Egyptian Journal of Plant
Protection Research Institute
www.ejppri.eg.net

Biochemical effects of three insect growth regulators against cotton leafworm, *Spodoptera littoralis* (Lepidoptera: Noctuidae)

Thabet, M. M.; Elawdy, S. M.; El-Ansari, M. and Megahed, M. M. M.
Plant Protection Department, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt.

ARTICLE INFO
Article History
Received: 24/1 /2024
Accepted:19 /3/2024

Keywords

Biochemical effects, insect growth regulators (IGRs), and *Spodoptera littoralis.*

Abstract

The current study was carried out to evaluate the biochemical effects of 4th and 6th instar larvae of *S. littoralis* provided with treated castor bean leaves for three different insect growth regulators **(**IGRs) (Novaluron, Pyriproxyfen, and Cyromazine). To determine the effect of these IGRs on total protein, total carbohydrate, total lipids, the activity of chitinase, the activity of acetylcholinesterase and carbohydrates hydrolyzing enzymes (Amylase, invertase, and trehalase activities) at LC_{50} concentrations of this treatment. The results showed that total proteins, lipids, and carbohydrate content were significantly decreased with all tested IGRs, except for an increase in total carbohydrates with Novaluron and Cyromazine for the 6th instar larvae of *S. littoralis***.** Cyromazine induced a significant decrease in chitinase activity, followed by Pyriproxyfen then Novaluron. The tested IGRs significantly increased the acetylcholinesterase activity. A significant decrease in the activity of amylase and Invertase activity was induced by the tested IGRs, except with Novaluron for 6th instar larvae in the case of amylase and increased with all treatments for 6th instar larvae of Invertase, in contrast, all tested IGRs led to an increase in the activity of Trehalasec except Cyromazine decreased the enzyme activity.

Introduction

The cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae), is a great polyphagous pest attacking a wide range of economic crops such as cotton in Egypt. It has a minimum of 7-9 generations during the cotton season, additionally infesting more than 29 other crops and vegetables (El-Din and El-Gengaihi, 2000).

Cotton leafworm (CLW) larvae mainly feed on leaves and stems and can extremely postpone growth or decrease cotton production. Furthermore, during heavy infestations, CLW can also penetrate flowers and bolls, causing a substantial loss of up to 50% reduction in yield (Russell *et al.*, 1993). Although the level of field infestations of the Egyptian cotton leafworm, *S. littoralis* has decreased in recent years, it is still considered one of the most destructive insect pests in cotton growing areas not only in Egypt but also in Africa, Asia, and Europe (Smagghe and Degheele, 1997).

Physiological differences in herbivores are relatively easy and are often the first step in studies of differential host use (Pashley *et al.*, 1995), although some farmers in Egypt laboriously, and pick the egg

batches to control *S. littoralis* population, most farmers prefer using chemical pesticides, which are detrimental to natural enemies, pollinators and all other non-target insects. At present, using insect growth regulators (IGRs) is considered as the possible alternative way of synthetic insecticides for controlling this pest (Raslan, 2002).

Insect growth regulators (IGRs) received great attention as a hope for controlling insects in the future that showed diverse effects against *S. littoralis*, and caused large selectivity to beneficial insects (Raslan, 2002). Using IGRs pesticides could result in growth reduction, moulting inhibition, anatomical abnormalities as well as mortality, in a wide range of insect species, most of them belonging to Order Lepidoptera, where its action depends on insect species and the applied concentration (Khedr *et al.,* 2005).

An insect growth regulator (IGR) is a chemical that interferes with the growth and development of an insect and in turn inhibits its life cycle. An IGR does not always have to be toxic to its target organism, instead, it may cause various abnormalities (Siddall, 1976). Pyriproxyfen, methoxyfenozide, novaluron, cyromazine, tebufenozide, diflubenzuron, hydroprene, methoprene etc. are some examples of IGRs. The ongoing research objective was evaluating the effects of IGRs against CLW 4th and 6th larval instars and the influence of the latent effect of tested IGRs on some biochemical aspects of CLW.

Materials and methods

1. Insect rearing:

The strain of Egyptian cotton leafworm, *S. littoralis* larvae used in the present study was obtained from the Plant Protection Research Institute, Agricultural Research Center, Doqqi, Giza, Egypt. These larvae were reared in the laboratory at the Department of Plant Protection, Faculty of Agriculture, Al-Azhar University, Cairo. Larvae were reared under laboratory-controlled conditions (25±2^{o}C, 65±5% R.H., photoperiod 12 h L: 12 h D) as described by El-Defrawi *et al*. (1964). The 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 emerging adults were provided with a 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 insect growth regulators:

The selected IGRs common names, trade names, percentage of active ingredients, formulation types, manufacturer, and mode of action.

2.1. Common name: Novaluron
Trade name: Roxy® 10%EC
Chemical name: 1-[3-chloro-4-(1,1,2-trifluoro-2-trifluoromethoxyethoxy) phenyl]-3- (2,6- difluorobenzoyl) urea.
Mode of action: Chitin Synthesis Inhibitor.
Chemical formula: $C_{17}H_9ClF_8N_2O_4$

2.2. Common name: Pyriproxyfen
Trade name: Admafin® 10% EC
Chemical name: 4-phenoxyphenyl (*RS*)-2-(2-pyridyloxy) propyl ether 2-[1-(4-phenoxyphenoxy) propan-2- yloxy] pyridine.
Mode of action: Juvenile Hormone Mimics
Chemical formula: $C_{20}H_{19}NO_3$

2.3. Common name: Cyromazine
Trade name: Trigard®75% WP
Chemical name: [N-cyclopropyl-1,3,5-triazine-2,4,6-triamine]
Mode of action: Moulting Inhibitors
Chemical formula: $C_6H_{10}N_6$

3. Biochemical bioassays:

3.1. Sample preparation:

Three samples of 4th and 6th larval instars of *S. littoralis* provided with treated castor bean leaves, were placed in plastic

tubes kept in a deep freezer set at -17±1ºC until needed for biochemical assays. These larvae were homogenized in phosphate buffer (pH 7) using a Teflon tissue homogenizer. The insects were homogenized in distilled water (50 mg./ml.) The homogenates were centrifuged at 8000 rpm for 10 min at 5 ºC in a refrigerated centrifuge. The deposits were discarded, and the supernatant was kept in a deep freezer until needed for the determination of the following:

3.2. Determination of total protein content:

Total proteins were estimated by the method described by Bradford (1976) using standard Bovine serum albumin.

3.3. Determination of total carbohydrate content:

Total carbohydrates were determined by the method described by Singh and Sinha (1977) using an anthron reagent.

3.4. Determination of total lipid content:

Total lipid content in *S. littoralis* larval homogenate was estimated according to the method described by Knight *et al*. (1972) using phosphovanillin reagent and standard curve.

3.5. Determination of chitinase activity:

Chitinase was assayed using 3,5-dinitrosalicylic acid reagent to determine the free aldehydic groups of hexosamine liberated on chitin digestion according to the method described by Ishaaya and Casida (1974).

3.6. Determination of acetylcholinesterase enzyme activity:

The activity of acetylcholine esterase enzyme (AChE) was measured according to Simpson *et al*. (1964) using acetylcholine bromide (AChBr) as substrate.

3.7. Determination of carbohydrate hydrolyzing enzyme activities:

The method was based on the digestion of trehalose, starch, and sucrose by trehalase, amylase, and invertase, respectively, according to the method described by Ishaaya and Swirski (1976).

4. Statistical analysis:

Mortality data was corrected using Abbott's Formula (Abbott, 1925), and the LC_{50} value was expressed as ppm with fiducial limits (FL) and slope which was calculated using probit analysis (Finney, 1971). Data were subjected to ANOVA by using "Costat" program (1988) and significant differences among the treatments were portioned by LSD test at probability levels of (P= 0.05) according to Duncan (1955).

Results and discussion

1. Total protein content (µU/mg protein):

Protein has always been an interesting biochemical tool for insect biochemists because of its potential role in growth, development, morphogenesis, and many intermediaries of the metabolic pathway of insects (Kar *et al.*, 1994).

The present study showed a significant decrease in the total proteins at all the tested IGRs, compared with that of the control. total proteins in larvae treated with Cyromazine were significantly dropped following the treatment with Pyriproxyfen and Novaluron (Table 1). The total protein content of the 4th and 6th instar larvae of *S. littoralis* was decreased with all treatments. The total protein for the fourth larval instar was 23.86, 17.52, and 15.28 (uU/mg protein) with Novaluron, Pyriproxyfen, and Cyromazine, respectively as compared with control 25.32 (uU/mg protein). The results obtained were that the total protein content of *S. littoralis* for 6th larvae was 21.90, 20.16, and 18.60 (uU/mg protein) with Novaluron, Pyriproxyfen, and Cyromazine, respectively as compared with control 21.52 (µU /mg protein).

These results are in agreement with that obtained by Awadalla *et al.* (2017) who noticed that the content of total protein was decreased in the 4th larval instar of *S. littoralis* treated with lufenuron belonging to the IGRs group. El-Gabaly (2015) in Egypt, indicated

that the lufenuron at their LC_{50} values caused a decrease in total protein content of the 4th larval instar of *S. littoralis* relative to control. This reduction in the protein content may be due to the inhibition of DNA and RNA synthesis.

The decrease of the total protein in the treated 4th larval instar may reflect the decrease in the enzymatic activities of various enzymes. These results are those demonstrated by Abd El-Aziz *et al*. (2007). Assar *et al.* (2016) indicated that the total protein content was decreased when treated 4th larval instar of *S.littoralis* with teflubenzuron and hexaflumuron as insect growth regulators. Total proteins are the major biochemical components necessary for an organism to develop, grow, and perform its vital activities. The reduction of protein content may be due to inhibition of DNA and RNA synthesis (Elbarky *et al.*, 2008).

Extensive work has been carried out to determine how various toxic agents affect protein synthesis. A diminution in the rate of ATP synthesis and inhibition of RNA synthesis are also the main causes of decreased total protein content (Nabih *et al.*, 1990). Ahmed *et al.* (1993) and Rawi *et al.* (1995) reported that protein leakage during intoxication might arise from reduced body weight, conversion of protein to amino acids, degradation of protein to release energy, or the direct effect of the tested compounds on the amino acid transport of the cell. Protein helps to synthesize microsomal detoxifying enzymes which assist in the detoxification of toxicants that into the insect body. Proteins are the most important components of the biochemical of insects that bind the foreign compounds. In general, the problem of protein synthesis is intimately related to the metabolism of nucleic acids (Wilkinson, 1976).

Table (1): Effect of LC_{50} of the three tested IGRs on the total proteins (µU /mg protein) of 4th and 6th instar larvae of *Spodoptera littoralis.*

Treatments		**4th instar larvae**	**6th instar larvae**
Control		25.32±0.41	21.52±0.76
Novaluron		23.86±0.58	21.90±0.39
Pyriproxyfen		17.52±0.23	20.16±2.18
Cyromazine		15.28±0.94	18.60±0.19
LSD at 5%	**Treat. + Cont.**	1.12*	2.21*
	Treat.	1.29*	2.57ns

2. Total carbohydrate content (mg/g body weight):

Carbohydrates play a major role in insect development metabolism, metamorphosis, development of flight muscles, reproduction, and embryonic development (Chapman, 1998). Results given in Table (2) indicated that all tested treatments led to significant changes in total carbohydrates which were more obvious compared with the control. Total carbohydrate content decreased significantly for the 4th instar larvae were 3.77, 2.19, and 2.22 (mg/g.b.wt.) for Novaluron, Pyriproxyfen, and Cyromazine, respectively, while it was 5.15 (mg/g.b.wt) with control. While the value of total Carbohydrates in the 6th instar larvae was 4.60, 2.30, and 3.52 (mg/g.b.wt.) for Novaluron, Pyriproxyfen, and Cyromazine, respectively, while it was 2.33 (mg/g.b.wt.) with control. These results are like those of Awadalla *et al.* (2017) who observed this significant decrease in carbohydrate content at *S. littoralis* in the 4th larval instar after treatment with lufenuron.

Table (2): Effect of LC_{50} of the three tested insect growth regulators on the total carbohydrates (mg/g body weight) on 4th and 6th instar larvae of *Spodoptera littoralis.*

Treatments		4th instar larvae	6th instar larvae
Control		5.15±0.83	2.33±0.35
Novaluron		3.77±0.26	4.60 ± 0.56
Pyriproxyfen		2.19±0.31	2.30±0.20
Cyromazine		2.22±0.14	3.52±0.24
LSD at5%	**Treat. + Cont.**	0.87*	0.68*
	Treat.	0.49*	0.73*

Abdel-Aal (2012) studied the insecticidal and biological effects of three insect growth regulators (Chlorfluazuron, Tebofenozoid, and pyriproxyfen) were evaluated on 4th instar larvae of *S. littoralis*, furthermore, different levels of significant changes in the total protein, carbohydrate contents of the female ovaries pre-treated as 4th instar larvae by LC_{50} of used IGRs were recorded. Moreover, different abnormal histological structures of the ovary were noticed.

3. Total lipid content (mg/gram):

Data in Table (3) revealed that the total lipids were decreased by treatment with Cyromazine, Pyriproxyfen, and Novaluron compared with untreated larvae. Total lipids content decreased significantly for the 4th instar larvae were 2.11, 3.79, and 4.38 (mg/g.b.wt.) for Cyromazine, Pyriproxyfen, and Novaluron, respectively, as compared with control 8.73 (mg/g.b.wt). While the value of total lipids in the 6th instar larvae was 3.52, 3.64, and 4.78 (mg/g.b.wt.) for Cyromazine, Pyriproxyfen, and Novaluron, respectively, while it was 5.60 (mg/g.b.wt.) with control. Assar *et al.* (2016) indicated that the total lipids content was decreased when treated 4th larval instar of *S.littoralis* with teflubenzuron and hexaflumuron as insect growth regulators. Similar results were obtained by El-Sheikh *et al.* (2013) on teflubenzuron as an insect growth regulator. Kiran *et al.* (1998) reported that the fat body synthesizes a number of proteins and releases them into the haemolymph during the active larval period. Hochachka and Somero (1973) revealed the increase in the contents of total lipids, free fatty acids, and phospholipids to increase food consumption. Many authors Ellis *et al.* (2002) and Costamagna and Landis (2004) reported that the orders Lepidoptera and Orthoptera use lipids and stored carbohydrates as the main energy source. Regarding the total lipid content, a number of toxic agents have been found to cause disturbances of fats in different body organs of both vertebrate and invertebrate animals (Rawi *et al.*, 1995). Lipids are the most suitable reserves for the storage of energy. Compared to carbohydrates, lipids can supply as much as eight times more energy per unit weight (Beenakkers *et al.*, 1985). They suggested that the reason for the lower fat content in larvae could be due to the extended larval period of the treated insects and blocked food ingestion, and the fat reserves might have been utilized for maintenance during the extended larval period.

Table (3): Effect of LC_{50} of the three tested insect growth regulators on the total Lipids (mg/g body weight) on 4th and 6th instar larvae of *Spodoptera littoralis.*

Treatments		4th instar larvae	6th instar larvae
Control		8.73±0.90	5.60±2.19
Novaluron		4.38±1.64	4.78±25.26
Pyriproxyfen		3.79±1.33	3.64±1.88
Cyromazine		2.11±0.14	3.52±0.44
LSD at5%	**Treat. + Cont.**	2.16*	3.09 ns
	Treat.	1.86*	3.11 ns

4. Chitinase activity determination (µg NAGA/min/mg protein):

Results given in Table (4) indicated that all tested compounds led to a reduction in chitinase activity ranging between 37.55, 22.63, and 15.31(µg NAGA/min/mg protein) for Novaluron, Pyriproxyfen, and Cyromazine, respectively less than the control that was 58.53(µg NAGA/min/mg protein) for the 4th instar larvae. Also, data in Table (4) showed that reduction in the chitinase activity for the 6th instar larvae was induced by the tested compounds Novaluron, Pyriproxyfen, and Cyromazine, the values were 18.66, 14.04, and 9.42 (µg NAGA/min/mg protein), respectively, as compared with control 49.98 (µg NAGA/min/mg protein). The tested compounds caused inhibition to the production of chitin; therefore, the larvae are unable to successfully moult into the next stage (Kassem *et al*., 1986). To gain preliminary information on the biochemical effect of enzymes affecting cuticle sclerotization and chitin deposition, such as phenoloxidase and chitinase, and of the cuticle protein and chitin levels (Ishaaya and Casida 1974). Locke (1964) stated that, in the normal molting process, chitinase is compartmentalized in the molting fluid for the digestion of the old cuticle, and the products of cuticle digestion become available for the synthesis of the new cuticle.

Table (4): Effect of LC_{50} of the three tested insect growth regulators on the activity of chitinase (µg NAGA/min/mg protein) on 4th and 6th instar larvae of *Spodoptera littoralis.*

Treatments		4th instar larvae	6th instar larvae
Control		58.53±31.50	49.98±9.33
Novaluron		37.55±4.02	18.66±5.38
Pyriproxyfen		22.63±4.03	14.04±4.22
Cyromazine		15.31±4.14	9.42±1.90
LSD at 5%	**Treat. + Cont.**	30.38	11.03*
	Treat.	8.11*	12.00

5. Acetylcholinesterase activity determination (µU/mg protein):

Acetylcholinesterase (AchE) was tabulated in Table (5) which significantly activated treated 4th instar larvae of *S. littoralis* with Pyriproxyfen and Cyromazine 2,008 and 1,364 (µU/mg protein), respectively, followed by a moderate increase with Novaluron 909 as compared with control 900 (µU/mg protein). while it was significantly activated treated 6th instar larvae of *S. littoralis* with Cyromazine, Pyriproxyfen, and Novaluron 2.419, 1.450, and 1.248 (µU/mg protein), respectively, as compared with control 952 (µU/mg protein).

AChE has a key role in neurotransmission by hydrolyzing the neurotransmitter acetylcholine (AChE) in cholinergic synapses of the nervous system and is the target site of several neurotoxic insecticides (Salgado *et al.,* 1998 and Rashwan, 2013).

Table (5): Effect of LC_{50} of the three tested insect growth regulators on the activity of acetylcholinesterase (µU/mg protein) on 4th and 6th instar larvae of *Spodoptera littoralis.*

Treatments		4th instar larvae	6th instar larvae
Control		900±28	952±37
Novaluron		909±24	1,248±15
Pyriproxyfen		2,008±46	1,450±22
Cyromazine		1,364±49	2,419±62
LSD at 5%	**Treat. + Cont.**	160.18*	969.91
	Treat.	193.68*	983.360

6. Carbohydrate hydrolyzing enzyme activities:

Data presented indicated that the activities of amylase, Invertase, and Trehalase enzymes were affected by all tested compounds in the treated 4th and 6th instar larvae of *S. littoralis.*

6.1 Amylase activity (µg glucose/min /g body weight):

Kandil *et al.* (2005) found that the two insect growth regulators i.e. diflubenzuron and chlorfluazuron reduced the activity of amylase in larvae of the pink bollworm *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) showed a significant reduction in the activity of Amylase for the 4th instar larvae was induced by the tested compounds Cyromazine, Novaluron, and Pyriproxyfen, the values were 147.20, 530.29, and 694.54 (µg glucose /g.b.wt), respectively as compared with control 1,177.23 (µg glucose /g.b.wt). A decrease in the activity of Amylase for the 6th instar larvae was also observed induced by the tested compounds Cyromazine and Pyriproxyfen, the values were 339.20, and 526.39 (µg glucose /g.b.wt), respectively, except increase with Novaluron 1.049 as compared with control 761.33 (µg glucose /g.b.wt) (Table 6).

Table (6): Effect of LC_{50} of the three tested insect growth regulators on Amylase activity (µg glucose/min /g body weight) on 4th and 6th instar larvae of *Spodoptera littoralis.*

Treatments		4th instar larvae	6th instar larvae
Control		1,177.23±0.92	761.33±206.91
Novaluron		530.29±97.52	1,049.63±43.10
Pyriproxyfen		694.54±92.04	526.39±59.38
Cyromazine		147.20±36.80	339.20±49.96
LSD at 5%	**Treat. + Cont.**	130*	211*
	Treat.	160*	102*

6.2. Invertase activity (µg glucose/min /g body weight):

Data in Table (7) shows a significant reduction in the activity of Invertase of the 4th instar larvae was induced by the tested compounds Pyriproxyfen, Novaluron, and Cyromazine, the values were 1982.42, 3836.4, and 3864 (µg glucose /g.b.wt), respectively, as compared with control 4799.33 (µg glucose /g.b.wt). While significantly activated treated 6th instar larvae of *S. littoralis* the highest value of Invertase was recorded on Cyromazine, Novaluron, and Pyriproxyfen the values were 5400, 4719.6, 4606.13, and 3864 (µg glucose /g.b.wt), respectively, as compared with control 3867 (µg glucose /g.b.wt).

Table (7): Effect of LC_{50} of the three tested insect growth regulators on Invertase activity (µg glucose/min /g body weight) on 4th and 6th instar larvae of *Spodoptera littoralis.*

Treatments		4th instar larvae	6th instar larvae
Control		4799.33±295	3867±150
Novaluron		3836.4±361	4719.6±419
Pyriproxyfen		1982.42±148	4606.13±510
Cyromazine		3864±220	5400±306
LSD at 5%	**Treat. + Cont.**	1445 ns	1352 ns
	Treat.	1435	1413 ns

6.3 Trehalase activity (µg glucose/min /g body weight):

Enzymes are synthesized and secreted only in response to the presence of food in the gut (Barnard, 1973) and, at the end of digestion, the enzymes are largely reabsorbed and transferred to the pancreas, from which they are again secreted at the next

meal (Liebow and Rothman, 1975). Trehalase was found to be the most important enzyme that plays a major role in the digestion and metabolism of carbohydrates in insects (Wigglesworth, 1972 and Wyatt, 1967).

Data in Table (8) showed the effect of LC_{50} concentration of tested compounds on Trehalase activity in treated and untreated 4th instar larvae in treated larvae with untreated ones. The highest effect on Trehalase was recorded in larvae treated with Pyriproxyfen 4,083.57±713.02 (µg glucose/min/g.b.wt) compared with untreated larvae, while, the lowest was recorded in larvae treated with Cyromazine (1,993.33±170.22 µg glucose/min/g.b.wt) recorded increase compared with control 3,962.13±394.24 (µg glucose/min/g. b.wt.)

In the same direction, the results of LC_{50} concentration of tested compounds on Trehalase activity for 6th instar larvae. The highest effect on Trehalase was recorded in larvae treated with Novaluron 4,477.33±475.41 (µg glucose/min/g.b.wt) compared with control, while, the lowest was recorded in larvae treated with Cyromazine (2,514.67±341.06 µg glucose/min/g.b.wt) recorded increase compared with control 2,572.93±547.41(µg glucose/min / g. b.wt.)

Table (8): Effect of LC_{50} of the three tested insect growth regulators on Trehalase activity (µg glucose/min /g body weight) on 4th and 6th instar larvae of *Spodoptera littoralis.*

Treatments		**4th instar larvae**	**6th instar larvae**
Control		3,962.13±394.24	2,572.93±547.41
Novaluron		3,998.93±664.53	4,477.33±475.41
Pyriproxyfen		4,083.57±713.02	4,455.87±1,030.00
Cyromazine		1,993.33±170.22	2,514.67±341.06
LSD at 5%	**Treat. + Cont.**	1445 ns	1228 *
	Treat.	1435	1366

Terra and Ferreira (1981) reported that Trehalase activity decreases during starvation and increases after feeding, suggesting Trehalase activity depends upon the presence of food and not on haemolymph Trehalase tires. Shukla *et al.* (2015) mentioned that trehalose is the major blood sugar in insects playing a crucial role as an instant source of energy and in dealing with abiotic stresses. The hydrolysis of trehalose is under the enzymatic control of Trehalase. The enzyme Trehalase is gaining interest in insect physiology as it regulates energy metabolism and glucose generation via trehalose catabolism. The two forms of insect Trehalase namely, Tre-1 and Tre-2, are important in energy supply, growth, metamorphosis, stress recovery, chitin synthesis, and insect flight.

References

Abbott, W. S. (1925): A method of computing the effectiveness of an insecticide. J. Econ. Entomol., 18: 265-267.

Abd El-Aziz, H.S.; Hanan, S.; Mansy, M.S.; Desuky, W.M.; and Elyassaki, S.M. (2007): Toxicity evaluation and biochemical impacts of some *Bacillus thuringiensis* formulation on *Spodoptera littoralis* larvae. J. Egypt. Acd. Soc. Environ. Develop., 8(1): 1-10.

Abdel-Aal, A. E. (2012): Effect of three insect growth regulators (Chlorfluazuron, Tebofenozoid and pyriproxyfen) on fecundity of *Spodoptera littoralis*, histopathological and some biochemical aspects of moth ovary. Egyptian Academic Journal of

Biological Sciences, F. Toxicology & Pest Control, 4(1): 49-59.

Ahmed, N. A.; Rawi, S. M.; Radwan, N. M. and Attia, M. M. (1993): Comparative effects of hostathione, endrin, lannate and sumicidine on the transaminase activity of liver, brain and plasma of the frog Rana ridibunda. Bull. Fac. Sci. Cairo Univ., 6: 93-110.

Assar, A.A.; Abo El-Mahasen, M.M.; Dahi, H.F. and Amin, H.S. (2016): Biochemical effects of some insect growth regulators and bioinsecticides against cotton leafworm, *Spodopter alittoralis* (Boisd.) (Lepidoptera: Noctuidae). Journal of Bioscience and Applied Research, 2(8):587-594.

Awadalla, S. S.; El-Mezayyen, G. A.; Bayoumy, M. H., and EL-Mowafy, N. E. (2017): Bioinsecticidal Activity of some Compounds on the Cotton Leafworm, *Spodoptera littoralis* (Boisd.) under Laboratory Conditions. Journal of Plant Protection and Pathology, 8(10): 525-528.

Bakr, R.F.A.; El-Barky, N.M.; Abd Elaziz, M.F.; Awad, M.H. and Abd El-Halim, H.M.E. (2010): Effect of Chitin synthesis inhibitors (flufenoxuron) on some biological and biochemical aspects of the cotton leaf worm *Spodoptera littoralis* Bosid. (Lepidoptera: Noctuidae). Egypt. Acad. J. Biolog. Sci., (F.Toxicology and Pest control), 2(2): 43-56.

Barnard E. A. (1973): Digestion. In Comparative Animal Physiology (Ed. by PRSSEK C. L.) pp. 133-I 39. Saunders. Phjladelphia.

Beenakkers, A.M.T., D.J. Vander Horst, and W.J.A. Van Marrewijk, (1985). Biochemical processes directed to flight muscle metabolism. In: G. A. Kerkut and L. I. Gilbert (eds.), Comparative Biochemistry and Physiology, 10: 451-486.

Bradford, M.M. (1976): A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem., 7(72):248-254. Doi: 10.1006/abio.1976.9999. PMID: 942051.

Chapman, R. F. (1998): The insects: Structure and Function. Cambridge Univ. press, Cambridge, UK, pp. 770.

Costamagna, A. C. and Landis, D. A. (2004): Effects of food resources on adult *Glyptapantales militaris* and *Meteorus communis* (Hymenoptera: Braconidae), parasitoids of *Pseudaletia unipuncta* (Lepidoptera: Noctuidae). Environ. Entomol., 33: 128-137.

Duncan, D B. (1955): Multiple range and multiple biometrics, 11 (1):1-42.

Elbarky, N. M.; Dahi, H. F. and El-Sayed, Y. A. (2008): Toxicicological evaluation and biochemical impacts for radiant as a new generation of spinosyn on *Spodoptera littoralis* (Boisd.) larvae. Egyptian Academic Journal of Biological Sciences. A, Entomology, 1(2): 85-97.

El-Defrawi, M. E.; Toppozada, A.;Mansour, N. and Zeid, M. (1964): Toxicological studies on the Egyptian cotton leafworm, *Prodenia litura*. I. Susceptibility of different larval instars of Prodenia to insecticides. J. Econ. Entomol., 57: 591-593.

El-Din, M. M., and El-Gengaihi, S. E. (2000): Joint action of some botanical extracts against the Egyptian cotton leafworm *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). Egyptian Journal of

Biological Pest Control, 10 (1/2): 51-56.

El-Gabaly, A.R.M. (2015): Effect of some bioagents and chemical insecticides on biology and physiology of cotton leafworm, *Spodoptera littoralis* (Boisd.). Ph.D.Thesis.Fac.Agric., Banha University.

Ellis, J. R.J. D.; Neumann, R.H. and Elaz, P. J. (2002): Longevity and Success of *Aethina tumida* (Coleoptera: Nititulidae) fed different natural reproductive diets. J. Econ. Entomol., 95:902-907.

El-Sheikh, T.A.A.; Rafea, H. S.; El-Aasar, A.M. and Ali, S. H. (2013): Biological and biochemical effects of *Bacillus thuringiensis*, serratiamarcescens and teflubenzuron on cotton leafworm. Egypt. J. Agric. Res., 91 (4): 1327.

Finney, D. J. (1971): Probit analysis, 3rd ed. Cambridge University Press, London.

Ghoneim, K.S. (1985): Physiological studies on endocrine and reproductive systems of the cotton leafworm *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). Ph.D. Thesis, Fac. of Sci., Al-Azhar University.

Hochachka, P. W. and Somero, G. N. (1973): Strategies of biochemical adaptation. W. B. Saunders, Philadelphia,16.

Ishaaya, I. and Casida, J. E. (1974): Dietary TH 6040 alters composition and enzyme activity of housefly larval cuticle. Pestic. Biochemi. Physiol., 4(4):484-490.

Ishaaya, I. and Swirski, E. (1976): Trehalase, invertase, and amylase activities in the black scale, *Saissetia oleae*, and their relation to host adaptability. Journal of Insect Physiology, 22(7): 1025-1029.

Kandil, M.A.; El-Zaher, T.R.A. and Rashad, A.M. (2005): Some biological and biochemical effects of chitin synthesis inhibitors on pink bollworm *Pectinophora gossypiella* (Saunders). Annals of Agricultural Science, Moshtohor., 43(4): 1991-2002.

Kar, P. K.; Srivasta, P. P. ; Sinha, R. K. and Sinha, B. R. R. P. (1994): Protein concentration in the pupal haemolymph of different races and F1 stop cross of *Antheraea myllitta* D. Indian J. Service, 33(2):174- 175.

Kassem, S.; Sherby, S.M.; Aly M.I.; Zeid, M.I. and El-Sebae, A.H. (1986): Studies on toxic effects of endotoxin, chitin synthesis inhibitor and mixtures of diflubenzuron with different insecticides on Egyptian cotton leaf worm *Spodoptera littoralis* (Boisd.). Alexandrian J. Agric. Res., 31: 327-336.

Khedr, M.; Desuky, W. M.; El-Shakaa, S. A. D. and Khalil, S. I. (2005): Toxicological and biochemical studies on the effect of some insect growth regulators on *Spodoptera littoralts* (Boisd.) larvae. Egyptian Journal of Agricultural Research, 83(2): 539-561.

Kiran, K. N.; Ismail, S. M. and Dutta, A. (1998): Different tail uptake of storage protein by the fat body of Rice moth, *Corcyra cephalonica* during the larval pupal Develop. Entomol., 23: 83-90.

Knight, J.A. ; Anderson, S.; Rawle, J.M. (1972): Chemical basis of the sulfophospho-vanillin reaction for estimating total serum lipids. Clin. Chem., 18: 199-202.

Liebow C. and Rotiman, S. S. (1975): Enteropancreatic ctrculation of digestive enzymes. Sciel cc, 189, 473476. Lottce u. and Hicinbothamn.

(19791 Trampporf in Plmrs. Springer. New York.

Locke, M. (1964): The structure and formation of the integument in insects. In *Physiology of* Insecta (ed. M. Rockstein), Vol. lII, pp. 379-470. Academic Press, New York.

Nabih, L.; El-Dardiri, Z.; El-Ansary, A. and Ahmed, S. A. (1990): Kinetic properties of two transaminases and lactate dehydrogenase in freshwater snails specific intermediate host for human schistosomiasis. Cell. Mol. Biol., 3: 63-75.

Pashley, D. P.; Hardy, T. N. and Hammond, A. M. (1995): Host effects on development and reproductive traits in fall armyworm strains (Lepidoptera: Noctuidae). Ann. Entomol. Soc. Amer., 88 (6): 748-755.

Rashwan, M. H. (2013): Biochemical impacts of rynaxypyr (Coragen) and spinetoram (Radiant) on Spodoptera littoralis (Boisd.). Nature and Science, 11(8): 40-47.

Raslan, S. A. A. (2002): Preliminary report on initial and residual mortality of the natural product, Spinosad, for controlling cotton leaf worm egg masses. In Egypt. 2nd Inter. Conf., Plant Prot. Res. Inst., Cairo, Egypt, 1: 635-637.

Rawi, S. M.; El-Gindy, H.; Haggag, A. M.; Abou El Hassan, A. and Abdel Kader, A. (1995): Few possible molluscicides from calendula *Micrantha officinalis* and *Ammi majus* plants. I. Physiological effect on *B. alexandrina* and *B. truncatus.* J. Egypt. Ger. Soc. Zool, 16: 69-75.

Russell, D. A.; Radwan, S. M.; Irving, N. S.; Jones, K. A. and Downham, M. C. A. (1993): Experimental assessment of the impact of defoliation by *Spodoptera littoralis* on the growth and yield of Giza'75 cotton. Crop Protection, 12(4): 303-309.

Salgado, V.L.; Sheets, J.J. ; Watson, G.B. and Schmidt, A.L. (1998): Studies on the mode of action of spinosad: the internal effective concentration and the concentration dependence of neural excitation, Pesticide and Biochem. Physiol., 60 (2):103-110.

Shukla, E.; Thorat, L. J.; Nath, B. B. and Gaikwad, S. M. (2015): Insect trehalase: physiological significance and potential applications. Glycobiology, *25*(4): 357-367.

Siddall, J. B. (1976): Insect growth regulators and insect control: A critical appraisal. Environmental Health Perspectives, 14**:** 119-126.

Simpson, D.R.; Bulland, D.L, and Linquist, D. A. (1964): A Semimicrotechnique for estimation of cholinesterase activity in boll weevils. Ann. Ent. Soc. Amer., 57:367-371.

Singh, N.B. and Sinha, R.N. (1977): Carbohydrates, lipids and protein in the developmental stages of *Sitophillus oryzea* and *Sitophillus grannarius*. Ann. Entomol. Soc. Am., 107–111.

Smagghe, G. and Degheele, D. (1997): Comparative toxicity and tolerance for the ecdysteroid mimic tebufenozide in a laboratory and field strain of cotton leafworm *S. littoralis* (Lepidoptera: Noctuidae). J. Econ. Entomol., 90(2): 278-282.

Terra, W. R. and Ferreira, C. (1981): The physiological role of the peritrophic membrane and trehalase: digestive enzymes in the midgut and excreta of starved larvae of *Rhynchosciara*. J. Insect physiol., 27(5): 325-331.

Wigglesworth, V.B. (1972): The principles of insect physiology 7th ed. Chapman and Hall Ltd.

Wilkinson, F. (1976): Insecticide biochemistry and physiology. Plenum Press, New York.

Wyatt, G.R. (1967): The biochemistry of sugars and polysaccharides in insects Adv. Insect Pysiol., 4:287-360.