

## **Disruptive Effects of Some Novel Chitin Synthesis Inhibitors on the Transaminase Activity in Larval Tissues of *Spodoptera littoralis* (Lepidoptera: Noctuidae)**

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**Abstract:** Objective of the present study was to investigate the effects of novel CSIs, viz., Novaluron, Cyromazine, and Diofenolan, on the activities of glutamic oxaloacetic (GOT) and glutamic pyruvic (GPT) transaminases in larval haemolymph and fat bodies of the economically dangerous insect, *Spodoptera littoralis*.  $LC_{50}$  of each CSI (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. Cyromazine and Diofenolan exhibited a prevalent enhancing effect on GOT activity in haemolymph of larvae of all ages. Novaluron exhibited a similar inducing effect on the enzyme activity except in 4-day old larvae. With regard to fat bodies, remarkably decreasing GOT activity was determined in larvae of all ages, regardless the CSI. Regarding GPT activity, CSIs exhibited contradictory effects in haemolymph of larvae since Novaluron elaborately promoted it, with an exceptional case of 6-day old larvae while Cyromazine and Diofenolan treatments resulted in conspicuously declined enzyme level, with few exceptions. Dealing with fat bodies, Cyromazine and Diofenolan enhanced the enzyme activity while Novaluron failed to exhibit an effect, irrespective of the larval age.

**Keywords:** Cyromazine, Diofenolan, fat body, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, haemolymph, larva, Novaluron

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### **1. INTRODUCTION**

Several conventional synthetic insecticides have been used to control the population of Egyptian cotton leafworm, *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 [1-7]. Consequently, alternative ways of controlling *S. littoralis* are very important. In this regard, insect growth regulators (IGRs) have captured the interest of entomologists [8]. At present, using IGRs is considered as the possible alternative way of synthetic insecticides for controlling this pest [9].

IGRs are considered as the possible alternative way of conventional insecticides for controlling *S. littoralis* [10] 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 [11]. 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) [12].

Novaluron is a relatively new benzoylphenyl urea CSI with good activity against the Colorado potato beetle [13, 14] and low mammalian toxicity [15]. As reported by many authors [16-41], 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 [42]. Also, it has no cross-resistance with conventional insecticides, the juvenile hormone mimic pyriproxyfen and neonicotinoids [43]. Cyromazine is a triazine IGR used as alternative to insecticides and acaricides. As reported by many authors [44-60], Cyromazine exhibited various degrees of success for controlling different pests such as flies, stored product insect pests and

leafminers. It is harmless to parasitoids [61, 62] as well as to mammalian and poultry [63]. 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 [64]. Diofenolan is a CSI used for the control of several pests, such as lepidopterous species and scale insects [65- 67], *Papilio demoleus* [68], *Musca domestica* [69-72], *Rhynchophorus ferrugineus* [73] and *Schistocerca gregaria* [74-77]. It did not affect the survival of beneficial parasitoids and predators of some pests such as *Chrysoperla carnea* [78].

Transamination has been demonstrated in a number of insect tissues, particularly that concerning glutamate, aspartate and alanine [79]. The Glutamic oxaloacetic transaminase (GOT, official name: aspartate aminotransferase, AST) and Glutamic pyruvic transaminase (GPT, official name: alanine aminotransferase, ALT) are key enzymes in the formation of non-essential amino acids, in metabolism of nitrogen waste, gluconeogenesis and correlated with protein anabolism and catabolism [80]. Moreover, transaminases, especially GPT, acts as a catalytic agent in carbohydrates metabolism [81].

The noctuid *S. littoralis* is distributed throughout the world but it is native to Africa [82]. 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 [83] and the Mediterranean area [84-89]. Objective of the present study was to investigate the effects of novel CSIs, viz. Novaluron, Cyromazine and Diofenolan, on the GOT and GPT activities in two larval tissues of this economically major pest.

## 2. MATERIALS AND METHODS

### 2.1. 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 ( $27\pm 2^{\circ}\text{C}$ ,  $65\pm 5\%$  R.H., photoperiod 14 h L and 10 h D). Rearing procedure was carried out according to Ghoneim [90] and improved by Bakr *et al.* [91]. 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.

### 2.2. Larval Treatments with CSIs

Novaluron (Rimon, Pestanal<sup>®</sup>) [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<sup>®</sup>)[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<sub>50</sub> 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<sub>50</sub> of each compound, GOT and GPT activities were determined in haemolymph and fat bodies of the successfully moulted last instar larvae of different ages.

### 2.3. Larval Tissue Preparation

#### 2.3.1. Haemolymph

For the determination of the enzyme activities, haemolymph was collected from treated and control 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.

### **2.3.2. 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.

### **2.4. Determination of Transaminase Activities**

GOT and GPT activities were determined in the larval tissues according to the method of Harold [92] using a kit of Bioadwic. The enzyme was measured at wave length 546 nm by spectrophotometer.

### **2.5. Statistical Analysis of Data**

Data obtained were analyzed by the Student's *t*-distribution, and refined by Bessel correction [93] for the test significance of difference between means.

## **3. RESULTS**

### **3.1. Effects on GOT Activity**

Depending on the data arranged in Table (1), Novaluron enhanced larvae during the first half of the instar to gain elevated level of GOT in haemolymph (27.28 and 21.43% increments in 0- and 2-day old larvae, respectively) but prohibited larvae of 4-day old in significantly reduced enzyme activity ( $28.33 \pm 1.03$ , compared to  $31.67 \pm 2.12$  U/mL of control larvae) and failed to affect the late-aged ones. With regard to Cyromazine, data of Table (2) clearly show a strong prevalent inducing effect since the enzyme activity was unexceptionally increased in haemolymph (72.78, 42.86, 15.79 and 25.02% increments in 0-, 2-, 4- and 6-day old larvae, respectively). As obviously seen in Table (3), Diofenolan failed to affect GOT activity in haemolymph of newly moulted larvae but promoted other larvae to gain slightly or considerably increasing activity (14.32, 5.24 and 25.02% increments in 2-, 4- and 6-day old larvae, respectively).

In connection with the GOT activity in fat bodies, data assorted in the previously mentioned tables exiguously reveal a powerful prohibiting action of all compounds on larvae of all ages to achieve remarkably decreasing GOT activity. In some detail, the enzyme activity was pronouncedly decreased in 47.37, 50.11, 48.00 and 28.55% reductions in larvae of 0-, 2-, 4- and 6-day old, respectively, in case of Novaluron (see Table 1). Cyromazine caused 57.89, 50.01, 48.00 and 28.55% reductions in larvae of the same ages, respectively (Table 2). Also, Diofenolan treatment resulted in tremendously declined GOT activity (42.10, 32.14, 32.01 and 21.43% reductions in larvae of the same ages, respectively (Table 3).

### **3.2. Effects on GPT Activity**

According to data presented in Table (4), Novaluron promoted 0-, 2- and 4-day old larvae to obtain slightly or considerably raising GPT level (0.69, 100.05 and 5.24% increments, respectively) but late-aged ones were prohibited to attain normal level of GPT activity (30.0% reduction). After treatment with Cyromazine, data of determined GPT activity were listed in Table (5). Depending on these data, GPT activity was conspicuously elevated in haemolymph of only 2-day old larvae ( $41.67 \pm 1.11$ , compared to  $18.33 \pm 1.18$  U/mL of control larvae) but remarkably decreased in other larvae (23.60, 15.79 and 45.00% reductions in 0-, 4- and 6-day old larvae, respectively). As evidently shown in Table (6), Diofenolan failed to affect the GPT activity in haemolymph of 2-day old larvae but insignificantly or significantly inhibited it in larvae of other ages (65.27, 31.58 and 19.98% reductions in 0-, 4- and 6-day old larvae, respectively).

In respect of fat bodies, Novaluron exhibited no effect on larvae of 0-, 2- and 6-day old but stimulated 4-day old larvae to achieve elaborately high level of GPT ( $91.67 \pm 3.14$  vs.  $83.33 \pm 1.88$  U/mL of control larvae, see Table 4). A predominant enhancing effect was exhibited by both Cyromazine and Diofenolan on larvae of all ages to gain increasing GPT activity. In some detail, Cyromazine treatment resulted in 30.22, 31.92, 36.00 and 1.13% increments in fat bodies of 0-, 2-, 4- and 6-day old larvae, respectively (see Table 5) and Diofenolan treatment resulted in 30.22, 57.45, 78.00 and 3.41% increments in fat bodies of the congeners, respectively (see Table 6).

**Table1.** GOT Activity in Last Instar Larvae of *S. littoralis* after Treatment with LC<sub>50</sub> of Novaluron

Tissue			Larval age			
			0-day old	2-day old	4-day old	6-day old
Treated	Haemolymph (U/ml)	mean±SD	23.33±2.89 d	28.33±1.03 d	28.33±1.03 b	33.33±2.00 a
		Change (%)	+27.28	+21.43	-10.55	0.00
	Fat body (U/ml)	mean±SD	33.33±0.88 d	23.33±2.89 d	21.67±1.61 d	16.67±1.75 d
		Change (%)	-47.37	-50.11	-48.00	-28.55
Control	Haemolymph (U/ml)	mean±SD	18.33±1.13	23.33±0.93	31.67±2.12	33.33±1.50
	Fat body (U/ml)	mean±SD	63.33±2.22	46.67±1.17	41.67±1.01	23.33±0.66

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

**Table2.** GOT Activity in Last Instar Larvae of *S. littoralis* after Treatment with LC<sub>50</sub> of Cyromazine

Tissue			Larval age			
			0-day old	2-day old	4-day old	6-day old
Treated	Haemolymph (U/ml)	mean±SD	31.67±1.45 d	33.33±2.04 d	36.67±2.88 c	41.67±2.13 d
		Change (%)	+72.78	+42.86	+15.79	+25.02
	Fat body (U/ml)	mean±SD	26.67±2.80 d	23.38±0.96 d	21.55±1.75 d	16.44±0.75 d
		Change (%)	-57.89	-50.01	-48.00	-28.55
Control	Haemolymph (U/ml)	mean±SD	18.33±1.13	23.33±0.93	31.67±2.12	33.33±1.50
	Fat body (U/ml)	mean±SD	63.33±2.22	46.67±1.17	41.67±1.01	23.33±0.66

(c): highly significantly different ( $P < 0.005$ ). (d): See footnote of Table (1).

**Table3.** GOT Activity in Last Instar Larvae of *S. littoralis* after Treatment with LC<sub>50</sub> of Diofenolan

Tissue			Larval age			
			0-day old	2-day old	4-day old	6-day old
Treated	Haemolymph (U/ml)	mean±SD	18.33±0.66 a	26.67±1.46 c	33.33±1.04 a	41.67±2.00 d
		Change (%)	0.00	+14.32	+5.24	+25.02
	Fat body (U/ml)	mean±SD	36.67±1.88 d	31.67±0.52 d	28.33±1.52 d	18.33±1.10 d
		Change (%)	-42.10	-32.14	-32.01	-21.43
Control	Haemolymph (U/ml)	mean±SD	18.33±1.13	23.33±0.93	31.67±2.12	33.33±1.50
	Fat body (U/ml)	mean±SD	63.33±2.22	46.67±1.17	41.67±1.01	23.33±0.66

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

**Table4.** GPT Activity in Last Instar Larvae of *S. littoralis* after Treatment with LC<sub>50</sub> of Novaluron

Tissue			Larval age			
			0-day old	2-day old	4-day old	6-day old
Treated	Haemolymph (U/ml)	mean±SD	48.33±1.55 a	36.67±2.66 d	33.33±0.96 a	23.33±0.89 a
		Change (%)	+0.69	+100.05	+5.24	-30.00
	Fat body (U/ml)	mean±SD	71.67±2.45 a	78.33±1.68 a	91.67±3.14 b	146.67±2.67 a
		Change (%)	0.00	0.00	+10.01	0.00
Control	Haemolymph (U/ml)	mean±SD	48.00±2.89	18.33±1.18	31.67±1.37	33.33±2.16
	Fat body (U/ml)	mean±SD	71.67±3.18	78.33±2.89	83.33±1.88	146.67±2.67

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

**Table5.** GPT Activity in Last Instar Larvae of *S. littoralis* after Treatment with LC<sub>50</sub> of Cyromazine

Tissue			Larval age			
			0-day old	2-day old	4-day old	6-day old
Treated	Haemolymph U/ml)	mean±SD	36.67±2.89 d	41.67±1.11 d	26.67±1.91 c	18.33±2.03 d
		Change (%)	-23.60	+127.33	-15.79	-45.00
	Fat body (U/ml)	mean±SD	93.33±2.99 d	103.33±2.11 d	113.36±3.05 d	148.51±2.77 a
		Change (%)	+30.22	+31.92	+36.00	+1.13
Control	Haemolymph U/ml)	mean±SD	48.00±2.89	18.33±1.18	31.67±1.37	33.33±2.16
	Fat body (U/ml)	mean±SD	71.67±3.18	78.33±2.89	83.33±1.88	146.67±2.67

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

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**Table6.** GPT Activity in Last Instar Larvae of *S. littoralis* after Treatment with LC<sub>50</sub> of Diofenolan

Tissue			Larval age			
			0-day old	2-day old	4-day old	6-day old
Treated	Haemolymph U/ml	mean±SD	16.67±1.55 d	18.33±0.97 a	21.67±1.12 d	26.67±2.33 c
		Change (%)	-65.27	0.00	-31.58	-19.98
	Fat body (U/ml)	mean±SD	93.33±2.65 d	123.33±3.44 d	148.33±2.17 d	151.67±2.89 a
		Change (%)	+30.22	+57.45	+78.00	+3.41
Control	Haemolymph (U/ml)	mean±SD	48.00±2.89	18.33±1.18	31.67±1.37	33.33±2.16
	Fat body (U/ml)	mean±SD	71.67±3.18	78.33±2.89	83.33±1.88	146.67±2.67

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

#### 4. DISCUSSION

Because GOT (=AST) and GPT (=ALT) help in the production of energy and serve as a strategic link between the carbohydrate and protein metabolism, they are being altered during various physiological and pathological conditions [94, 95]. They may also play an important role in the insecticidal poisoning [96]. Various IGRs (including CSIs), were reported as disruptive agents on GOT and/or GPT activities in different insects. Some IGRs prohibited the enzyme activities while other IGRs enhanced them. Moreover, same IGR may enhance or prohibit the enzyme activities depending on the developmental stage of the insect and its tissue [97-100]. Generally, it is assumed that the control of transaminase activities in the insect body is achieved through secondary feed-back or homeostatic mechanisms adapted to spontaneous or hormonally induced alterations [101].

##### 4.1. Disturbed GOT Activity in Larvae of *S. littoralis* by CSIs

In the present study on *S. littoralis*, each of Cyromazine and Diofenolan exhibited a prevalent enhancing effect on GOT activity in haemolymph of last instar larvae of all ages. Novaluron exhibited a similar inducing effect on the enzyme activity except in 4-day old larvae which contained declined level of activity. With regard to fat bodies, remarkably decreasing GOT activity was determined in larvae of all ages, regardless the CSI. The enhanced GOT activity in haemolymph is, to some extent, in agreement with the increasing activity reported for the same insect species after treatment with several IGRs or insecticides, such as hexaflumuron [102], pyriproxyfen, flufenoxuron or teflubenzuron [98], hexaflumuron alone or its binary mixture with chlorpyrifos [103], pyriproxyfen, flufenoxuron or chlorfluazuron [104] and flufenoxuron [105]. In addition to *S. littoralis*, some IGRs enhanced GOT activity in *Pectinophora gossypiella* [106] and *M. domestica* [107] by pyriproxyfen as well as in *Culex pipiens* by Cyromazine [59].

On the other hand, the predominant inhibitory effect of CSIs on GOT activity in fat bodies of larvae, in the present study, is consistent with some of the reported results of decreasing enzyme activity in the same insect species after treatment with diflubenzuron or triflumuron [108], chlorfluazuron [109], hexaflumuron [102], pyriproxyfen, flufenoxuron or chlorfluazuron [104], chlorfluazuron or tebufenozide [110], flufenoxuron or chlorfluazuron [105], etc. Also, GOT activity was prohibited in other insects by some IGRs, such as *P. gossypiella* and *Earias insulana* by pyriproxyfen [106], *Agrotis ipsilon* [111] and *Bombyx mori* [95] by the same IGR; *M. domestica* by hexaflumuron or lufenuron [107], *C. pipiens* by Cyromazine [59]. The general declination of GOT activity in fat bodies of *S. littoralis* after treatment with CSIs, in the present study, may be due to the difficulty in formation of dissociable enzyme-inhibitor complexes which reduce the specific enzyme activity [112] or they disturbed the link between the carbohydrate and protein metabolism.

##### 4.2. Disturbed GPT Activity in Larvae of *S. littoralis* by CSIs

In the present study, CSIs exhibited contradictory effects on GPT activity in haemolymph of last instar larvae of *S. littoralis* since Novaluron elaborately promoted it, with an exceptional case of 6-day old larvae while Cyromazine and Diofenolan treatments resulted in conspicuously declined enzyme level, with few exceptions. Dealing with fat bodies, Cyromazine and Diofenolan enhanced the

enzyme activity while Novaluron failed to exhibit an effect, irrespective of the larval age. Enhanced GPT activity in haemolymph of larvae by Novaluron or in fat bodies by Cyromazine and Diofenolan is, to a great extent, in accordance with the increasing activity in the same insect by some IGRs and CSIs, such as hexaflumuron [102] and pyriproxyfen, flufenoxuron or chlorfluazuron [104]. Also, stimulated GPT activity was reported for some of other insects by different IGRs, such as *P. gossypiella* and *E. insulana* [106] and *M. domestica* [107] by pyriproxyfen as well as *Bactrocera zonata* by malathion, diazinon, methoxyfenozide or lufenuron [113] and *C. pipiens* by Cyromazine [59].

On the other hand, declined level of GPT in haemolymph of *S. littoralis* larvae by Cyromazine and Diofenolan, in the present study, agrees with the decreased activity in the same insect by several IGRs and CSIs, such as triflumuron [108], chlorfluazuron [109], hexaflumuron [102], pyriproxyfen, flufenoxuron or teflubenzuron [98], pyriproxyfen, flufenoxuron or chlorfluazuron [104] and flufenoxuron [105]. In other insect species, decreasing GPT activity was reported in *P. gossypiella* and *E. insulana* by pyriproxyfen [106], *A. ipsilon* by the same IGR [95], *M. domestica* by hexaflumuron or lufenuron [107] and *C. pipiens* by Cyromazine [59]. The inhibited GPT activity in haemolymph of larvae of *S. littoralis* by Cyromazine and Diofenolan, in the present study, can be understood since pyruvate is the precursors of Krebs cycle compounds, concerned with the mitochondrial oxidation phenomenon and ATP products [94]. However, diverse effects of the tested CSIs on GPT activity in larvae may be due to their effects on the synthesis or functional levels of this enzyme directly or indirectly by altering the cytomorphology of the cells [114] or the neurosecretory hormonal pattern.

The increasing activity of transaminases, in the current work, may be attributed to the occurrence of reversible binding between the tested CSIs and enzymatic site of action on the enzyme surface. This may be due to the fact that the relationships between protein synthesis and transaminase levels were affected by the hormonal control of protein synthesis and neurosecretory hormones which involved in the regulation of transaminase levels [115]. Thus, the present CSIs may intervene in the hormonal control of protein synthesis and neurosecretory hormones involved in the regulation of transaminase levels [116]. However, the exact mode of action of the tested CSIs on transaminase regulation is still controversial until now!!

## 5. CONCLUSION

The disturbance of transaminase activities by the present CSIs, in the present study, may be lead to disturbance of protein metabolism and synthesis of some specific compounds. Thus, these CSIs will disrupt many physiological functions and ultimately lead to death, i.e., they can be used as a part in the integrated pest management program against this dangerous pest.

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