Zagazig J. Agric. Res., Vol. 36 No. (4) 2009 815-824

TOXICOLOGICAL AND BIOCHEMICAL STUDIES ON SOME COMPOUNDS AGAINST COTTON LEAFWORM

Ali, Yasmin, A.A.[1], K.A. Guhar[1], R.M. Sherif[1], A.A. Aioub[1] and W.M.M.Desuky[2]

1. Plant Protection Dept., Faculty of Agric., Zagazig Univ.

2. Plant Protection Res. Inst., Agric. Res. Center, Dokki, Giza.

Accepted 16 /8/2009

ABSTRACT: Toxicological and biochemical studies of chlorpyrifos, leufenuron, tebufenozid and neem T5% were carried out on 2nd and 4th instar larvae of cotton leaf worm, *Spodoptera Littoralis* under laboratory conditions. LC_{50}'s , in case of 2nd instar larvae, were 5.4 × 10^{-5}, 4.64 × 10^{-4}, 1.266 and 13.952 ppm, whearas, in case of 4th instar larvae, values were 9.104, 0.025, 16.964 and 215.015 ppm for the above mentioned compounds, respectively.

Glutamic oxalacetic transaminase (GOT) levels in homogenated treated-larvae with the four tested compounds at LC_{50} were 8, 9, 48.6 and 15 unit/L. after 1 day post treatment, in case of 2nd instar larvae, wheareas (GOT) values in case of 4th larvae were 12, 37.6, 70 and 41.3 unit /L., respectively.

Glutamic pyruvic transaminase (GPT) levels in 2nd and 4th instar larvae were 5.33, 41.66; 35.0 , 43.33; 35.66, 55.33 and 25 and 53.86 unit/L., after 1 day of treatments with chlorpyrifos, leufenuron, tebufenozid and neem T5%, respectively.

Cholin estrase activity (CHEA) levels in 2nd instar larvae were 235.5, 704, 235 and 293.5 unit/L. the erspective values in 4th instar larvae, were 293.5, 1408, 469 and 527.5 unit/L. after 1 day of treatment with the tested compounds, respectively.

Highly significant and significant differences between days after treatment were noticed.

Key words: Posticides, chlorpyrifas, leufenuron, tebufenozid neem, biochemical changes, cotton leafworm.

INTRODUCTION

Egyptian cotton leafworm, *Spodoptera littoralis* (Bosid.) is one of the major cotton pests in Egyptian agriculture. Chemical control of such pest have consisted largely at application of cholinesterase-inhibiting organophosphate (OP) and carbamate insecticides. Intensive use of these chemicals, the target of which is the enzyme acetylcholinestrase (ACHE), may result in insecticide resistance of the pest species, conferred by an altered form of such enzyme (Bonning and Hemingway, 1991). Insect growth regulators (IGRs) are insecticides acting on various insect order by disrupting chitin synthesis. The major effect of members of this group is upon those periods of the life cycle where chitin is being formed and where its incorrect or insufficient production can lane to malformation of lator stage of the life cycle. Many authors investigated IGR's effect on *S. Littoralis* (Ishaaya *et al.*, 1986; El-Deeb *et al.*, 1991 and Khedr *et al.*, 1991 and Aioub *et al.*, 2002). Lufenuron (Match) is an insect growth inhibitor interfering with chitin synthesis. Synthetic insecticides are often a part of management programs to control cotton leaf worm in Egypt. The use of synthetic pesticides resulted in potential hazards for mammals, disturbances of the environment, pest resistance of pesticides and all mall effects on non-target organisms, natural enemis and agro eco systems (Prakash and Rao, 1987). Therefore, it must be use some plant extracts like azadirachtin (neem) to minimize harmful effects for these chemicals.

The present investigation aimed to study the toxicological and biochemical effects of chlorpyrifos, lufenuron, tebufenozid and neem on 2nd and 4th instar larvae of cotton leafworms.

MATERIALS AND METHODS

Tested Pesticides

Organophorus compound

Chlorpyrifos (Dursban 48%, EC) O, O-diethyl O-(3, 5, 6-trichloro-2-pyridinyl) phosphorothioate.

Insect growth regulators

a- Lufenuron (Match 5%, EC): N (((2,5-dichloro-4-(1,1,2,3,3,3-hexafluoropropoxy)-phenyl] amino] carbonyl] -2.6 difluorobenzamide].

b- Tebufenozide (Mimic 24%, 5C):3.5-dimethylbenzoic acid 1-(1.1-dimethylethyl)-2-(4-ethyl benzoyl) hydrazide.

Plant extracts (Neem T 5%)

Dimethyl [2aR-[2aα, 3β, 4β (1aR*, 2S*. 3aS*, 6aS*,7S*, 7aS*) 4aβ, 5α, 7aS*, 8β(E) 10β, 10aα,10bβ]]-10-(acetyloxy) octahydro-3,5-dihydroxy-4-methyl-8-[(2-methyl-1-oxo-2-butenyl) oxy]-4-(3a, 6a, 7, 7a-tetrahydro-6a-hydroxy-7a-methy2, 7-methanofuro[2.3-6] oxireno [e] oxepin-1a(2H)-yl)-1H, 7H-naphtho [1, 8-bc4, 4a-c] difuran-5, 10a (8H)-dicarboxylate.

Formulated samples of tested compounds were supplied by Ministry of Agriculture, Egypt.

Rearing of the Egyptian Cotton Leaf Worm *Spodoptera littoralis* (Boisd.)

The stock culture of susceptible Egyptian cotton leaf worm *Spodoptera littoralis* (Boisd.) was feod on castor leaves *Ricinus communis* (L.) for several generations at laboratory conditions at 27 ± 1°C and 70 ± 5% RH according to the method described by El-Defrawi *et al.*, 1964).

Bioassay Test

For studying the effect of chlorpyrifos, lufenuron, tebufenozide and Neem T 5% against the 2nd and 4th instar larvae of *Spodoptera littoralis,* serial concentrations for each compound were used.

The bioassay tests were done by dipping the castar bean leaves in each of the different concentrations for 30 seconds. Then the leaves were left for air dryness. Each concentration, including the check one, was replicated 3 times using, 10 larvae for each replicate, check larvae were offered castor leaves immersed in distilled water. After feeding period of 48 hs., mortality, percentages were recorded and the LC_{50} was calculated according to the method described by Finney. (1952).

Biochemical Studies

Preparation of sample for biochemical assay

Samples of survival larvae (2nd and 4th instar larvae) were collected at 1, 3, 7 and 10 days after exposure with LC_{50} of pesticides tested and kept in clean jars. Samples were homogenized in distilled water using Teflon homogenize. The homogenates were centrifuged at 500 r.p.m for 10 minutes at 5°C. The supernatants were immediatly assayed to determine the activities of acetyl cholinesterase (ACHE), (GOT) and (GPT).

Determination of enzymes activities

Choline estrase activity was determined according to whereas (GOT) and (GPT) levels were determined according to the method described by Reitman and Frankel (1957).

Statistical Analysis

Data obtained were statistically analyzed using the analysis of variance (breakdown one way (ANOVA) followed by a least significant difference, LSD test (Anonymous, 1990).

RESULTS AND DISCUSSION

Toxicity of Pesticides

Data in Table 1 show the lethal concentrations values of the tested pesticides against 2nd and 4th instar larvae of cotton leaf worm. LC_{50} values against 2nd instar larvae were 5.4×10^{-5}, 4.64×10^{-4}, 1.266 and 13.952 pp_m whereas these values were 9.104, 0.025, 16.964 and 215.015 pp_m in case of 4th stage larvae for chlorpyrifos, leufenuron, tebufenozid and neem T5%, respectively. Results demonstrated that chlorpyrifos was the toxic compound and neem T5% was the least one. Leufenuron and tebufenozid pesticides were moderatly toxic.

Biochemical Changes

Results in Tables 2, 3 and 4 show biological changes of (GOT, GPT) and Acetyl Choline Estrase levels after the treatment of 2nd and 4th instar larvae of cotton leaf worm with LC_{50} of the abovementioned pesticides at different intervals i.e. 1, 3, 7 and 10 days post treatments.

(GOT) levels Table 2 in 2nd instar larvae were 8, 9, 48.6 and 15 unit/L. after 1 day post treatments with chlorpyrifos, leufenuron, tebufenozide and Neem T5%, respectively, compared with 16 unit/L in check. The respective values with 4th instar larvae were 12, 37.6, 70 and 41.3 unit/L. compared with 52 unit/L in check. After 3 days of treatments, (GOT) values were 14, 50, 50.3 and 18 unit/L in 2nd instar larvae whereas these figures were 46.6, 52.6, 84.7 and 54.3 unit/L. in case of 4th instar larvae after treatments with the four tested pesticides, respectively. Data demonstrate that chlorpyrifos, lufenuron and neem T5% decrease the levels of (GOT) but tebufenozide in two instars and lufenuron in 2nd instar increase the activity of (GOT) compared with

Table1. Acute toxicity (LC_{50}) of some pestieides against 2^{nd} and 4^{th} instar larvae of cotton leaf worm

Pesticides	Larvalages	LC 50(PP_m)
Chlorpyrifos	2^{nd}	5.4×10^{-5}
	4^{th}	9.104
Leufenuron	2^{nd}	4.64×10^{-4}
	4^{th}	0.025
Tebufenozid	2^{nd}	1.266
	4^{th}	16.964
Neem T 5 %	2^{nd}	13.952
	4^{th}	215.015

Table 2. Effects of some pesticides at the level of LC_{50} on the aetivties of glutamic oxaloacetic transaminase (unit/L.) in homogenated 2^{nd} and 4^{th} instar larvae of cotton leaf warm

Days after Treatment	Control		Pesticides							
			Chlorpyrifos		Lufenuron		Tebufenozide		Neem T 5%	
	2^{nd}	4^{th}	2^{nd}	4^{th}	2^{nd}	4^{th}	2^{nd}	4^{th}	2^{nd}	4^{th}
1	16.0	52.0	8.0	12.0	9.0	37.6	48.6	70.0	15.0	41.3
3	27.0	80.3	14.06	46.6	50.0	52.6	50.3	84.7	18.0	54.3
7	46.6	73.0	-	27.0	-	-	-	-	47.0	17.0
10	73.0	-	-	21.6	-	-	-	-	41.0	13.0
F. test	**	**	**	**	**	*	N.S	**	**	**
L.S.D	32.07	16.51	3.67	8.56	26.15	10.21	-	6.58	9.28	11.49

(-) Not carried out because all individuals dead
(**) Highly significant differences (*) Significant differences
L.S.D leaf significant differences

control treatment. After 7 and 10 days of treatments, most treated-individuals were dead. Highly significant differences were noticed in all treatments except tebufenozid pestiacide in case of 2nd instar larvae, results were not significant.

(GOT) levels Table 3 were 5.33, 35, 35.66 and 25 unit/L. in case of 2nd instar larvae whereas values were 41.66, 43.33, 55.33 and 53.86 unit/L in case of 4th instar larvae after 1 day of treatments with chlorpyrifos, lufenuron, tebufenozide and neem T5% compounds, compared with 9.3 and 29.3 unit/L in control treatments, respectively. After 3 days post treatment, GPT levels in case of 2nd instar larvae were 12.33, 35.66, 43.33 and 35.66 unit /L for the foure tested compounds, respectively. In case of 4th instar larvae, values were 62, 48, 67 and 58.66 unit/L. after the treatments with tested chemicals, respectively, compared with 15.3 and 88 unit/L. for the two tested instars in control treatments, respectively. After 7 and 10 days post treatment, most treated-individuals were dead. Significant and highly significant differences between the treatments were noticed. While in case of lufenuron pesticide, not significant differences was noticed.

Choline estrase levels Table 4 were 235, 704, 235 and 293.5 unit/L. in case of 2nd instar larvae whereas these values were 293.5, 1408, 469 and 527.5 unit/L. for the 4th instar larvae after 1 day post treatments with chlorpyrifos, lufenuron, tebufenozide and neem T5% compounds, compared with 235 and 469 unit/L. in for the two tested instars in control treatments, respectively. After 3 days post treatment, choline estrase levels in case of 2nd instar larvae were 352, 1114.5, 352 and 704 unit/L after treatments with the four tested compounds, respectively. The respective values in case of 4th instar larvae were 586.5, 1700.5, 527.5 and 879.5 unit/L compared with 352 and 497.5 unit/L., in control treatments, After 7 and 10 days post treatments, most treated-individuals were dead. Data in the same table revealed that highly significant differences between treatments were noticed.

Our results are in agreement with many investigators (El-awa and Hashem, 2000 and Khedr *et al.*, 2005). El-awa and Hashem (2000) studied the effects of profenofos insecticide on some biochemical changes of cotton leaf worm. The outhors reported that the determination of the head ACHE activity at different

Table 3. Effects of some pesticides at the level of LC_{50} on the aetivties of glutamic pyruvic transaminase (unit/L.) in homogenated 2nd and 4th instar larvae of cotton leaf warm

Days after Treatment	Pesticides									
	Control		Chlorpyrifos		Lufenuron		Tebufenozide		Neem T 5%	
	2nd	4th	2nd	4th	2nd	4th	2nd	4th	2nd	4th
1	9.3	29.3	5.33	41.66	35.00	43.33	35.66	55.33	25.00	53.6
3	15.3	88.0	12.33	62.00	35.66	48.00	43.33	67.00	35.66	58.66
7	57.0	86.33	-	49.00	-	-	-	-	47.66	43.00
10	62.0	00.00	-	35.66	-	-	-	-	68.66	38.66
F. test	**	**	*	**	N.S	N.S	*	*	**	**
L.S.D	24.06	31.65	4.11	15.49	-	-	6.11	5.27	27.14	10.58

(-) Not carried out because all individuals dead
(**) Highly significant differences (*) Significant differences
L.S.D leaf significant differences

Table 4. Effects of some pesticides at the level of LC_{50} on the activities of Choline esterase (unit/L.) in homogenated 2nd and 4th instar larvae of cotton leaf warm

Days after Treatment	Pesticides									
	Control		Chlorpyrifos		Lufenuron		Tebufenozide		Neem T 5%	
	2nd	4th	2nd	4th	2nd	4th	2nd	4th	2nd	4th
1	235.0	469.0	235.0	293.5	704.0	1408.0	235.0	469.0	293.5	527.5
3	352.0	497.5	352.0	586.0	1114.5	1700.5	352.0	527.5	704.0	879.5
7	527.5	762.2	527.5	762.5	-	-	-	-	-	1231.5
10	997.0	-	410.5	704.0	-	-	-	-	-	1466.5
F. test	**	**	**	**	**	**	**	*	**	**
L.S.D	152.54	92.52	86.14	96.45	85.19	106.12	76.15	41.66	121.17	116.21

(-) Not carried out because all individuals dead
(**) Highly significant differences (*) Significant differences
L.S.D leaf significant differences

intervals of day time showed distinct fluctuations in male and female moths. The maximal percentages of *in vivo* inhibition of head AchE, which occurred 48 hrs post-exposure to 1350 ppm. Profenofos, were 39.5% and 35.75% for male and females, respectively Khedr *et al.* (2005) studied the biochemical effects of five insect growth regulators (IGRs) namely Cascade, Atabron Consult, Match and Mimic against 2nd and 4th instar larvae of *spodoptera littoralis* (Boisd.) under laboratory conditions. Larvae were fed on castorbean leaves treated with 9 successive concentrations. The obtained results indicated that: A tabron proved to be the most potent insect growth regulator, whereas Mimic was the least toxic one among the tested IGRs against both 2nd and 4th instar larvae. The 4th instar larvae proved to be more sensitive to all the tested IGRs than the 2nd one at all tested concentrations. The tested IGRs decreased the activity of (GOT) while it increased (GPT) activity at 2 days post treatment the 2nd instar larvae. The inverse was true at 5 days post treatment. In case of the 4th in star, the tested IGRs increased the activity of the two enzymes after 2nd 5 days of treatment.

REFERENCES

Aioub, A.A.A., S.A. Raslan, E.A. Gomaa, W.M. Desuky and A.A. Zaki. 2002. Effectiveness of certain insect growth inhibitors on cotton leaf worm. Zagazig. J. Agric. Res.; Vol. 29 No. (1) 247 – 267.

Anonymous. 1990. Castat statistical software, micro-computer program analysis version 4.20 Coharts of tware-Berkeley. GA.

Bonning, B.C. and J. Heming Way. 1991. The efficacy of acetyl. Cholinesterase in organophosphorus and carbamate resistance *inculex pipiens L.* from Italy. Pestic Biochem. Physiol. 40(2): 143 – 148.

El-Deeb, W.M.H., Y.F.E. Ghouneim and E.L. Helmy. 1991. potential use of the Juvenile hormone mimic pyriproxyfen for the control of spodoptera littoralis (Boisd.) 4th Arab congress plant prot. Cairo; 1-5 Dec.

El-Defrawi, M.E., A. Toppozada, N. Mansour and M. Zeid. 1964. Toxicological studies on the Egyptian cotton leaf worm *prodenia litura*. 1. Susceptibility of different larval instar to insecticides. J. Econ. Entomol; 57: 591 – 593.

El-Awa, M.A. and M. Hashem. 2000. Toxicological and biochemical studies on moths of cotton leaf worm, *Spodoptera littoralis* (Boisd.) Alex. J. Agric. Resea. 45 (2): 153 – 166.

Finney. D.J. 1952. Probit analysis astatistical treatment of the sigmodresponse curve. Cambridge Unipress.

Ishaaya, L., A. Novon and E. Gurevitz. 1986. Comparative toxicity of chlorfluazuron (1k1-7899) and cypermethrinto *Spodoptera littoralis*, *Lobesia botrana and Drosophila melanogaster.* Crop protection; 6: 885-388.

Khedr, A.A., S.M. Radwan, S.A. Emava, S.A. Raslan, and S.A. Helalia. 1991. Relative susceptibility of the cotton leaf worm *spodoptera littoralis* (Boisd.). Againsť some chitin synthesis inhibitors. 4th Arab congress plant prot. Cairo; 1-5 Dec.

Khedr, M.M.A., W.M.H. Desuky, S.M.A. El-Shkaa and S.I.Y. Khalil. 2005. Toxicoligical and Biochemical studies on the effect of some insect growth regulators on *spodoptera littoralis* (Boisd). Larvae. Egypt. J. Agric. Res.; 83(2): 539.

Prakash, A. and J., Rao. 1987. Use of chemical as grain protectans in storage ecosystem and its consequences. Bull. Graintech., 25(1): 65-69.

Reitma, S.M.D. and S. Frankel. 1957. A colorimetric method for the determination of serum glutamic Oxaloacetic and glutamic-pyruvic transaminase. Ann. J. Clin-pathol. 28: 56-62.

دراسات توكسيكولوجية وبيوكيميائية لبعض مبيدات الآفات ضد دودة ورق القطن

ياسمين أحمد أحمد[١] – كمال عبد الرازق جوهر[١] – رفعت مصطفي شريف[١]

علـي أحمـد أيـوب[١] – وحيـد محمـود دسوقـي[٢]

١– قسم وقاية النبات – كلية الزراعة – جامعة الزقازيق.

٢– معهد بحوث وقاية النباتات – مركز البحوث الزراعية – الدقي – الجيزة.

أجريت دراسة توكسيكولوجية وبيوكيميائية لمركبات كلوروبيريفوس، ليوفينيورون، تيبيوفينوزيد والنيم ٥% علي العمر اليرقي الثاني والرابع لدودة ورق القطن تحت الظروف المعملية، دلت النتائج علي أن قيم التركيزات التي تقتل ٥٠% من الأفراد المعاملة LC_{50} للعمر اليرقي الثاني كانت ٥,٤ x ١٠$^{-٥}$، ٤,٦٤ x ١٠$^{-٤}$، ١,٢٦٦ و ١٣,٩٥٢ جزء في المليون بينما كانت قيم LC_{50} ليرقات العمر الرابع ٩,١٠٤، ٠,٠٢٥ و ١٦,٩٦٤ و ٢١٥,٠١٥ جزء في المليون للمركبات السابق ذكرها علي التوالي . وكانت مستويات GOT في يرقات العمر الثاني بعد يوم واحد من المعاملة ٨، ٩، ٤٨,٦، ١٥,٠، وحدة/لتر بينما كانت هذه القيم في يرقات العمر الرابع ١٢,٠، ٣٧,٦، ٧٠,٠ ، ٤١,٣ وحدة/لتر نتيجة المعاملة بالمبيدات السابقة علي التوالي. وقيم GPT بعد يوم واحد من معاملة العمر اليرقي الثاني ٥,٣٣، ٣٥,٠ ، ٣٥,٦٦ و ٢٥,٠٠ وحدة/لتر. بينما في يرقات العمر الرابع كانت ٤١,٦٦، ٤٣,٣٣، ٥٥,٣٣ و ٥٣,٨٦ وحدة/لتر للمركبات السابق ذكرها علي الترتيب. وكانت مستويات ChEA في يرقات العمر الثاني بعد يوم واحد من المعاملة ٢٣٥,٠، ٧٠٤,٠، ٢٣٥,٠ ، ٢٩٣,٥ وحدة/لتر بينما في يرقات العمر الرابع كانت القيم ٢٩٣,٥، ١٤٠٨,٠، ٤٦٩,٠، ٥٢٧,٥ وحدة/لتر علي التوالي. لوحظ وجود فروق معنوية وعالية المعنوية بين فترات المعاملة.