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# Disturbed protein content in Egyptian cotton leafworm *Spodoptera littoralis* (Boisd.)(Lepidoptera: Noctuidae) by some novel chitin synthesis inhibitors.

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**Abstract**

The present study was carried out to investigate the effects of chitin synthesis inhibitors, Novaluron, Cyromazine and Diofenolan, on the protein content in larvae and pupae of *S. littoralis*. After treatment of penultimate instar larvae with $LC_{50}$ values (2.71, 74.44 and 7.65 ppm, respectively), the total protein content was determined in haemolymph and fat bodies of successfully moulted last instar larvae (of different ages) as well as in homogenates of early-, mid- and late-aged pupae. Increasing protein content in haemolymph and fat bodies of last instar larvae of all ages was determined after treatment with Cyromazine or Diofenolan. Novaluron treatment resulted in increasing protein content in haemolymph of larvae, except of the early ages whose fat bodies only contained increasing protein content. Novaluron exhibited an inhibitory effect on this metabolite in haemolymph, but not in fat bodies, of the early aged larvae. With regard to the developed pupae, all CSIs seriously suppressed the protein content, regardless the age.

**Keywords:** Cyromazine, Diofenolan, fat body, haemolymph, larvae, Novaluron, pupa

## Introduction

The Egyptian cotton leafworm *Spodoptera littoralis* (Boisduval) is a polyphagous insect. Approximately112 plant species belonging to 44 families are reported as hosts of this pest in tropical and temperate zones of the old world (Magd El-din and El-Gengaihi, 2000) or 73 species recorded from Egypt (Moufied *et al*., 1960). In Egypt, this destructive phytophagous lepidopterous pest attacks cotton and various vegetable and field crops all over the year (Hosny *et al*., 1986; Shonouda and Osmam, 2000; El-Khawas and Abd El-Gawad, 2002; Adham *et al*., 2009). When large numbers of the pest are present complete crop loss is possible (Khalil, 1988).

To control the attacks of *S. littoralis*, several types of insecticides have been used, including synthetic pyrethroids, organophosphates, and non-steroidal compounds (Casida and Quistad, 1998). The extensive use of these insecticides has caused resistant insect strains to emerge making their control even more difficult (Smagghe *et al*., 1999; Miles and Lysandrou, 2002; Abo-El Ghar *et al*., 2005; Aydin and Gurkan, 2006; Davies *et al*., 2007, Mosallanejad and Smagghe, 2009) in addition to serious toxicological problems of the conventional synthetic pesticides to humans and the environment (Costa *et al*., 2008; Relyea, 2009). Owing to the socioeconomic importance of *S. littoralis*, the insect is subject to extensive research,

much of which is focused on finding new ways to control it as a pest and to improve the effects of known pest control methods (Hussain, 2012).

At present, using insect growth regulators (IGRs) is considered as the possible alternative agents of conventional synthetic insecticides for controlling this pest (Raslan, 2002). IGRs are regarded as a third generation of insecticides or biorational pesticides because they differ in their mode of action from other insecticides and have low toxicity to non-target organisms (Zhou *et al*., 2003). Because of their desirable characteristics, such as low toxicity, less environmental pollution, high selectivity, and low impact on natural enemies and people, IGRs are used to control various insect pests (Wu, 2002; Cedric, 2005; Wang and Wang, 2007). Chitin synthesis inhibitors (CSIs) are classified in IGRs (Tunaz and Uygun, 2004).

Novaluron is a relatively new benzoylphenyl urea CSI with good activity against the Colorado potato beetle (Cutler *et al*., 2005a,b, 2007; Alyokhin *et al*., 2009) and low mammalian toxicity (Barazani, 2001; Ishaaya and Horowitz, 2002). Novaluron was found as an deteriorating effective CSI on survival and development (Ghoneim *et al*., 2015) and adult performance of *S. littoralis* (Hamadah *et al*., 2015)**.** Its residues tend to dissipate with half-life of 2.08 days and the safe use of it on tomatoes, and possibly on other crops in Egypt was established (Malhata *et al*., 2014). Cyromazine is a triazine IGR used as alternative to insecticides and acaricides. It is used in veterinary medicine for the protection of animals, such as sheep and lamps, against flies (Emea, 2001). As reported by many authors (Kanno *et al*., 1981; Saito, 1988; Reynolds and Blakey, 1989; Levot and Sates, 1998; Tomlin, 2000; Vazirianzadeh *et al*., 2007**)**, Cyromazine exhibited various degrees of success for controlling different pests such as house flies and leafminers. It exhibited remarkable toxic and inhibitory effects on growth of *S. littoralis* (Tanani *et al*., 2015). Diofenolan is a CSI used for the control of several pests, such as lepidopterous species and scale insects (Streibert *et al*., 1994; Paloukis and Navrozidis, 1995; Dhadialla *et al*., 1998), *Papilio demoleus* (Singh and Kumar, 2011), *Musca domestica* (Ghoneim *et al*., 2001, 2003; Amer *et al*., 2006; Al-Dali, 2008), *Rhynchophorus ferrugineus* (Ghoneim *et al*., 2004) and *Schistocerca gregaria* (Bakr *et al*., 2008; 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. Also, proteins are integrated in the cell as a structural element at the same time as the carbohydrates and the lipids. In addition, proteins in all viable cells, as nucleoproteins, are essential to the cell division and as enzymes and hormones are essential to control many chemical reactions in the cell metabolism. Ecdysteroids and juvenoids were reported to be associated with changes in the rates of protein synthesis in insects (Locke *et al*., 1982). However, many factors had been implicated in the control of protein synthesis (Carlisle *et al*., 1987).

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 insect's body (Pugazhvendan and Soundararajan, 2009). The exposure of an organism to xenobiotic product can modify the synthesis of certain metabolite and disturb the functionality of the organisms (Rodriguez-Ortega *et al*., 2003). On the other hand, the fat body in insect 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). The present study was carried out aiming to investigate the effects of novel CSIs, *viz*., Novaluron, Cyromazine and Diofenolan, on the protein content in haemolymph and fat bodies of larvae as well as in the pupal homogenate of *S. littoralis*.

## Materials and Methods

### 1. Experimental insect

A sample of *S. littoralis* pupae was kindly obtained from the culture of susceptible strain maintained for several generations in Plant Protection Research Institute, Agricultural Research Center, Doqqi, Giza, Egypt. In laboratory of Entomology, Faculty of Science, Al-Azhar University, Cairo, a culture was reared under laboratory controlled conditions (27±2°C, 65±5% R.H., photoperiod 14 h L and 10 h D). Rearing procedure was carried out according to Ghoneim (1985) and improved by Bakr *et al*. (2010). Larvae were provided daily with fresh castor bean leaves *Ricinus communis*. The emerged adults were provided

with 10% honey solution on a cotton wick as a food source. Moths were allowed to lay eggs on branches of *Nerium oleander*, then the egg patches were collected daily, and transferred into Petri dishes for another generation.

## 2. Larval treatments with CSIs

Novaluron (Rimon, Pestanal®) [1-[chloro-4-(1,1,2-trifluoromethoxyethoxy) phenyl] -3- (2,6-difluorobenzoyl) urea] was purchased from Sigma-Aldrich Chemicals (https://www.sigmaaldrich.com), Cyromazine (Larvadex, Trigard, Vetrazin) [N-cyclopropyl-1, 3, 5-triazine-2, 4, 6-triamine] was purchased from Sigma-Aldrich Chemicals (https://www.sigmaaldrich.com) and Diofenolan (CGA 59205, Aware®)[2-ethyl-4-[(4-phenoxyphenoxy) methyl]-1,3-dioxolane] was obtained from Agricultural research center, laboratory of pesticides, Doqqi, Giza, Egypt. In a preliminary experiment, $LC_{50}$ values of Novaluron, Cyromazine and Diofenolan were calculated, after treatment of penultimate instar larvae of *S. littoralis*, in 2.71, 74.44 and 7.65 ppm, respectively. After treatment of these larvae with $LC_{50}$ of each CSI, total protein content was determined in haemolymph and fat bodies of the successfully moulted last instar larvae (of different ages) as well as in homogenate of the developed early- (0-day old), mid- (4-day old) and late-aged (7-day old) pupae.

## 3. Tissue preparation

### 3.1. Larval haemolymph

For the determination of the total protein content, haemolymph was collected from treated and control 6$^{th}$ (last) instar larvae (of different ages: 0-, 2-, 4-, and 6-day old). The haemolymph was obtained by 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 were never mixed.

### 3.2. Larval fat body

For the determination of the total protein content, fat bodies (parietal and visceral) were carefully collected from the treated and control 6$^{th}$ (last) instar larvae (of different ages: 0-, 2-, 4-, and 6-day old). 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.

### 3.3. Pupal homogenate

For the determination of the total protein content, healthy treated and control pupae (of different ages: 0-, 4-, and 7-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.

## 4. Determination of the total protein content

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 Biodiagnostics. The method depends on the protein forms a violet complex with cupric ions in alkaline medium, and then measured the absorbance at 550 nm using a spectrophotometer.

## 5. Statistical analysis of data

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

# Results

## 1. Effect of Novaluron on protein content in *S. littoralis*

Depending on the data assorted in Table (1), protein content in haemolymph on control larvae gradually increased with the age but *vice versa* in fat body.

Similar trends of protein content had been detected in the two tissues with the age of Novaluron-treated larvae. With regard to the effect on protein content, Novaluron slightly prohibited the newly moulted larvae to attain normal proteins in haemolymph (2.61% reduction) while it enhanced larvae of other ages to gain increasing proteins. The most powerful enhancing effect of Novaluron was exhibited on 4-day old larvae (5.52±0.05 vs. 5.04±0.04 g/dL in control larvae). Concerning the fat body, Novaluron slightly promoted larvae of 0- and 2-day old to gain more proteins (1.00 and 3.88%, respectively) while it prohibited larvae of 4- and 6-day old to attain normal protein content (5.33 and 0.80% reduction, respectively). The strongest inhibitory effect of Novaluron on fat body proteins was exhibited on 4-day old (49.77±0.54 vs. 52.57±0.43 mg/g in control larvae). An extended effect of Novaluron on the protein content was observed in the pupal stage as obviously shown in Table (2). According to data of this table, a remarkable predominant reducing action of Novaluron was exerted on the proteins in pupal homogenate. Moreover, its potency increased with the age (13.71, 16.38 and 25.87% reduction in early-, mid- and late-aged pupae, respectively).

**Table 1: Total protein content in last instar larvae as influenced by treatment of newly moulted penultimate instar larvae of *S. littoralis* with $LC_{50}$ of Novaluron.**

| Tissue | | | Larval age | | | |
|---|---|---|---|---|---|---|
| | | | 0-day old | 2-day old | 4-day old | 6-day old |
| Treated | Haemolymph (g/dL) | mean±SD | 4.86±0.07 a | 5.01±0.07 a | 5.52±0.05 b | 6.13±0.04 a |
| | | Change (%) | -2.61 | +0.00 | +9.52 | +4.25 |
| | Fat body (mg/g) | mean±SD | 60.60±0.50 a | 56.23±0.38 a | 49.77±0.54 b | 49.59±0.61 a |
| | | Change (%) | +1.00 | +3.88 | -5.33 | -0.80 |
| Control | Haemolymph (g/dL) | mean±SD | 4.99±0.07 | 5.01±0.11 | 5.04±0.04 | 5.88±0.21 |
| | Fat body (mg/g) | mean±SD | 60.08±0.36 | 54.15±0.42 | 52.57±0.43 | 49.90±0.38 |

Mean ± SD followed with the letter (a): insignificantly different (P >0.05), (b): significantly different (P<0.05).

**Table 2: Total protein content in pupae as influenced by treatment of newly moulted penultimate instar larvae of *S. littoralis* with $LC_{50}$ of Novaluron.**

| Homogenate | | Pupal age | | |
|---|---|---|---|---|
| | | 0-day old | 4-day old | 7-day old |
| Treated | mean mg/g±SD | 23.54±0.68 b | 30.94±0.30 c | 13.87±0.30 d |
| | Change (%) | -13.71 | -16.38 | -25.87 |
| Control | mean mg/g±SD | 27.28±1.04 | 37.00±1.08 | 18.71±0.66 |

b: See footnote of Table (1). Mean ± SD followed with the (c): highly significantly different (P<0.01), (d): very highly significantly different (P<0.001).

## 2. Effect of Cyromazine on protein content in *S. littoralis*

As easily seen in Table (3), protein content in haemolymph on control larvae gradually increased with the age but *vice versa* in fat body. Similar trends of protein content had been almost detected in the two tissues with the age of Cyromazine-treated larvae. In the light of data assorted in this table, Cyromazine exhibited a slightly inducing effect on larvae to gain insignificantly protein increase in haemolymph (1.20, 4.19, 4.76 and 0.85% enlargement in 0-, 2-, 4- and 6-day old larvae, respectively). A similar stimulatory effect was exhibited in larvae of the same ages to gain more proteins in fat bodies. The strongest stimulatory

effect was detected in the 0-day old larvae (76.51±0.59, compared to 60.08±0.36 mg/g in control larvae). Data included in Table (4) clearly reveal a reversed action of Cyromazine on pupae since pronouncedly declined protein levels were achieved, regardless the age (5.73, 7.50 and 12.35% reduction in proteins of early-, mid- and late-aged pupae, respectively). Moreover, the protein reduction runs parallel to the pupal age.

**Table 3: Total protein content in last instar larvae as influenced by treatment of newly moulted penultimate instar larvae of *S. littoralis* with $LC_{50}$ of Cyromazine.**

| Tissue | | | Larval age | | | |
|---|---|---|---|---|---|---|
| | | | 0-day old | 2-day old | 4-day old | 6-day old |
| Treated | Haemolymph (g/dL) | mean±SD | 5.05±0.07 a | 5.29±0.03 a | 5.28±0.05 a | 5.93±0.07 a |
| | | Change (%) | +1.20 | +4.19 | +4.76 | +0.85 |
| | Fat body (mg/g) | mean±SD | 76.51±0.59 d | 57.24±0.46 a | 52.87±0.40 a | 51.86±0.60 a |
| | | Change (%) | +27.50 | +5.73 | +0.57 | +3.81 |
| Control | Haemolymph (g/dL) | mean±SD | 4.99±0.07 | 5.01±0.11 | 5.04±0.04 | 5.88±0.21 |
| | Fat body (mg/g) | mean±SD | 60.08±0.36 | 54.15±0.42 | 52.57±0.43 | 49.90±0.38 |

a: See footnote of Table (1). d: See footnote of Table (2).

**Table 4: Total protein content in pupae as influenced by treatment of newly moulted penultimate instar larvae of *S. littoralis* with $LC_{50}$ of Cyromazine.**

| Homogenate | | Pupal age | | |
|---|---|---|---|---|
| | | 0-day old | 4-day old | 7-day old |
| Treated | mean mg/g±SD | 23.99±0.81 b | 34.22±0.33 b | 16.40±0.54 b |
| | Change (%) | -5.73 | -7.50 | -12.35 |
| Control | mean mg/g±SD | 27.28±1.04 | 37.00±1.08 | 18.71±0.66 |

b: See footnote of Table (1).

### 3. Effect of Diofenolan on protein content in *S. littoralis*

Data arranged in Table (5) show that protein content in haemolymph of control larvae gradually increased with the age but *vice versa* in fat body. Similar trends of protein content had been almost detected in the two tissues with the age of Diofenolan-treated larvae. According to data of this table, Diofenolan induced larvae to obtain slightly increased proteins in haemolymph, regardless the age (1.00, 1.20, 2.98 and 1.89% increments in 0-, 2-, 4- and 6-day old larvae, respectively). In respect of the fat bodies, data of the same table clearly reveal a similar prevalent promoting effect of Diofenolan on larvae to gain more proteins. However, the profoundly enhanced larvae were those of 0- and 2-day old since protein content conspicuously increased (8.17 and 8.87% increase, respectively). On the contrary, Diofenolan seriously suppressed the pupae, as obviously shown in data of Table (6), since their protein content was drastically depleted, regardless the age (10.52, 12.38 and 15.45% reduction in early-, mid- and late-aged pupae, respectively).

**Table 5: Total protein content in last instar larvae as influenced by treatment of newly moulted penultimate instar larvae of *S. littoralis* with $LC_{50}$ of Diofenolan.**

| Tissue | | | Larval age | | | |
|---|---|---|---|---|---|---|
| | | | 0-day old | 2-day old | 4-day old | 6-day old |
| Treated | Haemolymph (g/dL) | mean±SD | 5.04±0.04 a | 5.07±0.03 a | 5.19±0.06 a | 5.14±0.17 a |
| | | Change (%) | +1.00 | +1.20 | +2.98 | +1.89 |
| | Fat body (mg/g) | mean±SD | 64.96±1.31 b | 58.94±0.92 b | 54.68±2.36 a | 52.27±0.45 a |
| | | Change (%) | +8.17 | +8.87 | +4.00 | +4.61 |
| Control | Haemolymph (g/dL) | mean±SD | 4.99±0.07 | 5.01±0.11 | 5.04±0.04 | 5.08±0.21 |
| | Fat body (mg/g) | mean±SD | 60.08±0.36 | 54.15±0.42 | 52.57±0.43 | 49.90±0.38 |

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

**Table 6: Total protein content in pupae as influenced by treatment of newly moulted penultimate instar larvae of *S. littoralis* with $LC_{50}$ of Diofenolan.**

| Homogenate | | Pupal age | | |
|---|---|---|---|---|
| | | 0-day old | 4-day old | 7-day old |
| Treated | mean mg/g±SD | 24.41±1.18 b | 32.42±0.66 b | 15.82±0.98 b |
| | Change (%) | -10.52 | -12.38 | -15.45 |
| Control | mean mg/g±SD | 27.28±1.04 | 37.00±1.08 | 18.71±0.66 |

b: See footnote of Table (1).

## Discussion

### 1. Enhanced protein content in *S. littoralis*

Although the protein content was unaffected by some IGRs, as recorded in *M. domestica* after treatment with diflubenzuron or triflumuron (El-Kordy, 1985), many available literatures documented the influenced protein content in several insects by various IGRs and CSIs. Enhanced protein content in *S. littoralis* was reported after treatment with hexaflumuron, alone or combined with chlorfluazuron (Ghoneim, 1994), as well as with pyriproxyfen and chlorfluazuron (Farag, 2001; Abdel-Aal, 2003). Also, increasing protein content was determined in other insect species, such as *M. domestica* by methoprene or triflumuron (Bakr, 1986), *Muscina stabulans* by chlorfluazuron or hexaflumuron (Basiouny, 2000), *S. gregaria* by chlorfluazuron or pyriproxyfen (El-Sokkary, 2003) and *Bactrocera cucurbitae* by methoprene (ul Haq *et al*., 2010). To some extent, enhancing effects of Novaluron, Cyromazine and Diofenolan on the protein content in *S. littoralis* larvae, in the present study, ran in accordance with the previously reported results. In other words, increasing protein content in haemolymph and fat bodies of last instar larvae of all ages was estimated after treatment with Cyromazine or Diofenolan. Novaluron treatment resulted in increasing protein level in haemolymph of larvae, except ones of the early ages whose fat bodies only contained increasing proteins. Generally, changes in protein content probably reflect the balance between synthesis, storage, transport and degradation of structural and functional nutrients during ontogeny as well as response to particular physiological conditions (Shoukry *et al*., 2003). The increasing protein content, in the current work, could be in relation with the transport of this metabolite in haemolymph, released from the mobilization of the reserves, destined for the synthesis of new cuticle (Willis, 2010). This induction of 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 explained 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).

## 2. Suppressed protein content in *S. littoralis*

Inhibition of the total protein content in some tissues of *S. littoralis* was evidently reported for several IGRs and CSIs, such as diflubenzuron and triflumuron (Abdel-Hafez *et al*., 1988); ecdysteroid agonist RH-5849 (Smagghe and Degheele, 1992); pyriproxyfen, flufenoxuron and triflumuron (Mostafa, 1993); chlorfluazuron (Ghoneim, 1994); hexaflumuron (Sokar, 1995); pyriproxyfen and diflubenzuron (Ahmed, 2001); flufenoxuron and chlorfluazuron (Abdel-Aal, 2003, 2006); teflubenzuron (El-Sheikh *et al*., 2013); etc. In addition, depleted proteins had been reported in other insect species by various compounds, such as *S. gregaria* by fenoxycarb (El-Gammal *et al*., 1989), pyriproxyfen (Ghoneim *et al*., 2012) or flufenoxuron (Hamadah, 2014); *M. domestica* by diflubenzuron, triflumuron and methoprene (Bakr *et al*., 1991) or ecdysteroid agonist 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); *S. litura* by pyriproxyfen (Perveen and Miyata, 2000); *Tenebrio molitor* by ecdysteroid agonist Halofenozide (RH-0345) (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; etc. In agreement with these results of inhibited protein content by different compounds, decreasing protein content was determined in larvae and pupae of *S. littoralis*, in the present study. In other words, Novaluron exhibited an inhibitory effect on this metabolite in haemolymph, but not in fat bodies, of the newly moulted larvae. Moreover, this metabolite was reduced by Novaluron in fat bodies of other larvae. With regard to the developed pupae, all CSIs seriously suppressed the protein content, regardless the age.

Proteins are the known biological compounds which regulate and integrate several physiological and metabolic processes in the body through hormones, enzymes and nucleoproteins. However, reduction of the protein content in larval fat bodies by Novaluron or in pupae by all CSIs, *viz*, Novaluron, Cyromazine and Diofenolan, can be interpreted in the light of some acceptable suggestions. 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, 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 (Yu and Terriere, 1975; Hajjar and Casida, 1979) and affecting the metabolism (De Mark and Bennett, 1989) or protein synthesis in insects (Padmaje and Rao, 2000).

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).

## Conclusion

Because protein synthesis is necessary for the insect development and reproduction, the inhibited protein content in pupal stage of the present pest, *S. littoralis*, predicts an impaired adult performance and imperfect reproductive capability. Also, the tested CSIs may adversely affect the detoxifying enzymes in this pest. Therefore, the tested CSIs have a good potential in formulating novel IGR-based control agents against this pest in an environmentally-friendly manner to ecosystem.

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