008). As clearly seen in the available literature, some authors reported elevated carbohydrate content in some insect species as a response to the action of different IGRs, while others reported opposite results. These contradictory findings may be due to differences in the species sensitivity, the potency of the IGRs themselves, or the developmental stage under treatment of determination of carbohydrate content (Ghoneim *et al*., 2003).

In the present study on *P. unionalis*, treatment of newly moulted last instar larvae with LC_{50} values of Novaluron, Methoxyfenozide and Pyriproxyfen resulted in dramatically declined carbohydrate content in haemolymph of larvae, regardless the age. Novaluron exerted the strongest reducing action but both Methoxyfenozide and Pyriproxyfen exerted the least reducing action. In fat bodies of treated larvae, all of the tested IGRs exerted suppressing actions on the carbohydrate content. Pyriproxyfen exhibited the strongest reducing effect while Novaluron exhibited the least reducing effect. In the developed pupae, carbohydrate content had been slightly or considerably decreased, regardless of the tested IGR or the pupal age. These results are in corroboration with those reported results of reduction in carbohydrates in larvae of *P. gossypiella* after treatment of 1-day old eggs with Lufenuron, Chlorfluazuron and Chromafenozide (Kandil *et al*., 2012) and after treatment of full grown larvae with Novaluron and Diofenolan (Tanani *et al*., 2017) as well as depletion of carbohydrate level in different developmental stages of other insects had been caused by various IGRs, such as *S. littoralis* by Pyriproxyfen, Diflubenzuron and Flufenoxuron (Ahmed, 2001; Farag, 2001; Abdel-Aal, 2003); *S. gregaria* by Pyriproxyfen, Teflubenzuron and Lufenuron (Tanani *et al*., 2012); *M. domestica* by Methoprene (Abou El-Ela *et al*., 1990), Lufenuron, and Diofenolan (Ghoneim *et al*., 2006) or Buprofezin (Assar *et al*., 2010); *Rhynchophorus ferrugineus* pupae by Lufenuron and Diofenolan (Ghoneim *et al*., 2003); *Agrotis ipsilon* by pyriproxyfen (El-Sheikh, 2002); *C. pipiens* by Spiromesifen (Bouabida *et al*., 2017); *etc*.

In contrast, results of declined carbohydrate content in larvae and pupae of *P. unionalis*, by the tested IGRs (*viz*. Novaluron, Methoxyfenozide and Pyriproxyfen), in the present study, disagreed with those reported results of increasing carbohydrate content in certain tissues of different developmental stages of various insect species after treatment with several IGRs, such as *S. littoralis* by Chlorfluazuron (Ghoneim, 1994) and Teflubenzuron (El-Sheikh *et al*., 2013); *T. molitor* pupae and adults by Diflubenzuron (Soltani-Mazouni *et al*., 1999); *M. domestica* pupae by methoprene (Abou El-Ela *et al*., 1990) or Lufenuron and Diofenolan (Ghoneim *et al*., 2006); *S. gregaria* by Pyriproxyfen (El-Sokkary, 2003) or Flufenoxuron (Hamadah, 2014); *C. longiareolata* (Bouaziz *et al*., 2011); *G. pyloalis* by Lufenuron (Aliabadi *et al*., 2016); *C. javanus* by Buprofezin, Novaluron and Flubendiamide (Saha and Joy, 2016); *etc*.

It is interesting to mention that the production or utilization of the main body metabolites, such as carbohydrates, are controlled by juvenile hormone (Gade, 2004; Sugumaran, 2010) or are related to various hormonal systems and neurosecretion (Gade *et al*., 1997). Thus, the prevalent reduction of the carbohydrate content in larvae and pupae of *P. unionalis*, in the present study, may be due to interference of the tested IGRs with the hormonal regulation of carbohydrate metabolism (Imboden and Luscher, 1976) or to their effects on the carboxylase activity (Mukherjee and Sharma, 1996). Also, the alimentary canal may be damaged or ruptured and thus the larvae were unable to assimilate

the food or any metabolite (Lohar and Wright, 1993). Furthermore, the carbohydrate reduction may be due to prohibiting effects of the tested IGRs on glycogen and/or trehalose or interference with the glycolytic path-way. On the other hand, it is suggested that this carbohydrate depletion may be due to the utilization of the reserved glucose sources of the larval tissues as a result of IGRs' stresses (Sharma *et al*., 2011).

Disturbed Lipid Content in *P. unionalis* by IGRs:

Quantity of lipids available for the reserves seems to be the result of a balance between the catch of food and the requests for reserves by processes such as maintenance, growth and reproduction, and this balance is disturbed by any toxic product (Canavoso *et al*., 2001). In the currently available literature, many research works reported the decreasing lipid content in certain tissues of larvae and/or other developmental stages of different insects after treatment of larvae with sublethal concentrations of various IGRs, such as *S. littoralis* by Diflubenzuron (Ahmed, 2001), Flufenoxuron (Abdel-Aal, 2003) and Teflubenzuron (El-Sheikh *et al*., 2013); *Corcyra cephalonica* by Pyriproxyfen (Mandal and Chaudhuri, 1992), *Choristoneura fumiferana* larvae by Fenoxycarb (Mulye and Gordon, 1993), *Agrotis ipsilon* larvae by Flufenoxuron (El-Sheikh, 2002), *Rh. ferrugineus* pupae of early- and late-age by Lufenuron and Diofenolan (Ghoneim *et al*., 2003); *Periplaneta americana* nymphs by Peram-AKH II (synthetic adipokinetic hormone) (Michitsch and Steele, 2008), *Plodia interpunctella* larvae by 20-Hydroecdysone (Rharrabe *et al*., 2008) or Pyriproxyfen (Ghasemi *et al*., 2010), *E. integriceps* nymphs by the latter IGR (Zibaee *et al*., 2011), *S. gregaria* nymphs and adult females by Pyriproxyfen, Tebufenozide and Lufenuron (Hamadah *et al*., 2012) or Flufenoxuron (Hamadah, 2014); *P. gossypiella* larvae by Diflubenzuron (Rashad *et al*., 2006), Chlorfluazuron and Hexaflumuron (Kandil *et al*., 2013), Teflubenzuron (Rashad *et al*., 2015), Chromafenozide and Diflubenzuron in adults (Salem, 2015), and Novaluron and Diofenolan in larvae and pupae (Tanani *et al*., 2017); *G. pyloalis* by Lufenuron (Aliabadi *et al*., 2016); *C. javanus* by Buprofezin, Novaluron and Flubendiamide (Saha and Joy, 2016); *C. pipiens* by Spiromesifen (Bouabida *et al*., 2017); *etc*. Results of the present investigation were, to some extent, in agreement with those previously reported results of decreasing lipids, since the treatment of newly moulted last instar larvae of *P. unionalis* with LC_{50} values of Novaluron, Methoxyfenozide and Pyriproxyfen resulted in remarkably decreasing lipid content in haemolymph of treated larvae, regardless the age. Also, the lipid content in the successfully developed pupae was dramatically reduced after larval treatment with Pyriproxyfen.

In contrast, treatment of *P. unionalis* larvae with the tested IGRs, in the present study, promoted to increase the lipid content in fat bodies of treated larvae. Also, treatment with Novaluron or Methoxyfenozide resulted in pronouncedly increasing lipids in the developed pupae. The current results were, to a great extent, in accordance with those reported results of increasing lipids in some insects by various IGRs, such as *P. gossypiella* after-treatment of the newly hatched larvae with LC_{50} of Diflubenzuron and Chlorfluazuron (Kandil *et al*., 2005) as well as in pupae and adult females of *T. molitor* by Diflubenzuron (Soltani-Mazouni *et al*., 1999); mid-aged pupae of *Rh. ferrugineus* by Lufenuron and Diofenolan (Ghoneim *et al*., 2003); late-aged pupae of *M. domestica* by Diofenolan (Amer *et al*., 2005); 4th instar larvae of *C. longiareolata* (Bouaziz *et al*., 2011) and *C. pipiens* (Djeghader *et*

al., 2013) by Novaluron; early-aged nymphs of last instar and 4-day old adult females of *S. gregaria* by Pyriproxyfen, Tebufenozide and Lufenuron (Hamadah *et al*., 2012); early-aged nymphs of last instar and newly emerged adult females of the same locust after treatment of nymphs with Flufenoxuron (Hamadah, 2014); *etc*.

To interpret the reduction of lipids in larvae and pupae of *P. unionalis*, in the current work, it is important to point out that the lipid turnover in insects is regulated by neuroendocrine-controlled feed-back loops (Downer, 1985). Although the first site of action of IGRs, in general, is the endocrine system, many biochemical and physiological changes have been reported to occur in different metabolism pathways (Leonardi *et al*., 2001; Kim *et al*., 2002; Etebari *et al*., 2007). The impaired synthesis of lipids in insects has been resulted in disruptively affected physiology and subsequently deranged vital functions of growth and reproduction. Therefore, the decreased lipid content in larvae and pupae of *P. unionalis* may be due to the inhibitory effects and stress of the tested IGRs on various hormonal systems and neurosecretion (Gade *et al*., 1997; Bouaziz *et al*., 2011). Also, the declined lipid level may be due to the shift in energy metabolism towards lipid catabolism as a result of physiological stress induced by these IGRs (El-Sherif, 1995).

Conclusions:

The protein synthesis is necessary for the insect development and reproduction, carbohydrates are the main sources of energy during insect metamorphosis and lipid disturbance resulted in disruptively affected physiology and subsequently deranged vital functions of growth and reproduction. In the present study, Novaluron, Methoxyfenozide and Pyriproxyfen adversely affected these metabolites in larvae and pupae of *P. unionalis via* disturbance of the detoxifying enzymes or hormonal regulation of several vital processes. Therefore, each of the tested IGRs has good potential for the formulation of novel IGR-based control agents against this pest in an environmentally-friendly manner to the ecosystem.

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