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Biochemical effects of some insect growth regulators and bioinsecticides against cotton leafworm, *Spodoptera littoralis* (Boisd.)(Lepidoptera: Noctuidae)

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Abstract

The current work was carried out to evaluate the biochemical effects of LC_{50} of four compounds; emamectin and spinetoram as bioinsecticides, hexaflumuron and teflubenzuron as Insect growth regulators (IGR's) against the 4th instar larvae of *Spodoptera littoralis* to determine the effects of these compounds on total carbohydrates, proteins, lipids, acetylcholinesterase, chitinase, phenoloxidase, carbohydrates hydrolyzing enzymes, non-specific esterases, phosphatases and transaminase enzymes.

The obtained results indicated that total proteins and lipids content were significantly decreased with all tested insecticides, except slightly increase in total protein with spinetoram, in contrast, all tested insecticides led to an increase in total carbohydrates. The tested insecticides significantly increased the invertase activity except emamectin decreased the enzyme activity. A significant decrease in the activity of trehalase and amylase activity was induced by the tested insecticides, except with emamectin and teflubenzuron in case of amylase. The tested insecticides significantly decreased the activity of acid (AcP) and alkaline (AlP) phosphates. It is clearly noticed that teflubenzuron and spinetoram significantly increased alpha esterases in contrast, decreased with hexaflumuron and emamectin. A highly significant decreased in beta esterases was induced by teflubenzuron and hexaflumuron and increased with spinetoram and emamectin. All tested insecticides induced a significant inhibitory effect on aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activity except with teflubenzuron.

Acelylcolinesterase (AchE) activity significantly increased with emamectin and teflubenzuron while decreased with hexaflumuron and spinetoram. Phenoloxidase and Chitinase activity significantly increased with all tested insecticides.

Keywords: IGRs, Acid phosphatase, Alkaline phosphatase, Aspartate aminotransferase, Alanine aminotransferase, Acetylcholinesterase.

1 Introduction

The cotton leafworm *S. littoralis* is one of the most destructive pests in the tropical and subtropical areas of the world (Hill, 1987). It attacks plants in 44 families containing at least 112 species of plants of varying economic importance (Sarto and Monteys, 1988).

Over the last few decades, the intensive use of broad-spectrum insecticides against the Egyptian cotton leafworm, *S. littoralis* has led to the development of resistance to many registered pesticides making their control even more difficult (Miles and Lysandrou, 2002; Aydin and Gurkan, 2006). Insect growth regulators (IGR's) is considered as the possible alternative way of conventional synthetic insecticides for controlling this pest (Raslan, 2002). They have novel mode of action which disrupt the physiology and development of the target pest. Such compounds tend to be selective and generally less toxic to non-target organisms than conventional insecticides (Gurr *et al*., 1999). Depending on the mode of action, IGR's had been recently grouped in chitin synthesis inhibitors (CSIs) and substances that interfere with the action of insect hormones (i.e. juvenile hormone analogues, and ecdysteroids) (Tunaz and Uygun, 2004). CSIs interfere

with chitin biosynthesis in insects (Gijswijt *et al*., 1979) and thus prevent moulting, or produce an imperfect cuticle (Hammock and Quistad, 1981). They also affect the hormonal balance in insects, thereby resulting in different physiological disturbances (Soltani *et al*., 1984). These compounds have no appreciable effects on parasitoids and has probably a mild effect on other natural enemies (Ishaaya *et al*., 2002). Also, it has low mammalian toxicity (Barazani, 2001). The present work was carried out to evaluate the biochemical effects of LC_{50} of four compounds; emamectin and spinetoram as bioinsecticides, hexaflumuron and teflubenzuron as Insect growth regulators (IGR's) against the 4th instar larvae of *Spodoptera littoralis.*

2 Materials and Methods

1.Rearing technique:

Eggs of the cotton leafworm *S. littoralis* were obtained from laboratory strain maintained at the cotton pest research department, Plant Protection Research Institute, Agricultural Research Center, Dokki; Giza. These eggs were kept in glass jar covered with gauze under laboratory condition of 27±2°C and 65±5% R.H. until hatching. The larvae were reared on fresh leaves of castor bean *Ricinus commanis* till the fourth larval instar described by (Ghoneim, 1985).

2- Bioinsecticides tested:

2.1. Spinetoram (Radiant 12% SC)

Major component (3'ethoxy, 5,6-dihydro spinosyn J):(2R,3aR,5aR,5bS,9S,13S,14R,16aS,16bR)-13-{[(2S,5S,6R)-5-(dimethylamino)-6-methyltetrahydro-2Hpyran- 2-yl]oxy}-9-ethyl-14-methyl-7,15-dioxo-2,3,3a,4,5,5a,5b,6,7,9,10,11,12,13,14,15,16a,16b-octadecahydro-1H-as-indaceno[3,2-d]oxacyclododecin-2-y 6-deoxy-3-O-ethyl-2,4-di-O-methyl-beta-L-mannopyranoside was obtained from Dow Agro Sciences,Egypt

2.2. Emamectin benzoate (Emaskem 1.9%EC)

Emamectin benzoate consists of a mixture of at least 90% 4"-epi-methylamino-4"-deoxyavermectin B1, and a maximum of 10% 4"-epi-methylamino-4"-deoxyaverrnectin Blb benzoate was obtained from Egypt Agricultural Development

2.3. Hexaflumuron (Cameron 10%EC)

Hexaflumuron is a benzoylphenyl urea-type insecticide and is the common name for N-(((3,5- dichloro-4-(1,1,2,2-tetrafluoro-ethoxy)phenyl)amino)carbonyl)-2,6-difluorobenzamide was obtained from Cam Agricultural Chemicals

2.4. Teflubenzuron (Nomolt 15% SC)

IUPAC:1-(3,5-dichloro-2,4-difluorophenyl)-3-(2,difluorobenzoyl) ureaCA:N-[[(3,5-dichloro-2,4difluorophenyl)amino]carbonyl]-2,6-difluorobenzamide was obtained from Basev Limited ,Egypt

3-Biochemical studies:

The following biochemical studies were carried out for the 4th instar larvae of *S. littoralis* following their treated with the LC_{50} of each of the tested insecticides.

- Preparation of insects for analysis:-

The insects were prepared as described by (Amin, 1998**)**. They were homogenized in distilled water (50 mg /1 ml). Homogenates were centrifuged at 8000 r.p.m. for 15 min at 2 ºC in a refrigerated centrifuge. The deposits were discarded and the supernatants, was stored at least one week without appreciable loss of activity when stored at 5^0C.

1.Total carbohydrates were determined according to (Dubois *et al.*, 1956)

2.Total proteins were determined according to (Bradford, 1976)

3.Total lipids were determined according to (Knight *et al.*, 1972)

4. Digestive enzymes were determined according to (Ishaaya and Swirski, 1976)

5.Acetylcholinesterase was determined according to (Simpson *et al.*,1964)

6.Chitinase activity was determined according to (Bade and Stinson, 1981)

7.α- and β-esterases were determined according to (Van Asperen, 1962)

8.Phenoloxidase was determined according to (Ishaaya, 1971)

9.Phosphatases were determined according to (Powell and Smith, 1954)

10.Transaminases were determined according to (Reitman and Frankle, 1957)

4-Statistical analysis:

The results of biochemical determinations were pooled from triplicate determinations. The results were analyzed by one – way analysis of variance (ANOVA) using constant statistical software (cohort software, Berkeley). When the ANOVA statistics were significant ($P < 0.01$), means were compared by the Duncan's multiple range test.

3 Results and Discussion

1-Effect of tested insecticides on total carbohydrates, proteins and lipids

Results given in Table (1) indicated that all tested insecticides led to increase in total carbohydrates which more obvious with emamectin compared with control. Total carbohydrates content were 7.45, 7.14, 7.17 and 6.69 (mg/g.b.wt) for emamectin, spinetoram, hexaflumuron and teflubenzuron, respectively, while it was 6.54 (mg/g.b.wt) with control.

Carbohydrates play a major role in insect development like metabolism, metamorphosis, development of flight muscles, reproduction and embryonic development (Chapman, 1998).

The total haemolymph protein content of 4th instars of *S. littoralis* was decreased with all tested insecticides. The total protein were 30.3, 27.9 and 26.9 (mg/g.b.wt) with emamectin, hexaflumuron and teflubenzuron respectively, except increase with spinetoram 36.8 (mg/g.b.wt) as compared with control 35.7 (mg/g.b.wt). This increase may be due to the natural increase of protective hydrolytic and

detoxifying enzymes that usually take place shortly after treatment.

Similar results were obtained by (Mostafa, 1993) and (Sokar, 1995) for the total haemolymph protein of the same species treated with triflubenuron and hexaflumuron, respectively. Different results were obtained by (El-Barky *et al.*, 2008) stated that total proteins significantly decreased using spinetoram on *S.littoralis*. The protein pool of the haemolymph functions as a reserve source of protein synthesis need for growth and development of the adult stage during pupal life (Florkin and Jeanuiaux, 1964).

Wilkinson (1976) stated that protein help to synthesize microsomal detoxifying enzymes which assist in the detoxificaition of toxicants that enter into the insect body. Proteins are the most important components of biochemical of insect that bind the foreign compounds. In general, the problem of protein synthesis is intimately related to metabolism of nucleic acids.

All tested insecticides led to decrease in lipids which more obvious with emamectin than other compounds. It was 2.8, 3.5, 3.2 and 3.3 (mg/g.b.wt) for emamectin, spinetoram, hexaflumuron and teflubenzuron, respectively, as compared with control 3.9 (mg/g.b.wt). Hill and Izatt (1974) reported that lipid accumulation is more likely to be related directly to lack of juvenile hormone. The administration of tested insecticide does not act on the site of secretion of juvenile hormone, i.e., corpora allata.

Table (1): Effect of LC_{50} of the tested insecticides on the total carbohydrates, proteins and lipids on 4th instar larvae of *S. littoralis* after 3 days of treatment

Insecticides	Total Carbohydrates (mg/ml) ± SE	Total Proteins (mg/gm tissue) ± SE	Total Lipids (mg/gm tissue) ± SE
control	6.54±0.1	35.7±0.6	3.9±0.09
emamectin	7.45±0.07	30.3±0.4	2.8±0.09
spinetoram	7.14±0.09	36.8±0.7	3.5±0.09
hexaflumuron	7.17±0.09	27.9±0.6	3.2±0.05
teflubenzuron	6.69±0.04	26.9±0.8	3.3±0.08
F value	23.6 *	33.7 *	40 *

*: significant

2-Effect on carbohydrates hydrolyzing enzyme

Three digestive enzymes; amylase, trehalase and invertase were determined in 4th instar larvae of *S. littoralis* which treated with LC_{50} of the tested insecticides. Data in table (2) showed that increased the invertase activity which was 332, 319.3 (µg glucose /g.b.wt) with spinetoram, teflubenzuron, respectively, except with emamectin which exhibited decreased the enzyme activity was 299.3 while it was 313 with control.

Also data in (Table 2) showed that a significant decrease in the activity of trehalase was induced by the tested insecticides spinetoram, hexaflumuron, Teflubenzuron, the values were 118.7, 118 and 109.3(µg glucose /g.b.wt), respectively, except increase with emamectin 131.7. as compared with control 121(µg glucose /g.b.wt). Furthermore, the activity of amylase was high in case of emamectin 78.7 followed by hexaflumuron 74 than other compounds which teflubenzuron caused inhibition of the activity of amylase 45.3 as compared with control 52.3(µg glucose /g.b.wt).

Similar findings were also reported by El-Barky *et al.* (2008) and Rashwan(2013) using spinetoram on *S.littoralis* with trehalase and amylase; (El-Sheikh *et al.*, 2013) Using teflubenzuron with fluctuated changes in trehalase on *S.littoralis*.The rapid decrease of glucose concentration at the end of last larval instar of the cotton leafworm, *S. Littoralis* was probably caused by high metabolic activity of the epidermis, which is known as a tissue with low trehalase, so it is unable to utilize trehalose (Florkin and Jeanuiaux, 1964)

3-Effect on phosphatase enzymes

The data in Table (3) showed that the all tested insecticide significantly decreased the activity of alkaline phosphatase (AlP) as compared with control, hexaflumuron gave a lowest decrease (248.7 $Ux10^3$/g.b.wt) followed by emamectin, teflubenzuron and spinetoram (309.7, 310 and 445.7$Ux10^3$/g.b.wt), respectively, as compared with control (611 $Ux10^3$/g.b.wt).

The tested insecticides also caused inhibition in the activity of acid phosphatase (AcP) as compared with control (Table 3), hexaflumuron and teflubenzuron induced more inhibition in the activity of acid phosphatase (58.2 and 63.2 $Ux10^3$/g.b.wt) respectively, than emamectin and spinetoram (66.5 and 73.9 $Ux10^3$/g.b.wt) respectively, as compared with control (84.2 $Ux10^3$/g.b.wt).

These results are in agreement with those obtained on *S.littoralis* by [(El-Barky *et al.*, 2008) and (El-Sheikh, 2012)] using spinetoram with significant decrease in both acid and alkaline phosphatases. On the other hand, some increase in the activity of acid phosphatase in the same insect by (Sokar, 1995) using hexaflumuron.

Sridhara and Bhat (1963) stated that the increase or decrease of both phosphatase enzymes development is reflected in increase or decrease in acid-soluble phosphorus content.

Table (2): Effect of LC_{50} of the tested insecticides on the carbohydrates hydrolyzing enzymes on 4th instar larvae of *S. littoralis* after 3 days of treatment

Insecticides	Invertase	Trehalase	Amylase
	Mean enzyme activity (µg glucose /g.b.wt) ± SE		
control	313±3.8	121±2.2	52.3±1.7
emamectin	299.3±3.6	131.7±4.1	78.7±1.5
spinetoram	332±3.3	118.7±0.9	51.7±0.9
hexaflumuron	313.7±1.2	118±2.1	74±0.9
teflubenzuron	319.3±1.8	109.3±2.3	45.3±1.5
F value	10.74 *	6.6 *	99.4 *

*: significant

Table (3): Effect of LC_{50} of the tested insecticides on the activity of phosphatase (AcP and AlP) on 4th instar larvae of *S. littoralis* after 3 days of treatment

Insecticides	Alkaline phosphatase	Acid phosphatase
	Mean enzyme activity ($U*10^n$ /g.b.wt) ± SE	
control	611±5.4	84.2±2.5
emamectin	309.7±3.5	66.5±1.5
spinetoram	445.7±5.9	73.9±1.0
hexaflumuron	248.7±3.3	58.2±1.2
teflubenzuron	310±7.8	63.2±1.1
F value	532.6 **	30.9 *

*: significant **: highly significant

4-Effect on α- and β-esterases

Values of alpha and beta esterases are tabulated in Table (4). Alpha esterases was activated with spinetoram (1488 ug α- naphthol/min/g.b.wt) as compared with control 1366.7 and activation occur with emamectin 388.3 in case of beta esterases, while it was 327 ug α-naphthol/min/g.b.wt with control. In both alpha and beta esterases is highly inhibited with hexaflumuron 807 and 171.7 ug (α- naphthol/min/g.b.wt), respectively.

It is clearly noticed that IGR's may be cause different levels of significant changes in alpha and beta esterases on *S.littoralis* (Bakr *et al.*, 2013), spinetoram slightly increased compared with control in case of alpha esterases (Rashwan, 2013) and emamectin highly increased in case of beta esterases.

Table (4): Effect of LC_{50} of the tested insecticides on the activity of α-and β- esterases on 4th instar larvae of *S. littoralis* after 3 days of treatment

Insecticides	Alpha esterase	Beta esterase
	Mean enzyme activity (ug α-or β-naphthol/min/g.b.wt) ± SE	
control	1366.7±12.9	327±4.6
emamectin	1268±14.1	388.3±4.8
spinetoram	1488±13.6	329±1.4
hexaflumuron	807±9.2	171.7±3.1
teflubenzuron	1377±7.4	250.7±6.8
F value	602.1 **	216.3 **

**: highly significant

5-Effect on transaminases enzymes

The obtained results (Table 5) revealed that all tested insecticides induced a significant inhibitory effect on aspartate aminotransferase (AST) activity were 5730, 7033.3, 5960 and 5126.7 ($Ux10^3$/g.b.wt) for emamectin, spinetoram, hexaflumuron and teflubenzuron, respectively, while it was 7510 with control. Also inhibition occurs in alanine aminotransferase (ALT) activity except activation occur with teflubenzuron (3676.7 $Ux10^3$/g.b.wt) as compared with control 3556.7 ($Ux10^3$/g.b.wt).

It is clearly noticed that pronounced inhibition of AST compared with control in the same insect by (Abd El-Aziz, 2014); (Amin and Fahmy, 2011) using spinetoram reduced both GOT and GPT. On the other hand, (Mostafa, 1993) reported a decrease in GPT activity and an increase in GOT activity of haemolymph 4th larval instar of *S. Littoralis* after treatment with hexaflumuron. Emamectin on the same insects increased both GOT and GPT Abd El-Hafez and Osman (2013).

Table (5): Effect of LC_{50} of the tested insecticides on the activity of transminases on 4th instar larvae of *S. littoralis* after 3 days of treatment

ALT(GPT)	AST(GOT)	Insecticides
Mean enzyme activity ($UX10^n$/g.b.wt) ± SE		
3556.7±119.4	7510±211.4	control
3106.7±84.4	5730±83.8	emamectin
3393.3±56.2	7033.3±125.3	spinetoram
2766.7±58.2	5960±100.3	hexaflumuron
3676.7±93	5126.7±124.4	teflubenzuron
10.96 *	30.5 *	F value

*: significant

6-Effect on acelylcolinesterase, chitinase and phenoloxidase

Acelylcolinesterase (AchE) was tabulated in Table (6) which significantly activated with emamectin and teflubenzuron 427.3 and 310 (ug AchBr/min/g.b.wt), respectively, while it was inhibited with hexaflumuron and spinetoram 209.7 and 224.7, respectively, as compared with control 231.7(ug AchBr/min/g.b.wt).

Similar findings were also reported in the same insect by (Abd El- Mageed and Shalaby, 2011) using IGR's. Reduction in acetylcholinesterase appeared (Mostafa, 1993) using teflubenzuron. On other hand spinetoram induced moderate increase in activity of acetylcholinesterase by [(El-Barky *et al.* (2008), Fahmy and Dahi (2009) and Rashwan, (2013).

Chitinase values were 1853, 1993.3, 1270.3 (µg NAGA/min/g.b.wt) with emamectin, spinetoram and hexaflumuron, respectively, and slightly decreased with teflubenzuron 1203.3, while it was 1238 with control (µg NAGA/min/g.b.wt).

These results are in agreement with those obtained on *S.littoralis* by (Rashwan, 2013) using spinetoram stated that pronounced increase in activity of chitinase; fluctuated changes recorded in chitinase by (El-Sheikh *et al.*, 2013) using teflubenzuron and spinetoram on *S.littoralis.*

Phenoloxidase activity was highly significant activated with three tested insecticides 16.9, 14.6 and 11.3 (O.D. units/min/g.b.wt) with emamectin, hexaflumuron and teflubenzuron, respectively, and more obvious with spinetoram 18.5, as compared with control 11(O.D. units/min/g.b.wt). It is clearly noticed that pronounced changes in the same insect by (Abd El- Mageed and Shalaby, 2011) using IGRs. On other hand, phenoloxidase activity was reduced by (Mostafa, 1993) using teflubenzuron on *S. littoralis*.

In response to microbial infection, insects mount several defense reactions including the induction of proteolytic cascades that lead to localized melanization and coagulation. Melanization requires the activation of prophenoloxidase (pro po) to its active form phenoloxidase (po) a key enzyme that leads to the formation of melanin at wound sites and around intruding microorganisms in the haemolymph Revenis (2011)

Table (6): Effect of LC_{50} of the tested insecticides on the activity of acetylcolinesterase, chitinase and phenoloxidase on 4th instar larvae of *S. littoralis* after 3 days of treatment

phenoloxidase	Chitinase	AchE	Insecticides
Mean enzyme activity (O.D. units/min/g.b.wt) ± SE	**Mean enzyme activity (µg NAGA/min/g.b.wt) ± SE**	**Mean enzyme activity (µg AchBr/min/g.b.wt) ± SE**	
11±0.3	1238±16.8	231.7±4.9	**control**
16.9±0.9	1853±20.7	427.3±11.4	**emamectin**
18.5±0.6	1993.3±28.3	224.7±2.4	**spinetoram**
14.6±0.4	1270.3±4.7	209.7±4.5	**hexaflumuron**
11.3±0.2	1203.3±5.5	310±10.3	**teflubenzuron**
33.9 **	248.99 **	80.7 *	**F value**

*significant **: highly significan

4 References

Abd EL-Aziz, H. S. (2014). Effect of some insecticides on certain enzymes of *Spodoptera littoralis* (Boisd.). Egypt. J. Agric. Res., 92 (2):58-71.

Abd El-Hafez, H. F. and Osman, H. H. (2013). Effects of pyridalyl and emamectin benzoate on some biological and biochemical parameters of *Spodoptera littoralis* (Boisd.). and Albino rat. Egypt. Acad. J. Biol. Sci., 6 (3): 59-68.

Abd El-Mageed, A. E. and Shalaby, S. E. (2011).Toxicity and biochemical impacts of some new insecticide mixtures on cotton leafworm, *Spodoptera littoralis* (Boisd.). Plant Protect. Sci., 47: 166–175.

Amin, T. R. (1998). Biochemical and physiological studies of some insect growth regulators on the cotton leafworm, *Spodoptera littoralis* (Boisd.) .Ph.D. Thesis, Faculty of Science, Cairo Univ. Egypt.

Amin, T. R. and Fahmy, N. M. (2011).Impact of spinetoram on some nitrogenous components related to protein metabolites in the cotton leafworm, *Spodoptera littoralis* (Boisd.). Egypt. Acad. J. Biol. Sci., 3(1):51–64.

Aydin, M. H. and Gurkan, M. O. (2006). The efficacy of spinosad on different strains of *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). Turkish J. Biol.; 30 (1): 5-9.

Bade, M. L. and Stinson, A. (1981). Biochemistry of insect differentiation. A system for studying the mechanism of chitinase activity in vitro. Archs Biochem. Biophys.; 206: 213-221.

Bakr, R. F.; Hafez, J. A.; Khamiss, O. A. and Zyaan, O. H. (2013). Biochemical studies on the effect of chitin synthesis inhibitor, (flufenoxuron) and *Spli*MNPV on the cotton. Egypt. Acad. J. Biol. Sci., 6(2): 29 -38.

Barazani, A. (2001). Rimon, an IGR insecticide. Phytoparasitica, 29: 59-60.

Bradford, M. M. (1976). A rapid and sensitive method for the quatitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., 72: 248-254.

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

Dubios, M. ; Gilles, K. A.;Hamilton, J. K.; Rebers, P. A. and Smith, F. (1956). Colorimetric method for determination of sugars and related substances. Analyt.Chem., 28:350-356.

El-Barky, 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. Egypt. Acad. J. Biol. Sci., 1(2): 85 – 97.

El- Sheikh, E. A. (2012). Biological, biochemical and histological effects of spinosad, *Bacillus thuringiensis var. kurstaki* and cypermethrin on the cotton leafworm, *Spodoptera littoralis* (Boisd.). Egypt. Acad. J. Biol. Sci., 4 (1) 113-124.

El-Sheikh, T. A.; Rafea, H. S.; El-Aasar, A. M. and Ali, S. H. (2013). Biochemical studies of *Bacillus thuringiensis* var.kurstaki, *Serratia marcescns* and teflubenzurone on cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptea: Noctuidae) . Egypt. Acad. J. Biol. Sci., 5 (1):19-30.

Fahmy, N. M.; and Dahi, H. F. (2009). Changes in detoxifying enzymes and carbohydrate metabolism associated with spinetoram in two field-collected strains of *Spodoptera littoralis* (Boisd.). Egypt. Acad. J. Biol. Sci., 1 (1): 15 – 26.

Florkin, M. and Jeanuiaux, C. H. (1964). Haemolymph composition. In "Physiology of Insecta": (Edited by Rockstein, M.). Academic Press, New York & London., (3): 109-152.

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 Univ., Cairo, Egypt.

Gijswijt, M. J.; Deul, D. H. and DeJong, B. J. (1979). Inhibition of chitin synthesis by benzoylphenylurea insecticides, III. Similarity in action in *Pieres brassicae* (L.) with polyxin D. Pestic. Biochem. Physiol., 12:84-94.

Gurr, G. M.; Thwaite, W. G. and Nicol, H. I. (1999). Field evaluation of the effects of the insect growth regulator (tebufenozide) on entomophagous arthropods and pests of apples. Austr. J.Entomol., 38: 135-140.

Hammock, C. D. and Quistad, G. B. (1981).Metabolism and mode of action of juvenile hormone, juvenoids and other insect growth regulators. In: "Progress in pesticide Biochemistry" (Hutson, D.H. and Roberts, T.R. eds.). John Wiley & Sons Ltd., (1): 1-85

Hill, D. S. (1987). Agricultural insect pests of temperate regions and their control. Cambridge University Press.

Hill, L. and Izatt, E. G. (1974). The relationships between corpora allata and fat body and hemolymph lipids in the adult female desert locust. J. Insect Physiol., 20: 2143-2156.

Ishaaya , I. (1971). Observation on the phenoloxidase system in the armored scale *Aonidiella aurantii* and *Chrysomphalus aonidum* . Comp. Biochem . Physiol . , 39 B , 935-943.

Ishaaya, I.; Horowitz, A. R.; Tirry, L. and Barazani, A. (2002). Novaluron (Rimon), a novel IGR-Mechanism, selectivity and importance in IPM programs. 54th Intern. Symp. on Crop Protection, Part II, Gent, Belgium, 67 (3): 617-626.

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

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

Miles, M.; and Lysandrou, M. (2002). Evidence for negative cross resistance to insecticides in field collected *Spodoptera littoralis* (Boisd.) from Lebanon inlaboratory bioassays. Mededelingen (Rijksuniversiteitte Gent. Fakulteit van de LandbouwkundigeenToegepaste Biologische Wetenschappen), 67: 665.

Mostafa, S. A. (1993). Biochemical effect of some chemical compounds on *Spodoptera littoralis* (Boisd.). Ph.D. Thesis, Fac. Agric., Al-Azhar Univ., Egypt.

Powell, M. E. and Smith M. J. (1954). The determination of serum acid and alkaline phosphatase activity with 4- aminoantipyrine . J.Clin. Pathol . ,7 : 245-248 .

Rashwan M. H. (2013). Biochemical Impacts of rynaxypyr (coragen) and spinetoram (radiant) on *Spodoptera littoralis* (Boisd.). Nat. Sci ; 11(8):40-47.

Raslan, S. A. (2002). Preliminary report on initial and residual mortality of the natural product, spinosad, for

controlling cotton leafworm egg masses. In: Egypt.2nd Inter. Conf., Plant Prot. Res. Inst., Cairo, Egypt, 1: 635-637.

Reitman, S. and Frankel, S. (1957). Colourimetric method for aspartate and alanine transaminases. Amer. J. Clin. Pathol., 28 : 56 .

Revenis, C. E. (2011). Role and importance of phenoloxidase in insect hemostasis. J. Innate. Immun.;3(1): 28-33.

Sarto, I. and Monteys, V. (1988). Lepidopterous species recognised as pests in Catalina and the affected crops. Resumnese I JournadaIberica de Lepidopterologia Madrid 19 Nov.

Simpson, D. R.; Bull D. L. and Lindquist, D. A. (1964). A semimicro technique for the estimation of cholinesterase activity in bollweevil. Ann. Entomol. Soc. Amer., 57 (3): 367- 377.

Sokar, L. A. (1995). Possible alternatives to classical insecticides in management program of *Spodoptera littoralis* (Boisd.). Ph. D. Thesis, Zagazig Univ., Egypt.

Soltani, N.; Besson, M. T. and Delachambre, J. (1984). Effects of diflubenzuron on the pupal-adult development of *Tenebrio molitor* (L.) (Coleoptera: Tenebrionidae): growth and development cuticle secretion, epidermal cell density, and DNA synthesis. Pestic. Biochem. Physiol., 21:256-264.

Sridhara, S. and Bhat, J. V. (1963). Alkaline and acid phosphatases of the silkworm, *Bombyx mori* L. J. Insect Physiol., 9: 693-701.

Tunaz, H. and Uygun, N. (2004). Insect growth regulators for insect pest control. Turkish J. Agricultural. Forestry, 28: 337-387.

Van Asperen, K. (1962). A study of housefly esterase by means of sensitive colourimetric method. J. Insect Physiol., 8: 401-416.

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