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Perturbation of main body Metabolites in the Pink Bollworm *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) by the chitin synthesis inhibitors Novaluron and Diofenolan

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ABSTRACT: The pink bollworm *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) is one of the most destructive insects attacking cotton fields world-wide. It acquired resistance against most of the conventional pesticides. Therefore, the objective of the present study was to investigate the disturbances of the main body metabolites (proteins, carbohydrates and lipids) in homogenates of larvae (6 hr post-treatment) as well as in early- aged pupae (1-day old), mid-aged pupae (3-day old) and late-aged pupae (7-day old), after treatment of full grown larvae with LC_{50} of Novaluron (0.765 ppm) and Diofenolan (0.036 ppm). Treatment with Novaluron or Diofenolan resulted in a considerable reduction in the protein content of larvae. Novaluron exceptionally enhanced the early- and mid-aged pupae to gain remarkably increasing proteins but a reducing action was exerted on the late-aged pupae. Diofenolan exhibited a predominant reducing effect on the protein content in pupae of all ages. Carbohydrates had been drastically declined in larvae. Diofenolan was stronger than Novaluron for reducing this metabolite. With regard to pupae, both CSIs exerted prevalent reducing actions, regardless the age. Novaluron and Diofenolan suppressed the lipid content in larvae. Diofenolan was comparatively stronger than Novaluron for reducing this metabolite. The successfully developed pupae suffered powerful inhibitory effects on their lipid contents.

Keywords: carbohydrate, homogenate, larva, lipid, protein, pupa.

INTRODUCTION

Worldwide, the pink bollworm *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) is one of the most destructive insect pests that cause terrible damage to the cotton (Patil, 2003). In Egypt, this insect pest causes serious damage to cotton bolls resulting in enormous reduction in quantity and quality arising to one million kentar annually (Khidr *et al.,* 1996; El-Aswad and Aly, 2007; Kandil *et al*., 2012).In Egypt, also, *P. gossypiella* has recently developed resistance to several classes of insecticides currently used in cotton fields because it has been known for its ability to detoxify these chemicals (Khurana and Verma,1990; Abd-Elhady and Abd El-Aal, 2011). In addition, the intensive and discriminate uses of many conventionally synthetic pesticides led to several drastic problems, such as the environmental pollution, hazards to human and animals like birds, fishes and mammals, destruction of the natural enemies, like parasites, predators(Quarles, 2001; Rose, 2001; Sabry and Abdel-Aziz, 2013). Therefore, alternative materials have been initiated recently to minimize the pesticide hazards (Derbalah *et al*., 2014) and to delay the resistance development in *P. gossypiella* (Salama *et al*., 2013).

During the last few decades, a new class of comparatively safe pesticides have been developed and known as insect growth regulators (IGRs) (Dhadialla *et al*., 1998; Khan and Qamar, 2012). In contrast to the classical chemical insecticides, IGRs are not directly toxic, but act selectively on the development, metamorphosis or reproduction of the target insect (Hoffmann and Lorenz, 1998; Martins and Silva, 2004). Therefore, IGRs are used as control agents against various insect pests, and can assist in the development of sustainable agriculture (Raslan, 2002; Zhou *et al*., 2003; Cedric, 2005; Wang and Wang, 2007). Chitin synthesis inhibitors (CSIs) are usually classified in IGRs (Tunaz and Uygun, 2004) interfering with chitin biosynthesis in insects and thus prevents moulting, or produces an imperfect cuticle (Hammock and Quistad, 1981). These compounds affect, also, the hormonal balance in insects, thereby resulting in physiological disturbances (Soltani *et al*., 1984).

Novaluron is a relatively new benzoylphenyl urea CSI with low mammalian toxicity (Barazani, 2001; Ishaaya and Horowitz, 2002). It was found as a deteriorating effective compound on survival and development (Ghoneim *et al*., 2015), adult performance (Hamadah *et al*., 2015) and protein content (Basiouny *et al*., 2016) of Spodoptera littoralis. As reported by many authors (Ishaaya *et al*., 2003; Cutler *et al*., 2005;Cetin *et al*., 2006; Mascari *et al*., 2007;Kostyukovsky and Trostanetsky, 2008; Jambulingam *et al*., 2009; Martin *et al*., 2010; Bouaziz *et al*., 2011; Kamminga *et al*., 2012; Djeghader *et al*., 2013, 2014), Novaluron inhibits the chitin formation in larvae of various insects classified in Lepidoptera, Coleoptera, Homoptera and Diptera. Diofenolan is a CSI used for the control of several pests, such as lepidopterous species and scale insects (Paloukis and Navrozidis, 1995; Dhadialla *et al*., 1998), *Papilio demoleus* (Singh and Kumar, 2011), *Musca domestica* (Ghoneim *et al*., 2001,2003), *Rhynchophorus ferrugineus* (Ghoneim *et al*., 2004) and *Schistocerca gregaria* (Ghoneim *et al*., 2012; Hamadah *et al*., 2012; Tanani *et al*., 2012).It did not affect the survival of beneficial parasitoids and predators of some pests, such as *Chrysoperla carnea* (Sechser *et al*., 1994).

As reported by many authors (Hassan, 2002; Chapman, 2004; 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, especially CSIs (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). Taking all of these considerations into account, 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. gossypiella* after treatment of full grown larvae with LC_{50} values of Novaluron and Diofenolan.

MATERIALS AND METHODS

A. Experimental insect

A culture of the pink bollworm *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) was originated by a sample of newly hatched larvae from the susceptible culture maintained for several generations along some years in Plant Protection Research Institute, Doqqi, Giza, Egypt. It was reared under constant conditions (27±2°C and 75±5% R.H.) at Department of Zoology and Entomology, Faculty of science, Al-Azhar University, Cairo. Larvae were provided with an artificial diet as described by Abd El-Hafez *et al*. (1982). For rearing details and manipulation of all developmental stages under controlled conditions in the laboratory, see Ghoneim *et al*. (2017).

B. CSIs and larval treatment

Novaluron (Rimon) [1-[chloro-4-(1,1,2-trifluoromethoxyethoxy) phenyl] -3- (2,6-difluorobenzoyl) urea] has the molecular formula $C_{17}H_9ClF8N_2O_4$.Diofenolan(Aware®)(2S,4R)-2-Ethyl-4-[(4-phenoxyphenoxy) methyl]-1,3-dioxolanehas the molecular formula $C_{18}H_{20}O_4$. Both compounds were supplied by Sigma-Aldrich Chemicals (https://www.sigmaaldrich.com).In a preliminary experiment on full grown larvae of *P. gossypiella*, LC_{50} values were estimated in 0.765 and 0.036 ppm of Novaluron and Diofenolan, respectively.

C. Homogenate preparation

After treatment of full grown larvae, homogenate samples of larvae (6 hr post-treatment) and pupae of three ages: 1-day old (early-aged pupae), 3-day old (mid-aged pupae) and 7-day old (late-aged pupae) were prepared. Control larvae and pupae were treated with distilled water only and homogenate samples of larvae and pupae were prepared as treated congeners.

The treated and control larvae were homogenized in saline solution (50 larvae/5 ml saline solution) using a fine electric homogenizer. Homogenates were centrifuged at 4000 r.p.m. for 15 min. under 2°C in a refrigerated centrifuge. The supernatant was used directly or stored at -20ºC until the use for determining the metabolites. For the determination of the main metabolites in pupae, treated pupae of each age and equal number of control pupae of the same ages were homogenized in saline solution (50 pupae/5 ml saline solution) using an fine electric homogenizer. Homogenates were centrifuged and the supernatant was manipulated as done with larvae.

D. Determination of the main body metabolites

Quantitative determination of the total protein content was conducted in the larval and pupal homogenates according to the method of Weichselbaum (1946) using the kit of Diamond diagnostics. The method depends 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 and pupal homogenates using the anthrone reagent according to Singh and Sinha (1977) by 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) and using the Spectrophotometer at 520 nm.

E. 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

In a preliminary experiment, LC_{50} values of the CSIs Novaluron and Diofenolan were calculated, after treatment of full grown larvae (just before prepupae) of *P. gossypiella*, in 0.765 and 0.036 ppm, respectively.

After treatment of full grown larvae with LC_{50} of Novaluron and Diofenolan, total contents of the main body metabolites (proteins, carbohydrates and lipids) were determined in larvae (6 hr post-treatment) as well as in early- aged pupae (1-day old), mid-aged pupae (3-day old) and late-aged pupae (7-day old).Technically, it was very difficult to obtain adequate samples of haemolymph or fat body. Therefore, a total body homogenate was prepared for determining these main metabolites.

A. Effects of CSIs on the protein content in larvae and pupae

Depending on data assorted in Table 1, treatment of full grown larvae of *P. gossypiella* with LC_{50} of Novaluron resulted in considerable reduction of the protein content in larval homogenate (6 hr post-treatment) (54.68% reduction). A similar prohibiting action was exerted by Diofenolan on larvae (37.72% reduction). With regard to the pupal stage, data of the same table obviously revealed no certain trend of protein content in the control pupae.

Table 1: Total protein content in the body homogenate of *P. gossypiella* as influenced by treatment of full grown larvae with LC_{50} values of CSIs.

CSI		Full-grown larvae*	Pupal age		
			1-day old pupae	3-day old pupae	7-day old pupae
Novaluron	Mean (g/dL)±SD	1.55±0.08 d	1.99±0.14 b	1.64±0.08 b	1.29±0.08 b
	Change (%)	-54.68	+15.03	+19.71	-16.77
Diofenolan	Mean (g/dL)±SD	2.13±0.13 d	1.29±0.07 d	1.20±0.01 b	1.51±0.08 a
	Change (%)	-37.72	-25.43	-12.41	-2.58
Control	Mean (g/dL)±SD	3.42±0.16	1.73±0.01	1.37±0.08	1.55±0.08

Mean±SD followed by letter (a): not significantly different (P>0.05), (b): significantly different (P<0.05), (c): highly significantly different (P<0.01), (d): very highly significantly different (P<0.001).*: 6 hr post-treatment.

On the other hand, the protein content gradually decreased with the pupal age (1.99±0.14, 1.64±0.08 & 1.29±0.08 g/dL, in the early-, mid- & late-aged pupae, respectively), after treatment with Novaluron. On the basis of statistical analysis, Novaluron enhanced the early- and mid-aged pupae to gain considerably increasing proteins (15.03 & 19.71% increments, respectively) but a contradictory action was exhibited on the late-aged pupae since protein content was remarkably declined (1.29±0.08 in Novaluron-treated pupae, compared to 1.55±0.08 g/dL in control pupae). Treatment of full grown larvae with Diofenolan resulted in a continuous decrease of the protein content in the developed pupae. Although Diofenolan exerted a predominant reducing effect on this metabolite, its strength gradually decreased with the pupal age (25.43, 12.41 & 2.58% reduction, in the early-, mid- & late-aged pupae, respectively).

B. Effects of CSIs on the carbohydrate content in larvae and pupae

After treatment of full grown larvae of *P. gossypiella* with LC_{50} of Novaluron or Diofenolan, data of the carbohydrate content were arranged in Table 2. As exiguously shown in this table, both compounds exhibited potent inhibitory effects on larvae (6 hr post-treatment) which failed to attain normal carbohydrate level since it was drastically suppressed (45.06 & 51.09% reduction, in Novaluron-treated and Diofenolan-treated larvae, respectively). As clearly seen, Diofenolan was stronger than Novaluron for reducing the carbohydrate content.

In respect of the pupae, data of the same table obviously revealed a gradual regression of carbohydrates in control pupae with the age (227.67±4.04, 196.33±15.01 & 115.67±5.69 mg/dL in early-, mid- & late-aged pupae, respectively). For detecting the effects of Novaluron and Diofenolan, data of the same table evidently presented prevalent reducing actions of both compounds on the carbohydrate content of pupae, regardless the age. In some detail, significantly decreasing carbohydrates had been determined in the pupae of all ages, after treatment with Novaluron (21.67, 19.86 & 75.79% reduction, in early-, mid- & late-aged pupae, respectively). These data clearly revealed a predominant inhibitory effect of Novaluron on carbohydrate content, but in no certain trend. Furthermore, Diofenolan exerted a similar inhibitory action on carbohydrates, the strength of which increased with the pupal age (10.98, 33.44 & 42.08% reduction, in early-, mid- & late-aged pupae, respectively).

Table 2: Total carbohydrate content in the body homogenate of *P. gossypiella* as influenced by treatment of full grown larvae with LC50 values of CSIs.

CSI		Full-grown larvae*	Pupal age		
			1-day old pupae	3-day old pupae	7-day old pupae
Novaluron	Mean (mg/dL)±SD	261.33±5.86d	178.33±2.52 d	157.33±5.69 b	28.00±1.73 d
	Change(%)	-45.06	-21.67	-19.86	-75.79
Diofenolan	Mean (mg/dL)±SD	232.67±9.71d	202.67±2.52 d	130.67±4.04 c	67.00±1.73 d
	Change(%)	-51.09	-10.98	-33.44	-42.08
Control	Mean (mg/dL)±SD	475.67±19.55	227.67±4.04	196.33±15.01	115.67±5.69

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

C. Effects of CSIs on the lipid content in larvae and pupae

Depending on the data distributed in Table 3, both Novaluron and Diofenolan exerted suppressing actions on the lipids in larvae of *P. gossypiella* (6 hr post-treatment). Diofenolan was comparatively stronger than Novaluron for reducing these lipids (65.67 & 48.51% reduction, by Diofenolan and Novaluron, respectively). The successfully developed control pupae attained gradually decreasing lipids with the age (4080.0±222.71, 3320.0±105.83 & 1800.0±120.0 mg/dL, in early-, mid- & late-aged pupae, respectively). On the other hand, the successfully developed pupae from treated larvae suffered powerful inhibitory effects of Novaluron and Diofenolan on their lipid contents. In some detail, data of the same table unambiguously demonstrated that the reducing intensity of Novaluron gradually decreased with the age of pupae (52.61, 44.18 & 42.96% reduction of lipids, in early-, mid- & late-aged pupae, respectively). With regard to Diofenolan, the reducing effect on the lipid content in pupae run in no certain trend (61.76, 69.88 & 45.19% reduction of lipids, in early-, mid- & late-aged pupae, respectively).

Table 3: Total lipid content in the body homogenate of *P. gossypiella* as influenced by treatment of full grown larvae with LC_{50} values of CSIs.

CSI		Full-grown larvae *	Pupal age		
			1-day old pupae	3-day old pupae	7-day old pupae
Novaluron	Mean (mg/dL)±SD	920.0±40.00 d	1933.33±83.27 d	1853.33±83.27 d	1026.67±100.66 c
	Change (%)	-48.51	-52.61	-44.18	-42.96
Diofenolan	Mean (mg/dL)±SD	613.33±23.09 d	1560.0±40.0 d	1000.0±80.0 d	986.67±23.09 d
	Change (%)	-65.67	-61.76	-69.88	-45.19
Control	Mean (mg/dL)±SD	1786.67±46.19	4080.0±222.71	3320.0±105.83	1800.0±120.0

c, d ,*: see footnote of Table (1).

However, the treated pupae had gradually decreasing lipid content with the age, regardless the compound, as detected also in control pupae.

DISCUSSION

The content of macromolecules (i.e. protein, carbohydrates and lipids) is good indicator of the level of metabolism in insects treated with chemicals (Zhu *et al*., 2012). It is very important to point out that the protein synthesis is necessary for the insect development and reproduction. Carbohydrates are the main sources of energy during insect metamorphosis. The impaired synthesis of lipids in insects has been resulted in disruptively affected physiology and subsequently deranged vital functions of growth and reproduction. As reported by many authors (Leonardi *et al*., 2001; Kim *et al*., 2002; Etebari *et al*., 2007), 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.

A. Disturbed protein content in P. gossypiella by CSIs.

Depending on the available literature, treatment of the newly hatched larvae of *P. gossypiella*, in Egypt, with LC_{50} of Diflubenzuron (Rashad *et al*., 2006), Chlorfluazuron (Kandil *et al*., 2005), Pyriproxyfen (Derbalah *et al*., 2014) or Teflubenzuron (Rashad *et al*., 2015) resulted in considerable reductions in the haemolymph protein content of 4th instar larvae. Also, treatment of the newly hatched larvae of the same insect with LC_{50} of Chromafenozide and Diflubenzuron resulted insignificant reductions in soluble protein content of adults (Salem, 2015). After treatment of 1-day old eggs of the same insect with LC_{50} values of Lufenuron, Chlorfluazuron and Chromafenozide, reduced protein content was estimated in larvae (Kandil *et al*., 2012).

Treatment of adult females of the same insect with Diflubenzuronled to high reduction in haemolymph protein content of the treated adults (Rabie *et al*., 2006).

Results of the present study are in agreement with those reported results of reduced proteins in *P. gossypiella,* since treatment of full grown larvae with LC_{50} values of Novaluron (0.765 ppm) or Diofenolan (0.036 ppm) resulted in drastically reduced protein content in larvae. With regard to pupae, Diofenolan exhibited a predominant reducing effect on the protein content along the pupal stage while Novaluron exhibited a similar effect only on the late-aged (7-day old) pupae. Also, the present results of reduced protein content in larvae or pupae (of some ages) are, to a great extent, in accordance with those reported results of declined protein level in some developmental stages of other insect species after treatment with various insect growth regulators (IGRs) or chitin synthesis inhibitors (CSIs), such as *Spodoptera littoralis* by pyriproxyfen, Flufenoxuron and Triflumuron (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). In addition, the available literature contains many reported results of prohibited proteins in other insects by different IGRs, such as *Schistocerca gregaria* by Pyriproxyfen (Ghoneim *et al*., 2012) or Flufenoxuron (Hamadah, 2014); *Musca domestica* by Diflubenzuron, Triflumuron and Methoprene (Bakr *et al*., 1991) or Methoxyfenozide(RH-2485) (Assar and Abo-Shaeshae, 2004); *Leptinotarsa decemlineata* by 20-Hydroxyecdysone or ecdysteroid agonists RH-5849 and tebufenozide (RH-5992) (Smagghe *et al*., 1999); *Spodoptera litura* by Pyriproxyfen (Perveen and Miyata, 2000);

Tenebrio molitor by Halofenozide (Soltani *et al*., 2002); Cephalopina titillator by Pyriproxyfen or 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).

On the contrary, treatment of full grown larvae of *P. gossypiella*, in the current work, with LC_{50} of Novaluron resulted in remarkably increasing protein content in the early-aged (1-day old) pupae and mid-aged (3-day old) pupae, i.e., pupae of these ages had been subjected to an enhancing action of Novaluron to gain more proteins than control pupae. This result 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, 2002) or 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) and Bactrocera cucurbitae by Methoprene (ul Haq *et al*., 2010).

The prevalent reducing effect of Diofenolan on proteins in *P. gossypiella* larvae (6 hr post-treatment) and pupae (of all ages), as well as the powerful inhibitory effect of Novaluron on proteins in larvae and pupae, of only 7-day old, in the present study, can be interpreted in the light of some conceivable suggestions, as follows. Proteins are the known biological compounds which regulate and integrate several physiological and metabolic processes in the body through hormones, enzymes and nucleoproteins. With regard to foreign compounds, proteins help insects to synthesize the microsomal detoxifying enzymes (Wilkinson, 1976), i.e. proteins can bind with foreign compounds and therefore the decrease in proteins may reflect the decrease in activity of these enzymes (Kyung and Kim, 1990).CSIs 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 stress, to provide intermediates to the Krebs cycle, by retaining free amino acid content in haemolymph (Nath, *et al*., 1997). Extensive work has been carried out in order to determine how various toxic agents affect protein synthesis. The protein reduction in the current work may, also, be due to the interference of tested CSIs with the insect endocrine system causing a hormonal imbalance (Hajjar and Casida, 1979) and affecting the metabolism (De Mark and Bennett, 1989) orprotein synthesis in insects (Padmaje and Rao, 2000).

On the other hand, Novaluron induced the early- and mid-aged pupae of *P. gossypiella* to gain increasing proteins, in the current investigation. This result can be acceptable, since proteins could be used to as biomarker of exposure which is the response to an interaction between a xenobiotic agent (such as CSIs, in the present study) and a molecule or target cell (Owa *et al*., 2010; Sugumaran, 2010). As affected by the tested CSIs, *S. littoralis* 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 synthesis of cuticular proteins and associated tanning under stress of insecticides or CSIs (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. The protein plays a major role in the synthesis of microsomal detoxifying enzymes and helps to detoxify the toxicants when entering into the animals (Wilkinson, 1976). Thus, the protein disturbance by the tested CSIs, in the present study, may be reflected on the insects' detoxification capability. For understanding the mode of action, the tested CSIs, in the present investigation, may either act on the hormonal level in the haemolymph to announce the synthesis, degradation or inhibition of proteins or on the neurosecretory cells which control endocrine organs (Bouaziz *et al*., 2011; Djeghader *et al*., 2013, 2014).

B. Disturbed carbohydrate content in P. gossypiella by CSIs

The carbohydrates, as energy elements, play a crucial role in the physiology of the insects (Kaufmann and Brown, 2008). Some authors reported elevated carbohydrate content in some insect species as a response to the action of different IGRs or CSIs, while others reported opposite results. These contradictory findings may be due to differences in species sensitivity, the potency of the IGRs, or the developmental stage (Ghoneim *et al*., 2003).

In the present study, treatment of full grown larvae of *P. gossypiella* with LC_{50} values of Novaluron (0.765 ppm) or Diofenolan (0.036 ppm) resulted in considerably decreased carbohydrate content in the treated larvae (6 hr post-treatment). However, Diofenolan was stronger than Novaluron for reducing this metabolite. In respect of the successfully developed pupae, both compounds exerted prevalent reducing actions on carbohydrates, regardless the pupal age. These results are in corroboration with those reported results of reduction in carbohydrates in larvae of the same insect after treatment of 1-day old eggs with LC_{50} values of Lufenuron, Chlorfluazuron and Chromafenozide (Kandil *et al*., 2012) as well as the declined carbohydrate levels in different developmental stages of other insects by various IGRs (including CSIs), 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 suppressed carbohydrate content in larvae and pupae of P. gossypiella, by Novaluron and Diofenolan, in the present study, disagree with those reported results of increasing carbohydratecontent in certain tissues of different developmental stages of various insect species after treatment with several IGRs, such as S. littoralis by Kinoprene (Fouda and Amer, 1990), Chlorfluazuron, alone or combined with Mevalonic acid (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 Chlorfluazuron (El-Gammal *et al*., 1993), 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 important to point out that the production or utilization of main body metabolites, such as carbohydrates, are suggested to be 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 carbohydrate content in larvae and pupae of *P. gossypiella*, in the present study, may be due to interference of Novaluron and Diofenolan with 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 CSIs 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 utilization of the reserved glucose sources of the larval tissues as a result of CSIs stresses (Sharma *et al*., 2011).

C. Disturbed lipid content in P. gossypiella by CSIs.

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/orother developmental stages of different insects after treatment of larvae with sublethal concentrations or doses of various IGRs or CSIs, such as *S. littoralis* by Diflubenzuron (Ahmed, 2001), Flufenoxuron (Abdel-Aal, 2003) and Teflubenzuron (El-Sheikh *et al*., 2013); *C. cephalonica* by Pyriproxyfen (Mandal and Chaudhuri, 1992), Ch. 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), *P. 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) and Chromafenozide or Diflubenzuronin adults (Salem, 2015); *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. In the present investigation, the obtained results of declined lipids in *P. gossypiella* are, to some extent, in agreement with those previously reported results of decreasing lipids in the same insect and some of other insects, since both CSIs suppressed the lipid content in homogenates of larvae (6 hr post-treatment) and pupae of different ages, after treatment of the full grown larvae with LC_{50} concentrations of Novaluron (0.765 ppm) and Diofenolan (0.036 ppm). Moreover, Diofenolan was comparatively stronger than Novaluron for reducing this metabolite.

On the contrary, recorded results in the present study disagree with those reported results of increasing lipids in the same insect, after treatment of newly hatched larvae with LC_{50} of Diflubenzuron and Chlorfluazuron (Kandil *et al*., 2005).Our results disagree, also, with those reported results of elevated lipid levels in some other insects, by various IGRs,such as 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 this reduction of lipids 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). Moreover, many biochemical and physiological changes in insects have been reported to occur in different metabolism pathways under control of hormones (Leonardi *et al*., 2001; Kim *et al*., 2002; Etebari *et al*., 2007). Therefore, the decreased lipid content in larvae and pupae of *P. gossypiella* may be due to the inhibitory effects and stress of Novaluron and Diofenolan on various hormonal systems and neurosecretion (Gade *et al*., 1997; Bouaziz *et al*., 2011). Also, the declined lipid content may be due to shift in energy metabolism towards lipid catabolism as a result of physiological stress induced by IGRs (El-Sherif, 1995).

CONCLUSION

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 and Diofenolan exhibited predominant reducing effects on these metabolites in larvae and pupae of *P. gossypiella*. Therefore, each of these compounds hase a good potential in formulating novel IGR-based control agents against this pest in an environmentally-friendly manner to ecosystem.

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