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RESEARCH ARTICLE

Deteriorated acid and alkaline phosphatase activities in certain tissues of *Spodoptera littoralis* (Lepidoptera: Noctuidae) by some novel chitin synthesis inhibitors.

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Abstract

Objective of the present study was to investigate the effects of novel chitin synthesis inhibitors (CSIs), *viz*., Novaluron, Cyromazine, and Diofenolan, on the activities of acid (ACP) and alkaline (ALP) phosphatases in two larval tissues of *S. littoralis*. Each of LC_{50} values of these CSIs (2.71, 74.44 and 7.65 ppm, respectively) was applied on the penultimate instar larvae and the enzyme activities were determined in the successfully moulted last instar larvae of different ages. All CSIs enhanced larvae to gain increasing ACP activity in haemolymph, with few exceptions. In fat bodies, each of Novaluron and Cyromazine enhanced larvae to gain remarkably increasing enzyme activity, regardless the age. In contrast, Diofenolan exhibited an inhibitory effect on the enzyme activity, with an exception of 6-day old larvae. All CSIs promoted larvae to achieve elevated level of ALP in haemolymph, with few exceptions. On the contrary, they exhibited inhibitory effects on the enzyme activity in fat bodies of larvae, regardless the age.

Introduction

Several conventional synthetic insecticides have been used to control the population of Egyptian cotton leaf worm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae). Over the last three decades, the intensive use of broad-spectrum insecticides against this pest has led to the development of resistance against many registered pesticides, detrimental effects on the natural enemies, pollinators and all other non-target insects, and serious toxicological problems to humans and the environment (Miles and Lysandrou, 2002; Abo-El Ghar *et al*., 2005; Aydin and Gurkan, 2006; Davies *et al*., 2007; Costa *et al*., 2008; Relyea, 2009; Mosallanejad and Smagghe, 2009). For avoiding these problems, agrochemical research has focused on the discovery of alternative selective compounds which interfere with the pest insect growth and development and less toxic to non-target animals and environment. (Dhadialla *et al*., 2005). In this regard, insect growth regulators (IGRs) have captured the interest of entomologists (Mondal and Parween, 2000). At present, using IGRs is considered as the possible alternative way of synthetic insecticides for controlling this pest (Hussain, 2012).

IGRs are considered as the possible alternative way of conventional insecticides for controlling *S. littoralis* (Raslan, 2002) because they differ widely from the commonly used insecticides, as they exert their insecticidal effects through their influence on development, metamorphosis and reproduction of the target insects by disrupting the normal activity of the endocrine system (Oberlander *et al*., 1997). IGRs can be grouped according to their mode of action as chitin synthesis inhibitors (CSIs) and substances that interfere with the action of insect hormone (i.e. juvenile hormone analogues, ecdysteroids) (Tunaz and Uygun, 2004).

Novaluron is a relatively new benzoylphenyl urea CSI with good activity against the Colorado potato beetle (Cutler *et al*., 2007; Alyokhin *et al*., 2009) and low mammalian toxicity (Ishaaya and Horowitz, 2002). As reported by many authors (Tomlin, 1997; Ishaaya and Horowitz, 1998; Cutler *et al*., 2005a,b; Ishaaya *et al*., 2001, 2002, 2003; Mulla

et al., 2003; Su *et al*., 2003; Arrendondo-Jimenez and Valdez-Delgado, 2006; Cetin *et al*., 2006; Tawatsin *et al*., 2007; Mascari *et al*., 2007; Kostyukovsky and Trostanetsky, 2006; Jambulingam *et al*., 2009; Martin *et al*., 2010; Nwankwo *et al*., 2011; Bouaziz *et al*., 2011; Fontoura *et al*., 2012; Kamminga *et al*., 2012; Arthur and Fontenot, 2012; Portilla *et al*., 2012; Rajasekar and Jebanesan, 2012; Djeghader *et al*., 2013, 2014), Novaluron inhibits the chitin formation in larvae of various insects classified in Lepidoptera, Coleoptera, Homoptera and Diptera. This CSI generally is selective in favor of non-target organisms, such as natural enemies (Cutler *et al*., 2006). 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). Also, it has no cross-resistance with conventional insecticides, the juvenile hormone mimic pyriproxyfen and neonicotinoids (Ishaaya *et al*., 2005). Cyromazine is a triazine IGR used as alternative to insecticides and acaricides. As reported by many authors (Awad and Mulla, 1984; Binnington, 1985; Saito, 1988; Reynolds and Blakey, 1989; Keiding *et al*., 1992; Viñuela *et al*., 1993; Viñuela and Budia, 1994; Levot and Sates, 1998; Tomlin, 2000; Emea, 2001; Kamaruzzaman *et al*., 2006; Vazirianzadeh *et al*., 2007; Darriet *et al*., 2008; Mediouni-Ben Jemâa and Boushih, 2010; Al-Mekhlafi *et al*., 2011; Assar *et al*, 2012; Taylor *et al*., 2012**)**. Cyromazine exhibited various degrees of success for controlling different pests such as flies, stored product insect pests and leafminers. It is harmless to parasitoids (Beitia *et al*., 1991; Schuster, 1994) as well as to mammalian and poultry (Graf, 1993). It exhibited remarkable toxic and inhibitory effects on growth of *S. littoralis* (Tanani *et al*., 2015). Because of its inhibitory effects on the moulting process, it is possible to suggest that the mode of action is related to the developmental hormone, 20-hydroxyecdysone. However, the precise mode of Cyromazine action remains unknown (Wouw *et al*., 2006**)**. 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).

Acid phosphatase (ACP, E.C.3.1.3.2), known as a lysosomal marker enzyme (Csikos and Sass, 1997), is active in guts (Ferreira and Terra, 1980), Malpighian tubules (Srivastava and Saxena, 1967) and is also abundant in the disintegrating tissues and organs subjected to cytolysis (Sahota, 1975). This enzyme hydrolyzes a variety of orthophosphate esters and is capable of transphosphorylation reactions to increase the phosphate pool for synthesizing higher energy compounds as adenosine triphosphate (ATP), ATP ase, and genetic materials (DNA or RNA) (Hollander, 1971). Alkaline phosphatase (ALP, E.C.3.1.3.1) is primarily found in the intestinal epithelium of animals and its major function is to provide phosphate ions from mononucleotide and ribonucleo-proteins for a variety of metabolic processes. In insects, ALP is a brush border membrane marker enzyme (Ferreira and Terra, 1980; Wolfersberger, 1984) and is especially active in tissues with active membrane transport, such as intestinal epithelial cells (Sakharov *et al*., 1989; Caglayan, 1990), Malpighian tubules (Etebari and Matindoost, 2004 a, b) and haemolymph (Etebari *et al*., 2007). It is responsible for cytolysis of tissues during the insect development (Dadd, 1970). Its primary function is to provide phosphate ions from mononucleotide and ribonucleo-proteins for a variety of metabolic processes (Etebari *et al*., 2005). In addition to ACP, ALP may act as hydrolase during the final stages of digestion (Cheug and Low, 1975), gonad maturation and metamorphic moults (Rhadha and Priti, 1969). Its activity is low during the larval moulting stage and increased gradually after moulting (Miao, 1988).

The noctuid *S. littoralis* is distributed throughout the world but it is native to Africa (Shonouda and Osmam, 2000). It is a serious or major pest of cultivated crops primarily in tropical and subtropical regions, in Africa, Southern Europe, Middle East and Asia Minor (Brown and Dewhurst, 1975) and the Mediterranean area (Hosny *et al*., 1986; Bayoumi *et al*. 1998, Salama *et. al*., 1990; Azab *et al*., 2001; El-Aswad *et al*. 2003; Pineda *et al*., 2007). The present study was carried out aiming to investigate the effects of novel CSIs, *viz*. Novaluron, Cyromazine and Diofenolan, on ACP and ALP activities in two larval tissues of this economically major pest.

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^{o}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 by 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, Acid (ACP) and alkaline (ALP) phosphatase activities were determined in haemolymph and fat bodies of the successfully moulted last instar larvae of different ages.

3. Tissue preparation:

3.1. Larval haemolymph:

For the determination of the enzyme activities, haemolymph was collected from treated and control last (6^{th}) 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 enzyme activities, fat bodies (parietal and visceral) were carefully collected from the treated and control last instar larvae of the same ages. 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.

4. Determination of phosphatase activities:

ACP activity was determined in the larval tissues according to the method of Tietz (1986) using a kit of Biodiagnostics. The enzyme was measured at wave length 405 nm by spectrophotometer. ALP activity was determined in the larval tissues according to the method of Klein *et al*. (1960) using a kit of Biodiagnostics. The enzyme was measured at wave length 550 nm by 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. Effects on ACP activity:

ACP activity in haemolymph and fat bodies of last instar larvae was remarkably disturbed after treatment of penultimate instar larvae with LC_{50} of Novaluron. Data arranged in Table (1) obviously reveal a depressed activity in haemolymph of only newly moulted larvae (34.13±0.81 vs. 41.34±0.82 U/L in control larvae). Larvae of other ages had been stimulated to gain slightly or seriously elevated enzyme activity in haemolymph (0.73, 114.3 and 69.88% increments in 2-, 4- and 6-day old larvae, respectively). As easily seen in the same table, Novaluron continuously promoted larvae to attain significantly increasing ACP activity in fat bodies, regardless the age. The most potent enhancing effect of Novaluron was detected in late-aged larvae (100.27±0.43, compared to 43.99±0.48 U/L in control larvae).

With regard to the effects of Cyromazine on ACP activity, data assorted in Table (2) exiguously reveal a similar trend of decreasing and increasing activities. In some detail, ACP activity in haemolymph of newly moulted larvae was slightly reduced (40.42±0.63 vs. 41.34±0.82 U/L in control congeners) but larvae of other ages had been

stimulated to obtain pronouncedly raised level of activity (5.76, 118.63 and 69.47% increments in 2., 4- and 6-day old larvae, respectively). As clearly shown, the most powerful stimulatory effect of Cyromazine was exhibited on the 4-day old larvae. Also, ACP activity was insignificantly or significantly increased in fat bodies of larvae as a response to a prominent enhancing effect of Cyromazine (39.92±0.17, 41.15±0.53, 93.95±0.65 and 112.34±0.55 U/L in 0-, 2-, 4- and 6-day old larvae, respectively). As obviously seen, the strongest promoting action was exerted on the late-aged larvae (155.38% increment).
Data of altered ACP activity, as a response to Diofenolan, were presented in Table (3). Depending on these data, the enzyme activity was drastically regressed in haemolymph of newly moulted larva (13.16% reduction) but slightly or remarkably elevated in the same tissue of larvae of other ages (1.02, 116.24 and 65.98% increments in 2-, 4- and 6-day old larvae, respectively). In addition, Diofenolan exhibited an effect, in reciprocal trend, on ACP activity in fat bodies. In some detail, only late-aged larvae were enhanced to attain elaborately increasing activity while larvae of other ages suffered a prohibiting action of Diofenolan since ACP activity was tremendously reduced (61.28, 38.55 and 18.06% reductions, respectively).

2. Effects on ALP activity:
In the light of data contained in Table (4), late-aged larvae had been subjected to a strong inhibitory effect of Novaluron causing dramatically reduced ALP activity in haemolymph (24.07±0.16 vs. 51.42±0.71 U/L of control larvae) but to a powerful enhancing effect as detected in evidently increasing enzyme activity in fat body (132.76±0.82 vs. 122.34±1.57 U/L of control larvae). A reversal trend of effect was easily detected in larvae of other ages, depending on data of the same table, because ALP activity seriously increased in haemolymph (56.03, 35.62 and 2.69% increments, in 0-, 2- and 4-day old larvae, respectively) but dangerously suppressed in fat bodies (58.18, 50.00 and 14.62% reductions in 0-, 2- and 4-day old larvae, respectively).

Concerning the effects of Cyromazine on ALP activity, data presented in Table (5) clearly show considerably raising activity in haemolymph of 0-, 2- and 4-day old larvae (106.54, 57.55 and 35.24% increments, respectively) but severely descended activity in haemolymph of late-aged congeners (32.59±0.25 vs. 51.42±0.71 U/L of control larvae). In fat bodies, a prevalent prohibiting action of Cyromazine was exerted on ALP activity, regardless the larval age.

Data included in Table (6) exiguously reveal enhancing and inhibitory effects of Diofenolan in a similar trend as seen for Cyromazine. In other words, Diofenolan prohibited the late-aged larvae to achieve normal level of ALP in haemolymph (21.31% reduction) but promoted other larvae to gain increasing ALP activity in haemolymph (6.40, 6.76 and 17.23% increments in 0-, 2- and 4-day old larvae, respectively). In fat bodies, a prevalent inhibitory effect of Diofenolan was exhibited on ALP activity, regardless the larval age (for detail, see Table 6).

Table 1: ACP activity in the last instar larvae of *S. littoralis* after treatment with LC_{50} of Novaluron.

Tissue			Larval age			
			0-day old	2-day old	4-day old	6-day old
Treated	Haemolymph (U/L)	mean±SD	34.13±0.81 c	41.45±0.41 a	107.00±0.54 d	111.48±0.42 d
		Change (%)	-17.44	+0.73	+114.30	+69.88
	Fat body (U/L)	mean±SD	38.74±0.42 c	43.76±0.35 b	62.57±0.24 d	100.27±0.43 d
		Change (%)	+13.11	+11.21	+46.23	+127.94
Control	Haemolymph (U/L)	mean±SD	41.34±0.82	41.15±0.53	49.93±0.89	65.63±0.61
	Fat body (U/L)	mean±SD	34.25±0.75	39.35±0.37	42.79±0.48	43.99±0.48

Mean±SD followed with the letter (a): insignificantly 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).

Table 2: ACP activity in the last instar larvae of *S. littoralis* after treatment with LC_{50} of Cyromazine.

Tissue			Larval age			
			0-day old	2-day old	4-day old	6-day old
Treated	Haemolymph (U/L)	mean±SD	40.42±0.63 a	43.52±0.64 a	109.16±1.33 d	111.22±0.41 d
		Change (%)	-2.23	+5.76	+118.63	+69.47
	Fat body (U/L)	mean±SD	39.92±0.17 b	41.15±0.53 a	93.95±0.65 d	112.34±0.55 d
		Change (%)	+16.55	+4.47	+119.56	+155.38
Control	Haemolymph (U/L)	mean±SD	41.34±0.82	41.15±0.53	49.93±0.89	65.63±0.61
	Fat body (U/L)	mean±SD	34.25±0.75	39.35±0.37	42.79±0.48	43.99±0.48

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

Table 3: ACP activity in the last instar larvae of *S. littoralis* after treatment with LC_{50} of Diofenolan.

Tissue			Larval age			
			0-day old	2-day old	4-day old	6-day old
Treated	Haemolymph (U/L)	mean±SD	35.90±0.54 c	41.57±0.58 a	107.97±0.57 d	108.93±0.40 d
		Change (%)	-13.16	+1.02	+116.24	+65.98
	Fat body (U/L)	mean±SD	13.26±0.46 d	24.18±0.22 d	35.02±0.45 c	113.26±0.46 d
		Change (%)	-61.28	-38.55	-18.06	+157.47
Control	Haemolymph (U/L)	mean±SD	41.34±0.82	41.15±0.53	49.93±0.89	65.63±0.61
	Fat body (U/L)	mean±SD	34.25±0.75	39.35±0.37	42.79±0.48	43.99±0.48

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

Table 4: ALP activity in the last instar larvae of *S. littoralis* after treatment with LC_{50} of Novaluron.

Tissue			Larval age			
			0-day old	2-day old	4-day old	6-day old
Treated	Haemolymph (IU/L)	Mean±SD	44.14±0.35 d	41.31±0.48 d	34.68±0.23 a	24.07±0.16 d
		Change (%)	+56.03	+35.62	+2.69	-53.19
	Fat body (IU/L)	Mean±SD	37.07±0.75 d	49.06±0.82 d	94.28±0.94 d	132.76±0.82 d
		Change (%)	-58.18	-50.00	-14.62	+8.52
Control	Haemolymph (IU/L)	Mean±SD	28.29±0.24	30.46±0.65	33.77±0.66	51.42±0.71
	Fat body (IU/L)	Mean±SD	88.64±1.24	98.12±0.98	110.42±1.30	122.34±1.57

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

Table 5: ALP activity in the last instar larvae of *S. littoralis* after treatment with LC_{50} of Cyromazine.

Tissue			Larval age			
			0-day old	2-day old	4-day old	6-day old
Treated	Haemolymph (IU/L)	mean±SD	58.43±0.67 d	47.99±0.59 d	45.67±0.38 d	32.59±0.25 d
		Change (%)	+106.54	+57.55	+35.24	-36.62
	Fat body (IU/L)	mean±SD	25.78±0.59 d	39.85±0.95 d	48.59±0.98 d	51.80±0.95 d
		Change (%)	-70.92	-59.39	-56.00	-57.66
Control	Haemolymph (IU/L)	mean±SD	28.29±0.24	30.46±0.65	33.77±0.66	51.42±0.71
	Fat body (IU/L)	mean±SD	88.64±1.24	98.12±0.98	110.42±1.30	122.34±1.57

d: See footnote of Table (1).

Table 6: ALP activity in the last instar larvae of *S. littoralis* after treatment with LC_{50} of Diofenolan.

Tissue			Larval age			
			0-day old	2-day old	4-day old	6-day old
Treated	Haemolymph (IU/L)	mean±SD	30.10±0.20 a	32.52±0.24 a	39.59±0.28 c	40.46±0.15 d
		Change (%)	+6.40	+6.76	+17.23	-21.31
	Fat body (IU/L)	mean±SD	20.53±0.34 d	38.32±0.49 d	45.30±0.72 d	48.81±0.23 d
		Change (%)	-76.84	-60.95	-58.97	-60.10
Control	Haemolymph (IU/L)	mean±SD	28.29±0.24	30.46±0.65	33.77±0.66	51.42±0.71
	Fat body (IU/L)	mean±SD	88.64±1.24	98.12±0.98	110.42±1.30	122.34±1.57

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

Discussion:

Detoxification enzyme in insects is generally demonstrated as the enzymatic defense against foreign compounds and play significant roles in maintaining their normal physiological functions (Li and Liu, 2007). Four types of detoxifying enzymes have been found to react against insecticides, or compounds exhibi9ting insecticidal activities. These enzymes include general esterases, glutathione S-transferase and phosphatases (Zibaee *et al*., 2011). Induction of detoxification metabolic system plays an important role in insect's detoxification mechanism (Terriere, 1984). Acid (ACP) and alkaline (ALP) phosphatases are the hydrolytic enzymes, which hydrolyze phosphate monoesters under acid or alkaline milieus, respectively (Janda and Benesova, 1991). In insects, ACP and ALP are responsible for cytolysis of tissues during the insect development (Dadd, 1970). Also, they may act as hydrolases during the final stages of digestion (Cheug and Low, 1975), gonad maturation and metamorphic moults (Tsumuki and kanehisa, 1984). In general, these hydrolyzing enzymes are responsible for removing phosphate groups from many types of molecules, including nucleotides, proteins, and alkaloids in alkaline and acidic conditions, respectively under the name of dephosphorylation (Zibaee *et al*., 2011).

1. **Disturbed ACP activity in larvae of *S. littoralis* by CSIs:**

In larvae of *S. littoralis*, ACP activity increased after treatment with several IGRs and CSIs, such as diflubenzuron and triflumuron (Abdel-Hafez *et al*., 1989), hexaflumuron (Sokar, 1995), chlorfluazuron, flufenoxuron and pyriproxyfen (Abdel-Aal, 2003), diflubenzuron, hexaflumuron, flufenoxuron, chlorfluazuron, lufenuron and tebufenozide (Anwar and Abd el-Mageed, 2005), chlorfluazuron (Zohry, 2006), etc. Enhancement of ACP activity had been reported in other insects, such as *Heliothis armigra* by hexaflumuron and chlorfluazuron or binary mixtures of each with some insecticides (Abdeen *et al*., 1986); *Pectinophora gossypiella* and *Earias insulana* (Anan *et al*., 1993) and *Agrotis ipsilon* (El-Sheikh, 2002) by pyriproxyfen; *Culex pipiens* by the same IGR (El-Bassal, 1993) or Cyromazine (Assar *et al*., 2012); *Spodoptera litura* by sublethal doses of methoxyfenozide (Tian-jun and Tian, 2009); *M. domestica* by buprofezin, hexaflumuron, lufenuron, tebufenozide and pyriproxyfen (Assar *et al*., 2010); *Ephestia kuehniella* by pyriproxyfen (Sharifi *et al*., 2013); etc. In the present study on *S. littoralis*, Novaluron, Cyromazine and Diofenolan enhanced ACP activity in haemolymph of last instar larvae, with few exceptions. Also, remarkably increasing enzyme activity was determined in fat bodies of larvae after treatment with Novaluron or Cyromazine. The enhanced ACP activity may be attributed to increasing lysosome number as a response to the tested CSIs, as suggested for ecdysone which is responsible for increase of lysosomal ACP enzyme (Van Pelt-Verkuil, 1979; Bassal and Ismail, 1985). This result can be also interpreted since ACP, directly or indirectly, interferes with the food digestion and absorption (Smirle *et al*., 1996; Senthil Nathan *et al*., 2004).

In contrast, ACP activity was inhibited in larvae of *S. littoralis* by various IGRs and CSIs, such as 20-Hydroxyecdysone (Prasada, 1990), pyriproxyfen (Mostafa, 1993), triflumuron (El-Bermawy, 1994), higher concentration levels of chlorpyrifos (Eid, 2002), tebufenozide (Anwar and Abd el-Mageed, 2005), LC_{25} and LC_{70} of chlorfluazuron (Zohry, 2006), flufenoxuron (Bakr *et al*., 2010), tebufenozide and lufenuron (Bakr *et al*., 2013). Also, decreasing ACP activity was reported in other insects, such as *M. domestica* by methoprene (Qureshi *et al*., 1983), diflubenzuron (Shafi *et al*., 1986), triflumuron or pyriproxyfen (El-Bermawy, 1994; Hassanein *et al*., 1996); *A. ipsilon* (El-Sheikh, 2002) and *Bombyx mori* (Etebari *et al*., 2007) by pyriproxyfen; etc. In the current investigation on *S. littoralis*, treatment with Diofenolan, not with Novaluron or Cyromazine, resulted in decreasing activity of ACP in fat bodies of last instar larvae except 6-day old ones. This declined ACP level may be due to strong inhibitory effect of Diofenolan on the phosphorus libration for energy metabolism, rate of metabolism as well as rate of transport of ACP regulation (Senthil Nathan *et al*., 2005).

2. **Disturbed ALP activity in larvae of *S. littoralis* by CSIs:**

In the present work on *S. littoralis*, Novaluron, Cyromazine and Diofenolan promoted larvae to gain elevated level of ALP in haemolymph of last instar larvae, with few exceptions. This increasing ALP activity is, to a great extent, in agreement with the reported enzyme increase in the same insect species after treatment with pyriproxyfen (Abdel-Aal, 2003) and diflubenzuron or lufenuron (Anwar and Abd el-Mageed, 2005) as well as in other insects, such as *Anthonomus grandis* by diflubenzuron (Thompson and Korowisk, 1982); *P. gossypiella* (Mostafa, 1993), *E. insulana* (Anan *et al*., 1993) and *E. kuehniella* (Sharifi *et al*., 2013) by pyriproxyfen; *M. domestica* by buprofezin, hexaflumuron, lufenuron or tebufenozide (Assar *et al*., 2010); *Schistocerca gregaria* by pyridalyl (Teleb *et al*., 2012); etc. However, increasing ALP activity in haemolymph of *S. littoralis* larvae by tested CSIs, in the present study, may indicate the involvement of this enzyme in detoxification process against these CSIs (Shekari *et al*., 2008).

On the other hand, the available literature contains some reports of inhibited ALP activity in *S. littoralis* larvae by some IGRs and CSIs, such as chlorfluazuron and flufenoxuron (Anwar and Abd el-Mageed, 2005) and in larvae of other insect species, such as *C. pipiens* by diflubenzuron (Yan and Wu, 1990) or Cyromazine (Assar *et al*., 2012), and *B. mori* by pyriproxyfen (Etebari *et al*., 2007). In accordance with these reported results, Novaluron, Cyromazine and Diofenolan exhibited inhibitory effects on ALP activity in fat bodies in *S. littoralis* larvae, in the present study. However, this inhibition of ALP activity may be attributed to the effects of tested CSIs, directly or indirectly, on the juvenile hormone and ecdysone regulation (Sridhara and Bhat, 1963). It may be, also, explicated by some developmental disturbance as appreciably suggested by Wu (1990) for *C. pipiens* after treatment with Diflubenzuron.

It is important to point out that inhibition of the detoxifying enzymes, including ACP and ALP, indicates that these enzymes play no role in the detoxification of tested compounds and may increase the susceptibility of insect pest against these compounds (Abd-Elaziz and El-Sayed, 2009). On the other hand, increasing activities of these phosphatases denoted an increasing capability of the insect to detoxify the tested compounds (Sharifi *et al*., 2013). The ineffectiveness of IGRs in controlling insect pests is attributed to the increased levels of enzymatic detoxification, (Biddinger *et al*., 1996).

Conclusion:

Because the induction of detoxification metabolic system plays an important role in insect's detoxification mechanism, enhanced ACP and ALP activities in haemolymph of *S. littoralis* larvae by the present CSIs denote an increasing capability of the insect to detoxify them. On the other hand, inhibited enzyme activities in fat bodies indicate that these CSIs may not be detoxified by these enzymes. Therefore, tested CSIs, Diofenolan in particular, may be effective and can be used as a part in the integrated pest management of this pest.

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